PROMOTION PACKAGE

For promotion from the rank of Assistant to Associate Professor

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http://faculty.washington.edu/sr320

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PERSONAL STATEMENT

This statement is intended to provide the context for the remainder of the promotion package. It consists of a brief introduction followed by sections that describe my contributions in the areas of education, service, and research.

Introduction

As an undergraduate I became interested in fish physiology and today I consider myself a comparative physiologist with my focus and approach having evolved over the past decade. Early on, my research interest revolved around better understanding fundamental processes associated with reproduction and growth in finfish. In general, this was accomplished using an experimental approach that provided alternative phenotypes that could be examined at the molecular level to identify underlying mechanisms. For example, a major component of my PhD dissertation work involved characterizing the role of myostatin in somatic growth and development. I was the first to isolate two isoforms of this gene and went on to elucidate the mechanism of action at the transcript and protein level. As part of my USDA Post-Doctoral Fellowship and subsequent position at the Marine Biological Laboratory, I transferred this research framework to shellfish, particularly bivalves. During this time DNA sequencing technology was evolving with the promise of "next-generation sequencing" technology (i.e. Solexa, SOLiD, *454*). Whereas my PhD thesis essentially focused on a single gene (myostatin), today we are able to sequence entire transcriptomes. This transition was also marked by a greater inclusion of questions driving my research. Early on, the majority of my research was based on increasing our understanding of agriculturally important traits. While this impetus continues, studying bivalves has opened up a complementary set of questions. Foremost is how environmental change impacts organismal physiology and in turn, ecological relationships. In the remainder of this personal assessment statement I will highlight accomplishments, discuss challenges, and outline future directions.

Education: Teaching Philosophy

In all the courses I teach, I give the students an opportunity to decide what and how they learn. I find that this improves engagement and students are likely to appreciate other topics in the class. The 300-level course I teach (Biology of Shellfishes) has the least flexibility given the vast amount of material that is covered, however over the past few years I have given students the opportunity to design research projects on the effects of ocean acidification on shellfish. They then go on throughout the quarter to write a paper on their research. This project has always received positive feedback. Other times finding the balance of freedom and structure has been challenging. An example of this is the graduate level course I teach, FISH546: Bioinformatics for Environmental Sciences. The second time this course was offered (2010) I shifted teaching it from a gene-centric point of view to more global analysis of

transcriptomic and genetic differences. I also allowed faculty and post-docs to sit in on the class and let everyone select their data set for analysis. While this experience was great for some students, there was too much variation in baseline knowledge coming into the class that resulted in a relatively low student evaluation. I was able to learn from this and the next time the course was offered (2012) I provided more structure. Though students still had the ability to select their biological focus, I grouped them into two general "pipelines" and set numerous milestones to ensure students kept up to speed. I also facilitated an open science, peer educating system so students could easily share successful workflows. A similar success was realized in FISH441: Integrative Environmental Physiology. For the lab section of this course students work in small groups through the entire quarter to characterize the response of aquatic organisms to environmental stress at the molecular level. This past year (2011) I limited the number of projects to three and spent a lot of time in their development module to ensure the experiment was robust enough to obtain novel results. The students did an excellent job in the course, I received my highest teaching evaluation to date (4.9), and a manuscript was just accepted based on one of the research projects in that course.

Related to my philosophy to provide students with freedom, I also strive to provide students with the confidence that they can educate others either from life experiences or with material they learn in my courses. In lectures I do this by asking questions that are not necessarily content based but asking them to draw an analogy from their own experiences. In other words, the questions are framed where anyone could answer and no responses are incorrect. On the occasions where their responses are limited in their relevancy, I make sure to redirect them without discouraging them from engaging in class discussion in the future. Often there are several students that can add value to class beyond my knowledge base. I make an effort to point this out to the class which I believe helps me become more approachable when students have questions. This teaching approach did take a some time to develop as I recall my first year with limited student interaction. Now students have a clear understanding of my teaching style from the first day of class. There are some students that might not be vocal in lecture but still have plenty to offer their peers. I try to facilitate this by setting up different venues for them to educate their class, as well as the general public. One example is in FISH310: Biology of Shellfishes, where we have a discussion board where I make it clear this is the primary means of class communication. While I monitor it, I rarely respond to questions as other students (often those not necessarily vocal in lecture) will accurately respond. Students also begin to share experiences related to class (visits to beaches, online videos, *etc.*) and engage in insightful conversations. In other courses it teach, students are actively engaging in open science by either having their lab notebooks online, draft manuscripts online, or sharing content of the class using social media. In these instances not only are they educating their peers, but are gaining confidence in the fact that they have knowledge to positively contribute to the science community.

In the end, while I believe I am an effective instructor, I am always looking for new ways to teach students. This has included attending the University of Washington Faculty Fellows Program (2007), Annual Teaching and Learning Symposium (2008-2010), and a Workshop on Discussion in the Classroom (2011). All of these experiences were extremely educational as I have had the chance to interact with teachers from across the campus. In 2009, I presented on my use of *Open Access Electronic Notebook in a Bench Science Laboratory Class* at the Annual Teaching and Learning Symposium (video interview- http://goo.gl/7pH4n). This year I also attended ScienceOnline 2012 in Raleigh, NC which was an amazing opportunity to interact with other scientists, teachers, journalists, librarians, students, bloggers and others "interested in the way the World Wide Web is changing the way science is communicated, taught, and done". I was able to learn about numerous effective tools and ideas used in teaching. Examples include educating undergraduates early on about their ability to teach others, the value of sharing data and performing science in the open, and the implementation of new tools for students to manage data in the classroom. I am continually looking for new educational experiences so that I can continue to improve as an instructor.

Education: Mentoring Graduate Students

Initially I was concerned about the ability to recruit students interested in genomics to a 'fisheries' school. However, I have been pleasantly surprised to have recruited a very good cohort of graduate students in my lab. I started my position in December 2006, following the graduate student application deadline and therefore missing the recruitment window. As a result, my initial graduate student (Mackenzie Gavery) was not able to begin until the Fall of 2008. Each year since the lab has grown in size and I currently serve as thesis advisor for six students (2 Ph.D and 4 Masters). One student (Metzger) has graduated and is currently in the PhD program at the University of British Columbia and a second student (Storer) is expected to successfully defend her Master's thesis this Summer 2012. In mentoring graduate students, my goal is to first provide them with the tools to answer scientific questions, then make sure they do not lose sight of the big picture and keep in mind how their research relates to larger environmental phenomenon. I find the best means to accomplish this is through effective communication.

There is regular communication among our lab facilitated in part by our open access electronic notebooks that are updated on a daily basis. This ensures that I am aware of the research directions as well as any issues which might arise. It is common for me to meet with students on an individual basis once a week. The schedule of lab meetings has varied through the years dependent on the quarter. Recently we have been meeting on a weekly basis. This is an increase in frequency, in part due to the number of students (graduate and undergraduates) and external collaborators. Another form of communication I have implemented with graduate students is having them post their monthly goals on our lab blog

(genefish.tumblr.com). This serves a variety of purposes, but foremost forces the students to think about how they are going to be spending their time.

Graduate students have been very successful in their research efforts. They have presented their research at a number of scientific conferences and two papers have been published with a graduate student as first author. Another paper with a graduate student as first author is in press, and two more have been accepted with minor revisions. Graduate students have also been successful in obtaining awards, fellowships, and funding. Outside of funding from my grants, students have received fellowships from EPA-STAR, NSF OACIS, and the School of Aquatic and Fishery Sciences. Below is a list of graduate students whom I serve as thesis advisor, along with their major accomplishments organized by year.

Graduate Student Advisees

Claire Ellis - Masters (2012-)

Characterizing the DNA methylome in the Pacific oyster

Doug Immerman - Masters (2012-)

Reproductive biology and gamete cryopreservation in sablefish

Andy Jasonowicz - Masters (2012-)

Population genetic characterization of sablefish in the North Pacific

David Metzger - Masters (2010-2012)

Impacts of elevated pCO2 conditions on *Ruditapes philippinarum* larval and juvenile transcriptome

Emma Timmins-Schiffman - PhD (2009-)

The physiological effects of ocean acidification on multiple life history stages of the Pacific oyster, *Crassostrea gigas*

Caroline Storer - Masters (2009-2012) co-chair - Dr. Jim Seeb

Genetic and phenotypic variation in Sockeye salmon

Mackenzie Gavey - PhD (2008-)

The role of epigenetic processes in regulating the response of Pacific oysters (*Crassostrea gigas*) to xenobiotic exposure

Graduate Student Accomplishments

2012

Thurlow C. Nelson Award for Outstanding Student Presentation, 104th National Shellfisheries Association Meeting, Seattle, WA. (Gavery)

NOAA-NWFSC Outstanding Innovation Award (Immerman)

RocketHub Project: *Save oysters from ocean acidification!* Fully Funded - $5,175 http://rkthb.co/6330 (Timmins-Schiffman)

Presentation: Epigenetic Mechanisms as a Source of Phenotypic Plasticity in the Pacific Oyster *Crassostrea gigas*. National Shellfisheries Association, 104th Annual Meeting. March 2012. Seattle, WA. (Gavery)

Presentation: Ocean Acidification Alters Larval Pacific Oyster Growth and Physiology. Association For The Sciences Of Limnology And Oceanography, Salt Lake City, UT. (Timmins-Schiffman)

Presentation: Finding the Physiological Limit: Exposure to Ocean Acidification and Heat Stress in the Pacific Oyster. National Shellfisheries Association, 104th Annual Meeting. March 2012. Seattle, WA. (Timmins-Schiffman)

Presentation: Finding the Physiological Limit: Impacts of elevated pCO2 conditions on the *Ruditapes philippinarum* larval transcriptome. National Shellfisheries Association, 104th Annual Meeting. March 2012. Seattle, WA. (Metzger)

2011

EPA Science to Achieve Results (STAR) Fellowship (Gavery)

NSF OACIS GK-12 Fellowship (Storer)

Presentation: Exploring the Role of DNA Methylation as a Source of Phenotypic Variation in *Crassostrea gigas*. ESF-EMBO Symposium – Epigenetics in Context: From Ecology to Evolution. September 2011. San Feliu de Guixols, Spain (Gavery)

Presentation: Investigating the Role of DNA Methylation as an Epigenetic Mechanism in the Pacific oyster (*Crassostrea gigas*). National Shellfisheries Association, 103nd Annual Meeting. March 2011. Baltimore, MD. (Gavery)

Presentation: Beyond the Genome: Epigenetic Regulation in the Pacific Oyster. Plant and Animal Genome Conference. January 2011 (Gavery)

Presentation: The Effects of Climate Change on Physiology: Pacific Oyster (*Crassostrea gigas*) Larval Response to Environmental Change. Society For Integrative And Comparative Biology, Salt Lake City, UT. (Timmins-Schiffman)

Presentation: Effects of 3 Levels of pCO2 on Early Development of the Pacific Oyster. World University Network Ocean Acidification Workshop, Friday Harbor, WA. (Timmins-Schiffman)

Presentation: Assessment of Manila Clam larval survival and physiology at increased pCO2 levels. World University Network Ocean Acidification Workshop, Friday Harbor, WA. (Metzger)

Presentation: Gene Expression as an Indicator of Environmental Stress in the Pacific Oyster, *Crassostrea gigas*. American Fisheries Society, Seattle, WA. (Timmins-Schiffman)

Presentation: The Effects of Ocean Acidification on Pacific Oyster Larval Development and Physiology. Pacific Coast Shellfish Growers Association, Salem, OR. (Timmins-Schiffman)

Presentation: The development of molecular tools to monitor the physiological response of shellfish to ocean acidification. Pacific Coast Shellfish Growers Association, Salem, OR. (Metzger)

Presentation: Rank and order: evaluating the performance of sockeye salmon SNP assays. American Fisheries Society Annual Meeting. Seattle, WA. (Storer)

2010

Faculty Merit Award, M.S. student, University of Washington School of Aquatic & Fishery Sciences (Gavery)

Student Endowment Travel Award, National Shellfisheries Association (Gavery)

Victor and Tamara Loosanoff Fellowship & John G. Peterson Scholarship, School of Aquatic and Fisheries Science, University of Washington (Gavery)

Summer Institute in Statistical Genetics Student Scholarship (Storer)

SNP Workshop III Young Investigator Award (Storer)

Presentation: DNA Methylation Patterns & Epigenetic Regulation in the Pacific Oyster. PCSGA Annual Meeting. September 2010. Tacoma, WA (Gavery)

Presentation: Pacific oysters & ecosystem health. Aquaculture 2010 / National Shellfisheries Association, 102nd Annual Meeting. March 2010. San Diego, CA (Gavery)

Presentation: Investigations into the Effects of Multiple Stressors on Marine Organisms. Ocean Sciences, 2010, Portland, OR. (Timmins-Schiffman)

Presentation: Characterizing the Response of *Vibrio tubiashii* to Changes in Environmental Conditions. Aquaculture 2010, San Diego, CA. (Timmins-Schiffman)

Presentation: Pacific Oyster Physiological Response to Disease under Variable Environmental Regimes. Pacific Coast Shellfish Growers Association, Tacoma, WA. (Timmins-Schiffman)

Presentation: Endocrine control of growth in coho salmon: validation of a multiplex gene expression assay an quantification of relations between messenger RNA levels and proteins during feeding and fasting. Society for Integrative and Comparative Biology meeting, Seattle, WA. (Metzger)

Presentation: Rapid senescence in sockeye salmon: insights from telomeres. Alaska Salmon Program Symposium, University of Washington. Seattle, WA. (Storer)

Presentation: Genetic and epigenetic variation in sockeye salmon. SAFS Graduate Student Symposium, University of Washington. Seattle, WA. (Storer)

Presentation: Global application of novel SNPs in sockeye salmon, *Oncorhynchus nerka*. SNP Workshop III. Blaine, WA. (Storer)

Presentation: Searching for SNPs: Mining the sockeye transcriptome for novel molecular markers. WA-BC AFS Annual Meeting. Nanaimo, BC. (Storer)

2009

Student Scholarship Award for Applied Science, Pacific Coast Shellfish Growers Association (Gavery)

Best Graduate Student Presentation, Pacific Coast Shellfish Growers Association, Portland, OR (Gavery)

Student Endowment Travel Award, National Shellfisheries Association (Gavery)

Victor and Tamara Loosanoff Fellowship & John G. Peterson Scholarship, School of Aquatic and Fisheries Science, University of Washington (Gavery)

William H. Pierre Sr. Fellowship, School of Aquatic and Fisheries Science, University of Washington (Gavery)

NSF Graduate Research Fellowship Runner-Up (Storer)

Presentation: Pacific oysters and ecosystem health. SAFS Graduate Student Symposium. Nov 2009. Seattle, WA. (Gavery)

Presentation: Pacific oysters as indicators of ecosystem health. PCSGA Annual Meeting. September 2009. Portland, OR. (Gavery)

Presentation: Characterization of prostaglandin pathway genes of the Pacific oyster (*Crassostrea gigas*): Evidence for a role in immune response. National Shellfisheries Association 101st Annual Meeting. March 2009. Savannah, GA. (Gavery)

Presentation: Mining the sockeye transcriptome for novel molecular markers. Alaska Salmon Program Symposium, University of Washington. Seattle, WA. (Storer)

2008

William H. Pierre Sr. Fellowship, School of Aquatic and Fisheries Science, University of Washington (Gavery)

Presentation: Characterization of prostaglandins in the Pacific oyster *Crassostrea gigas*: evidence for a role in the immune response. SAFS Graduate Student Symposium. Nov 2008. Seattle, WA (Gavery)

Education: Mentoring Undergraduate Students

One of my priorities in education is providing opportunities for undergraduates to gain hands-on experience in the lab, working with faculty, research scientists, and graduate students. Based on my own experience I know these opportunities can be significant in a student's chosen professional career. In some cases this means students realize what they do not want to do, and in other cases students often get exposed to new types of science and career options. Below is a list of the undergraduate students I have mentored while at the University of Washington including their project title. Other details such as their current status or awards are also indicated. A complete compilation of student presentations, research proposals, and research papers can be viewed online on our website: faculty.washington.edu/sr320

2012

Harry Podschwit; UW Applied Mathematics Independent Study
Developing new computational approaches for pattern discovery in the oyster genome

Bradley Chi; UW SAFS Capstone
The transcriptomic response of Olympia oysters to altered photoperiod and mechanical stress

Derek Brady; UW SAFS Capstone
Acute exposure to 17-alpha-ethinyl-estradiol and its effect on estrogen receptor and vitellogenin expression in the pacific oyster

Manel Khan; UW Post-graduate
Vitellogenin expression in oysters
- Pharmacy School

2011

David Berman; UW SAFS Capstone
A Study in Hydroponics and Aquaculture Integration

Herschel Cox; UW SAFS Capstone
Defensin: An Oysters First Defense

Lexie Miller; UW Biology Independent Study
QPX Virulence Factors

Jason Tayag; UW SAFS Capstone
The effects of low pH on telomerase gene expression in juvenile sockeye salmon, *Oncorhynchus nerka*

Sonia Albin; UW SAFS Capstone
DNA Methylation Characterization of Sockeye Salmon

2010

Zac Hall; UW Work-Study
Shellfish husbandry

Amanda Davis; UW Biology Independent Study
Effect of 5-azacytidine on Global DNA Methylation

Christina Miller; UW Biology Independent Study
Ocean acidification and mechanical stress
Rony Thi: UW Work-Study
Response of fish to low pH

2009

Anna Fabrizio; UW SAFS Capstone
Chemotaxis And Foraging Behavior in *Octopus rubescens*
Kevin Jeong; UW Biology Independent Study
Hemocyte characterization in oysters
- Medical School
Rachel Thompson; UW SAFS Capstone
Development of non-invasive stress biomarkers in octopuses
- Awarded Mary Gates Research Scholarship; USGS Scientist
Leslie Jensen; UW SAFS Captonse
Rapid senescence in sockeye salmon (*Oncorhynchus nerka*)
Christin McLemore; UW SAFS Captone
Identification and Isolation of Stress Related Genes in Grey and Fin Whales
- Fish and Wildlife State Agency

2008

Tatyana Marushchak; UW Chemistry Independent Study
Vibrio tubiashii proteomic analysis
Stephannie Spurr; UW SAFS Independent Study
Microbial characterization in black abalone exposed to withering syndrome
Katie Fulkerson; UW SAFS Capstone
A Comparison of Growth and Gene Expression in Two Species of Oysters
Cullen Taplin; UW SAFS Capstone
Characterization of a toll-interacting protein gene in black abalone (*Haliotis cracherodii*)
- Medical School

2007

Juliann Clark; UW Work-Study
Educational Shellfish Collection Database - http://goo.gl/ahHan
Tushara Saint Vitus; UW SAFS Capstone
Interleukin-17 expression in *Crassostrea gigas* following *E. coli* exposure
Lindsay Braun; Visiting Undergraduate - Santa Clara University
Immune-related gene discovery and expression in black abalone exposed to withering syndrome

Education: Outreach and Science Communication

While maybe not traditionally considered a component of faculty responsibilities, I consider engaging with persons outside of Academia to be a core aspect of my program. This is an aspect that I try to integrate into research, classroom teaching, and mentoring. Central to this is the *open science* approach that is followed in our research and teaching labs. Everyone in my lab (including myself) maintains online lab notebooks that are accessible to the public at genefish.wikispaces.com. Other platforms that are maintained include blogs and twitter accounts. A summary of venues where we strive to share our research and data with the public is provided below. My current approach to outreach is to simply "raise the curtain" on what we are already doing. For instance, we document our research, produce data, teach others, and read interesting science articles - why not share them? As scientists in the "big data" era, it is likely others can make use of our datasets, and vice-versa. My hope is that by educating others on the value of *open science*, we will be able to address more complex and exciting research questions. In the future I would like to make time to be able to dedicate efforts to produce more end-user directed activities and provide more opportunities for the public and stakeholders to engage with our lab. One of the challenges to this will be time and resources. To that end, I am currently serving on the *College of the Environment Communication Task Force* to identify and prioritize recommendations for the Dean's Office to facilitate science communication in the College.

Platform	Purpose	URL	Metrics
Website	Primary Lab Website	faculty.washington.edu/sr320	2 months - 216 unique visitors
Wiki	Share online lab notebooks and protocols, lab communication	genefish.wikispaces.com	2012 - 20k views
Blog	Lab Tumblr	genefish.tumblr.com	12 months - 6137 visits
Facebook	Shares Lab Tumblr posts	goo.gl/pOitK	47 likes; 11k Friends of Fans
Blog	Blog specifically documenting our Ocean Acidification Research	safsoa.wordpress.com	2012 - 1645 views
Twitter	Automatic lab feed - notebook entries, posts, events	twitter.com/genefish	17,330 tweets
Twitter	Personal twitter account	twitter.com/sr320	1491 tweets
Flickr	Sharing research images	goo.gl/yzwE7	2726 photos
Youtube	Primarily host instructional videos	youtube.com/user/srlab	7351 views
Scribd	Share documents, primarily undergrad student papers	scribd.com/sr320	9908 reads
Figshare	Sharing datasets and documents	goo.gl/umGFg	4947 views
Slideshare	Sharing presentations	slideshare.net/sr320	49 slideshares

Service

I have had the privilege of serving various roles in professional and scientific organizations. These multi-year commitments include serving on the Board of Directors for the Pan-American Marine Biotechnology Association, Executive Committee for the National Shellfisheries Association, and Steering Committee for the NSF Research Coordination Network: Evaluating the Impacts of a Changing Ocean on Management and Ecology of Infectious Marine Diseases. My longest tenure has been with the Pan-American Marine Biotechnology and has been particularly rewarding, as much of our activity focuses on providing information and financial awards to members with limited resources. Over the past several years I have also been selected by federal agencies and industry groups to serve on review panels and organize national workshops. This has included the 2010 Meeting of USDA WERA099: Broodstock Management, Genetics and Breeding Programs for Molluscan Shellfish (rebranded "Genetics and Breeding of Shellfish Workshop" in 2012). This is a yearly meeting of shellfish growers and scientists from the United States and overseas where we discuss common issues and ways we can share information to advance the shellfish aquaculture industry in a sustainable manner.

Within the School of Aquatic and Fishery Sciences I have served on all major committees (see *Curriculum Vitae* for details). My most significant contribution to the School's operations has been serving on the Recruitment and Scholarship Committee from 2008-2011 and as Chair of the Computing Committee since 2010. In the Computing Committee my goal has been to simplify our computing policy and make resources more available to our students, staff, and faculty. Similar to my approach with outreach and research, I have made our discussion (meeting minutes), policies, and resources more easily accessible online.

As part of the my research program moving to a systems-based approach (see *Research* section for details), coupled with advances in technology that facilitates greater access to genomic data, there was a need for local infrastructure within our School. In collaboration with a group of faculty (Seeb, Seeb, Naish, Hauser), we successfully acquired a computer cluster and associated software. My role in the venture included the identification of appropriate resources. Since installation in 2008 I have been solely responsible for continued maintenance and providing services to the SAFS community. This includes regular updates, disk storage management, and managing user accounts for our major suites of software.

Our school has been fortunate to have received a number of valuable shellfish collections prior to my arrival. As part of my interest in invertebrates and teaching FISH310: Biology of Shellfishes, I developed on online database portal catalog so persons outside of the school are able to learn and see some of the over 4000 specimens in the Colton Memorial Shellfish Collection (http://genefish.wikispaces.com/Colton). This has included scanning original documentation and photographing select samples. The collection database has been a great resource for teaching purposes and has also garnered external interest. For example, this

Spring I received a request from the Natural History Museum of Los Angeles County to have access to samples they found on our website. I was able to supply detailed photographs and documentation regarding the location of collection. In the future I would like to make this collection more accessible to the public and am currently considering ways to use new web platforms to disseminate images and information more effectively.

Research

The focus of my research program has been to gain a better understanding of aquatic organism physiology using a comparative approach. This has included research into basic biological function as well as complex responses to large scale environmental change. We are constantly riding the wave of technological innovation that is allowing us to make novel discoveries at a much finer resolution and examine biology at a truly integrative level. Below I provide a list of what I consider to be my most significant research contributions while at the University followed by research summaries and future research directions.

Significant research contributions

- Development of genomic resources and approaches for numerous aquatic species
- Describing DNA methylation patterns in shellfish
- Producing a novel theory on the role of epigenetics in phenotypic plasticity
- Integrating genomic approaches into traditional ecological studies

One of my significant research contributions is the development and implementation of high-throughput sequencing technology to characterize transcriptomes in non-model organisms. This effort began with finfish and in collaboration with other researchers examining issues related to genetic signatures of the Exxon Valdez Oil Spill, single nucleotide polymorphism marker discovery, and immune function of erythrocytes. Each of these cases represents a collaboration of researchers from complementary disciplines (*i.e.* population geneticists, immunologists, physiologists, fish biologists). These initial studies used 454 pyrosequencing technology, one of the three original "next-generation" DNA sequencing technologies. Products from these research efforts include the first large scale sequencing effort for a member of the teleostean order Clupeiformes, a novel role of piscine red blood cells in immune function, and a basis for phenotypic differentiation in lake trout.

A significant milestone in the area of genomic resource development was demonstrating ultra-short read sequencing technology (*i.e.* SOLiD) can provide an effective means for gene discovery and expression analysis in organisms with limited genomic resources. We showed that it is technically possible and efficient to use this approach to 1) generate transcriptomic resources, 2) identify novel genes, and 3) perform RNA-Seq analysis. Even though there have been a number of Sanger-based transcriptome sequencing projects, novel transcripts were still identified in our model system- the Pacific oyster. In this research, RNA-Seq analysis was carried out on oyster populations exposed to varying degrees of anthropogenic impact. Gene enrichment analysis determined that in addition to biological processes predicted to be associated with anthropogenic influences (*e.g.* immune response), other processes play important roles including cell recognition and cell adhesion. Impact of this manuscript is evident as *it is the #1 most downloaded paper in Comparative Biochemistry and Physiology, Part D*

during 2012. Currently our research group is using this approach to elucidate the physiological response of a number of marine invertebrates (*i.e.* clams, abalone, oysters, corals) to environmental stressors.

Related Manuscripts

Roberts SB, Hauser L, Seeb LW, Seeb JE (2012) Development of genomic resources for Pacific herring through targeted transcriptome pyrosequencing. PLoS ONE *7*(2): e30908. doi:10.1371/journal.pone.0030908

Gavery MR* and Roberts SB. (2012) **Characterizing short read sequencing for gene discovery and RNA-Seq analysis in *Crassostrea gigas*.** Comparative Biochemistry and Physiology Part D: Genomics and Proteomics, Available online 29 December 2011. doi:10.1016/j.cbd.2011.12.003

Morera D, Roher N, Ribas L, Balasch JC, Doñate C, Callol A, Boltaña A, Roberts SB, Goetz G, Goetz FW, Mackenzie SA. (2011) RNA-Seq Reveals an integrated immune response in nucleated erythrocytes. PLoS ONE 6(10): e26998. doi:10.1371/journal.pone.0026998

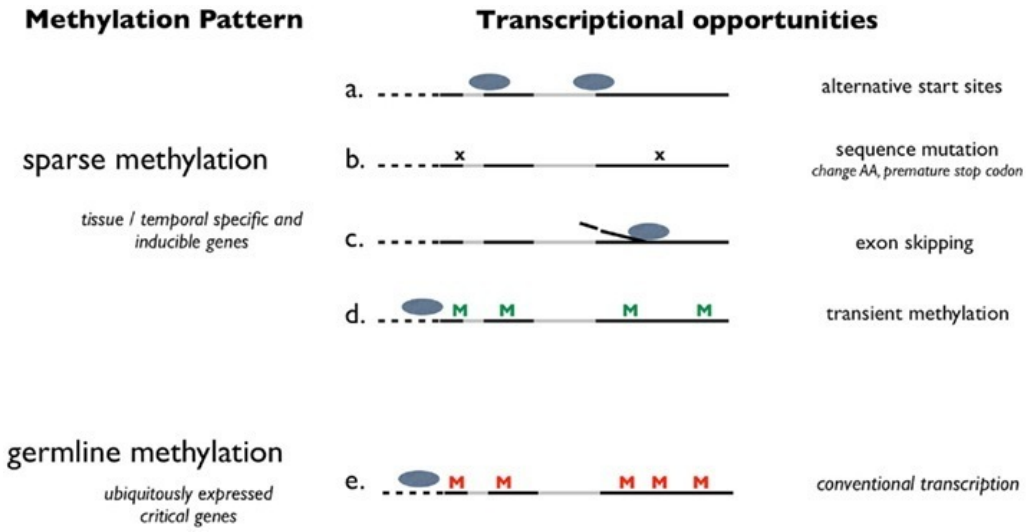
Goetz FW, Rosauer D, Sitar S, Goetz G, Simchick C, Roberts SB, Johnson R, Murphy C, Bronte C, Mackenzie S. (2010) A genetic basis for the phenotypic differentiation between siscowet and lean lake trout (*Salvelinus namaycush*). Molecular Ecology, 19 176–196

Seeb JE, Pascal CE, Graue ED, Seeb LW, Templin WD, Harkins T, Roberts SB. (2010) Transcriptome sequencing and high-resolution melt analysis advance single nucleotide polymorphism discovery in duplicated salmonids. Molecular Ecology Resources.

Overall, my research into epigenetic processes is the most significant contribution I have made and will have the largest impact to science in the long-term. Epigenetics refers to processes capable of inducing changes in genetic activity without altering the underlying DNA sequence. DNA methylation is one type of epigenetic process and is currently the focus of our research. DNA methylation is prevalent across taxa, however the occurrence, landscape, and function is amazingly diverse. In 2010 we reported the first investigation into DNA methylation profiles in the genome of the Pacific oyster. We also demonstrated a relationship between predicted methylation status and gene function, suggesting that DNA methylation performs important regulatory functions in *Crassostrea gigas*.

Based on the discovery that DNA methylation patterns were strongly associated with gene function and the fact that methylation was present in a mosaic, intergenic pattern I developed a theory regarding the putative function of DNA methylation. This theory, referred to as the Methylation Enhanced Random Variation Theory, suggests that the absence of germline methylation in genes involved in effectively adapting to heterogeneous conditions, facilitates random variation that contributes to phenotypic plasticity and increased adaptive potential. In other words, in species that can be exposed to a wide variety of selective pressure, the DNA methylation system has evolved to increase survival. It is expected that genes that lack germline methylation will have a larger number of "transcriptional opportunities" as compared to the ubiquitously expressed, critical genes. One "opportunity" or variation that is expected is

the production of alternative transcripts that might arise from 1) alternative start sites, 2) alternative stop codons, and/or 3) alternative combinations of exons. We also expect that there will be more sequence variation in this suite of genes, which could contribute to increased phenotypic plasticity. In addition, there is the opportunity for these genes to be transiently methylated, which would also influence transcription. Conversely, germline methylation limits mutation and alternative splicing in critical genes that are core to survival.



Schematic representation of a how DNA methylation potentially influences transcriptional activity in invertebrate species. This theory proposes the absence of germline methylation (sparse methylation) contributes to adaptive potential by allowing for multiple transcriptional opportunities. Transcriptional opportunities are diagrammed for genes with sparse methylation (a–d) and genes methylated at the germline (e). Dashed lines represent the 5′ UTR, solid lines represent exons and gray lines indicate introns. "M" designates a methylated CpG. "x" Represents a sequence mutation. Ovals represent putative promoter complexes. [Figure reproduced from Roberts and Gavery 2012]

The theory proposes that the absence of germline methylation affords the organism an increased adaptive potential by facilitating random variation in a portion of the genome responsible for maintaining homeostasis under selective pressure. The mechanisms described under this theory may only pertain to select lineages. These mechanisms would be advantageous in species such as marine invertebrates, where planktonic larvae are at the mercy of the currents and adults live in fluctuating, heterogeneous environments. On a larger time scale, DNA methylation will likely play a significant role in the ability of species to respond to global climate change by increasing the probability of successful adaptation compared to the expectations based on conventional genetic theory alone. Thus, a better understanding of the phenomenon and evaluation of this working theory will not only provide important information on molecular processes but will also improve our ability to predict

ecosystem responses. Research designed to test the Methylation Enhanced Random Variation Theory was recently funded by the National Science Foundation (*DNA Methylation as a Mechanism to Increase Adaptive Potential in Invertebrates*). This is a two year award that will support multiple graduate students and use a combination of high-throughput sequencing and microarray approaches to investigate DNA methylation using the Pacific oyster as a model system. The grant was my first application to the National Science Foundation for funding as a principal investigator. A detailed review of this theory was recently published in Frontiers in Physiology (*Is there a relationship between DNA methylation and phenotypic plasticity in invertebrates?* Roberts and Gavery 2012). In short I predict that for certain species, epigenetics will dramatically alter how we consider population genetics and organismal physiology. In addition to the ongoing NSF funded research, a research proposal to the NOAA Aquaculture program was recently funded (September 2012). This project is designed to evaluate the epigenetic population structure associated with local adaptation in the native oyster, *Ostrea lurida*.

Related Manuscripts

Roberts SB and Gavery MR (2012) Is there a relationship between DNA methylation and phenotypic plasticity in invertebrates? Frontiers in Physiology. 2:116. doi:0.3389/fphys.2011.00116

Gavery MR* and Roberts SB. (2010) DNA methylation patterns provide insight into epigenetic regulation in the Pacific oyster (*Crassostrea gigas*). BMC Genomics 11:483

Research into epigenetic processes increased the molecular expanse of my research program. In the past, the transcript (or transcript product) was the primary focus. In characterizing the epigenome there was a need to go beyond exons and interrogating non-coding regions. This meant that we essentially had to assemble the genome. I constructed draft genomes using publicly available raw sequencing reads and our labs own data. While extremely crude in nature, I was able to develop a series of draft genomes that have been very helpful in our analyses. I have made these assemblies and associated genomic feature sets publicly available (http://genefish.wikispaces.com/crassostreome), and gave a presentation on this effort on how it could be used by other researchers at the National Shellfisheries Association Conference this year. The ultimate goal of this effort is to be able to visualize a variety of data including methylation, expression patterns, and genetic features so that functional relationships can be identified. Currently there is an undergraduate student from the University of Washington's Applied and Computational Mathematics program (Harry Podschwit) who is developing new computational approaches for pattern discovery.

My research program going forward will continue to incorporate new levels of characterization expanding on the relationship of the transcriptome, genetics, and epigenetics.

This is an exciting time in the discipline of comparative environmental physiology as technological innovations are allowing us to abandon the gene-centric approach and study processes from the system level. Granted this will come with challenges but in the end we will have a better understanding of the organism physiology what we can use to infer ecological change, particularly in dynamic environments. Not only will we be looking at entire transcriptome changes on an individual basis in the near future, but we will be overlaying individual epigenomes and genetic landscapes to determine the connection. From there, we will delve down the spatial and temporal scale to examine the dynamics at the cellular level and throughout developmental stages.

The more we learn about epigenetics, the more we will see that these processes are key targets for species in their ability to adapt. I have outlined above how DNA methylation is theorized to play a role in phenotypic plasticity, not readily quantified before, but this is just scratching the surface. We have yet to characterize how short RNAs, histone modifications, and other epigenetic phenomenon regulate biological process in aquatic invertebrates.

Just as I indicated six years ago, when I was interview for this position- the line that divides "field" and "bench" biologist will continue to disappear. It will be accepted that if you want to understand the ecological relevance of salmon senescence you should be characterizing telomere length; if you want to assess changes in benthic communities related to hypoxic events you should measure transcriptomic responses; if you want to define fisheries management units you should know the DNA methylation patterns of surrounding stocks. I believe these are good examples of a fundamental shift in the research community and am currently working with colleagues to achieve this *new* paradigm in environmental science.

CURRICULUM VITAE – STEVEN BEYER ROBERTS

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web: faculty.washington.edu/sr320

Education

Ph.D. – University of Notre Dame (South Bend, IN) – 2002
Biological Sciences
Dissertation title: Characterization of growth hormone in yellow perch and myostatin in several teleost species

B.S. – North Carolina State University (Raleigh, NC) – 1997
Natural Resources – Concentration in Marine and Coastal Resources

Employment

2007-Present · Assistant Professor
University of Washington, Seattle,WA

2007-Present · Adjunct Assistant Scientist
Marine Biological Laboratory, Woods Hole, MA

2003-2006 · Assistant Research Scientist
Marine Biological Laboratory, Woods Hole, MA

Research Grants & Contracts

Summary

Total Research Funds - $3.5 Million
Research Funds to University of Washington - $2.5 Million
Primary Funding Agencies - USDA, NOAA, NSF
Graduate Students Supported - 8

Awards

Title: Alleviating Regulatory Impediments to Native Shellfish Aquaculture
Source of Support: NOAA Aquaculture Program
Award Amount: $427,371
Award Period: 9/1/2012 - 8/30/2014
Time Committed: 2.0 months
Student Support: New Student
Role: PI

Title: Sablefish Reproductive Life History and Genetics
Source of Support: NOAA / JISAO
Award Amount: $189,869
Award Period: 9/15/2012 - 6/30/2013
Time Committed: 0.4 months
Student Support: Doug Immerman, Andrew Jasonowicz
Role: PI

Title: DNA Methylation as a Mechanism to Increase Adaptive Potential in Invertebrates
Source of Support: National Science Foundation
Award Amount: $243,090
Award Period: 5/1/2012 - 4/30/2014
Time Committed: 3.0 months
Student Support: Claire Ellis
Role: PI

Title: Sablefish Broodstock Development and Functional Genomics
Source of Support: NOAA [Contract]
Award Amount: $349,407
Award Period: 9/15/2011 - 9/14/2013
Time Committed: 2.0 months
Student Support: Doug Immerman, Andrew Jasonowicz
Collaborators: Frederick Goetz
Role: PI

Title: Effects of Ocean Acidification on Declining Puget Sound Molluscan Calcifiers
Source of Support: Washington Sea Grant
Award Amount: $400,300
Award Period: 2/1/2010 - 6/30/2013
Time Committed: 1.0 months
Student Support: Dave Metzger, Liza Ray, Emma Timmins-Schiffman
Collaborators: Carolyn Friedman
Role: Co-PI

Title: Threats to Bivalve Aquaculture and Fisheries: The Influence of Emerging Diseases and Environmental Change
Source of Support: NOAA
Award Amount: $243,000
Award Period: 9/1/2009 - 2/29/2012
Time Committed: 2.0 months
Student Support: Elene Dorfmeier and Emma Timmins-Schiffman
Collaborators: Ralph Elston, Carolyn Friedman
Role: PI

Title: Evaluation of Putatively QPX-Resistant Strains of Northern Hard Clams Using Field and Genetic Studies
Source of Support: USDA-NRAC
Award Amount: $79,503
Award Period: 3/1/2008 - 2/28/2011
Time Committed: 1.0 months
Student Support: Dave Metzger and Caroline Storer
Collaborators: John Kraeuter, Dave Bushek, Scott Lindell
Role: Co-PI

Title: High Resolution SNPs for Sockeye Salmon
Source of Support: Alaska Sustainable Salmon Fund
Award Amount: $313,523
Award Period: 7/1/2009 - 6/30/2010
Time Committed: 1.0 months
Student Support: Caroline Storer
Collaborators: Jim Seeb, Lisa Seeb, Chris Habicht
Role: Co-PI

Title: High Density DNA Sequencing to Detect Population Structure of Pacific Herring
Source of Support: Exxon Valdez Oil Spill Fund
Award Amount: $40,000
Award Period: 7/1/2009 - 6/30/2010
Time Committed: 1.0 months
Collaborators: Jim Seeb, Lisa Seeb, Lorenz Hauser
Role: Co-PI

Title: Enumeration of viable organism
Source of Support: Ballast Water Management Demonstration Program
Award Amount: $200,216
Award Period: 10/1/2008 - 12/1/2009
Time Committed: 2.0 months
Collaborators: Russ Herwig
Role: Interim PI

Title: Genome Sequencing of *Cycloclasticus pugetti*, a widely distributed Marine PAH-Degrading Bacterium
Source of Support: Joint Genome Institute
Award Amount: In-kind Genome Sequencing
Award Period: 6/1/2008 - 6/1/2011
Time Committed: 0.5 months
Collaborators: Russ Herwig, Mark Strom
Role: Co-PI

Title: Development of tools to monitor and predict outbreaks of *Vibrio tubiashii*
Source of Support: UW SAFS
Total Award Amount: $24,644
Total Award Period: 7/30/2008 – 6/30/2009
Time Committed: 0.5 months
Student Support: Undergraduate Student
Collaborator: Carolyn Friedman
Role: Co-PI

Title: Development and implementation of quantitative assay for Dermo detection in oysters
Source of Support: USDA-NRAC (subaward from Marine Biological Laboratory)
Award Amount: $12,798
Award Period: 6/1/2008 − 7/16/2008
Time Committed: 1.0 months
Student Support: Mackenzie Gavery
Collaborators: Scott Lindell (MBL)
Role: PI

Title: Assessing withering syndrome resistance in California Black Abalone: Implications for conservation and restoration
Source of Support: California Sea Grant
Award Amount: $15,067
Award Period: 6/1/2007 - 5/30/2008
Time Committed: 0.5 months
Collaborators: Carolyn Friedman
Role: Co-PI

Title: Production of Myostatin Gene Knockouts in Zebrafish, and the Effects of Specific Myostatin Interacting Proteins on Salmonid Muscle Growth
Source of Support: USDA - NRI
Award Amount: $472,840 (UW - $92,255)
Award Period: 1/1/2005 - 11/30/2008
Time Committed: 4 months
Collaborators: Frederick Goetz, Paul Collodi
Role: Co-PI

----------Appointment at University of Washington

Title: Development of genetic markers to assess disease resistance in the eastern oyster
Source of Support: USDA / Northeastern Regional Aquaculture Center
Award Amount: $154,066

Award Period: 9/1/2006-1/30/2008
Time Committed: 4 months
Collaborators: Roxanna Smolowitz and Rick Karney
Role: PI

Title: The spread of lobster shell disease – genetic and social barriers
Source of Support: NOAA / Rhode Island Sea Grant
Role: Collaborator
Award Amount: $150,991
Award Period: 03/01/2007 – 12/31/2008
Time Committed: 0.2 months
Collaborators: Jella Atema and Gabriel Gerlach
Role: Co-PI

Title: Development of Diagnostic and Management techniques to select cod broodstocks and hatchery stocks free from nodavirus
Source of Support: USDA / Northeastern Regional Aquaculture Center
Award Amount: $124,612
Award Period: 10/01/2003 - 10/01/2005
Time Committed: 2 months
Role: PI

Title: Isolation and characterization of factors regulated during larval competence and metamorphosis in the bay scallop, *Argopecten irradians.*
Source of Support: USDA-NRICGP
Award Amount: $89,934
Award Period: 11/01/2002 - 10/31/2004
Time Committed: 24 months
Role: PI

Service

Profession-level

Executive Committee: National Shellfisheries Association; Member-at-large (2011-2014)
Board of Directors: Pan-American Marine Biotechnology Association (2009-present)
Panel Member: NSF-Evolutionary Processes (2011), USDA-SBIR (2007)
Review Panel Member: Visioning Workshop- Challenges and Opportunities for Genetic Improvement of the Pacific Oyster on the U.S. West Coast OSU/NIFA Molluscan Broodstock Program and USDA-ARS Shellfish Genetics Program.
Steering Committee: NSF Research Coordination Network: Evaluating the Impacts of a Changing Ocean on Management and Ecology of Infectious Marine Disease (2012-2015)

Service continued

Workshops and Conferences:

Plant and Animal Genome (PAG) XIX Workshop Program Committee 2013
Chair Elect

2012 Genetics and Breeding of Shellfish Workshop. March 28, 2012. Seattle, WA.
Co-Organizer

California-Current Acidification Network OA-Shellfish Workshop. December 13–14, 2011. Palo Alto, California.
Participant

Pacific Coast Shellfish Growers Association Annual Conference, September 19-22, 2011. Salem, Oregon.
Session Organizer and Moderator (Ocean Acidification)

2010 Meeting of USDA WERA099: Broodstock Management, Genetics and Breeding Programs for Molluscan Shellfish. Feb 28 - March 1, 2010 San Diego, CA. https://catalysttools.washington.edu/workspace/sr320/8608/
Organizer

SNP Workshop III, Blaine, WA. March 2010. http://www.snpworkshop.org/
Co-Organizer

Reviewer for:

Animal Genetics, AJP- Regulatory; Integrative and Comparative Physiology, BARD, the United States - Israel Binational Agricultural Research & Development Fund; Aquaculture, Aquaculture Research, Biology Letters; BMC Genomics; Comparative Biochemistry and Physiology; Ecological Applications; Fish Physiology and Biochemistry; Gene; Genome British Columbia: Science Opportunity Fund; Journal of Molluscan Studies; Journal of Shellfish Research; Journal of the World Aquaculture Society; Maine Sea Grant; Marine Biotechnology; Maryland Sea Grant; National Sea Grant College Program: Oyster Disease Program; New Hampshire Sea Grant; NOAA- Northwest Regional Office Internal Grants Program; National Science Foundation, PLoS ONE; Transactions of the American Fishery Society, USDA-ARS Internal Manuscript Review, USDA-SBIR Aquaculture Review Panel; USDA National Research Initiative: Animal Growth and Nutrient Utilization; USDA National Research Initiative: Animal Reproduction

Service continued

College-level

Communications Task Force 2012-present

School-level

Curriculum Committee 2007-2008; 2011-present
Recruitment and Scholarship Committee 2008-2011
Computing Committee 2010-present (*Chair*)
https://catalyst.uw.edu/workspace/sr320/18102
Graduation Committee 2010-present
Ken Chew Professorship Committee (2009-present)
SAFS Genomic Computing Cluster Maintenance 2009-present
Young Investigator Seminar Series Organizer 2008-2009
Graduate Student Symposium Judge 2009, 2010, 2011

Invited Presentations

•Short-read sequencing used for genomic characterization in aquacultured shellfish. National Shellfisheries Association Conference. Seattle, WA. March 2012.
http://goo.gl/zqWLI
Slideshare: 824 views

•Short-read sequencing used for genomic characterization in aquacultured shellfish. Plant and Animal Genome XX. San Diego, CA. January 2012.
http://goo.gl/QApPx
Slideshare: 578 views

•Shellfish as indicators of environmental health. Gordon Research Conference: Oceans and Human Health. University of New England, Biddeford, ME. June 2010
http://goo.gl/utD8C
Slideshare: 958 views

•SNP Discovery in Pacific Herring using transcriptome sequencing. SNP Workshop III, Blaine, WA. March 2010.
http://goo.gl/sz7vo
Slideshare: 499 views

•Changes in the environment and changes in expression: insight from oysters. USC Marine Environmental Biology Seminar Series. University of Southern California, Los Angeles, CA. February 2, 2010
Slideshare: 293 views

Invited Presentations *continued*

•Functional genomic approaches to better understand shellfish-pathogen-environment interactions. American Fisheries Society Annual Meeting. Nashville, TN. September 1, 2009. Slideshare: 396 views

•Genomic approaches in aquaculture research. pre-BIO 2009 Symposium; Opportunities for UW / University of Queensland Australia Collaboration, Seattle WA, May 14, 2009.

•Overview of Shellfish Activities at the University of Washington. USDA-WERA099: Broodstock Management, Genetics and Breeding Programs for Molluscan Shellfish. March 21, 2009. Savannah, GA

•Characterizing the response of *Vibrio tubiashii* to changes in environmental conditions: a funomic approach. National Shellfisheries Association Conference. Savannah, GA. March 23, 2009.
http://goo.gl/7Z8Sh
Slideshare: 1028 views

•Analysis of genes isolated from plated hemocytes of the Pacific Oyster. National Shellfisheries Association Conference. Savannah, GA. March 24, 2009.

•Characterization of trout myostatin interacting proteins on primary muscle cells. Eighth International Congress on the Biology of Fish. Portland OR. July 29 2008.

•Immune response in shellfish. USGS Western Fisheries Research Center, Seattle WA. March 4, 2008.

•Disease tolerance and immune response in oysters. Washington Resource Agencies: WA Department of Fish and Wildlife, WA Department of Natural Resources, and WA Department of Ecology. Olympia, WA. March 26, 2008.

•Gene expression profiling and cellular characteristics of *Crassostrea virginica* hemocytes: evaluating interactions of physical stress and disease exposure. Aquaculture 2007, San Antonio, TX. March 1, 2007.

•Characterizing myostatin function in salmonids: examining post- translational processes and protein interactions. Aquaculture 2007, San Antonio, TX. February 28, 2007.

•The potential of genomics in aquaculture: current science. 2006 MIT Environmental Fellows Retreat. Woods Hole Oceanographic Institution. Woods Hole, MA. November 3, 2006,

•Genomic approaches in characterizing shellfish disease: interrelationships between animal, human and ecosystem health. Cummings School of Veterinary Medicine at Tufts University,

Invited Presentations *continued*
Annual Symposium: Marine and Aquatic Medicine & Conservation. North Grafton, MA. April 22, 2006

•Characterization of differentially expressed genes from QPX: insight into possible virulence mechanisms. National Shellfisheries Association Annual Meeting, Monterey, CA. March 28, 2006 .

•Overview and application of bay scallop genomics resources. National Shellfisheries Association Annual Meeting, Monterey, CA. March 27, 2006.
http://goo.gl/7BNTr
Slideshare: 1572 views

•Molecular physiology and genetic characterization of aquatic organisms. Institute for Science and Interdisciplinary Studies (ISIS) Fellows Program: Southern Connecticut State University, Woods Hole, MA. March 11, 2005.

BIBLIOGRAPHY - STEVEN BEYER ROBERTS

I have published 36 peer-reviewed manuscripts and currently have 5 manuscripts in review. Of the published manuscripts, I am first author on 14 and principal author on 5 manuscripts. Publications where I am considered principal author are manuscripts where students (indicated with an asterisk) are first and I am the last author. In these cases I contributed to experimental design, data analysis, and writing of the manuscript. For manuscripts where I am neither first author or principal author I played a lesser role in the manuscript, usually contributing one aspect to a collaborative project and my role would involve limited experimental implementation as well as analysis and writing limited to my contribution. An example would be carrying out RNA-seq analysis in a larger project that also examined morphometrics, cell function, or population structure in fish. The latter would be carried out by my co-authors. To provide an indication of what areas I contributed to manuscripts, the following codes are used: I = Experimental or Conceptual Ideas, F = Funding, E = Experimental Implementation, A = Analysis, W = Writing and/or Critical Editing. A summary of my citation indices as provided by Google Scholar are listed as well as a list of all manuscripts organized by date. Where data was available I have provided metrics including manuscript views and citations.

Citation indices

	All	Since 2007
Citations	595	461
h-index	15	14
i10-index	19	16

In press

Timmins-Schiffman E*, O'Donnell MJ, Friedman CS, Roberts SB. Effects of elevated pCO2 on early growth and calcification of the Pacific oyster, *Crassostrea gigas*. Marine Biology [I F W]

Storer CG*, Pascal CE, Roberts SB, Templin WD, Seeb LW, Seeb JE. Rank and order: evaluating the performance of SNPs for individual assignment in a non-model organism. PLoS ONE (revised) [F A]

Timmins-Schiffman E* and Roberts SB. Evidence of a lipid signaling molecule involved in the stress response in the Pacific oyster, *Crassostrea gigas*. BMC Research Notes [I F W]

Metzger DC*, Pratt P, Roberts SB. (2012) Characterizing the effects of heavy metal and *Vibrio* exposure on hsp70 expression in *Crassostrea gigas* gill tissue. Journal of Shellfish Research. [I F A W]

Burge CA, Douglas N, Conti-Jerpe I, Weil E, Roberts SB, Friedman CS, CD Harvell. (2012). Friend or foe: the association of Labyrinthulomycetes with the Caribbean sea fan, *Gorgonia ventalina*. Diseases of Aquatic Organisms. doi:10.3354/dao02487 [I A]

2012

Roberts SB, Hauser L, Seeb LW, Seeb JE (2012) Development of genomic resources for Pacific herring through targeted transcriptome pyrosequencing. PLoS ONE 7(2): e30908. doi:10.1371/journal.pone.0030908 [I F E A W]
Pages: 10
Article Views: 1075
Supplemental Data Views: 3792 (*most viewed on Figshare*)

Roberts SB and Gavery MR (2012) Is there a relationship between DNA methylation and phenotypic plasticity in invertebrates? Frontiers in Physiology. 2:116. doi:0.3389/fphys.2011.00116 [I F E A W]
Pages: 5
Article Views: 651

Gavery MR* and Roberts SB. (2012) Characterizing short read sequencing for gene discovery and RNA-Seq analysis in *Crassostrea gigas*. Comparative Biochemistry and Physiology Part D: Genomics and Proteomics, Available online 29 December 2011. doi: 10.1016/j.cbd.2011.12.003 [I F E A W]
Pages: 6
Statistic: *#1 most downloaded article in journal January – March 2012*

2011

Morera D, Roher N, Ribas L, Balasch JC, Doñate C, Callol A, Boltaña A, Roberts SB, Goetz G, Goetz FW, Mackenzie SA. (2011) RNA-Seq reveals an integrated immune response in nucleated erythrocytes. PLoS ONE 6(10): e26998. doi:10.1371/journal.pone.0026998 [E A W]
Pages: 9
Article Views: 1680

Roberts SB, Sunila I, Wikfors G. (2011) Immune response and mechanical stress susceptibility in diseased oysters, *Crassostrea virginica*. Journal of Comparative Physiology B. 182:1 41-48, doi:10.1007/s00360-011-0605-z [I F E A W]
Pages: 8
Seeb JE, Carvalho G, Hauser L, Naish K, Roberts SB, Seeb LW. (2011) Single-nucleotide polymorphism (SNP) discovery and applications of SNP genotyping in nonmodel organisms. Molecular Ecology Resources, 11 1–8 [W]
Pages: 8
Citations: 22

2010

Seeb JE, Pascal CE, Graue ED, Seeb LW, Templin WD, Harkins T, Roberts SB. (2010) Transcriptome sequencing and high-resolution melt analysis advance single nucleotide polymorphism discovery in duplicated salmonids. Molecular Ecology Resources. [I F E A W]
Pages: 13
Citations: 20

Gavery M* and Roberts SB. (2010) DNA methylation patterns provide insight into epigenetic regulation in the Pacific oyster (*Crassostrea gigas*). BMC Genomics 11:483 [I F A W]
Article Views: 3798
Pages: 9
Citations: 9
Statistic: *Highly Accessed*

Mathger L, Roberts SB, Hanlon R. (2010) Evidence for distributed light sensing in the skin of cuttlefish, *Sepia officinalis*. Biology Letters. online April 14, 2010, doi: 10.1098/rsbl. [I E A] 2010.0223
Pages: 3
Citations: 4

Goetz FW, Rosauer D, Sitar S, Goetz G, Simchick C, Roberts SB, Johnson R, Murphy C, Bronte C, Mackenzie S. (2010) A genetic basis for the phenotypic differentiation between siscowet and lean lake trout (*Salvelinus namaycush*). Molecular Ecology, 19 176–196 [E A W]
Pages: 20
Citations: 23

2009

Defaveri J*, Smolowitz R, Roberts SB (2009) Development and validation of a real-time quantitative PCR assay for the detection and quantification of *Perkinsus marinu*s in the Eastern oyster, *Crassostrea virginica*. Journal of Shellfish Research. Vol. 28 No. 3 459-464 [I F A W]
Pages: 5

Roberts SB, Goetz G, White S, Goetz F (2009) Analysis of genes isolated from plated hemocytes of the Pacific Oyster, *Crassostrea gigas*. Marine Biotechnology. 11:24-44 [I F E A W]
Pages: 21
Citations: 23

2008

Roberts SB, Gueguen Y, de Lorgeril J, Goetz F. (2008) Rapid accumulation of an interleukin 17 homolog transcript in *Crassostrea gigas* hemocytes following bacterial exposure. Developmental and Comparative Immunology. 32, 1099-1104 [I F E A W]
Pages: 5
Citations: 15

2007

Lyons MM*, Lau Y-T, Carden WE, Ward JE, Roberts SB, Smolowitz R, Vallino J, Allam B. (2007) Characteristics of marine aggregates in shallow-water ecosystems: Implications for disease ecology. EcoHealth 4, 406–420 [I A]
Pages: 5

Weiss E*, Bennie M*, Hodgins-Davis A*, Roberts SB, Gerlach G. (2007) Characterization of new SSR-EST markers in cod, *Gadus morhua*. Molecular Ecology Notes, 7: 866–867. doi: 10.1111/j.1471-8286.2007.01731.x [I W]
Pages: 2

Hodgins-Davis A*, Roberts SB, Cowan D, Atema J, Avolio C, Defaveri J, Gerlach G. (2007) Characterization of SSRs from the American lobster, *Homarus americanus*. Molecular Ecology Notes. 7:330-332 [I W]
Pages: 3

Rodgers BD, Roalson EH, Weber GM, Roberts SB, Goetz FW. (2007) A proposed nomenclature consensus for the myostatin gene family. AJP- Endocrinology and Metabolism. 292(2):E371-2 [I]
Pages: 2

2006

Lyons MM*, Smolowitz R, Dungan C, Roberts SB. (2006) Development of a real-time quantitative PCR assay for the hard clam pathogen, Quahog Parasite Unknown (QPX). Diseases of Aquatic Organisms. 72(1):45-52
Pages: 13

2005

Biga PR, Roberts SB, Iliev DB, McCauley LA, Moon JS, Collodi P, Goetz FW. (2005) The isolation, characterization, and expression of a novel GDF11 gene and a second myostatin form in zebrafish, *Danio rerio*. Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology 141: 218-230
Pages: 13

Roberts SB, Romano C, Gerlach G. (2005) Characterization of EST derived SSRs from the bay scallop, *Argopecten irradians*. Molecular Ecology Notes. 5: 567-568
Pages: 2

Jentoft S, Topp N, Seeliger M, Malison JA, Barry TP, Held JA, Roberts SB, Goetz FW. (2005) Lack of growth enhancement by exogenous growth hormone treatment in yellow perch in four separate experiments. Aquaculture. 250:471-479
Pages: 9

2004

Hollis DM, Goetz FW, Roberts SB, Boyd SK. (2004) Acute neurosteroid modulation and subunit isolation of the GABAa receptor in the bullfrog, *Rana catesbeiana*. Journal of Molecular Endocrinology. 32(3):921-34

Biga PR, Cain KD, Hardy RW, Schelling GT, Overturf K, Roberts SB, Goetz FW, Ott TL. (2004) Growth hormone differentially regulates muscle myostatin1 and -2 and increases circulating cortisol in rainbow trout (*Oncorhynchus mykiss*). General and Comparative Endocrinology. Vol 138(1):32-41

Roberts SB, McCauley LAR, Devlin RH, Goetz FW. (2004) Transgenic salmon over-expressing growth hormone exhibit decreased myostatin transcript and protein expression. Journal of Experimental Biology. 207(Pt 21):3741-8

Kim H-W*, Mykles DL, Goetz FW, Roberts SB. (2004) Characterization of an invertebrate myostatin homologue from the bay scallop, *Argopecten irradians*. BBA – Gene Structure and Expression. 1679(2):174-9

Roberts SB, Barry T, Malison J, Goetz FW. (2004) Production of a recombinantly-derived growth hormone antibody and the characterization of growth hormone levels in yellow perch. Aquaculture. Vol. 232/1-4: 591-602

2003

Roberts SB, Goetz FW. (2003) Expressed sequence tag analysis of genes expressed in the bay scallop, *Argopecten irradians*. Biological Bulletin. 205: 227-228.

Roberts SB, Goetz FW. (2003) Myostatin protein and mRNA transcript levels in adult and developing brook trout. Molecular and Cellular Endocrinology. 210 (1-2): 9-20.

2001

Roberts SB, Goetz FW. (2001) Differential skeletal muscle expression of myostatin across teleost species, and the isolation of multiple myostatin isoforms. FEBS Letters. Vol 491, No. 3, pp. 212-216.

2000

Roberts SB, Langenau DM, Goetz FW. (2000) Cloning and characterization of prostaglandin endoperoxide synthase-1 and -2 from the brook trout ovary. Molecular and Cellular Endocrinology.160(1-2):89-97.

Moser ML, Roberts SB. (2000) Effects of nonindigenous ictalurids and recreational electrofishing on the ictalurid community of the Cape Fear River drainage, North Carolina. In Catfish 2000: Proceedings of the International Ictalurid Symposium; ER Irwin, WA Hubert, CF Rabeni, HL Schramm, Jr., and T Coon, editors. Davenport, IA. June 23-25, 1998. pp 479-485.

Roberts SB, Langenau DM, Goetz FW. (2000) Isolation through cloning of fish prostaglandin endoperoxide synthase (cyclooxygenase) in Proceedings of the 6th International Symposium on the Reproductive Physiology of Fish; B Norberg, OS Kjesbu, GL Taranger, E Andersson, and SO Stefansson, editors. Bergen, Norway. July 4-9, 1999. p 197.

1999

Langenau DM, Goetz FW, Roberts SB. (1999) The upregulation of messenger ribonucleic acids during 17a, 20b-dihydroxy-4-pregnen-3-one-induced ovulation in the perch ovary. Journal of Molecular Endocrinology. 23(2):137-52.

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Is there a relationship between DNA methylation and phenotypic plasticity in invertebrates?

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There is a significant amount of variation in DNA methylation characteristics across organisms. Likewise, the biological role of DNA methylation varies across taxonomic lineages. The complexity of DNA methylation patterns in invertebrates has only recently begun to be characterized in-depth. In some invertebrate species that have been examined to date, methylated DNA is found primarily within coding regions and patterning is closely associated with gene function. Here we provide a perspective on the potential role of DNA methylation in these invertebrates with a focus on how limited methylation may contribute to increased phenotypic plasticity in highly fluctuating environments. Specifically, limited methylation could facilitate a variety of transcriptional opportunities including access to alternative transcription start sites, increasing sequence mutations, exon skipping, and transient methylation.

Keywords: epigenetic, methylation, oyster, plasticity, adaptation

Epigenetics refers to processes capable of inducing changes in genetic activity without altering the underlying DNA sequence (Jablonka and Lamb, 2002). Histone modifications, DNA methylation, and non-coding RNA activity (e.g., miRNA) are the most commonly described epigenetic mechanisms. DNA methylation is one of the most studied mechanisms of epigenetic regulation and refers to the addition of a methyl group to position 5 of cytosine bases. DNA methylation is presumed to be evolutionarily ancient, and, while the mark itself is prevalent across taxa, the landscape of methylation patterning is incredibly diverse.

DNA methylation has been well-studied in mammals and plants, however surprisingly little is known about this mechanism in invertebrates. Recent research characterizing DNA methylation in a handful of species is providing evidence that the absence of DNA methylation could contribute to phenotypic plasticity by increasing the number of transcriptional opportunities. Evidence of a relationship between methylation patterns and transcriptional opportunities is found primarily in studies on the mollusk, *Crassostrea gigas*, and the eusocial insect *Apis mellifera*. Here we discuss this perspective and supporting research with a particular focus on the adaptive potential this phenomenon could have on species in highly fluctuating environments. In order to provide a broad view we first outline taxonomic trends in DNA methylation patterns and describe gene-associated DNA methylation characteristics in the limited number of invertebrate species where this has been examined. Molecular mechanisms that likely contribute to phenotypic plasticity are discussed followed by a summary of fundamental questions with respect to DNA methylation in invertebrates that remain unanswered.

DNA METHYLATION PATTERNS

The relative amount of DNA methylation varies significantly across taxa. In vertebrates, ∼70–80% of cytosines in CpG dinucleotides are methylated (Bird and Taggart, 1980), a pattern referred to as global methylation. In contrast, invertebrates display a wide range of DNA methylation. In fact, two common model organisms (*Drosophila melanogaster* and *Caenorhabditis elegans*) essentially lack DNA methylation (Simpson et al., 1986; Gowher et al., 2000). Other invertebrates have an intermediate level of methylation, including sea urchins (*Strongylocentrotus purpuratus*; Bird et al., 1979), sea squirts (*Ciona intestinalis*; Simmen and Bird, 2000; Suzuki et al., 2007), honey bees (*A. mellifera*; Lyko et al., 2010), and oysters (*C. gigas*; Gavery and Roberts, 2010). Among plants, not all species studied have methylated genomes, and related species can exhibit varying degrees of methylation. For example, a global methylation pattern is observed in maize (*Zea mays*; Palmer et al., 2003), whereas an intermediate level, similar to that seen in invertebrates, has been reported for *Arabidopsis thaliana* (Zhang et al., 2006).

The location of DNA methylation across the genome is also diverse among taxa. In vertebrates, the limited amount of the genome that is not methylated is often found in CpG rich gene promoter regions called CpG islands. Gene bodies are typically methylated in vertebrates, though the degree of methylation decreases in 5′ and 3′ regions. In invertebrates, tracts of methylated CpGs are interspersed with unmethylated regions across the genome, referred to as a mosaic pattern (Suzuki et al., 2007). Another example of spatial heterogeneity is the predominance of methylation in exons. This phenomenon has been observed in *A. mellifera* (Lyko et al., 2010), *C. intestinalis* (Suzuki et al., 2007), and *C. gigas* (Gavery and Roberts, 2010). This is in contrast to the blood fluke (*Schistosoma mansoni*) where methylation has been found in a highly repetitive intronic region (Geyer et al., 2011). In plants, methylation occurs predominantly on repetitive DNA elements and transposons (Zhang et al., 2006), though gene bodies are substantially methylated in some species (Zhang et al., 2006; Zilberman et al., 2006).

GENE-ASSOCIATED DNA METHYLATION IN INVERTEBRATES

If one considers the significant diversity of methylation across taxa it seems plausible that these marks could have different functions, and potentially different mechanisms of action, across organisms and evolutionary time. Here we will focus on a functional role of DNA methylation in invertebrate species where DNA methylation patterns are associated with transcript coding regions. A discussion of the functional relationship of DNA methylation in other taxonomic systems can be found elsewhere (Regev et al., 1998; Colot and Rossignol, 1999; Hendrich and Tweedie, 2003; Suzuki and Bird, 2008; Law and Jacobsen, 2010).

In contrast to well-studied mammalian and plant systems, there are limited studies on DNA methylation in invertebrates. Some of the first evidence supporting a regulatory role of intragenic DNA methylation in invertebrates comes from *in silico* analyses. Initial computational analysis revealed a relationship between gene body methylation and gene function. This analysis is based on the known hyper-mutability of methylated cytosines, which readily deaminate to thymine residues (Coulondre et al., 1978). The mutation is not easily corrected by DNA repair machinery and, as a result, consistently methylated regions of DNA are depleted of CpG dinucleotides over evolutionary time (Schorderet and Gartler, 1992). Consequently, regions of DNA with a low CpG observed versus expected ratio (denoted as CpG O/E) are predicted to be methylated at the germline, whereas regions with a high CpG O/E (approaching 1.0) are predicted to be sparsely methylated. Germline methylation refers to the methylation state that is inherited.

In *A. mellifera*, ubiquitously expressed critical genes were predicted to be methylated at the germline, whereas caste-specific genes were predicted to lack methylation (Elango et al., 2009; Foret et al., 2009). From their study, Elango et al. (2009), hypothesized that genes predicted to be unmethylated (caste-specific) might have greater epigenetic flexibility, which allows for higher regulatory control of these inducible classes of genes via transient methylation. In a previous publication, we described a similar relationship in the Pacific oyster, *C. gigas*. In *C. gigas*, genes predicted to be hyper-methylated are ubiquitously expressed critical genes such as those involved in DNA and RNA metabolism (Gavery and Roberts, 2010). Likewise, genes predicted to be sparsely methylated (i.e., higher CpG O/E) are associated with tissue specific and inducible expression, including those involved in general immune function (e.g., cell adhesion, cell–cell signaling, and signal transduction; Gavery and Roberts, 2010). These results suggest DNA methylation has regulatory functions in genes involved in stress and environmental responses.

In order to experimentally corroborate the *in silico* analysis that predicts hyper-methylated genes in oysters are ubiquitously expressed critical genes, our lab has recently performed deep sequencing of the methylated portion of the *C. gigas* genome. Methyl-CpG binding domain protein sequencing (MBD-seq; see Li et al., 2010) was carried out followed by Gene Ontology based analysis. Our results indicate that genes involved in DNA and protein metabolism were most prevalent in the MBD-library (thus having the highest amount of methylation) and the most underrepresented genes in the library are involved in cell adhesion

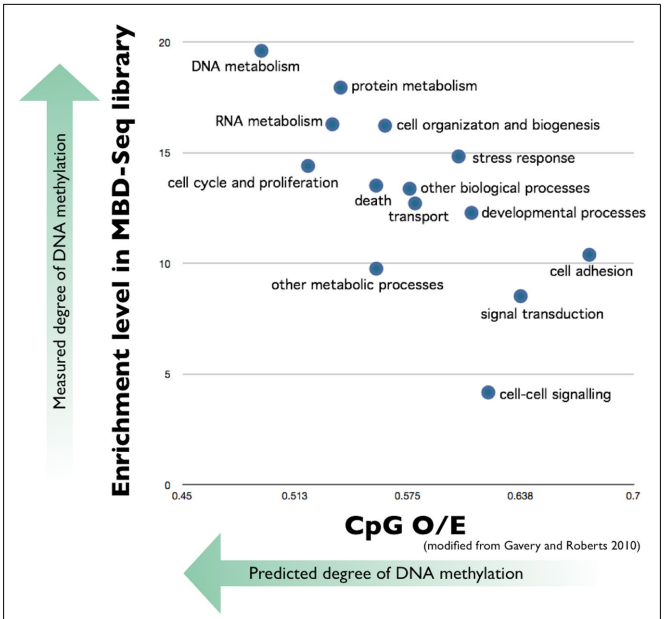

FIGURE 1 | Predicted methylation level of *C. gigas* genes categorized by biological processes compared to measured level of DNA methylation. Mean CpG O/E for 10,699 *C. gigas* genes categorized according to Biological Process Gene Ontology (GO) Slim terms are plotted on the *x*-axis (modified from Gavery and Roberts, 2010). DNA methylation was empirically measured by performing MBD-seq on the SOLiD 4 platform (Applied Biosystems). Genes identified in the MBD-library were associated with respective GO terms and enrichment analysis was performed based on the entire transcriptome (Fleury et al., 2009) using DAVID (Huang et al., 2009a,b). Results indicate the most underrepresented genes in the library are involved in cell adhesion and genes involved in DNA and protein metabolism were most prevalent in the MBD-library.

(**Figure 1**). These analyses are consistent with the results of the *in silico* analysis.

Direct measurements of DNA methylation patterns in *A. mellifera* have also been carried out. Using bisulfite treatment coupled with high-throughput sequencing, Lyko et al. (2010) found that methylated cytosines occur primarily in exons and that methylated genes had a higher degree of conservation across species than unmethylated genes. Just as with the oyster data, these results confirmed the inverse relationship between germline methylation and CpG O/E. Other trends that arose from this analysis were that (1) methylated cytosine clusters were associated with alternatively spliced exons and (2) genes containing introns were more likely to be methylated than those lacking introns. The authors also highlighted an example where an increased level of methylation in an alternatively spliced exon in the worker bee brain was associated with an increased expression of the variant lacking the exon. Lyko et al. (2010) concluded methylation may not be functioning as an "on/off" switch but instead allowing for "fine tuning" of transcriptional control of these conserved genes.

Another characteristic of gene-associated DNA methylation in invertebrates is that genes predicted to be methylated at the germline (i.e., low CpG O/E) have less genetic diversity compared to genes lacking germline methylation (i.e., high CpG O/E). One

source of evidence of this relationship comes from recent analyses in our own lab where high-throughput sequencing reads from a pooled oyster gill tissue cDNA library were mapped to the oyster transcriptome and single nucleotide polymorphisms characterized. There was as positive relationship among the mean number of polymorphisms per nucleotide and CpG O/E. This is consistent with results from Lyko et al. (2010) where they showed increased sequence conservation in low CpG O/E genes in *A. mellifera*.

DNA METHYLATION AND TRANSCRIPTIONAL OPPORTUNITIES

Given the similarities in DNA methylation patterns between *A. mellifera* and *C. gigas* it is possible that the mechanism of action is conserved at some level. However, given the dramatic differences in specific life history characteristics of the species, the role of DNA methylation in bees and oysters could have diverged over evolutionary time. In the following section we will primarily focus on a putative role of DNA methylation in the oyster, however a majority of the concepts discussed are in agreement with what has been observed in other taxa. For an in-depth review of the functional role of DNA methylation in insects see Glastad et al. (2011).

Based on what we currently know concerning DNA methylation in invertebrates, we propose the absence of germline methylation facilitates random variation that contributes to phenotypic plasticity and thus could increase adaptive potential. Another way to consider this is that in species that experience a diverse range of environmental conditions, processes have evolved to increase the number of potential phenotypes in a population in order to improve the chances for an individual's survival. This would be particularly important for estuarine species such as *C. gigas*, where a large number of planktonic larvae are dispersed by currents and can settle in a range of habitats. Germline methylation of genes essential for normal biological function, such as those involved in DNA and protein metabolism, essentially "protects" these genes from the inherent genome wide plasticity, as this would likely be lethal. Thus, as a result of their low methylation status, those genes involved in responding to environmental perturbation may be subject to one of several transcriptional opportunities.

Limited methylation might passively facilitate specific transcriptional opportunities including access to alternative transcription start sites, increasing sequence mutations, and exon skipping. Furthermore, there is the opportunity for these genes to be transiently methylated in somatic tissue, which could also influence transcription. Conversely, germline methylation limits transcriptional opportunities in critical genes. This theory provides an inclusive framework that suggests a suite of specific mechanisms that contribute to evolutionary success by increasing the number of phenotypes via gene-associated, random variation (**Figure 2**). This theory is consistent with what has been described in *A. mellifera* by Lyko et al. (2010) suggesting that methylation could "control which of several versions of a gene is expressed." Furthermore, researchers have shown a relationship between DNA methylation, alternative splicing, and sequence conservation (e.g., Lyko et al., 2010; Park et al., 2011) and suggested a role for DNA methylation in influencing ecologically important traits (e.g., Angers et al., 2010).

The absence of DNA methylation in genes that are induced in response to changing conditions could allow for multiple transcripts indirectly by providing access to alternative promoter sites. This explanation is consistent with the ability of DNA methylation to inhibit binding of transcription factors to response elements in mammalian promoter regions (Iguchi-Ariga and Schaffner, 1989). A recent mammalian study provided direct evidence of this, revealing that intragenic methylation limits the generation of alternate gene transcripts by masking intragenic promoters (Maunakea et al., 2010). Direct evidence of DNA methylation associated with alternative transcripts is also available in invertebrates (Lyko et al., 2010).

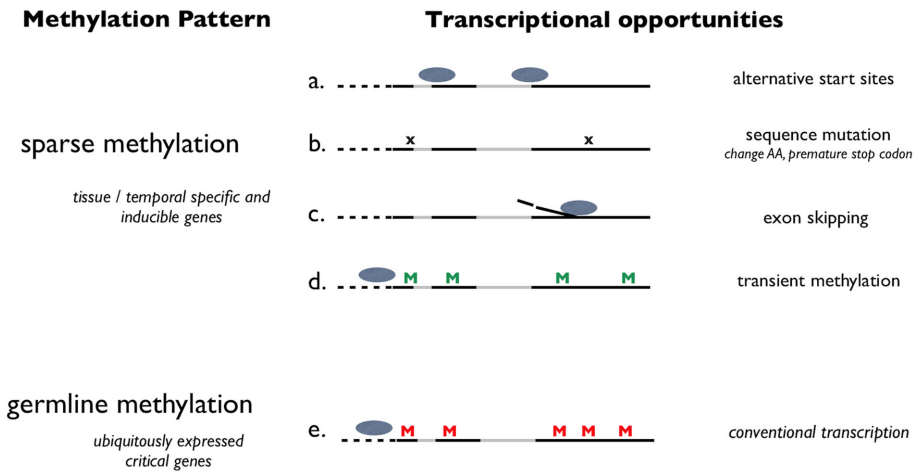

FIGURE 2 | Schematic representation of a how DNA methylation potentially influences transcriptional activity in invertebrate species. This theory proposes the absence of germline methylation (sparse methylation) contributes to adaptive potential by allowing for multiple transcriptional opportunities. Transcriptional opportunities are diagrammed for genes with sparse methylation (a–d) and genes methylated at the germline (e). Dashed lines represent the 5′ UTR, solid lines represent exons and gray lines indicate introns. "M" designates a methylated CpG. "x" Represents a sequence mutation. Ovals represent putative promoter complexes.

Sequence mutation is another important source of potential phenotypic variation. Transcript variations that could be associated with function include those that contribute to an alteration in amino acid or result in a premature stop codon. There are several instances of evidence supporting an inverse relationship between methylation density and sequence variation. As described above we have characterized this relationship in the oyster using high-throughput sequence analysis and a similar pattern has been reported in honey bees (Lyko et al., 2010). Furthermore, a recent study in the jewel wasp (*Nasonia vitripennis*) showed high CpG O/E ratios correspond with higher substitution rates between related species for synonymous, non-synonymous, and intron sites (Park et al., 2011). In other words, there was more genetic variation in genes lacking germline methylation.

Another means by which a transcriptional variant might be produced is through exon skipping, and there is evidence to suggest methylation is associated with this phenomenon in invertebrates. In *A. mellifera*, the gene GB18602 has two forms (long and short), which are distinguished by a cassette-exon being skipped in the long form (Lyko et al., 2010). This exon contains a stop codon that creates a shorter, alternative transcript. The researchers went on to find numerous examples of genes where the methylated CpGs were associated with differentially spliced exons (Lyko et al., 2010). This phenomenon would be consistent with the transient (or differential) methylation that could lead to alternative transcripts under different environmental conditions.

SUMMARY

Here we have set out to provide a perspective on the functional role of DNA methylation in invertebrates. We propose that an absence of germline methylation in genes involved in responding to fluctuating conditions facilitates phenotypic variation, which could contribute to increased adaptive potential. However, there are several questions that remain to be answered. Foremost is what contributes to the proposed promiscuous transcriptional nature in certain invertebrates that acts in concert with DNA methylation to enhance phenotypic plasticity? Here we suggest that the probability of a transcriptional opportunity occurring is random, however it is also possible that an environmental stressor could have a specific effect on methylation patterns that directly impacts the physiological response. Furthermore, it is not clear what mechanism(s) are responsible for transient methylation in invertebrates or how common transient methylation occurs. Finally, it is not known if DNA methylation patterns are heritable independent of genetic inheritance. Future research efforts will certainly begin to shed light on these questions as well as test the theory proposed here. Given the evidence we have to date, what we learn about DNA methylation and epigenetics in invertebrates has the potential to considerably change how we view organismal physiology and population biology.

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Characterizing short read sequencing for gene discovery and RNA-Seq analysis in *Crassostrea gigas*

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ABSTRACT

Advances in DNA sequencing technology have provided opportunities to produce new transcriptomic resources for species that lack completely sequenced genomes. However, there are limited examples that rely solely on ultra-short read sequencing technologies (e.g. Solexa, SOLiD) for transcript discovery and gene expression analysis (i.e. RNA-Seq). Here we use SOLiD sequencing to examine gene expression patterns in Pacific oyster (*Crassostrea gigas*) populations exposed to varying degrees of anthropogenic impact. Novel transcripts were identified and RNA-Seq analysis revealed several hundred differentially expressed genes. Gene enrichment analysis determined that in addition to biological processes predicted to be associated with anthropogenic influences (e.g. immune response), other processes play important roles including cell recognition and cell adhesion. To evaluate the effectiveness of restricting characterization solely to short read sequences, mapping and RNA-Seq analysis were also performed using publicly available transcriptome sequence data as a scaffold. This study demonstrates that ultra-short read sequencing technologies can effectively generate novel transcriptome information, identify differentially expressed genes, and will be important for examining environmental physiology of non-model organisms.

1. Introduction

High-throughput DNA sequencing technologies are providing new opportunities to generate genomic resources for non-model organisms. A widely used approach is transcriptome sequencing, which has the benefit of providing increased coverage as a result of the reduced representation of the genome. A primary platform being used to generate transcriptomic resources in non-model species is the Roche 454 GS-FLX (454) followed by de novo assembly of sequence reads. This approach has been used to characterize transcriptomes of diverse taxa including plants (e.g. Novaes et al., 2008), insects (e.g. Vera et al., 2008), corals (e.g. Meyer et al., 2009), molluscs (e.g. Craft et al., 2010) and fish (e.g. Fraser et al., 2011). One benefit of using the 454 platform is that reads are longer compared to other common high-throughput sequencing systems, such as the Illumina Genome Analyzer IIx (Solexa) and Applied Biosystems SOLiD (SOLiD). Compared to the approximately 350 bp read length from the 454 platform, Solexa and SOLiD provide 'ultra-short reads' that are commonly less than 75 bp. The benefits of the ultra-short read platforms include increased number of reads and decreased cost. Sequencing on these platforms can be up to 30 times less expensive compared to 454 sequencing (Shendure and Ji, 2008). Recently, researchers have begun to examine the applicability of using Solexa and SOLiD for generating de novo transcriptomes in non-model species. For example, a transcriptome was generated for the snail (*Radix balthica*) using Solexa (Feldmeyer et al., 2011). A study in sockeye salmon (*Oncorhynchus nerka*) used SOLiD to compare results of de novo assembly versus mapping to public expressed sequence tag (EST) databases (Everett et al., 2011). Everett et al. (2011) determined that assemblies using public EST databases had a higher percentage of mapped reads and higher coverage than de novo assemblies. These studies demonstrate that current sequence assembler performance is sufficient for producing accurate and functionally informative transcriptomes generated from ultra-short read platforms.

In addition to assembling transcriptomes, high-throughput sequencing can also be used to directly examine gene expression levels, a method referred to as RNA-Seq. In RNA-Seq, high throughput sequencing reads generated from cDNA libraries are aligned to a common reference sequence or scaffold (e.g. whole genome) to produce a transcriptome map that includes transcript abundance for each gene. RNA-Seq provides similar information as hybridization based microarray analysis, however, RNA-Seq has an increased dynamic range compared to hybridization-based methods (Wang et al., 2009). Furthermore, RNA-Seq is not limited to analysis of known sequences like qPCR and microarray technology, which makes RNA-Seq especially appropriate for non-model species.

The RNA-Seq approach has been primarily used in organisms with sequenced genomes, but very recently RNA-Seq has been applied in non-model organisms. For example, RNA-Seq was used to investigate

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the basis of phenotypic variation between lake trout (*Salvelinus namaycush*) ecotypes using the 454 platform (Goetz et al., 2010). RNA-Seq was also used to identify genes expressed in guppies (*Poecilia reticulata*) in response to predator cues using Solexa sequencing (Fraser et al., 2011). SOLiD transcriptome sequence reads have been used to investigate genes involved in response to temperature and settlement cues in coral larvae (Acropora millepora) (Meyer et al., 2011). In the latter two studies, Solexa or SOLiD short reads were mapped to a scaffold consisting of contigs generated from other sources (i.e. 454, ESTs). These studies conclude that this approach is effective in generating accurate and informative gene expression results. RNA-Seq analysis using one set of ultra-short read data as both the scaffold and individual reads for expression analysis would be the most cost efficient, especially for those organisms where genomic resources are limited. To date, a thorough evaluation of the effectiveness of this approach has not been performed.

The primary goal of this study was to evaluate the effectiveness of utilizing the SOLiD platform to both characterize the transcriptome and analyze gene expression patterns in the Pacific oyster (*Crassostrea gigas*). As part of this study, gene expression patterns between oyster populations exposed to varying degrees of anthropogenic impact were compared. RNA-Seq was performed using only the ultra-short read consensus sequences generated from de novo assembly as a scaffold. In order to evaluate the effectiveness of using solely ultra-short read data, RNA-Seq was also performed using publicly available transcriptome data as a scaffold and the results were compared. This work not only evaluates the use of limited ultra-short read sequence data for characterizing transcriptomes in non-model organisms, but also offers insight into the physiological responses of aquatic invertebrates in natural environments.

2. Materials and methods

2.1. Site selection

Oysters were collected from two locations in Puget Sound, Washington, USA. The sites were selected based on a difference in perceived degree of anthropogenic impact. The mouth of Big Beef Creek (BBC) in Hood Canal is a low impact site, and Drayton Harbor (DH), located in North Puget Sound, is an elevated impact site. The level of impact refers to water quality as determined by the Washington State Department of Ecology and Puget Sound Assessment and Monitoring Program (Newton et al., 2002). BBC has a relatively low population density compared to DH and routine monitoring by Washington State Department of Health shows low bacterial loads. DH is ranked as the number one shellfish growing area impacted by fecal coliform pollution (WSDOH, 2006). Additionally, the density of commercial dairies and animal keeping areas in the region surrounding DH is significantly higher than BBC (WSDOH, 2006), and a municipal wastewater treatment plant discharges in proximity to DH.

2.2. Sampling and library construction

Oysters were collected from both sites in April of 2009. At each site, gill tissue was immediately sampled from 16 oysters using sterile procedures and stored in RNAlater (Ambion). RNA was isolated from individual gill tissue samples (~50 mg) using Tri-Reagent (Molecular Research Center). To eliminate possible DNA carryover, total RNA was DNase treated using the Turbo DNA-free Kit (Ambion) according to the manufacturer's "rigorous" protocol. RNA from all individuals at a site ($n = 16$) was pooled in equal quantities (650 ng) to provide template for SOLiD libraries. Pooled samples were enriched for mRNA using the Ribominus Eukaryote Kit for RNA-Seq (Invitrogen) and MicroPolyA Purist Kit (Ambion). Libraries were prepared using the SOLiD Whole Transcriptome Analysis Kit (Applied Biosystems) and sequencing was performed using the SOLiD3 System (Applied Biosystems).

2.3. Sequence analysis

All sequence analysis was performed with CLC Genomics Workbench version 4.0 (CLC Bio). Initially, sequences were trimmed based on quality scores of 0.05 (Phred, Ewing and Green, 1998; Ewing et al., 1998) and the number of ambiguous nucleotides (>2 on ends). Sequences smaller than 20 bp were also removed. De novo assembly was carried out using the following parameters: limit = 8, mismatch cost = 2 and a minimum contig size of 200 bp. For comparison purposes, quality trimmed reads were also mapped to the 82,312 contigs in GigasDatabase (version 8) (Fleury et al., 2009). Parameters used for this reference mapping included: limit = 8 and mismatch cost = 2. Sequences and corresponding annotations from GigasDatabase were downloaded from the *C. gigas* Public Sigenae Contig Browser (http://public-contigbrowser.sigenae.org:9090/Crassostrea_gigas). Reference mapping, using the same parameters, was used to distinguish mitochondrial transcripts using the *C. gigas* mitochondrian genome (GenBank: AF177226).

Consensus sequences from the de novo assembly were compared to the UniProtKB/Swiss-Prot database (http://uniprot.org) in order to determine putative descriptions. Comparisons were made using the BLAST algorithm (Altschul et al., 1990). A cutoff E-value of 1E-05 was used for annotations. Associated GO terms (Gene Ontology database: http://www.geneontology.org) were used to categorize genes into parent categories and were assigned a functional group based on the MGI GO Slim database (URL: http://www.informatics.jax.org). The MGI GO Slim terms for 'other biological processes' and 'other metabolic processes' are not included in this analysis.

For RNA-Seq analysis, expression values were measured as RPKM (reads per kilobase of exon model per million mapped reads) (Mortazavi et al., 2008) with an unspecific match limit of 10 and maximum number of 2 mismatches. Statistical comparison of RPKM values between the BBC and DH libraries was carried out using Baggerly's test (Baggerly et al., 2003), and multiple comparison correction was performed using a false discovery rate. Genes were considered differentially expressed in a given library when 1) the p-value was less than or equal to 0.05 and 2) a greater-than-or-equal-to two-fold change in expression across libraries was observed. Galaxy was used for analysis (i.e. table joins) during annotation and RNA-Seq analysis (Blankenberg et al., 2010; Goecks et al., 2010). RNA-Seq analysis was performed using two different scaffolds including 1) the consensus sequences from de novo assembly of SOLiD reads and 2) contigs in GigasDatabase.

In order to identify enriched biological themes and GO terms, the Database for Annotation, Visualization and Integrated Discovery (DAVID) v6.7 was used (Huang et al., 2009a,b). Specifically, corresponding UniProt accession numbers for differentially expressed genes were used as the gene list, and compared to a complete list of the corresponding UniProt accession numbers of the respective transcriptome (i.e. results of de novo assembly or reference mapping) for the background. Biological Process terms (DAVID 'BP Level 2' categories) were considered significantly enriched when the p-value was less than 0.05.

3. Results

3.1. C. gigas SOLiD sequencing

After quality trimming, 20.7 and 24.6 million reads (average length: 40.6 bp) remained from the BBC and DH cDNA libraries, respectively. A majority of the reads (98%) corresponded to nuclear transcripts with the other 2% mapping to mitochondria protein coding genes. The quality trimmed reads from each library were combined for

de novo assembly and reference mapping. All sequence data has been submitted to the NCBI Short Read Archive under accession number: SRP007621.

3.1.1. De novo assembly

De novo assembly of reads from the combined libraries resulted in 18,510 consensus sequences with an average length of 276 bp. Twenty three percent of the reads assembled using this approach. The average number of assembled reads per consensus sequence was 454 and the mean coverage was 61.7x (Fig. 1).

3.1.2. Reference mapping (GigasDatabase)

SOLiD reads were also mapped to publicly available *C. gigas* transcriptomic resources (GigasDatabase v8). Reads from the combined libraries mapped to 64,645 of the 82,314 contigs in the database. The average number of reads per contig was 376 and the mean coverage was 15.8x (Fig. 1). See Table 1 for a full comparison of results of the de novo assembly compared to reference mapping.

3.1.3. De novo assembly: annotation

A total of 7724 consensus sequences could be annotated, 3931 of which could be classified using GO Slim terms. The most highly represented biological process was transport, followed by protein metabolism (data not shown). Of those consensus sequences associated with transport a majority were involved in protein and ion transport. Comparatively, 7296 of the GigasDatabase contigs with mapped reads were annotated with biological process GO terms. When the associated GO terms were evaluated, two of the most highly represented biological processes identified after binning into broader GO Slim terms included protein and RNA metabolism (data not shown).

3.1.4. De novo assembly: identification of novel transcripts

Short read consensus sequences generated from de novo assembly were compared to GigasDatabase v8 to identify novel sequences. Approximately 10% of the sequences (1776) did not have a significant match (E-value > 1.0E-01). Of these, 742 could be annotated (see Supplementary Table 1) and 690 could be classified using GO Slim. The 4 most highly represented biological processes included: transport, developmental processes, cell organization and biogenesis, and cell adhesion (Fig. 2).

Table 1
Summary of assembly and RNA-Seq statistics for de novo assembly and reference mapping (GigasDatabase v8).

		De novo assembly	Reference mapping
Assembly	Mapped reads	8,407,963	29,107,760
	Unmapped reads	36,944,698	16,244,901
	Contigs	18,510	77,433
	Average contig length	276	554
	Average contig coverage	62	16
	Contigs annotated to GO Slim	3931	7296
RNA-Seq	Differentially expressed genes	2991	427
	Enriched GO biological process	15	3

3.2. RNA-Seq analysis

3.2.1. De novo-based RNA-Seq

RNA-Seq analysis using the de novo assembled short read consensus sequences as the scaffold identified 2991 differentially regulated features. Most of these features represented moderately expressed transcripts (100–10,000 total reads), but 20% were rare transcripts (<100 total reads). Six consensus sequences were expressed uniquely in the BBC library and 5 were expressed only in the DH library. None of the uniquely expressed features could be annotated. Of differentially expressed features with reads in both libraries, 1200 were expressed higher in the BBC library and 1791 were expressed higher in the DH library. A subset of the differentially expressed features (751 in BBC and 313 in DH, respectively) could be annotated (see Supplementary Table 2). A majority of these annotated features represented a twofold difference, but overall differences ranged between 2 and 409 fold.

Functional enrichment analysis identified 15 biological processes that were overrepresented in the differentially expressed gene set (Fig. 3). The most significantly enriched process was cell adhesion (p-value = 8E-15), followed by cell recognition (p-value = 5E-5).

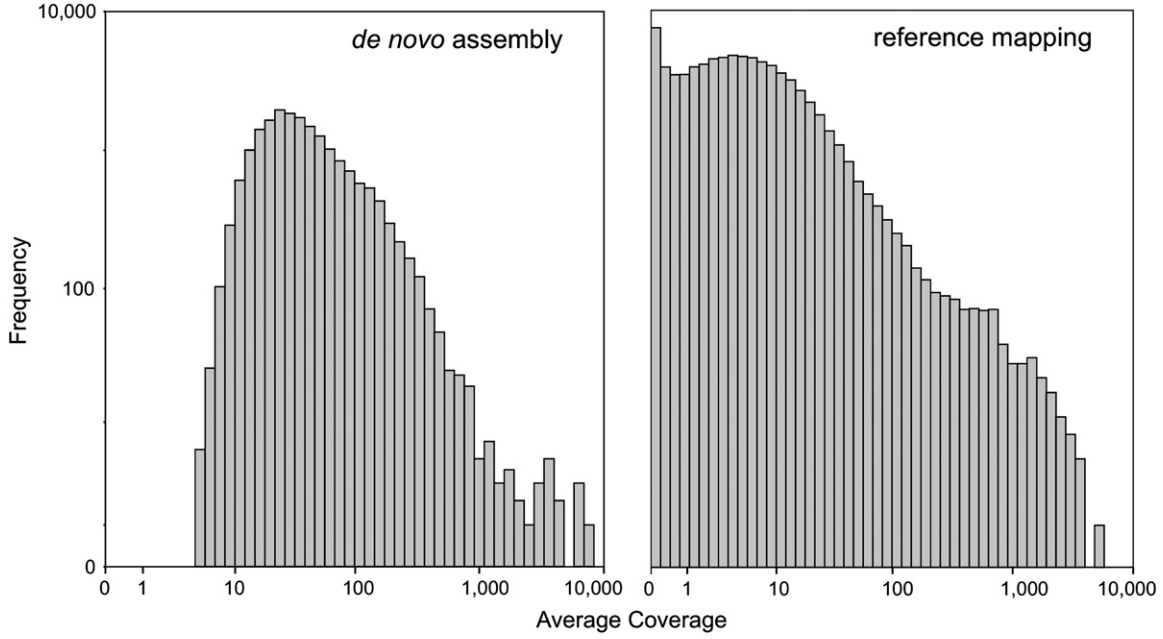

Fig. 1. Coverage distribution for de novo assembly and reference mapping. Histograms showing average read coverage for de novo assembly and reference mapping to GigasDatabase v8 for the combined *C. gigas* SOLiD transcriptome libraries.

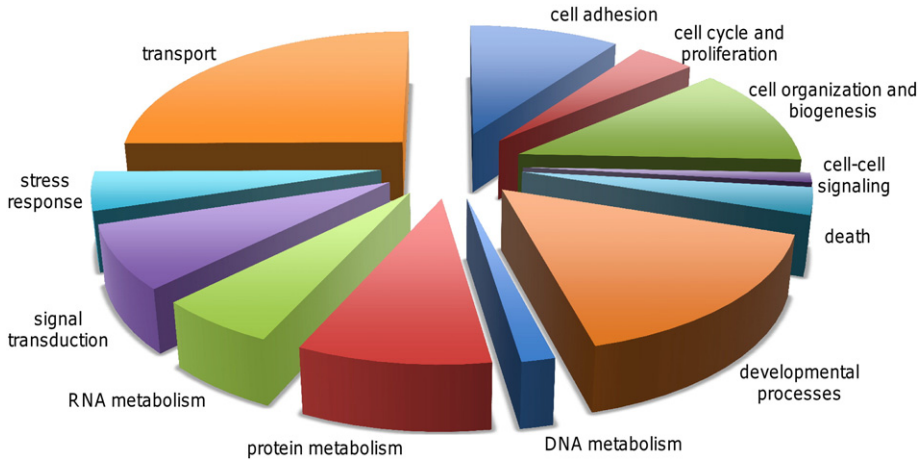

Fig. 2. Functional classification of novel transcripts identified by de novo assembly of the combined SOLiD transcriptome libraries. Categories represent 'GO Slim' terms associated with Biological Processes.

3.2.2. Reference-based RNA-Seq

For comparison, RNA-Seq was also performed using GigasDatabase v8 as the scaffold. In total, 427 differentially expressed features were identified. Of those, 239 were expressed higher in the BBC library and 189 were expressed higher in the DH library. Of these, 216 contigs could be annotated. Table 1 provides a comparison of data from both RNA-Seq procedures.

Functional enrichment analysis identified three biological processes that were enriched in the differentially expressed gene set. The most significantly enriched process was microtubule-based processes followed by oxidation reduction and cell recognition. One term, cell recognition (p value = 6E-3), overlapped between the de novo based and reference based RNA-Seq analysis. The other terms were unique to each analysis.

4. Discussion

This study evaluates the effectiveness of using high-throughput, short read sequencing technology to characterize the transcriptome of taxa with limited genomic resources. Specifically, SOLiD sequencing was carried out on cDNA libraries from Pacific oysters from two locations with differing anthropogenic influence. Sequence assembly and RNA-Seq analysis were carried out using resources generated solely as part of this study and compared to respective analyses using a publicly available transcriptome database. We found that limited ultra-short read sequence data can provide valuable information about transcriptome activity. Furthermore, we provide new genomic resources for *C. gigas* and have identified differences in oysters from areas that have experienced different degrees of human impact. These combined data significantly contribute to what we know about oyster biology but also offer a framework for efficiently characterizing transcriptomic differences in species lacking sequenced genomes. Advantages and limitations of using short read sequencing technology for gene discovery and RNA-Seq analysis are discussed.

4.1. Gene discovery

The number of Pacific oyster consensus sequences generated from de novo assembly is comparable to similar studies in sockeye salmon (Everett et al., 2011) and *R. balthica* (Feldmeyer et al., 2011). However, as expected, mean contig length (276 bp) was shorter than transcriptome

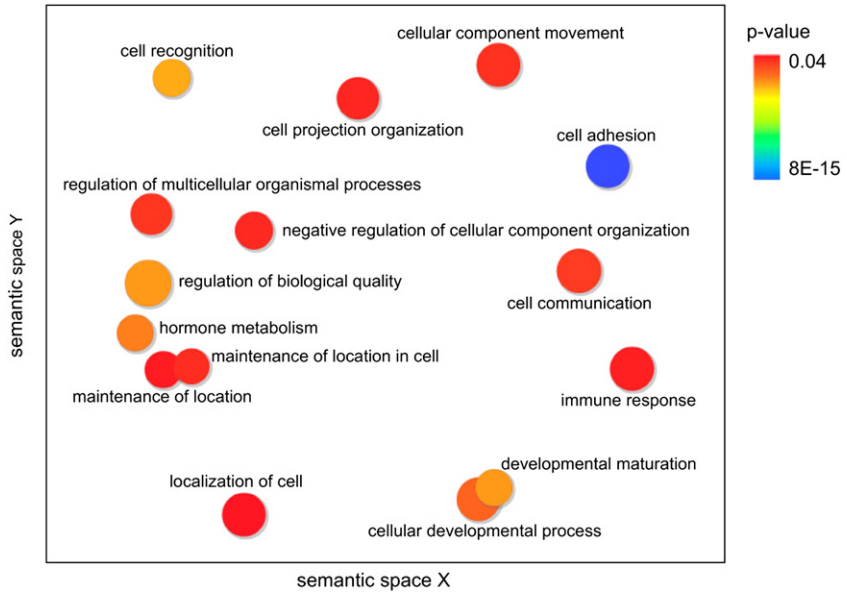

Fig. 3. Gene ontology categories overrepresented in the differentially expressed gene set. Color indicates p-value of the enrichment and size is proportional to the number of genes in the category. Spatial arrangement reflects a grouping of categories by semantic similarity.

characterizations that use 454 pyrosequencing. Recent studies in guppies (Fraser et al., 2011) and chum salmon (Seeb et al., 2010) produced mean contig lengths of 464 bp and 412 bp, respectively. In the current study our average coverage was 62x compared to 5x reported by Seeb et al. (2010). Dohm et al. (2008) have indicated greater than 20x coverage is sufficient to minimize effects of sequencing errors. We were able to annotate 42% of the consensus sequences generated from the de novo assembly. This included a large number of transcripts (742 contigs) not present in public databases. The number of novel sequences identified is slightly higher than reported in studies using Sanger sequencing for gene discovery in *C. gigas* (Gueguen et al., 2003; Roberts et al., 2008). The functional classification of the novels transcripts identified using SOLiD sequencing was highly diverse with a large proportion being involved in transport, developmental processes, stress response, and cell adhesion.

Several genes of interest were identified in the novel contigs, many of which are associated with response to stress. A number of these transcripts have been shown to be involved specifically in the immune response. For instance, a sequence with similarity to dual oxidase 2 was identified. In *Drosophila melanogaster* this protein regulates the production of reactive oxygen species in response to infectious and commensal microbes (Ha et al., 2009). The mitogen-activated protein kinase (MAPK) signaling pathway is involved in phagocytosis and the prophenoloxidase cascade in invertebrates (Lamprou et al., 2007). A subset of genes involved in this pathway has been previously identified in a *C. gigas* (Roberts et al., 2008). Here we identified a novel sequence in this pathway, mitogen-activated protein kinase kinase kinase 7 (M3K7). Another important component of the invertebrate immune system are bactericidal enzymes. A transcript similar to myeloperoxidase (MPO), which functions as a bactericide by generating hypochlorous acid (Harrison and Schultz, 1976), was present in the de novo consensus sequences. While this protein has been identified in molluscs based on its catalytic activity (Schlenk et al., 1991), this is the first time the nucleotide sequence has been reported in oysters. An additional sequence of interest possesses homology to a SAM domain and HD domain-containing protein, which has been shown to be involved in antiviral responses in humans (Rice et al., 2009).

Oysters and other coastal invertebrates are frequently exposed to xenobiotics. One of the first steps involved in the metabolism and subsequent exclusion of xenobiotics is binding of a ligand (i.e. aromatic hydrocarbon) to the aryl hydrocarbon receptor. As part of this sequencing effort we identified a transcript similar to aryl hydrocarbon receptor nuclear translocator (ARNT). ARNT encodes a protein that forms a complex with the ligand-bound aryl hydrocarbon receptor, and is required for receptor function (Hoffman et al., 1991). Activation of the aryl hydrocarbon receptor initiates transcription of cytochrome p450 oxidases. Several genes in this family have been previously reported in *C. gigas* (Roberts et al., 2008). Xenobiotic conjugates and metabolites are eventually excreted from the cell by membrane transporters in the multidrug resistance protein family. A contig generated as part of the de novo sequencing effort identified a transcript similar to multidrug resistance protein 1. Together the new sequences identified here demonstrate that limited ultra-short read sequencing provides an important resource for gene discovery.

When reference mapping was carried out, the proportion of reads that could be putatively annotated increased. While we have demonstrated that the sole use of a limited short read sequencing data set can provide cost-effective, valuable, novel genomic information, an available scaffold (i.e. EST contigs, genome) can provide benefits with respect to number of mapped reads and subsequent ability to annotate.

4.2. RNA-Seq

Using limited short read data we were able to effectively perform RNA-Seq analysis in the Pacific oyster. This is one of the first studies describing RNA-Seq analysis using solely ultra-short read data, along with other very recent publications in the crustacean *Pandalus latirostris* (Kawahara-Miki et al., 2011) and insect *Plutella xylostella* (Etebari et al., 2011). A similar approach is Tag-Seq, which utilizes short (<30 bp) tags, generally from the 3′ ends of transcripts to characterize differentially expressed genes. A recent study by de Lorgeril et al. (2011)) utilized Tag-Seq to identify approximately 4000 unique, immune responsive genes in *C. gigas*. In the current study, we were able to identify and annotate 1064 differentially expressed transcripts in *C. gigas* populations exposed to varying degrees of anthropogenic impact. Tag-Seq can be relatively less expensive than RNA-Seq with respect to coverage, however a reference scaffold is required. In addition, because tags are usually generated from a single end of a transcript, RNA-Seq analysis, as described here, has the advantage of identifying and quantifying novel transcripts (Cullum et al., 2011). In our RNA-Seq study, 18% of the differentially expressed transcripts were novel, representing a significant contribution to genomic resources. Together these studies demonstrate how advances in sequencing technology will continue improve our ability to characterize physiological responses in non-model organisms.

When comparing differentially expressed genes in oysters from the two sites, there was a large difference in the number of differentially expressed genes depending on whether the RNA-Seq was based on de novo or reference based assembly. Specifically, RNA-Seq performed using the de novo assembled consensus sequences reported seven-times as many differentially expressed genes as the RNA-Seq analysis using GigasDatabase v8. One possible explanation for this discrepancy is that using the de novo assembly as a scaffold may result in multiple sequences representing the same gene. In other words, the consensus sequences are relatively short and fragments representing different regions of the same gene may not overlap. As a majority of these differentially expressed genes could not be annotated, it is difficult to determine the precise impact of this possibility. However, 889 of the 1064 annotated, differentially expressed genes were deemed unique based on the protein identification code of the UniProt ID, suggesting there may be other factors contributing to this difference. As would be expected, based on the proportion of differentially expressed genes, the number of enriched GO biological processes identified was also different between the two analyses. It is likely that this difference is related to the scaffold itself, as all genes making up the scaffold are used as the "background" for the enrichment analysis. Therefore, it is possible that the de novo based enrichment analysis is more biologically relevant, as the background is a better representation of the genes expressed under similar conditions.

RNA-Seq analysis revealed that the set of transcripts differentially expressed between BBC and DH was most significantly enriched in genes associated with cell adhesion. In general, cell adhesion can be divided into to two general types. The first is a stable cell–cell adhesion that is critical for the organization of tissues. The second is a transient cell adhesion involved in processes such as cell adhesion between hemocytes and cell adhesion to pathogens. This transient type of cell adhesion is a critical part of invertebrate innate immunity by way of recognition of non-self particles, as well as chemotaxis and aggregation of hemocytes (reviewed by Johansson, 1999). The specific genes that are contributing to the difference between the two libraries include integrins, laminins and cadherins, which are expressed approximately 2–4 times higher in the DH library. While the precise biological role for this increased expression cannot be determined from this study, it could indicate the presence of specific contaminants in the environment. For instance, integrin expression increased in response to pathogen exposure in white shrimp (*Litopenaeus vannemai*) (Lin et al., 2010). In addition, estrogen exposure stimulates hemocyte binding to laminin 1 and collagen IV in mussels (*Mytilus galloprovincialis*) (Koutsogiannaki and Kaloyianni, 2011). While we can only speculate on the functional role, it is interesting to note that it is consistent with the environmental data from this locale, as DH is a site close to urban wastewater discharge and intensive agriculture exposure. However, additional research is

required to determine the role of genes associated with cell adhesion and environmental exposures in oysters.

5. Conclusions

Ultra-short read sequencing technology, such as SOLiD, provides a powerful and effective means for gene discovery and expression analysis in organisms with limited genomic resources. We have shown that it is technically possible and efficient to use this approach to 1) generate transcriptomic resources, 2) identify novel genes, and 3) perform RNA-Seq analysis. In terms of gene expression, de novo based RNA-Seq analysis does not rely on previous transcriptome information and results can be annotated at the biological process level. As high-throughput sequencing platforms continue to improve, they will serve as important tools for examining environmental physiology of non-model organisms.

Supplementary materials related to this article can be found online at doi:10.1016/j.cbd.2011.12.003.

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Development of Genomic Resources for Pacific Herring through Targeted Transcriptome Pyrosequencing

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Abstract

Pacific herring (*Clupea pallasii*) support commercially and culturally important fisheries but have experienced significant additional pressure from a variety of anthropogenic and environmental sources. In order to provide genomic resources to facilitate organismal and population level research, high-throughput pyrosequencing (Roche 454) was carried out on transcriptome libraries from liver and testes samples taken in Prince William Sound, the Bering Sea, and the Gulf of Alaska. Over 40,000 contigs were identified with an average length of 728 bp. We describe an annotated transcriptome as well as a workflow for single nucleotide polymorphism (SNP) discovery and validation. A subset of 96 candidate SNPs chosen from 10,933 potential SNPs, were tested using a combination of Sanger sequencing and high-resolution melt-curve analysis. Five SNPs supported between-ocean-basin differentiation, while one SNP associated with immune function provided high differentiation between Prince William Sound and Kodiak Island within the Gulf of Alaska. These genomic resources provide a basis for environmental physiology studies and opportunities for marker development and subsequent population structure analysis.

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Introduction

Many commercially exploited species face ecological and anthropogenic pressures in addition to fisheries, such as pollution, emerging diseases and climate change. Although demographic effects of such pressures are difficult to quantify, they are likely to affect both ecosystem structure and economic returns of dependent fisheries. Studies on the genetic and organismal effects of these pressures may provide insights into the phenotypic flexibility and the scope for adaptation that may allow resilience and resurgence of exploited populations. For example, Pacific herring (*Clupea pallasii*) in Prince William Sound (PWS) have collapsed after the *Exxon Valdez* oil spill in 1989, resulting in a closure of commercial and traditional fisheries. Despite two decades of research and extensive restoration efforts, Pacific herring is one of only two resources still classified as 'not recovered' [1]. Although herring spawning populations were large in 1989, the recruiting cohort was one of the weakest on record, and by 1993, the spawning population was reduced to about a quarter of its previous size [1]. Even now, the population has not recovered [2], and remains well below the recovery aim of 43,000 tons [1]. Because of the central position of herring in the marine food web and its importance as a commercially exploited species, such low biomass may affect the entire ecosystem as well local fishing communities.

The causes for the initial collapse of PWS herring are not well understood, and even the exact timeline of the collapse is under dispute [2], [3]. Nevertheless, exposure to oil pollution [4], disease [5], [6], predation/competition [7] and changes in the physical oceanography, or any combination of these factors, are potential culprits. Although it may not be possible to reconstruct the exact causes of the collapse, it is important to identify factors limiting or preventing recovery [1]. The combination of new molecular technologies allowing the sequencing of the entire expressed genome (transcriptome) of non-model species and novel computational approaches provide the opportunity for efficiently addressing potential causes underlying the lack of Prince William Sound herring recovery through the development of genomic resources. New sequencing technologies have greatly reduced the costs required for genomic resource development, though there are still challenges faced when working with non-model organisms [8], [9]. Short sequence read lengths and large quantities of data have to be analysed *de novo*, without the assistance of a reference genome that would be available for species such as humans, mice, and zebrafish. Following the initial steps of assembly and annotation, putative genetic markers can, however, be more easily compared to older sequencing technologies given the large quantity of sequencing reads. The large number of putative markers that can be identified greatly increases the potential to identify self-recruiting populations, even if the populations are large and connected by relatively high migration rates.

We report the sequencing of the herring hepatic and testicular transcriptome in order to provide a more comprehensive set of genomic resources for Pacific herring for population structure analysis and environmental physiology studies. This represents the first large scale sequencing efforts for a member of the teleostean order Clupeiformes. An annotated transcriptome is described, as well as a workflow for SNP discovery and validation. Furthermore,

we provide preliminary analysis on population structure at select genes and compare patterns of diversity and differentiation at loci developed from this effort to allozyme, microsatellite, and mitochondrial DNA markers screened in the same populations.

Results

Sequence Assembly and Annotation

In total, 2,117,781 raw sequencing reads were generated with an average length of 254 bp (Table 1). All data was submitted to NBCI's Short Read Archive under accession number SRX022719. After quality trimming, 96% of the data was retained for a total of 530 Mb of sequencing data. Quality trimmed reads from the liver and testes libraries were *de novo* assembled separately to generate 34,300 and 31,545 contiguous sequences (contigs), respectively [10], [11]. *De novo* assembly of all data resulted in 42,953 sequences with an average length of 728 bp. A majority, 81% of the contigs, were between 100 and 1000 bp in length (Figure 1). A majority of the contigs were classified as involved in protein metabolism, RNA metabolism, or other metabolic processes (Figure 2).

Library Comparison

To investigate the relative contribution of each library to the rate of gene discovery, RNA-seq analysis was performed. The testes tissue library included 15,401 features expressed at a higher level (>4-fold) with 13,379 features expressed at a higher level in the liver library (Figure 3). A large number of features, 8,346 in testes library and 11,185 in liver library, were expressed in only a single library.

The number of contigs per number of reads was lower in the liver library compared to the testes library. The percentage of reads that generated the contigs varied across libraries with the percentage ranging from 62% to 69% for the liver library and from 52% to 62% in the testes library. When sequencing effort was reduced *in silico* by approximately 50% (500,000 reads/library) 20,966 and 25,416 contigs were generated from the liver and testes libraries, respectively (Figure 4).

Table 1. Characteristics of Pacific herring hepatic and testicular transcriptome sequencing.

	Sequences (n)	Average Length
Raw Sequencing Reads		
Liver Library	1,195,565	278
Testes Library	982,216	233
Both Libraries	2,177,781	254
Quality Trimmed Reads		
Liver Library	1,109,404	284
Testes Library	837,401	257
Both Libraries	1,946,805	272
Contigs		
Liver Library	34,300	625
Testes Library	31,545	646
Both Libraries	42,953	728
Singletons		
Liver Library	749,929	284
Testes Library	315,852	266
Both Libraries	778,383	259

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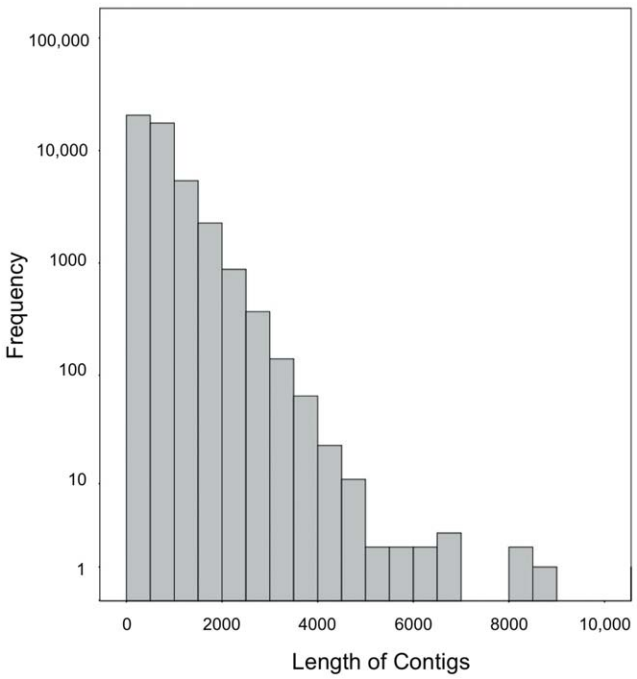

Figure 1. Histogram of contig sequence length. Contig sequences were generated from *de novo* assembly of both libraries (n = 42,953) and average length is 728 bp. Note logarithmic scale for frequency axis.
doi:10.1371/journal.pone.0030908.g001

SNP Discovery

SNP detection analysis revealed 10,933 potential SNPs in the combined herring transcriptome. A majority of the SNPs (60%) were transitions. A/T and C/G transversions were each present in 9% of the candidate SNPs while the G/T and A/C substitutions constituted 11% of the polymorphisms. Average coverage of putative SNPs was 16.6 (SD = 46.8), with 95% of the SNPs having coverage less than 25×.

Distinguishing synonymous and non-synonymous SNPs from high-throughput sequence data in species without sequenced genomes can be a challenge. Over both libraries, 161,059 potential open reading frames were identified. SNP detection using these open reading frames to map all quality trimmed reads revealed 4448 putative SNPs. Of those SNPs, 1610 resulted in a predicted amino acid substitution. After removing sequences with e-values greater than 1.0E-10 (Swiss-Prot database) and less than 10× coverage, 257 non-synonymous SNPs and 722 synonymous candidates remained (dn/ds = 0.356).

SNP Validation and Population Screening

Fifty candidate SNPs did not pass the initial primer testing; many of these were likely true SNPS adjacent to intron/exon boundaries and would not amplify. Sanger sequencing confirmed the presence of one polymorphism in 14 templates and two or more polymorphisms in 16 templates. The 14 templates with a single polymorphism were used for HRMA. Additional Sanger sequencing demonstrated the presence of more than one polymorphism in four of these sequences in other populations (Table 2). The 14 templates were originally identified based on functional annotation of the respective transcript. Some loci did not have significant BLAST hits when the targeted genomic region

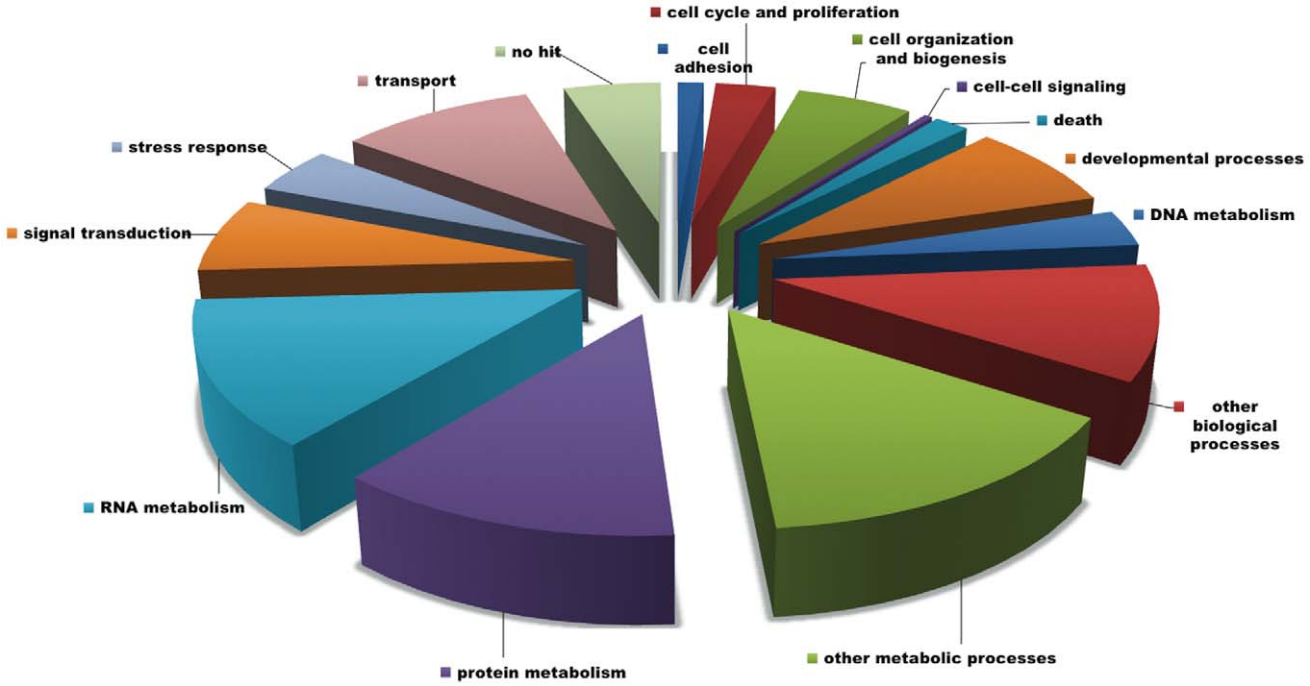

Figure 2. Functional categorization of contig sequences. Contig sequences were generated from *de novo* assembly of both libraries (n = 42,953) and annotations performed using BLASTx with Swiss-Prot and Gene Ontology databases.
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was examined, however several loci are likely associated with genes involved in immune and xenobiotic response (Table 3).

HRMA showed that eight of the fourteen tested loci conformed to HWE in all three samples (Table 4). Five loci deviated significantly from HWE in one sample (two loci because of heterozygote excess and three because of heterozygote deficiency). One locus deviated significantly from HWE in two samples (both heterozygote deficiency). Four loci deviated from HWE in Togiak herring, two loci in Prince William Sound and one locus in Kodiak Island fish. Average heterozygosity was lower in the Bering Sea ($H_e = 0.221$) than the Gulf of Alaska ($H_e = 0.339$) (Table 5). A hierarchical AMOVA showed that 8.7% of the variation was due to differences between populations, and most of that differentiation was due to differences between the Bering Sea (*i.e.* Togiak) and the Gulf of Alaska (Table 6). Eight of the 14 loci showed significant differentiation among populations before ($P <= 0.034$) and seven after Bonferroni correction ($P <= 0.001$). Differentiation between ocean basins was higher than within Bering Sea or Gulf of Alaska (Figure 5), but because of the small number of samples and the low power of permutation tests resampling entire collections between groups as carried out in Arlequin, that differentiation between ocean basins was not significant at any locus. However, one locus associated with virus response showed significant differentiation ($P = 0.042$) between PWS and Kodiak within the Gulf of Alaska (Figure 5).

Discussion

The large-scale sequencing effort characterized here was carried out to provide a foundation of such genomic information to assist in the research on the biology, ecology, and population genetics of herring. Prior to the completion of our sequencing project, there were less than 1000 publically available nucleotide sequences for the Pacific herring. Furthermore, sequence information from any species of the teleostean order Clupeiformes was limited, and zebrafish (*Danio rerio*) was the most taxonomically similar species with significant genomic resources. Here, we provide over 2 million nucleotide reads from the Pacific herring transcriptome. From these data, we were able to generate over 40,000 contigs with a 10× average coverage depth, identify thousands of putative SNPs, and demonstrate realistic levels of population diversity and differentiation in a small subset of these SNPs.

Pyrosequencing and non-model species

Pyrosequencing using the 454 platform on non-model organisms is increasingly proven to be an effective and efficient means to provide large scale transcriptomic information. Given the dynamic nature of gene expression, advances in technology and the variety of analytical techniques available make it difficult to directly compare studies, however generally our results are similar to other sequencing efforts. One of the initial applications of 454 pyrosequencing on non-model organisms was carried out on the butterfly, *Melitaea cinxia* [12]. More recently, this platform has been used to provide resources for aquatic organisms of ecological importance. In the lake sturgeon (*Acipenser fulvescens*), 47,060 reads were produced and assembled into 1831 contigs [13]. In chum salmon, two individual fish testes were sequenced and combined, resulting in 1.9 million reads and 118,546 contigs [8]. In both of these efforts, novel SNPs were characterized. In addition to using 454 pyrosequencing for gene discovery and SNP development, the platform provides the opportunity for large-scale expression analysis (RNA-Seq) in organisms of ecological importance. For example, in the lake trout (*Salvelinus namaycush*), 425,821 quality-trimmed reads from liver tissue were assembled into 2276 contigs that were then used for comparative transcriptomic analysis of two lake trout ecotypes [14]. In the studies listed above, methodologies other than pyrosequencing were employed for SNP validation (e.g. HRMA analysis and Sanger sequencing) and RNA-Seq analysis (e.g. quantitative reverse transcription PCR). Likewise, we present

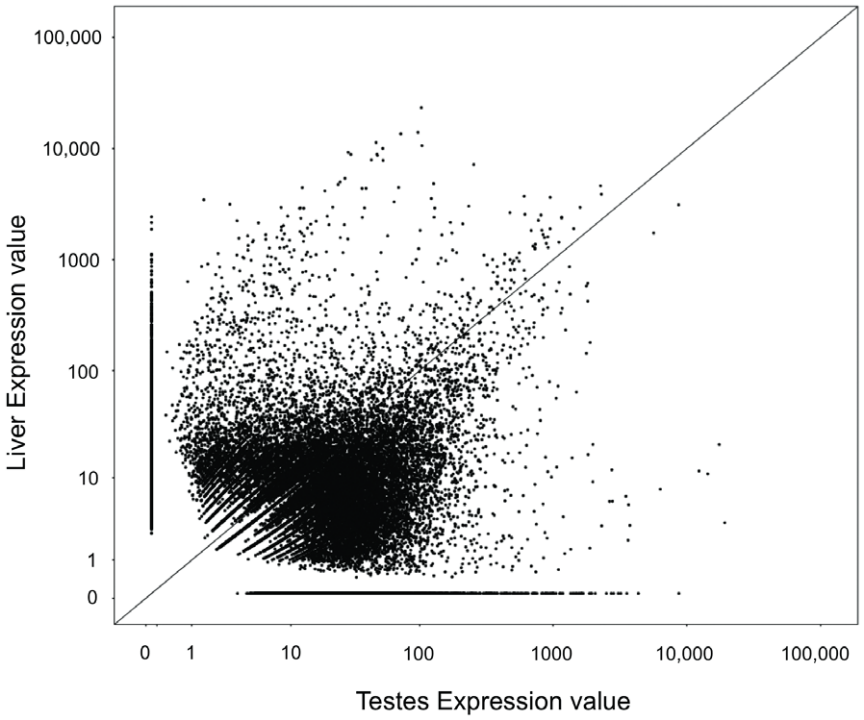

Figure 3. Relative expression (RPKM) of 454 sequenced transcriptome across liver and gonad tissue. Diagonal line represents equal expression in both tissue types. Note both axes are on the logarithmic scale.
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the use of HRMA and Sanger sequencing for the validation of SNPs in the Pacific herring. As sequence costs continue to decrease, it is likely that pyrosequencing and other sequencing technologies will be increasingly used for SNP validation and comprehensive RNA-Seq analysis in non-model species.

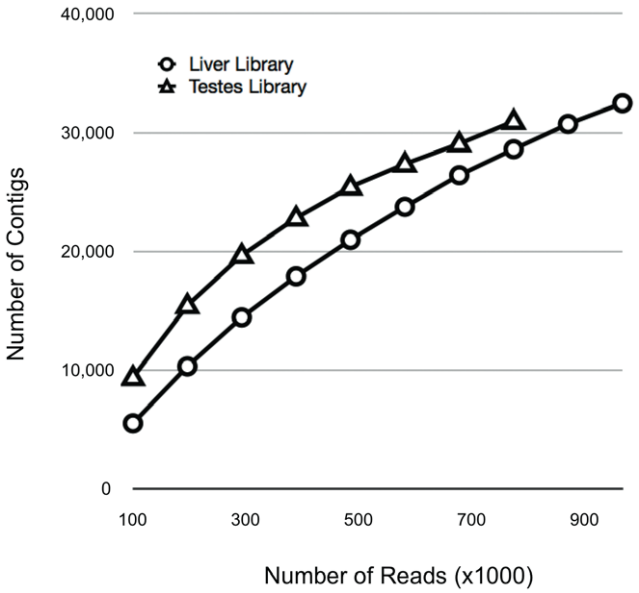

Figure 4. Rarefaction analysis of quality trimmed reads from each library. Rarefaction analysis was used to determine level of contig discovery relative to sequencing effort. Reads were sequentially sampled in 1×10^5 sequence read increments and *de novo* assembled.
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Rarefaction Analysis

The number of reads generated per given sequencing effort is relevant, particularly given any financial consideration. In order to characterize the benefit of additional reads to information yield, we examined the number of contigs generated for a given number of quality-trimmed sequence reads (Figure 4). This type of rarefaction analysis is similar to the approach taken by Hale et al. [13] to compare 454 library construction approaches for sturgeon transcriptome libraries. For the herring libraries, a decrease in rate of new contig discovery to below 10% of sequence reads was reached when between 500,000 and 700,000 reads were utilized. This would be expected and indicates with additional sequencing the amount of new information gained will decrease. When the same approach was used to compare incremental number of reads from chum salmon [8], the rate of increase of number contigs was higher, as was the total number of contigs (data not shown). This is likely related to genome duplication events present in the salmonid lineage. As described above, the dynamic nature of transcriptomes and laboratory techniques have to be taken into consideration when comparing libraries and planning sequencing effort. However, the use of rarefaction analysis does provide a simple way to characterize transcriptome diversity.

Tissue-Specific Gene Expression

While RNA-seq analysis was not used in the study to address ecological issues, the analytical techniques were utilized to evaluate expression differences between the two tissues examined, liver and testes. This approach can provide information on the benefit of the multiple tissues as well as information of functional importance. There was a large difference in the expression levels between tissue types, with over 67% of the features differentially expressed in one tissue. Furthermore, 45% of the features were only expressed in a

Table 2. Loci selected for HRMA and associated primers.

Loci ID	Primer 1	Primer 2	SNP Position	SNP
Cpa_28881	TCGTTCTGATTGGCTTACCC	GTTGGGGCTTGCCTAAAAAT	73, 93, 128	G/T, C/T, C/G
Cpa_RG9MTD2	CTGCCACAGTGTGTGTACCAT	CTCTCTGCCAGTGATGCTGA	84, 109	C/T, C/G
Cpa_ABCG5	CCACCGTCCAGTAGAGGAAT	TTTCCTGCACTCAGGGCTAT	75, 90, 121	G/T, G/T, A/G
Cpa_SOX11	TTGCTACAAAACGCAGATGG	GTGAATGGGTCCCACATAGC	90	A/C
Cpa_24210	TTGGACAAGCGTGTTGTGTT	GTAAGGAATGCCCACGTCTG	70	A/C
Cpa_28757	AAGGATGCCAACAGCACTCT	CCCTCAGAGGTTTCATGGTG	64, 77	A/G, A/G
Cpa_CYP2J5	TGTCTTTGGTGGCACTTCTG	GAGGAGATTGACCGTGTGGT	46	A/G
Cpa_UGT2A	CTCCTGAACTCCGTTCGTTC	AGGTCATCTGGAGGCATCTG	85	C/T
Cpa_11680	TCTTCGCACAATGACCACTC	GGCTTTAGCAATTAGCTGCAT	87	A/C
Cpa_11961	TCATCAGGCGTTGACAAAGA	GTCGACTGCTTGAGGAGACC	69	G/T
Cpa_PM20D1	GTGACTGTGTTGGGCATGAG	CCAACCCTGATGTCAGTTCC	49	C/T
Cpa_11785	CTGAGGGCTCTTTGGCTTTA	GGTTAAGAGGGCCGGTAAAA	63	A/G
Cpa_UPF0669	CACTTCGAGGACGATGATGA	GGCTGCTCATGTGTAGGATG	65	A/G
Cpa_APOB	TTGCAGTACCCTCAGTGGTG	AGGTGTCTGCCAAGGTCAAC	60	C/T

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single tissue. This was expected, given the functional difference in gonad and hepatic tissue, and illustrates tissue diversity is useful for discovery of markers at the genomic DNA level from transcriptome sequences.

Complement System

RNA-seq-based approaches also allow for the identification of genes associated with specific biological function. One group of genes of particular interest in immune physiology is components of the complement system (see [15] for review). The complement system is an important component of both the innate and acquired immune response. Three pathways are involved in the complement response, including the classical, lectin, and alternative pathways. The classical pathway is activated by antigen and antibody complexes, the lectin pathway requires interaction of lectins with sugar moieties on microbes, and the alternative pathway is initiated by the spontaneous activation (hydrolysis) of C3. Complement C3 in herring was identified in the herring liver and most similar to that of the complement C3 homolog in rainbow trout (Table S1). A complement C2 homolog was identified and is important for activation of both the classical and lectin pathways. Factor B is present, and together with complement C2, both found in the MHC III region in mammals [16]. In contrast to complement C2, Factor B is an important component in the alternative pathway. Activation of each pathway can lead to the assembly of the membrane attack complex (MAC).

Table 3. Annotation of selected loci based top BLAST hit and GO ontology.

Loci ID	Genomic BLAST Hit	Accession #	e-value	Gene Function
Cpa_28881	no hit			
Cpa_RG9MTD2	RNA methyltransferase domain-containing protein 2 (Human)	Q8TBZ6	9.55E-18	methyltransferase activity
Cpa_ABCG5	ATP-binding cassette sub-family G member 5 (Human)	Q9H222	9.57E-10	cholesterol homeostasis
Cpa_SOX11	Transcription factor Sox-11 (*Salmo salar*)	NM_001173797	2.00E-04	regulation of transcription
Cpa_24210	no hit			
Cpa_28757	no hit			
Cpa_CYP2J5	Cytochrome P450 2J5 (mouse)	O54749	1.34E-11	oxidation reduction
Cpa_UGT2A	UDP-glucuronosyltransferase 2A2 (Human)	Q9Y4X1	2.88E-14	sensory perception of smell
Cpa_11680	no hit			
Cpa_11961	no hit			
Cpa_PM20D1	Probable carboxypeptidase PM20D1 (zebrafish)	Q08BB2	1.62E-12	metal ion binding
Cpa_11785	no hit			
Cpa_UPF0669	UPF0669 protein C6orf120 homolog (zebrafish)	Q6NZZ3	6.53E-14	
Cpa_APOB	Apolipoprotein B-100 (human)	P04114	9.55E-18	response to virus

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Table 4. Minor allele frequency, expected heterozygosity, and inbreeding coefficient (F_{IS}) for select loci in three Pacific herring populations.

	Minor Allele Frequency			Expected Heterozygosity			Inbreeding Coefficient (FIS)		
Loci ID	**Kodiak**	**PWS**	**Togiak**	**Kodiak**	**PWS**	**Togiak**	**Kodiak**	**PWS**	**Togiak**
Cpa_28881	0.29	-	0.23	0.42	NA	0.36	−0.05	NA	−0.12
Cpa_RG9MTD2	0.33	-	0.21	0.45	NA	0.34	0.17	NA	**0.47**
Cpa_ABCG5	0.26	0.23	0.01	0.39	0.35	0.01	−0.04	−0.17	0
Cpa_SOX11	0.22	0.25	0.01	0.34	0.37	0.01	−0.08	0.15	0
Cpa_24210	0.25	0.27	0.02	0.37	0.4	0.04	−0.15	−0.15	**0.49**
Cpa_28757	0.26	0.22	0.03	0.39	0.34	0.06	−0.07	−0.08	0.32
Cpa_CYP2J5	0.13	0.14	0.38	0.23	0.25	0.47	**0.43**	**0.55**	0.16
Cpa_UGT2A	0.23	0.25	0.23	0.36	0.37	0.36	−0.21	−0.2	**−0.24**
Cpa_11680	0.32	0.28	0.20	0.44	0.41	0.32	−0.09	0.09	0.19
Cpa_11961	0.31	0.28	0.20	0.43	0.4	0.33	−0.05	−0.09	**0.38**
Cpa_PM20D1	0.28	0.25	0.12	0.41	0.37	0.21	−0.13	−0.15	−0.02
Cpa_11785	0.01	M	0.02	0.01	0	0.04	0	NA	−0.02
Cpa_UPF0669	0.20	0.19	0.17	0.32	0.31	0.28	0.11	−0.16	−0.05
Cpa_APOB	0.29	0.20	0.06	0.41	0.32	0.11	−0.18	**−0.25**	0.14

Numbers in bold represent values that deviated significantly from HWE.
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The membrane attack complex forms transmembrane channels in bacterial pathogens causing cell lysis and death. The membrane attach complex is composed of C5b, C6, C7, C8 and C9 components [17]. C5-C9 homologs were all identified in the herring liver transcriptome (Table S1).

Cytochrome p450 Superfamily

Several members of the Cytochrome p450 enzyme superfamily were identified in this pyrosequencing effort. In the consensus sequences generated from liver library reads, putative members of families CYP1, CYP2, CYP3, and CYP4 were all identified (Table S1). One of the most studied cytochrome p450 enzymes is CYP1A1. This enzyme is involved in phase I xenobiotic metabolism including dioxins, PCBs, and PAHs. CYP1A1 has served as a key biomarker for petroleum product exposure, as it has been shown to be induced by exposure to these compounds in aquatic species [18]. A 1400 bp consensus sequence was identified in the herring liver transcriptome that codes for CYP1A1. CYP1A1 expression is initiated by activation of the Aryl hydrocarbon receptor, which was also partially sequenced in this project. Partial sequence information of genes in this and other pathways of interest will facilitate research on Pacific herring and are of particular interest given the impacts of the oil spill.

Table 5. Unbiased heterozygosity of four genetic markers from Pacific herring; haplotype diversity was used for mtDNA.

	Bering Sea	Gulf of Alaska	Ratio[a]	
Allozymes	0.073	0.098	1.34	[22]
Microsatellites	0.851	0.905	1.06	[21]
MtDNA	0.778	0.883	1.13	[26]
SNPs	0.211	0.339	1.61	This study

[a]the ratio between the two values (Gulf of Alaska/Bering Sea).
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SNP Development

One primary motivation for the high-throughput sequencing effort on Pacific herring was the discovery of SNPs in coding regions that could be used for population studies on selection and local adaptation. Neutral molecular markers, such as allozymes and microsatellites, have provided powerful insights into population structure and demographic history of wild populations, but they cannot detect adaptive genetic variation. Large-scale genome sequencing efforts combined with outlier tests now have the potential to find genes that are important for adaptation in wild populations. Multiple strategies to increase coverage and target potentially selected genes are available, including exome capture [19], restriction enzyme-based selection [20], and transcriptomic sequencing [12]. For Pacific herring we chose to target the transcriptome of biologically relevant tissue, primarily as this approach provides a wealth of protein encoding sequence that can be used in physiological, ecological, and evolutionary studies and may reveal insights into biological and physiological reasons for the lack of recovery. A drawback to this approach is that, ultimately, population genetic markers will be characterized at the genomic DNA level, and the exclusion of introns in the transcriptome can contribute to marker "drop-out" during validation [8]. For instance, 96 putative SNPs were selected for further validation in this study and only 46 primer pairs produced a single PCR product. For those PCR reactions where no band was produced, it is likely that introns either prohibited primer annealing or resulted in a product too large for amplification. Another reason for the drop-out at this stage was non-specific amplification resulting in the presence of multiple amplicons. One way this could be mitigated in future projects would be to use other known species-specific sequence information to exclude primers without optimal specificity.

Another challenge in developing SNPs in non-model organisms is the determination whether a particular SNP will result in an alteration in amino acid. This information is particularly important when there is interest in selective environmental pressure that could be related to physiological responses and

Table 6. Locus by locus AMOVA of SNPS compared to allozyme, microsatellite and mtDNA.

F component		Allozyme[a]	F_{ST} Microsat[a]	R_{ST} Microsat[a]	MtDNA[a]	SNPs
within basin	FSB	0.003	0.01	0.03	0.013	−0.001
Between basins	FBT	0.241	0.023	0.209	0.169	0.087
Total	FST	0.244	0.033	0.233	0.178	0.086

[a]**From** [**26**].
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distinguish populations. Here, we developed and generalized a workflow that could be used for any species where there is an absence of an annotated genome. Essentially, a combination of open reading frame identification (based on absence of stop codons) and sequence similarity scores were used to identify SNPs that likely result in a predicted amino acid substitution. Based on the workflow described, we estimated that there were 26% non-synonymous SNPs. It should be pointed out we did not validate this prediction and only a fraction of SNPs (979 SNPs) were available for this form of characterization.

Preliminary population genetic analysis

Most loci conformed to HWE, even before Bonferroni correction, and significant deviations from HWE showed no clear concentration towards specific loci. However, the Bering Sea population (Togiak) had more loci that were out of HWE than populations from the Gulf of Alaska, which may be due to ascertainment bias or selection, but this needs further investigation. Ascertainment bias seems possible because even though sequences were obtained from a group of fish that included Bering Sea fish, three quarters of the fish originated from the Gulf of Alaska. It may be that in Bering Sea fish, these SNPs included a non-amplifying null allele more commonly known from microsatellite, though this would need to be confirmed by additional sequencing of homozygotes. Four templates had more than a single SNP, but three of the four showed no significant deviation from HWE in any of the populations, and the fourth only in the Togiak population. HRMA patterns were scored as biallelic loci, so even with multiple SNPs only a single polymorphism was considered, resulting in effective binning of rare alleles and conformance to HWE.

The distribution of genetic diversity within and between populations was remarkably similar for SNPs as for previously analyzed markers (allozymes, mtDNA and microsatellites, Tables 5 and 6). Almost all the genetic differentiation was between the two major basins, most likely due to secondary contact of western and eastern Pacific population groups after the last ice age [21], [22]. Similarly, the absence of stable genetic differentiation between Prince William Sound and Kodiak Island from microsatellites [21] was confirmed by SNPs. These patterns of genetic diversity suggest that the 14 loci surveyed here represent true genetic variation and not artifacts caused by paralogous loci or technical problems.

Genetic diversity was higher in the Gulf of Alaska than the Bering Sea (Tables 4 and 5), again conforming to previously reported results from other markers [21], [22]. However, the difference in genetic diversity was higher in SNPs than in the other markers (as measured by the ratio between heterozygosities in the two basins, Table 5). Average heterozygosity was intermediate in SNPs, therefore providing more opportunity for difference in diversity than at markers with very low (allozymes: $H_e = 0.10$) or

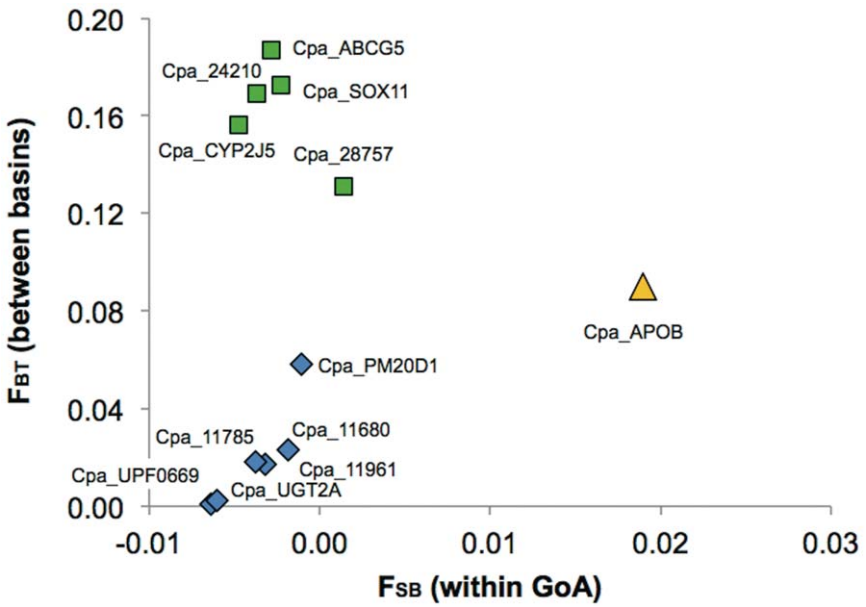

Figure 5. Variance components from a locus-by-locus AMOVA. Five loci (green) showed differentiation between the two ocean basins (high F_{BT}), while a single locus (yellow) significantly differentiated between Prince William Sound and Kodiak Island within the Gulf of Alaska (high F_{SB}, yellow).
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very high (microsatellites: $H_e = 0.91$) variability. Although the difference in diversity between Bering Sea and the Gulf of Alaska may have been due to ascertainment bias (17 of the 23 sequenced fish came from the Gulf of Alaska), the corresponding results between allozymes, microsatellites and SNPs suggest that differences in demographic history may have caused these genetic patterns, rather than a methodological issue in one of the three markers.

Although genetic patterns in SNPs are similar to microsatellites, the distribution of genetic differentiation among individual loci suggested some selective differentiation. Five of the 14 loci showed very high differentiation ($F_{ST}>0.1$) between the two ocean basins (Figure 5), and in four of these five loci genetic variability in the Bering Sea was extremely low ($H_e<0.06$, Table 4; *Cpa_CYP2J5* the exception). Two of these four loci had three and two SNPs, respectively (*Cpa_ABCG5*, *Cpa_28757*), and thus the differentiation may be due to any one of those SNPs. The fifth locus (*Cpa_CYP2J5*) demonstrated significant deficiencies of heterozygotes in both Gulf of Alaska samples. Despite this high differentiation, the variance component between ocean basins was not significant at any of the loci, likely because of the small number of populations (one in the Bering Sea, two in the Gulf of Alaska) and the consequent low power of permutation approaches randomizing entire samples between groups (Arlequin [23]). Nevertheless, the differentiation at these loci appears real and may either be due the different evolutionary history of the Bering Sea population [22] or due to selection in different environments. Further population genetic analyses are required to address this question in a genome scan approach; our data here provide the needed foundation for development of the necessary marker set.

Another locus showed high and significant differentiation between Kodiak Island and PWS (Figure 5; *Cpa_APOB*), and deficiency of heterozygotes in PWS, which may suggest selection within PWS. That locus is a virus response gene, which corresponds to the notion that infection by viral hemorrhagic septicemia virus may have at least contributed to the delayed recovery of Prince William Sound herring [24], but see [25]. However, data are too preliminary to reach any conclusions here, and additional loci need to be screened. Because of the limited number of markers and the biased selection of SNPs in genes coding for pollution and disease relevant genes, we did not attempt a formal outlier test. Nevertheless, these analyses show general correspondence of genetic patterns from SNPs with those of other genetic markers and suggest the value of expanding analyses to many more of the SNPs discovered in this study.

Conclusions

Targeted transcriptomic sequencing provides a valuable resource for genetic marker and gene discovery in non-model organisms. As part of one of the first large scale sequencing efforts for a member of the order Clupeiformes, we have characterized over 40,000 contigs and have described a workflow for SNP discovery and validation. Five SNPs supported a between ocean basin differentiation, while one SNP also showed significant differentiation between Prince William Sound and Kodiak Island within the Gulf of Alaska. These loci will provide a better understanding of Pacific herring population structure as well as insight into the dynamics of selection and local adaptation.

Methods

Tissue Collection

Liver and testes samples from sexually mature Pacific herring were provided by the Alaska Department of Fish and Game from four locations; three in the Gulf of Alaska (Kodiak Island, Prince William Sound and Sitka Sound) and one in the Bering Sea (Togiak Bay). Six fish each were sampled at Kodiak Island, Prince William Sound, and Togiak Bay, and five fish were sampled from Sitka. Tissue samples were immediately preserved in RNAlater (Ambion).

Sample Preparation

Individual tissue samples were transferred to TriReagent (Molecular Research Center), and total RNA was isolated as per manufacturer's instruction. Individual liver and testes RNA samples (four fish and three fish per location, respectively) were pooled in equal quantity for the construction of two Pacific herring transcriptome libraries. Messenger RNA was isolated from each total RNA pool with the MicroPoly(A) Purist Kit (Ambion) according to the manufacturer's protocol. The isolated mRNA was purified again with the MicroPoly(A) Purist Kit (Ambion) to further minimize residual rRNA carryover. Libraries were constructed by MOgene, LC (St. Louis, MO), following standard protocols from Roche Life Sciences.

Sequencing and Analysis

Both libraries were sequenced using the Genome Sequencer FLX System (Roche) at MOgene, LC (St. Louis, MO). Initially, all sequences were trimmed based on quality scores of 0.05 [27], [28] and a maximum allowance of two ambiguous nucleotides. Sequences smaller than 100 bp were removed. *De novo* assembly was carried out using CLC Genomics Workbench v3.7 (CLC Bio) with the following parameters: similarity = 0.98, length fraction = 0.9, insertion cost = 3, deletion cost = 3, mismatch cost = 2 and minimum size = 300. Where reference assemblies were performed, the same parameters were applied. Consensus sequences were compared to the Swiss-Prot database (http://uniprot.org) in order to determine putative annotation. Associated GO terms (Gene Ontology database: http://www.geneontology.org) were used to classify sequences based on biological process as well as categorize genes into parent categories (GO slim).

RNA-seq analysis was used to characterize the transcriptome tissue specificity (CLC Genomics Workbench v3.7 (CLC Bio)). Expression values were measured in RPKM (reads per kilobase of exon model per million mapped reads, see [29]). Parameters for RNA-seq analysis included an unspecific match limit of 10 and a minimum length fraction of 0.9. Given the resources necessary to perform pyrosequencing and the absence of the sequenced genome in herring, we evaluated the relative benefit of additional sequencing effort by rarefaction analysis. Specifically, quality trimmed reads from each library were sequentially sampled in 1×10^5 sequence read increments and *de novo* assembled as described.

SNP Discovery

Candidate SNPs were identified from assembled reads using CLC Genomics Workbench v3.7 (CLC Bio). Parameters were as follows: maximum gap and mismatch count = 2, minimum average quality = 15, minimum central quality = 20, minimum coverage = 4, minimum variant frequency (%) = 35.0, window length = 11. In order to determine whether a SNP resulted in a potential amino acid substitution, putative open reading frames were identified and SNP detection carried out as described. Specifically, open reading frames were identified in the consensus sequences from the *de novo* assembly of all reads based on the inclusion of start and stop codons, with a minimum size of 100 bp, using *getorf* (EMBOSS). All quality trimmed reads were then mapped back to these possible open reading frames, and SNP

detection was carried out as described (CLC Genomics Workbench v3.7 (CLC Bio)). Consensus sequences generated with *getorf* (EMBOSS) which contained putative SNPs were compared to the Swiss-Prot database.

SNP Selection and Primer Testing

Ninety-six putative SNPs were selected for validation. Selection stringency was increased from parameters described above to include a window length of 151. In addition, functional annotation was taken into consideration. PCR primers were designed using Primer3 [30] to amplify a template approximately 200 bp long that contained a single putative SNP in the ascertainment fish. A PCR test was done using 2× LightCycler480 High Resolution Melting Master (Roche Applied Science) following manufacturer's instructions. Primer pairs that produced a single, clean amplicon were sequenced. Specifically, six individuals were sequenced in both directions using ABI PRISM BigDye Terminator version 3.1 Cycle Sequencing Kit and analyzed on a 3730 DNA Analyzer (AB) by High-Throughput Sequencing Solutions (University of Washington, Department of Genome Sciences). Sequence chromatograms were aligned and visually screened for polymorphisms using Sequencher 4.9 (GeneCodes Corporation).

High Resolution Melt Analysis and Genotyping

Those SNPs validated using Sanger sequencing were genotyped using high resolution melt analysis (HRMA) [31], [32]. A total of 95 individuals from each of three populations sampled from Kodiak Island, Prince William Sound, and Togiak Bay, Alaska, were genotyped. Genomic DNA was extracted using DNeasy 96 Blood & Tissue Kits (QIAGEN) and quantified using Quant-iT PicoGreen dsDNA Assay Kit (Invitrogen) following manufacturer's instructions. Fluorescence was measured in a 200 µL reaction on a VICTOR3 multilabel microplate reader (Perkin Elmer). DNA concentrations were then normalized for HRMA. PCR was conducted in a 10 µL volume containing 10 ng of genomic DNA, 1× LightCycler 480 High Resolution Melting Master (Roche Applied Science), 3.5 mM MgCl2, and 0.2 µM each PCR primer. Primers used for HRMA are provided in Table 2. Thermal cycling was performed on a Veriti 384-Well Thermal Cycler (Applied Biosystems; AB) as follows: 95°C hold for 10 min followed by 45 cycles of 95°C for 15 sec, 60°C for 15 sec, and 72°C for 15 sec. The plates were transferred to a LightCycler 480 Real-Time PCR System (Roche Diagnostics) after PCR and heated to 95°C for 1 min, then cooled to 40°C for 1 min. HRMA data were collected between 62°C and 95°C at 25 acquisitions per °1C, using a ramp rate of 0.02°C per second. The amplicons were analyzed for the presence of discrete melt-curve families, signaling the presence of SNPs, using the LightCycler 480 Gene Scanning Software v. 1.5.0 SP1 (Roche Diagnostics). Genotypes were inferred from clearly resolved melt families [8], [32].

Data Analyses

Deviations from Hardy Weinberg Equilibrium (HWE) and significance of population differentiation were tested using Fisher's exact test in Genepop 4 [33]. A hierarchical locus-by-locus AMOVA was carried out in Arlequin v 3.5 [23], with ocean basins (Gulf of Alaska, Bering Sea) as groups.

Supporting Information

Table S1 Description of select contig sequences. Description is based on BlastX analysis using the Swiss-Prot database. E-values and organism associated with BlastX hits are provided as are the number of unique reads that map to each contig.
(XLS)

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Author Contributions

Conceived and designed the experiments: SBR LH LWS JES. Performed the experiments: SBR. Analyzed the data: SBR LH LWS. Contributed reagents/materials/analysis tools: SBR JES. Wrote the paper: SBR LH LWS JES.

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RESEARCH ARTICLE Open Access

DNA methylation patterns provide insight into epigenetic regulation in the Pacific oyster (*Crassostrea gigas*)

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Abstract

Background: DNA methylation is an epigenetic mechanism with important regulatory functions in animals. While the mechanism itself is evolutionarily ancient, the distribution and function of DNA methylation is diverse both within and among phylogenetic groups. Although DNA methylation has been well studied in mammals, there are limited data on invertebrates, particularly molluscs. Here we characterize the distribution and investigate potential functions of DNA methylation in the Pacific oyster (*Crassostrea gigas*).

Results: Methylation sensitive PCR and bisulfite sequencing PCR approaches were used to identify CpG methylation in *C. gigas* genes and demonstrated that this species possesses intragenic methylation. *In silico* analysis of CpGo/e ratios in publicly available sequence data suggests that DNA methylation is a common feature of the *C. gigas* genome, and that specific functional categories of genes have significantly different levels of methylation.

Conclusions: The Pacific oyster genome displays intragenic DNA methylation and contains genes necessary for DNA methylation in animals. Results of this investigation suggest that DNA methylation has regulatory functions in *Crassostrea gigas*, particularly in gene families that have inducible expression, including those involved in stress and environmental responses.

Background

Epigenetic mechanisms induce changes in gene activity without alteration to the underlying DNA sequence [1]. Common epigenetic mechanisms include DNA methylation, histone modifications and non-coding RNA activity. The most well-studied of these is DNA methylation, which refers to the addition of a methyl group to position 5 of cytosines. In animals, this reaction is catalyzed by a family of enzymes called DNA (cytosine-5) methyltransferases (DNMTs) and occurs almost exclusively in CpG dinucleotides. DNA methylation is typically associated with transcriptional repression, and is primarily achieved by methylation in gene promoters [2-4]. The functional significance of DNA methylation in vertebrates includes providing genomic stability [5], regulation of imprinted genes [6] and X-chromosome inactivation [7]. In mammals, DNA methylation is essential for development and cell differentiation [8] and defects or unintended changes in DNA methylation can have deleterious consequences such as embryonic lethality [9] and tumorgenesis [10]. DNA methylation, like many epigenetic marks, may be heritable, therefore unintended changes as a result of environmental exposures or other processes can be passed on for multiple generations [11].

The extent of cytosine methylation varies considerably among eukaryotes. In vertebrates, approximately 70-80% of cytosines in CpG dinucleotides are methylated [12], a pattern referred to as global methylation. Invertebrates display a wide range of DNA methylation, from very limited methylation in *Drosophilia melanogaster* [13] and *Caenorhabditis elegans* [14] to a mosaic pattern of methylation in the sea urchin (*Strongylocentrotus purpuratus*) [15] and *Ciona intestinalis* [16,17]. Bird and Taggart [12] concluded that there were three general types of methylation patterns: the 'insect-type' which shows little to no methylation, the 'echinoderm-type', the genomes of which contain both methylated and non-methylated fractions, and the heavily methylated 'vertebrate-type'. Recent studies in the honey bee (*Apis*

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mellifera) indicate these patterns may be more complex [18,19]. In contrast to *D. melanogaster*, which lacks most of the classical DNMTs [20] and shows limited cytosine methylation [21], *A. mellifera* has a fully functional set of DNA methylation enzymes and shows substantial methylation across its genome [18].

In vertebrates, regulation of transcription by DNA methylation is accomplished by differential patterns of methylation in intergenic regions, namely gene promoters [2-4]. In contrast, there are no significant differences reported in the methylation status of gene promoters in invertebrates, where methylation appears to be targeted specifically to transcription units [17,22]. Computational analyses of the methylation status of *A. mellifera* genes have provided some of the first evidence supporting a regulatory role of intragenic DNA methylation in invertebrates [19,23]. In these studies, genes associated with general metabolic or 'housekeeping' functions were predicted to be hyper-methylated, whereas caste-specific genes were preferentially hypomethylated. This functional clustering suggests DNA methylation serves to regulate gene transcription in *A. mellifera*, however, it is uncertain if this function is conserved across invertebrate taxa. Furthermore, it is unclear exactly how intragenic cytosine methylation directly affects transcription.

Studies in *A. mellifera* and others illustrate the diversity of DNA methylation patterns in invertebrate taxa and highlight gaps in our understanding of the evolutionary and functional significance of DNA methylation. One taxonomic group that has been notably absent from these investigations is the phylum Mollusca. Molluscs were first categorized as having 'echinoderm-type' DNA methylation patterns based on experimental evidence using the common mussel (*Mytilus edulis*) [12]. Since then, there has been little investigation of DNA methylation in molluscs with the exception of evidence suggesting the presence CpG methylation in the clam, *Donux truculus* [24]. In addition to increasing our understanding of the evolution of DNA methylation in invertebrate taxa, this study provides an opportunity to evaluate the Pacific oyster (*Crassostrea gigas*) as a model organism for analyzing DNA methylation in an aquatic species. Bivalve molluscs are important bioindicators [25] and elucidating the functional significance of DNA methylation in these organisms may prove valuable for understanding the effects of environmental stress in aquatic organisms. Here, we report the first investigation into DNA methylation profiles in the genome of the Pacific oyster. We confirm the presence of intragenic CpG methylation in *C. gigas*. We also demonstrate a relationship between predicted methylation status and gene function, suggesting that DNA methylation performs important regulatory functions in *C. gigas*. Implications of these findings are discussed in both an evolutionary and ecological context.

Results

Methylation Sensitive PCR

A Methylation Sensitive PCR (MSP) approach was used to identify specific methylated sites. Five genes associated with immune function were analyzed and methylation status determined (Table 1). Methylation status can be concluded based on the presence or absence of a PCR product in the methylation sensitive HpaII digest. Of the five genes analyzed, CpG methylation was confirmed for *heat shock protein 70* (*hsp70*), whereas no methylation was detectable at restriction site(s) for the other sequences examined. The CpG observed to expected ratios (see Methods for calculation) are included in Table 1 for each gene. It should be noted that *hsp70* has the lowest ratio of all the genes analyzed (0.57). This low ratio is predictive of a hyper-methylated status, which is confirmed here by MSP.

Bisulfite Sequencing PCR

In order to describe methylated cytosines outside of CCGG sites, Bisulfite Sequencing PCR (BSP) was used. Five genes predicted to be hyper-methylated, and five predicted to be hypo-methylated (based on CpG observed to expected ratio) were randomly selected for analysis. Valid PCR products were produced for two of the genes. This is a typical result as the conversion of unmethylated cytosines results in challenges for primer specificity. Four individual clones were sequenced for each of the two products. There was a 100% conversion rate for non-CpG cytosines for each of the clones sequenced. In the first fragment, a 136 bp fragment with homology to the amino terminal fragment of the human neuromedin-u receptor [Swiss-Prot: Q9GZQ4"], one of seven CpGs sites displayed methylation in 25% of the clones sequenced (Figure 1(a)). In a second fragment, one of two CpGs sites was determined to be methylated in 50% of the clones sequenced in a 93 bp region (Figure 1(b)). The latter sequence has significant homology to human bromodomain adjacent to zinc finger domain, 1A [Swiss-Prot: Q9NRL2].

In Silico Analysis of *C. gigas* Transcriptome

The ratio of observed to expected CpG dinucleotides (CpGo/e) was used to predict methylation status in the *C. gigas* transcriptome. This approach is based on the known hyper-mutability of methylated cytosines, which readily deaminate to thymine residues [26]. This CpG mutation is not easily corrected by DNA repair machinery, and as a result consistently methylated regions of DNA are depleted of CpG dinucleotides over evolutionary time [27]. Consequently, regions of DNA with a low

Table 1 Results of Methylation Specific PCR analysis for five *C. gigas* genes

Accession #	Best blast hit [Organism]	Undigested	HpaII	MspI	Number of restriction sites	CpG o/e
EW778441	heat shock protein 70 [*Crassostrea gigas*]	+	+	_	2	0.57
EW777519	heat shock protein 25 [*Danio rerio*]	+	_	_	3	0.81
EW778166	cytochrome P450 [*Haliotis diversicolor*]	+	_	_	1	0.85
EW778608	macrophage expressed protein 1-like protein [*Crassostrea gigas*]	+	_	_	6	1.08
EW778905	14-3-3 protein gamma (Protein kinase C inhibitor protein 1 [*Bos taurus*])	+	_	_	2	0.92

Results of methylation status of five genes associated with immune response by MSP. PCR was carried out on undigested, HpaII digested, and MspI digested DNA. Presence (+) or absence (-) of PCR product is indicated. Number of CCGG restriction sites in the indicated sequence and CpGo/e ratios are also provided.

CpGo/e are predicted to be methylated, whereas regions with a high CpGo/e (approaching 1.0) are predicted to be unmethylated. This approach has been used to reliably predict methylation status across many taxonomic groups [17,19,22,28].

A non-redundant *C. gigas* contig database, 'GigasDatabase' version 6 [29] was utilized for this analysis. To ensure only CpG (and not GpC) dinucleotides were being evaluated, analysis was limited to annotated sequences. The probability density function of the CpGo/e for 12,210 annotated *C. gigas* expressed sequence tag (EST) contigs is illustrated in Figure 2. We find that the data fit a bimodal mixture model (blue curve) significantly better than a unimodal distribution. The red curves represent the scaled, normal mixture components, which have means of 0.40 (± 0.12 SD) and 0.70 (± 0.21 SD) respectively (Figure 2). A majority of the contigs have a CpGo/e less than 1.0.

The ratio of observed to expected GpC dinucleotide frequencies (GpCo/e) was calculated in order to be assured that the bimodal distribution of CpGo/e was not biased toward G+C content of specific genes as there are no known mechanisms for preferential depletion of the GpC dinucleotide. As predicted, the ratio of observed to expected GpC's approaches 1.0 following a unimodal Gaussian distribution (Figure 2 inset). In addition, there is a significant negative correlation between CpGo/e and TpGo/e (p = 0.00) indicating that the depletion of CpG dinucleotides is associated with the conversion of methylated CpG sites to TpG dinucleotides.

In order to determine any functional difference that may exist among those genes with lower than expected CpGo/e ratios, data were analyzed in the context of each gene's biological process GO Slim term (Figure 3). Several biological processes have CpGo/e ratios that are significantly different from each other (see Additional file 1: Matrix of p-values for comparisons between GO Slim categories based on CpGo/e). Specifically, genes with lower CpGo/e ratios (predicted to be hyper-methylated) were associated with DNA metabolism, RNA metabolism, and cell cycle and proliferation. Biological processes with higher CpGo/e ratios (predicted to be hypo-methylated) include cell adhesion, cell-to-cell signalling and signal transduction. This analysis confirms that the normal mixture components described previously in Figure 2 are enriched with genes from particular functional categories.

Discussion

Results of methylation specific PCR and bisulfite sequencing PCR indicate that the Pacific oyster (*Crassostrea gigas*) genome is methylated. Further evidence supporting the presence and importance of methylation in *C. gigas* is the identification of genes that encode DNA methyltransferases (DNMT), the family of proteins responsible for the

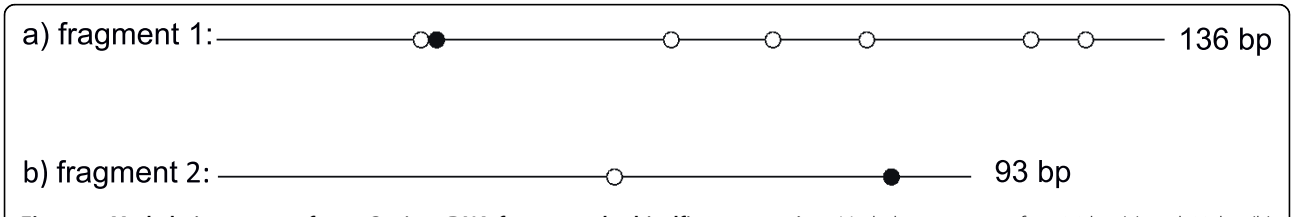

Figure 1 Methylation status of two *C. gigas* DNA fragments by bisulfite sequencing. Methylation status of a 136 bp (a) and 93 bp (b) fragment of *C. gigas* DNA as determined by bisulfite sequencing. Solid and open circles represent methylated and non-methylated CpG dinucleotides, respectively. One of four clones was determined to be methylated at the CpG indicated by the solid circle in (a) and 2 of 4 clones were determined to be methylated at the CpG dinucleotide indicated in (b).

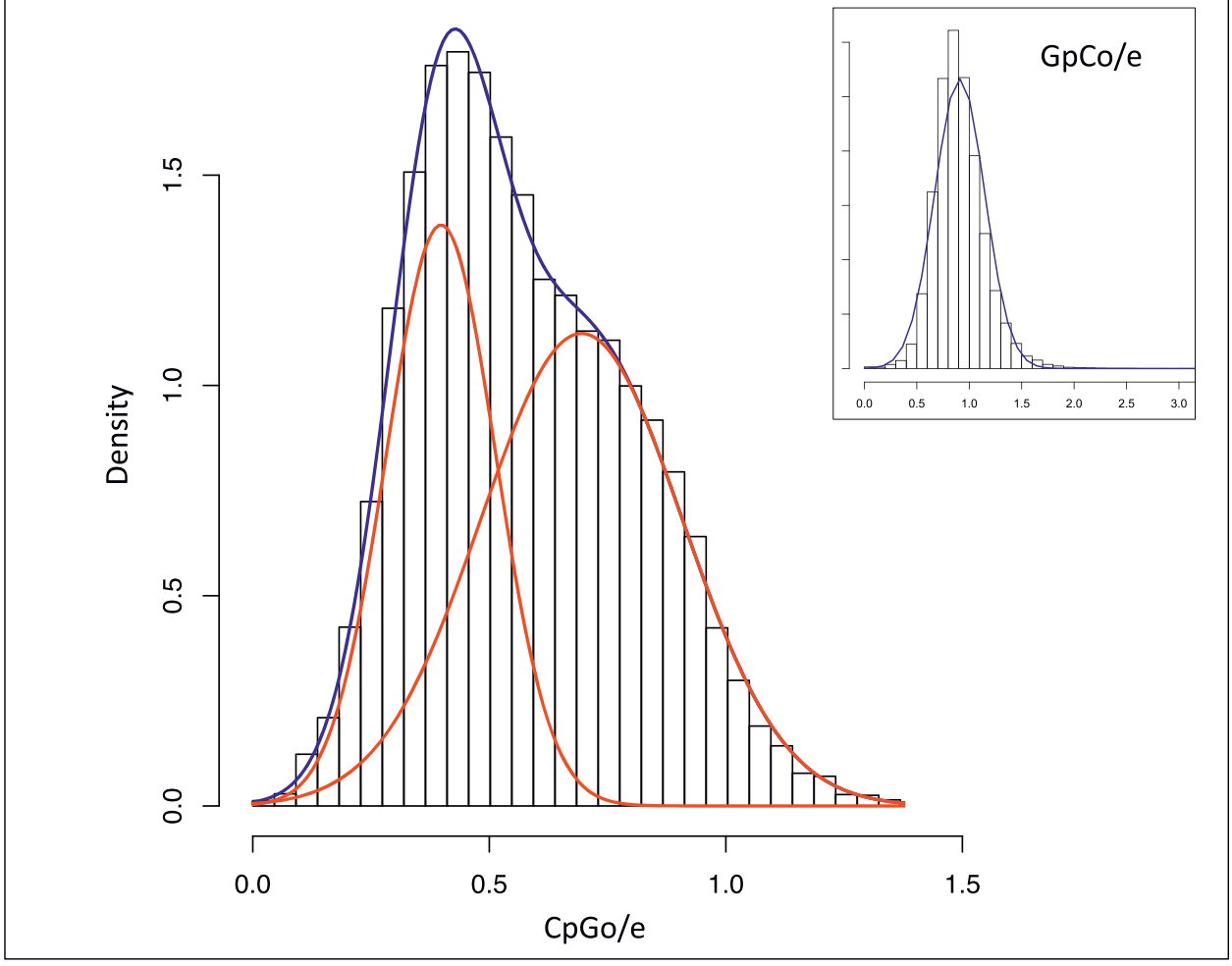

Figure 2 Distribution of predicted methylation status of 12,210 annotated *C. gigas* transcripts measured computationally by CpGo/e ratio. Probability density function of CpGo/e for 12,210 *C. gigas* contigs. Blue curve is fitted mixture model; red curves are scaled, normal mixture components with means of 0.40 and 0.70 respectively. For contrast, a control dinucleotide (GpCo/e) is also shown with the blue curve representing a normal, unimodal distribution (inset).

enzymatic conversion of cytosine to 5-methylcytosine. Animals that lack DNA methylation such as *C. elegans* also lack essential DNMTs, while invertebrates with an intermediate level of DNA methylation such as honey bees, sea urchins and urochordates have the full set of DNMT genes [30]. Sequences with high homology to DNMT3 (responsible for *de novo* methylation), DNMT1 (associated with maintenance methylation), and methyl-CpG-binding domain protein 2 (mediation of the effects of DNA methylation) are present in a publicly available *C. gigas* contig database, GigasDatabase version 6 [29]. These annotated sequences can be found in GigasDatabase with accession numbers CU684371.p.cg.6 (e-value 1e-61), CU994437.p.cg.6 (e-value 2e-26), and AM861084.p.cg.6 (e-value 1e-11), respectively. While a DNMT2 homolog has not been identified, it may not be required for DNA methylation in *C. gigas* as it functions primarily as a tRNA methyltransferase and shows only weak DNA methyltransferase activity *in vitro* [31]. DNMTs are an evolutionarily conserved group of proteins, but show structural diversity both within and among taxa [32]. The evolutionary diversity of DNA methylation within and among phylogenetic groups provides justification for further evaluation of the functions of this epigenetic mark.

The presence of intragenic methylation in *C. gigas* is similar to that of other invertebrates that primarily exhibit intragenic DNA methylation patterns [33,17], the roles of which have been largely unexplored. Studies of DNA methylation in mammals have generally focused on promoter regions, where hyper-methylation of promoters inhibits initiation of transcription [2]. In contrast, invertebrate genomes do not show differentially

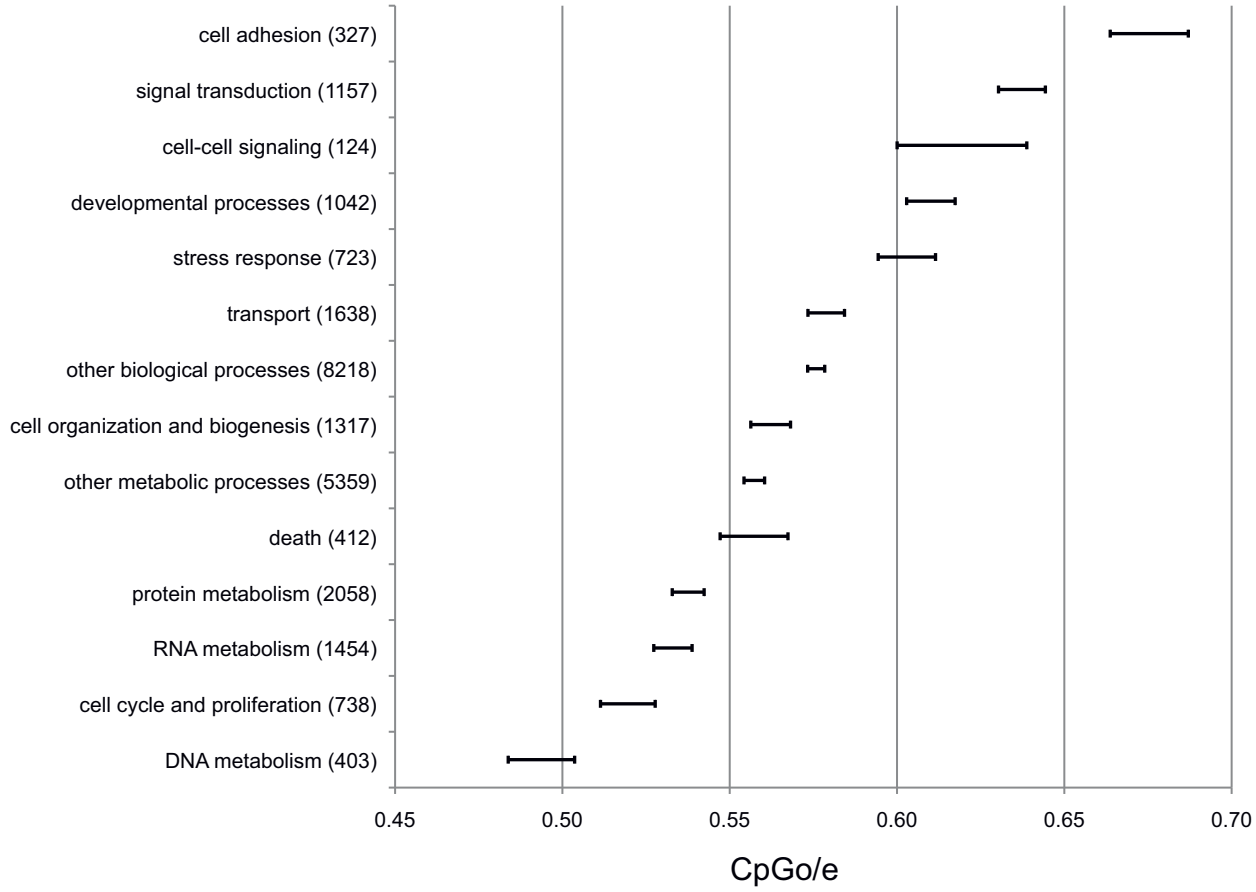

Figure 3 Differential methylation between categories of genes involved in discrete biological processes as measured by CpGo/e. Mean CpGo/e for 10,699 *C. gigas* contigs categorized by GO Slim term. Bars represent mean ± 1 standard error. The number of contigs in each category is listed in parenthesis.

methylated gene promoters [22]. One of the long-standing hypotheses is that intragenic DNA methylation prevents inappropriate initiation of transcription outside of promoter regions [34]; however new studies have begun to investigate a more active role for intragenic DNA methylation, namely in regulation of expression. For example, exonic DNA methylation has been shown to regulate transcription of the *phytochrome A* gene in *Arabidopsis thaliana* [35]. In humans, investigation of intragenic CpG islands (≥ 200 bp regions with G+C content of at least 50% and CpGo/e close to expected) has revealed that CpG islands in terminal exons may regulate transcription of non-coding RNAs [36]. Here, using BSP, we observed methylation variability in two CpG sites that may indicate cell-specific methylation. The function of intragenic DNA methylation in *C. gigas* cannot be conclusively determined from this study, but results of studies in other organisms suggest that it could be involved in either repression of transcription outside of transcription start sites and/or regulation of expression.

Within the transcriptome of the Pacific oyster, a significant difference in methylation pattern was observed across gene families. A majority of *C. gigas* genes analyzed were depleted in CpG dinucleotides (i.e. CpGo/e < 1.0) and show a significantly bimodal distribution, suggesting that DNA methylation is a common feature of the *C. gigas* transcriptome, and that certain groups of genes have significantly different levels of methylation. The bimodal distribution of CpGo/e is similar to the pattern observed in the honey bee *A. mellifera*, where authors reported a hyper-methylated fraction that was enriched in genes involved with general metabolic functions and a hypo-methylated fraction enriched with genes that are associated with caste-specific functions [19]. Similarly when *C. gigas* transcripts were clustered according to their functional annotations using GO Slim terms, we see that the two distributions are comprised of functionally distinct classes of genes with varying regulatory requirements. Specifically, genes predicted to be hyper-methylated are associated with housekeeping functions and those predicted to be hypo-methylated are

associated with general immune functions. Hyper-methylation of intragenic regions of housekeeping genes is consistent between *C. gigas* and *A. mellifera* [19], but stands in contrast to observations in vertebrates, where distinct hypo-methylation of housekeeping gene promoters is associated with global expression [37]. Constitutive DNA methylation in housekeeping genes in *C. gigas* could be important for repressing transcription outside of promoter regions as previously discussed. It has also been proposed that hyper-methylation of housekeeping genes in *A. mellifera* indicates epigenetic control of gene activity in housekeeping genes [23]. Further experiments will be required to determine whether hyper-methylation of housekeeping genes plays a passive role in preventing inappropriate transcription or a more active role in maintaining expression in *C. gigas*.

Highest CpGo/e ratios were observed in genes involved in the oyster's innate immune response, including categories of cell adhesion, cell-cell signaling, and signal transduction. Our experimental data using MSP supports the predicted hypo-methylation of this class of genes as only 1 of the 5 immune related genes were methylated. Our results do not indicate that DNA methylation is entirely absent from genes in the hypo-methylated group as CpG depletion is still observed (CpGo/e 0.7) which stands in contrast to the hypo-methylated genes in *A. mellifera* (CpGo/e >1.0). One explanation as to why it would be advantageous for this class of genes to be hypo-methylated is that it allows for greater epigenetic flexibility and higher regulatory control. Oysters have been shown to have high phenotypic plasticity in response to environmental changes and stress [38,39] and it could be postulated that an epigenetic mark, such as DNA methylation, could provide this level of control. DNA methylation has been generally considered to be a less dynamic epigenetic mark, however, it has been reported in plants that DNA methylation levels are involved in regulating gene expression in response to stress and show active methylation and demethylation in response to various stressors [40-42]. It has been hypothesized from these studies that DNA methylation is a possible mechanism to impart protection against local stresses in future generations [43]. The identification of genes involved in demethylation in *C. gigas* would be an important step toward uncovering the nature of these epigenetic marks.

DNA methylation patterns have been shown to be heritable in mammalian taxa [44], and changes in DNA methylation patterns can persist for multiple generations [45]. Little work has been done to investigate heritability of DNA methylation in invertebrates, although a recent study of the crustacean, *Daphnia magna*, has shown transgenerational heritability of DNA methylation patterns after exposures to 5-azacytidine [46]. If DNA methylation does play a role in regulation of transcription in *C. gigas* it may provide a mechanism not only for regulating responses to stress, but also for adapting to local stressors through heritability of DNA methylation patterns. Investigating the potential of epigenetic control in mechanisms of local adaptation may prove useful in understanding impacts of anthropogenic inputs in aquatic ecosystems and populations. Likewise, it is possible that epigenetic mechanisms may provide an explanation for other phenomena associated with heritability such as inbreeding depression and hybrid vigour.

Elucidating functional significance of DNA methylation in aquatic invertebrates may change the way we study impacts of environmental change in aquatic organisms. A range of factors such as diet [47,48], xenobiotic chemicals [49], and endocrine disruptors [11] have been shown to disrupt DNA methylation patterns. These epigenetic disruptions are increasingly associated with disease susceptibility, which in some cases can be passed on for multiple generations [50]. Although these investigations have been performed almost exclusively in mammalian species, recent studies have reported a dose dependent relationship between concentration of mercury and cadmium and total DNA methylation in *D. magna* [46,51]. Understanding which environmental factors can affect DNA methylation and elucidating the functional significance of DNA methylation in these important bioindicator species will be major steps toward clarifying the complex interactions between the environment, gene expression, and organismal responses.

Conclusions

The Pacific oyster genome displays methylation. *In silico* analysis reveals intragenic regions are targeted for methylation consistent with reports of methylation in other invertebrate species. Results of this investigation suggest that DNA methylation has regulatory functions in *Crassostrea gigas*, particularly in gene families involved in stress and environmental response. Experiments are underway in our lab to investigate relationships between the environment, DNA methylation, and control of gene expression to better characterize this process. In-depth analysis of methylation patterns in *Crassostrea gigas*, will help to advance the field of evolutionary epigenetics and will serve to illuminate functions of DNA methylation in invertebrates.

Methods

Animal collection & DNA isolation

Oysters used in this study were collected from naturalized *C. gigas* populations in Puget Sound, Washington. To isolate genomic DNA, 25 mg of gill tissue was processed according to the manufacturer's protocol using the DNeasy Blood & Tissue Kit (Qiagen, CA).

Methylation Sensitive PCR

Oyster genomic DNA was enzyme digested with either HpaII or MspI. Five immune related genes containing one or more CCGG recognition sites and covering a broad range of predicted methylation status (based on CpGo/e) were selected from a set of ESTs generated from a cDNA library of plated hemocytes [52]. PCR primers were designed to flank one or more restriction sites. Primer sequences are provided in Additional file 2: Primer Sequences. Quantitative PCR was performed using digested (HpaII or MspI) and undigested samples using 1× Immomix Master Mix (Bioline USA Inc., Boston, MA), 2 uM SYTO-13 (Invitrogen, Carlsbad, CA) and 0.2 uM forward and reverse primers in an Opticon 2 System (Bio-Rad, Hercules, CA) with the following cycling conditions: 10 min at 95C, followed by 37 cycles of 15 sec at 95C, 30 sec at 55C, and 1 min at 72C and a final extension at 72C for 10 min. Results were scored qualitatively based on the presence or absence of amplification as determined by fluorescence.

Bisulfite Conversion and Sequencing

Genomic DNA was bisulfite converted using the Epitect Bisulfite conversion kit (Qiagen, Carlsbad, CA). Briefly, 1.75 ug of DNA was subjected to treatment with sodium bisulfite at increased temperature to deaminate unmethylated cytosine residues to uracil. Following treatment, the solution was desulfonated on a column, washed and eluted.

To identify methylated cytosines in expressed regions of the oyster genome, Meth Primer [53] was used to design primers that flank multiple CpG sites, but do not contain CpGs. Primer sequences are provided in Additional file 2: Primer Sequences. The mean expected amplicon length for bisulfite primers was ~180 bp. PCR of bisulfite treated samples (54 ng/PCR reaction) was carried out using Taq DNA Polymerase Master Mix (Apex BioResearch Products, Research Triangle Park, NC) for 10 min at 95 C, followed by 40 cycles of 15 sec at 95 C, 30 sec at 55 C, and 1 min at 72C and a final extension at 72C for 10 min.

PCR products were separated using gel electrophoresis. Single bands were excised from the gel, purified using Ultra-DA purification columns (Ambion, Foster City, CA) and cloned using TOPO TA Cloning kit (Invitrogen). Four clones were sequenced for each primer pair. Methylation status was determined by comparing the sequence of bisulfite treated DNA to sequence of untreated DNA using Geneious 4.5.4 software (Biomatters Ltd., Aukland, NZ) and annotated using BLAST [54].

In Silico Analysis: Predicted DNA Methylation Status

For *in silico* analysis, the non-redundant *C. gigas* expressed sequence tag (EST) contig database, 'GigasDatabase' version 6 (http://public-contigbrowser.sigenae.org:9090/Crassostrea_gigas/index.html, [29]), was utilized. Analysis was limited to annotated sequences (n = 12,210) in order to be confident that sequences were reported in the 5' to 3'direction. It should be noted that this transcriptomic dataset is appropriate for predicting methylation status of the *C. gigas* genome as investigation into other invertebrate species shows that DNA methylation is specifically targeted to transcribed regions of the genome [17,12].

CpG observed/expected ratio (CpGo/e) was calculated using the following equation where l is the number of nucleotides in the contig:

$$\text{CpGo/e} = \frac{\text{number of CpG}}{\text{number of C}\times\text{number of G}} \times \frac{l^2}{l-1}$$

To evaluate the distribution of Pacific oyster contigs, a mixture model was fit to the CpGo/e ratios using the mixtools package [55] in R [56] yielding a two component mixture where $p_1 + p_2 = 1$. Hence the data C_i, are distributed as:

$$C_i \sim p_1 N(\mu_1, \sigma_1) + p_2 N(\mu_2 \sigma_2).$$

The log likelihood statistic of the bimodal mixture model was compared to the normal null model to test for a significant improvement in fit.

In order to evaluate the variation of CpGo/e within and among functional classes of genes, contigs from the GigasDatabase annotated with a biological process GO term (n = 10,699 contigs) were assigned a functional group based on the MGI GO Slim database http://www.informatics.jax.org[57]. Since each contig may have multiple GO terms, contigs were allowed to fall into multiple GO Slim bins. However, to avoid weighting within a single category, an individual contig was not allowed to be included more than once in a single GO Slim bin. The mean CpGo/e and standard errors were calculated for each GO Slim term. A one-way ANOVA followed by Tukey's test for multiple comparisons was performed using SPSS software (SPSS Inc., Chicago, IL). A significance level of $p < 0.05$ was accepted.

Additional material

Additional file 1: Matrix of p-values for comparisons between GO Slim categories based on CpGo/e. CpGo/e for GO Slim categories were compared with Tukey's multiple comparison test. This file contains the p-values of each comparison. Significant differences ($p < 0.05$) are highlighted.

Additional file 2: Primer Sequences. This file contains primer sequences used for methylation sensitive PCR and bisulfite sequencing PCR analysis.

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Authors' contributions
MG and SR conceived of the study and prepared the manuscript. MG carried out the laboratory procedures. All authors read and approved the final manuscript.

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Differential skeletal muscle expression of myostatin across teleost species, and the isolation of multiple myostatin isoforms

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Abstract Two myostatin (MSTN) isoforms were isolated from brook trout with 92% identity in corresponding regions at the nucleotide level. One isoform was isolated from muscle and brain and the second from ovarian tissue. To our knowledge this is the first time two MSTN isoforms have been isolated from a given vertebrate species. Within the brain, MSTN transcripts were localized to the optic lobes, hindbrain, and hypothalamus. In the trout ovary, MSTN transcripts were upregulated at ovulation in several females. MSTN cDNA fragments were also isolated from several other fish species and differential expression of MSTN among muscle fiber types was observed.

Key words: Myostatin; Trout; Muscle; Ovulation; Ovary

1. Introduction

Myostatin (MSTN), a member of the Transforming Growth Factor-β (TGF-β) superfamily, has been established as a regulator of development and growth in several vertebrates. MSTN, originally termed growth and differentiation factor-8, was first characterized in the mouse where it is expressed during embryogenesis in developing somites [1]. Adult mice that did not express the MSTN gene were 25–30% heavier than wild-type littermates and the individual muscles of the MSTN null mice were 2–3 times heavier. This increase in muscle mass was attributed to an increase in fiber number (hyperplasia) and size (hypertrophy). Subsequently, the double muscling phenotype found in Belgian Blue and Piedmontese cattle was attributed to mutations in the MSTN gene [2–4]. Research on cattle [2], pigs [5], and chickens [6] has indicated a similar developmental expression pattern of MSTN that begins early and continues through gestation. These studies also show that MSTN expression roughly coincides with primary and secondary muscle formation followed by reduced levels at birth. MSTN expression has been reported in adults of several vertebrate species [2–5]. During development and adulthood, MSTN is predominately expressed in skeletal muscle, though there have been reports of myostatin protein in cardiomyocytes and Purkinje fibers of the heart [7], as well as MSTN mRNA expression in the mammary gland [5]. Recently, Rodgers et al. [8] have also reported the presence of MSTN in a variety of tissues in tilapia.

Originally, the MSTN gene was cloned in representatives of several vertebrates including zebrafish [3]. The gene is highly conserved among species and the predicted amino acid sequences of human, rat, mouse, porcine, chicken, and turkey are identical within the active carboxy-terminal region [3]. Though MSTN has been cloned in representative lower vertebrates, MSTN gene expression has not been characterized in these animals. To understand the role that MSTN could have in the growth and development of fish, the present study examined MSTN gene expression in several fish species.

2. Materials and methods

2.1. Animals and tissue collection

Experiments and animal care were conducted according to the guidelines specified by the University of Notre Dame Institutional Animal Care and Use Committee. Mature brook trout (*Salvelinus fontinalis*) were purchased from a commercial hatchery in Grand Haven, MI and held in 300 gallon tanks in 12°C well water. Yellow perch (*Perca flavescens*) were obtained from the Lake Mills State Fish Hatchery in Lake Mills, WI. Mahi-mahi (*Coryphaena hippurus*), little tunny (*Euthynnus alletteratus*), and king mackerel (*Scomberomorus cavalla*) were obtained by hook and line in the Atlantic Ocean offshore of South Carolina. For obtaining ovaries, the reproductive stage of individual brook trout was determined by sampling follicles in vivo as previously described [9]. Ovarian tissue was collected from females before germinal vesicle breakdown, during ovulation (20–100% of the ovary ovulated at time of sampling), and 48 h after ovulation. In all cases, fish were overanesthetized and tissue samples were dissected and placed into ice-cold Tri-Reagent (Molecular Research Center Inc.). Tissue was homogenized with a TissueTearor (Biospec) and RNA isolation was completed as previously described [10,11]. When used, mRNA isolation was performed using the Poly-A-Tract mRNA Isolation System (Promega).

2.2. Cloning fish MSTNs

Two sets of degenerative primers were designed based on known MSTN sequences. These consisted of two forward primers: AA-(A,G)CCI(inosine)AA(A,G)TG(C,T) TG(C,T)TT(C,T)TT(C,T) [Fw1] and CAAAT(T,C)CT(T,C)AG(C,T)AAACT(C,G,T) [Fw2] and two reverse primers: (A,G)TGIGT(A,G)TGIGG(A,G)TA(C,T)TT(C,T)-TG [Rv1] and ATAATCCA(G,A)TCCCA(G,T)CCAAA [Rv2].

Total RNA from brook trout ovaries, brain, and red muscle, as well as red muscle from all other fish species, was reverse transcribed using AMV reverse transcriptase (Promega) and the resulting cDNA was used for PCR. Polymerase chain reactions were separated on agarose gels, visualized under UV light and the appropriate size band was cut, gel purified, and cloned in pCR 2.1 (Invitrogen). Positive clones were grown for plasmid preparation and the cDNAs were sequenced using a modified dideoxy chain termination method (SequiTherm EXCEL II Long-Read, Epicentre). The sequencing reactions were separated and analyzed using an ALFexpress Sequencer (Amersham Pharmacia Biotech).

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PII: S0014-5793(01)02196-2

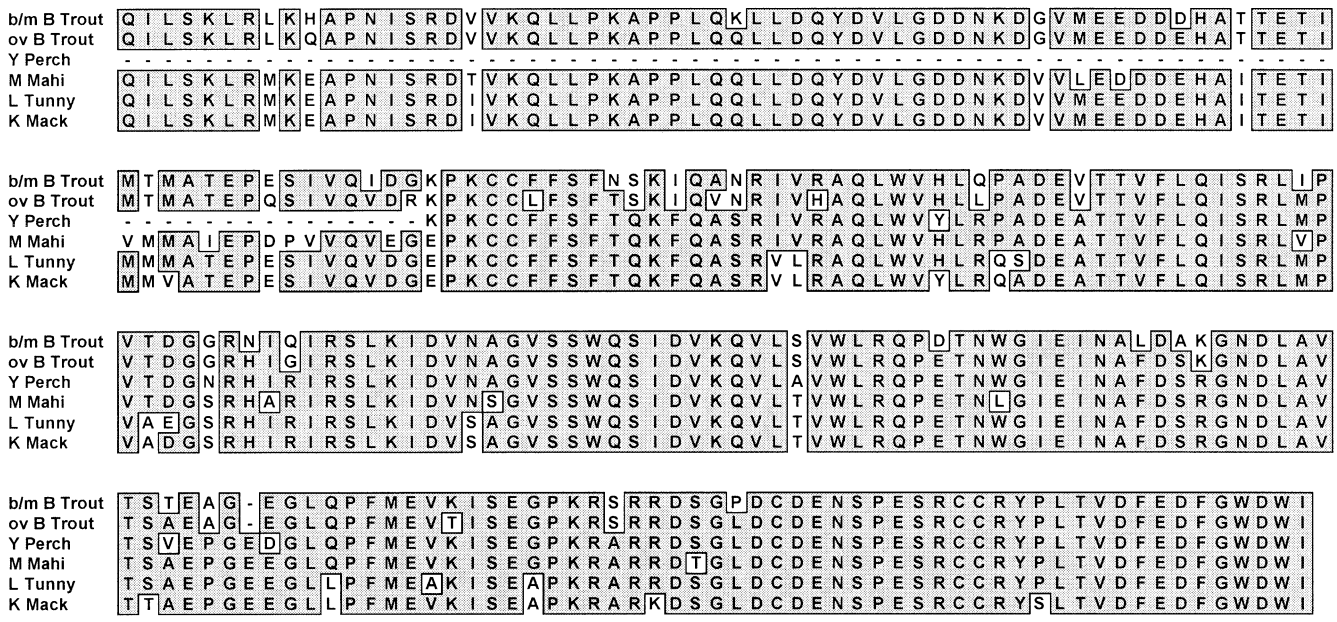

Fig. 1. Amino acid alignment of MSTN fragments from brook trout ovarian tissue (ov B Trout) (accession number AF313912), yellow perch red muscle (Y Perch) (accession number AF319959), mahi-mahi red muscle (M Mahi) (accession number AF317665), little tunny red muscle (L Tunny) (accession number AF317666), and king mackerel red muscle (K Mack) (accession number AF317667), compared with the corresponding section of the full-length brook trout brain/muscle MSTN (b/m B Trout) (accession number AF247650). Shading indicates amino acid identity.

A cDNA fragment obtained from RT-PCR of brain and muscle tissue (b/m BT MSTN) was used to screen a brook trout multiple tissue (liver, brain, testes, skin, and spleen) cDNA library constructed in Zap Express (Stratagene). Library screening was conducted under high stringency as previously described [12]. Positive clones were re-screened once to homogeneity, in vivo excised, and the resulting plasmids were completely sequenced on both strands as described above.

2.3. Northern analysis

Northern blot analysis was performed as previously described [12]. The full-length b/m BT MSTN clone obtained from library screening and the brook trout cDNA fragment from ovarian tissue (ov BT MSTN) were used to probe brook trout tissue blots. Northern blots of tissue from other fish species were probed using MSTN cDNA fragments cloned from each corresponding fish species.

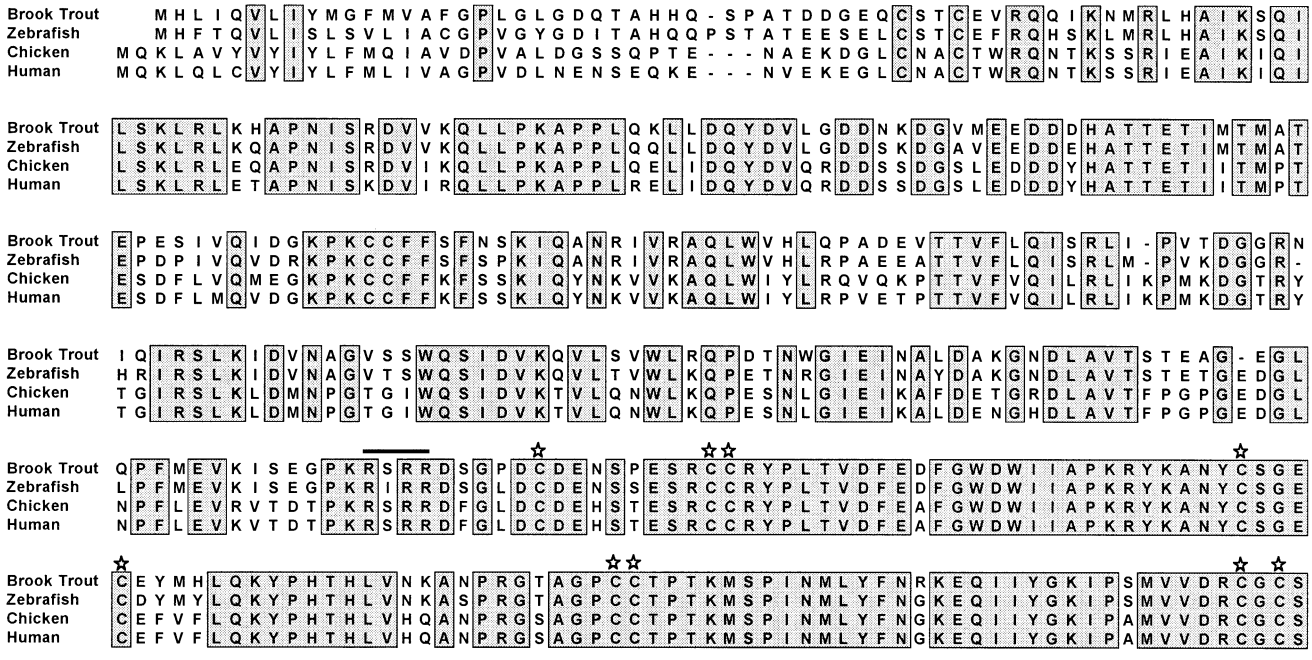

Fig. 2. Amino acid alignment of brook trout brain/muscle (accession number AF247650), zebrafish (accession number AF019626), chicken (accession number AF019621), and human MSTNs (accession number AF019627). Consensus sequence is shaded. Solid bar indicates RXXR proteolytic cleavage domain and conserved cysteines are indicated with stars.

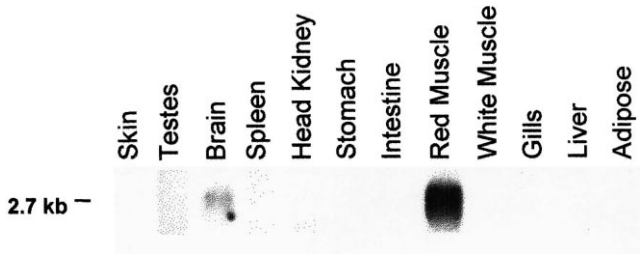

Fig. 3. Northern blot of mRNA (0.5 μg/lane) taken from various brook trout tissues. Blot probed with full-length b/m BT MSTN cDNA.

3. Results

The degenerative primer pair Fw2:Rv2 produced cDNA fragments of 716 bp using brook trout ovarian, muscle, and brain RNA. However, the fragment obtained from the ovary was different from that obtained from muscle and brain. The ov BT MSTN cDNA fragment was 91% identical to the corresponding sequence of b/m BT MSTN at the amino acid level (Fig. 1) and 92% identical at the nucleotide level. The identical ov BT MSTN form was independently isolated by RT-PCR performed on mRNA from ovaries taken from four different female brook trout. When the b/m BT MSTN fragment was used to screen the multiple tissue cDNA library, a 2278 bp clone was obtained. Compared to zebrafish, the full-length b/m BT MSTN clone is 84% identical throughout and 93% identical downstream of the proteolytic cleavage site (Fig. 2). Both human and chicken MSTNs are 65% identical to the brook trout MSTN and 88% identical downstream of the proteolytic cleavage site at the amino acid level (Fig. 2).

Based on known MSTNs, the degenerative primer pair Fw1:Rv1 produced an appropriate size (573 bp) cDNA band when used in PCR with RNA from yellow perch brain tissue. The Fw2:Rv2 primer pair produced cDNA fragments of 719 bp when used in PCR with mahi-mahi, little tunny, and king mackerel muscle RNA. As would be expected, the cloned fragments from king mackerel, little tunny, mahi-mahi, and yellow perch, all had high homology with one another and with b/m and ov BT MSTNs (Fig. 1). Just as other members of the TGF-β superfamily, all of the cDNAs have conserved cysteine residues and a RXXR proteolytic cleavage site. The one exception is that king mackerel MSTN contains the amino acids RARK in the corresponding site.

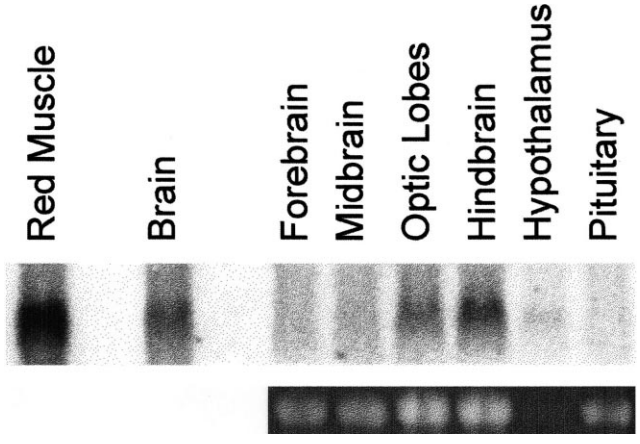

Fig. 4. Northern blot of brook trout red muscle and brain mRNA (0.5 μg/lane) (identical tissue samples as in Fig. 1) adjacent to samples of total RNA from specific regions of the brook trout brain (note: 'Midbrain' = midbrain–optic lobes). For all brain sections, 15 μg of total RNA was loaded per lane except for the hypothalamus for which as much RNA was loaded as was available. 18S rRNA bands of the brain tissue samples are shown below the blot. Blot probed with full-length b/m BT MSTN cDNA.

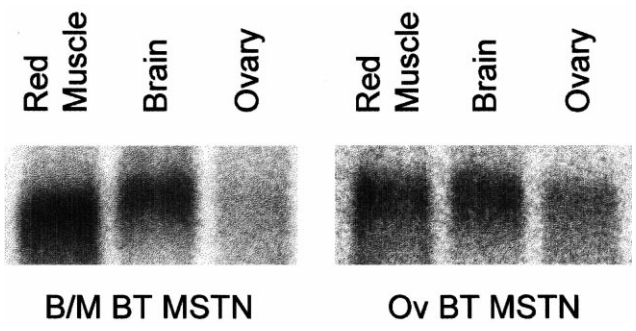

Fig. 5. Northern blots of brook trout red muscle, brain and ovarian mRNA (0.5 μg/lane). The ovarian sample is the same sample as in Fig. 6 (lane 5). A single gel was loaded in replicate with identical samples and after transfer, the blot was cut and replicates were separately hybridized with either the full-length b/m BT MSTN (2278 bp) or the ov BT MSTN cDNA fragment (716 bp).

On Northern blots, a 2.7 kb transcript was observed in red muscle when probed with the b/m BT MSTN cDNA (Fig. 3). A less abundant transcript was present in brain (Fig. 3). On Northern blots of brain tissue dissected into six distinct regions, the transcript was prominent in the optic lobes and hindbrain, and faint in the hypothalamus (Fig. 4). When the full-length b/m BT MSTN cDNA was used as a probe with ovarian tissue taken at ovulation, no hybridization was observed (Fig. 5). This was also true for Northern blots of ovarian tissue taken at stages prior to and following ovulation (results not shown). Transcripts were observed (Fig. 5), however, in red muscle, brain, and ovary when the ov BT MSTN cDNA fragment was used to probe a duplicate blot of the same tissues as probed with the b/m BT MSTN cDNA. On Northern blots of ovarian tissue taken at different reproductive stages and probed with the ov BT MSTN cDNA fragment, increased transcript levels were observed during ovulation in several individuals (Fig. 6).

To evaluate the distribution of MSTN in red and white muscle tissue across different species, the muscle of four teleosts was examined by Northern analysis (Fig. 7). In brook trout, king mackerel, and yellow perch, MSTN was predominately expressed in red muscle. In little tunny, higher levels of MSTN were observed in white muscle while in mahi-mahi MSTN expression was similar in both red and white muscle (Fig. 7).

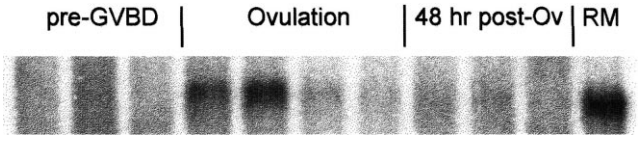

Fig. 6. Northern blot of brook trout ovarian mRNA (0.5 μg/lane) taken prior to germinal vesicle breakdown (pre-GVBD), during ovulation (Ovulation), and 48 h after ovulation (48 h post-Ov). Adjacent to the ovarian samples is a brook trout brain mRNA sample (0.5 μg/lane). Blot probed with the ov BT MSTN cDNA fragment.

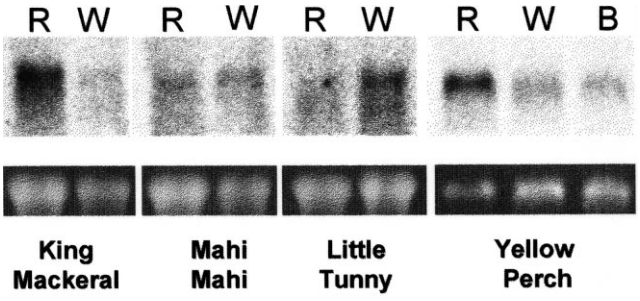

Fig. 7. Northern blots of total RNA (10 μg/lane) from red (fast) 'R' and white (slow) 'W' muscle fibers from four different fish species. Yellow perch blot also contains brain tissue 'B'. Each blot is probed with the corresponding species-specific MSTN cDNA fragment isolated from red muscle total RNA by RT-PCR. 18S rRNA bands for each lane are shown below the blot.

4. Discussion

Since the recent identification of MSTN there has been considerable knowledge gained concerning the role that MSTN plays in mammalian muscle development. Though MSTN has been cloned in representative lower vertebrates it is not known if MSTN has the same function in these animals as it does in higher vertebrates. The results of the present study clearly demonstrate several major differences between MSTN expression in fish and other vertebrates. To date, there has been only one MSTN cDNA cloned within a given vertebrate species. Surprisingly, in the present study two distinct cDNA clones were isolated from brook trout tissue using RT-PCR. These two clones were only 92% identical at the nucleotide level when comparing corresponding regions. When the specific cDNA fragment isolated from ovarian tissue (ov BT MSTN) was used as a probe, hybridization was observed in ovaries of several individuals undergoing ovulation. When the full-length b/m BT MSTN was used to probe the ovary of one of the same individuals undergoing ovulation no hybridization was observed and in other ovarian Northern blots we have not detected a signal using the b/m BT MSTN as a probe. While the MSTN isoform isolated from the brain and red muscle appears to hybridize only with those tissues, it should be noted that in RT-PCR reactions of ovarian mRNA one clone of the b/m BT MSTN form was isolated indicating that it may be in the ovary but expressed at low levels or at some reproductive stage not yet studied. The ov BT MSTN cDNA fragment did hybridize with red muscle and brain in addition to the ovary. Thus it is not known if the isoform isolated from the ovary is also found in other tissues or if hybridization was a result of using a highly homologous cDNA fragment (within the open reading frame) as a probe. Hybridization with brain and muscle might not occur if the full-length ov BT MSTN is used as a probe. However, so far we have been unable to clone this full-length cDNA. To our knowledge this is the first time two MSTN isoforms have been isolated from a given vertebrate species. At this time the role of MSTN in the ovary is unknown but the expression pattern indicates a possible role in ovulation.

A second difference between fish and other vertebrates is the presence of MSTN in multiple tissues. Originally it was believed that MSTN was limited to skeletal muscle. However, recently there have been reports of myostatin protein in cardiomyocytes and Purkinje fibers of the heart [7], as well as MSTN mRNA expression in the mammary gland [5]. In addition, MSTN has recently been isolated in multiple tissues from tilapia including the ovary and the brain [8]. In the current study, MSTN mRNA expression was observed in brook trout and yellow perch brains. Further, by dissecting the trout brain into distinct regions, Northern blots localized brook trout MSTN expression specifically to the optic lobe, hindbrain, and hypothalamus. The function of MSTN in the brain is unknown though other growth and differentiation factors in the TGF-β superfamily have been isolated in the brain of rodents [13–15] and *Xenopus* [16]. Expression of MSTN in the hypothalamus could indicate a possible endocrine function for MSTN. A MSTN immunoreactive substance has been found in human serum [17], suggesting a circulatory role for MSTN.

As expected, MSTN was expressed in the muscle tissue of all fish examined. However, what was unexpected was the differential expression of MSTN among fiber types within various fish species. In adult brook trout, king mackerel, and yellow perch, Northern analysis indicated that MSTN mRNA was specific to red muscle tissue (slow fibers). However, in other species MSTN was either expressed predominately in white muscle (little tunny) or equally in both fibers (mahi-mahi). During muscle unloading in mice, expression of MSTN mRNA is higher in fast muscle [18], and higher mRNA and protein concentrations were also observed in fast muscle in rats [19]. Recent research on rats has indicated that during muscle fiber damage MSTN protein is additionally expressed in slow fibers [20]. Most fish have distinct regions of muscle primarily containing fast twitch or slow twitch myofibers. In contrast, in most mammals there is a more heterologous arrangement of myofibers in muscle. The differences in MSTN expression in red and white muscle observed between fish species could be related to several aspects involving locomotion. Recently, evidence has been presented that MSTN plays a role in muscle regeneration [20–22] and loading/unloading processes [18,19,22] in mammals. It is likely that MSTN could also be involved in the same processes in fish. The species examined in the present study have slightly different red:white muscle ratios and use different strategies for locomotion. This, in conjunction with the fact that fish were taken from environments where varying degrees of muscle loading/unloading occur (e.g. tanks versus open ocean), might also explain the variation in MSTN expression in red and white muscle.

In conclusion, this study demonstrates the presence of MSTN in adult piscine skeletal muscle tissue and the differential expression in red and white muscle types. This study also provides evidence that MSTN is not limited to skeletal muscle, but is present in other tissues such as the brain and ovary. Finally, the data suggest the possibility of multiple MSTN isoforms that could be expressed in a tissue-specific manner.

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TEACHING SUMMARY

I have been an instructor in 5 courses at the University of Washington. Two of these courses I teach on a yearly basis- FISH310: Biology of Shellfishes and FISH441: Integrative Environmental Physiology. Both of these courses have a lecture and a lab component and are considered 5 credit courses. FISH310 is a required course for our School's major and is essentially an invertebrate zoology course with an emphasis on echinoderms, arthropods, molluscs, and cnidarians. FISH441 is course for upper level undergraduate students and graduate students where we study the physiological response of aquatic organisms to environmental change. I teach two other courses in alternating years- FISH546: Bioinformatics for Environmental Sciences and FISH510: Topics in Genetics and Physiology. FISH546 is a lecture and computer lab based course and FISH510 is a seminar course. I have also served as an instructor for BIO533: Ecology of Marine Infectious Diseases, a field course at Friday Harbor Laboratories. Below is a table summarizing my teaching effort with asterisks indicating classes that were co-taught. Official course descriptions are also provided. For detailed examples of my teaching philosophy please see my *Personal Statement*.

Year	Quarter	Course ID	Course Name	Student Evaluation	Enrollment
2007	Spring	FISH310*	Biology of Shellfishes	3.7	32
2007	Autumn	FISH546	Bioinformatics for Environmental Sciences	3.5	14
2008	Spring	FISH310*	Biology of Shellfishes	4.2	30
2008	Autumn	FISH510	Topics in Genetics and Physiology	4.2	10
2009	Winter	FISH441	Integrative Environmental Physiology	4.4	8
2009	Spring	FISH310	Biology of Shellfishes	3.9	36
2009	Autumn	FISH441	Integrative Environmental Physiology	4.2	10
2010	Winter	FISH546	Bioinformatics for Environmental Sciences	3.1	9
2010	Spring	FISH310	Biology of Shellfishes	4.2	36
2010	Summer	BIO533*	Ecology of Marine Infectious Diseases	4.3	12
2010	Autumn	FISH441	Integrative Environmental Physiology	4.2	13
2011	Winter	FISH510	Topics in Genetics and Physiology	4.1	16
2011	Spring	FISH310	Biology of Shellfishes	4	34
2011	Autumn	FISH441	Integrative Environmental Physiology	4.9	14
2012	Winter	FISH546	Bioinformatics for Environmental Sciences	4	10
2012	Spring	FISH310	Biology of Shellfishes	3.8	44
2012	Summer	BIO533*	Ecology of Marine Infectious Diseases		11
2012	Autumn	FISH441	Integrative Environmental Physiology		

Course Descriptions

FISH310: Biology of Shellfishes

website: http://goo.gl/xObyX

2007 - present

The course is intended to provide undergraduate students with an introduction to aquatic invertebrates with an emphasis on taxa with economic and cultural significance in the region. The class will expose students to the dramatic diversity of invertebrates and examine various mechanisms organisms employ to adapt to environmental conditions. Most of the content will focus on the morphology, life history, and physiology of arthropods and molluscs.

FISH546: Bioinformatics for Environmental Sciences

website: http://goo.gl/9qAJx

2008 - present (alternating years)

This is a course developed for biologists and ecologists that will cover computational analysis of molecular sequence data. Computational analysis of these data is a valuable tool to better understand biological processes and facilitates new discoveries. Bioinformatics can be considered a way of providing meaning (by means of computer algorithms) to the thousands upon thousands of genetic material continually being sequenced. In this course we will primarily focus on the resources for non-model organisms and will spend time on biology (*i.e.* reviewing central dogma), techniques (*i.e.* gene expression analysis) and computer science (*i.e.* sequence database, pairwise sequence comparisons). Various genomic resources that are publicly available will be reviewed along with web-based and stand-alone software that is used for analysis and functional annotation. Furthermore, we will examine modern techniques for gene expression analysis including advantages, disadvantages, and proper post-experiment processing.

FISH510: Innovations in Molecular Techniques

2007 - present (alternating years)

This course will provide students (both field and lab-centric) a forum to discuss innovations in molecular techniques that have recently evolved to play major roles in basic scientific research and natural resource management decisions.

FISH441/541: Integrative Environmental Physiology

website: http://goo.gl/nrSoG

2008 - present

Both freshwater and marine environments are continually changing in response to both natural processes and human activities, putting stress on aquatic organisms from microbes to marine mammals. This course will explore the surprising similarities and unique differences in

the physiological response organisms have to stress caused by factors as natural as tidal cycles, and as unnatural as excess pharmaceuticals. The course will take an integrative approach across disciplines linked to physiology, with an emphasis on molecular physiology and endocrinology; and assumes students have been introduced to basic physiological concepts in other coursework. The main focus will be on functional responses to system stressors; however, the course will also explore potential impacts at the population level, and the evolutionary implications of physiological response to environmental stress. Case studies and research papers will be used along with a primary textbook. The laboratory for this course will involve student working cooperatively to develop research projects.

BIO533: Ecology of Marine Infectious Diseases
website: http://goo.gl/tIzQ6
Summer 2010, 2012

This course will be a training program in invertebrate-pathogen ecology that will bring together and train the future leaders in this rapidly emerging, multidisciplinary field. The course will 1) survey host-pathogen interaction in the Friday Harbor region, 2) teach diagnostic tools for identifying viral, bacterial, protozoan and fungal infections of invertebrates, 3) teach approaches to examine the invertebrate innate immune response to different pathogens, and finally 4) use these methods to address ecological questions about the distribution of pathogenic interactions, and the experimental effects of temperature and increased acidification.

Student Evaluation of Instruction

Instructional Assessment System

FISH
AQUATIC & FISHERY SCIENCES
OCEAN AND FISHERY SCIENCES
University of Washington

Steven Roberts
Assistant Professor
Spring 2007

Department Copy

STUDENT EVALUATION OF INSTRUCTION

E=Excellent; VG=Very Good; G=Good; F=Fair; P=Poor; VP=Very Poor

	Respondents	PERCENTAGES [1] E (5)	VG (4)	G (3)	F (2)	P (1)	VP (0)	MEDIAN	Adjusted Median
1. The course as a whole was:	10	20	50	20	10			3.9	3.9
2. The course content was:	10	30	40	20	10			4.0	4.0
3. The instructor's contribution to the course was:	10	20	30	30	20			3.5	3.5
4. The instructor's effectiveness in teaching the subj. matter was:	10	20	10	30	40			2.8	2.8
COMBINED ITEMS 1-4	40	22	32	25	20			3.7	3.7
									Relative Rank
5. Course organization was:	10	20	30	30	20			3.5	10
6. Clarity of instructor's voice was:	10	20	30	20	30			3.5	16
7. Explanations by instructor were:	10	20	20	30	30			3.2	15
8. Instr's ability to present alternative explan. when needed was:	10	30	30	10	30			3.8	8
9. Instructor's use of examples and illustrations was:	10	30	50	10	10			4.1	5
10. Quality of questions or problems raised by instructor was:	10	30	20	40	10			3.5	13
11. Student confidence in instructor's knowledge was:	10	30	30	20	20			3.8	14
12. Instructor's enthusiasm was:	10	10	10	40	30	10		2.8	18
13. Encouragement given students to express themselves was:	10	10	30	20	30		10	3.0	17
14. Answers to student questions were:	10	20	30	20	30			3.5	12
15. Availability of extra help when needed was:	10	30	40	10	20			4.0	6
16. Use of class time was:	10	30	40	30				4.0	2
17. Instructor's interest in whether students learned was:	10	20	40	30	10			3.8	11
18. Amount you learned in the course was:	10	30	50	10	10			4.1	1
19. Relevance and usefulness of course content were:	10	40	30	10	20			4.2	3
20. Evaluative and grading techniques (tests, papers, etc.) were:	10	20	30	30	10	10		3.5	9
21. Reasonableness of assigned work was:	10	30	30	30	10			3.8	4
22. Clarity of student responsibilities and requirements was:	10	30	30	30	10			3.8	7
Relative to other college courses you have taken:		Much Higher (7)	(6)	(5) Average	(4)	(3)	(2)	Much Lower (1)	
23. Do you expect your grade in this course to be:	7	29	29	43				4.8	
24. The intellectual challenge presented was:	8	25	38	25	12			5.8	
25. The amount of effort you put into this course was:	8	25	38	25	12			5.8	
26. The amount of effort to succeed in this course was:	8	25	38	12	25			5.8	
27. Your involvement in course (assignments, attendance, etc.) was:	8	38	38	12	12			6.2	

28. On average, how many hours per week have you spent on this course?

Percent	
	Under 2
	2-3
12	4-5
	6-7
	8-9
62	10-11
12	12-13
	14-15
12	16-17
	18-19
	20-21
	22 or more

Respondents: 8
Class median: 10.7
Hours per credit: 2.14

29. From the total average hours spent, how many do you consider were valuable in advancing your education?

Percent	
	Under 2
12	2-3
	4-5
	6-7
	8-9
50	10-11
12	12-13
	14-15
12	16-17
	18-19
	20-21
12	22 or more

Respondents: 8
Class median: 11.0
Hours per credit: 2.20

30. What grade do you expect in this course?

Percent	
12	A (3.9-4.0)
25	A- (3.5-3.8)
38	B+ (3.2-3.4)
	B (2.9-3.1)
12	B- (2.5-2.8)
12	C+ (2.2-2.4)
	C (1.9-2.1)
	C- (1.5-2.1)
	D+ (1.2-1.4)
	D (0.9-1.1)
	D- (0.7-0.8)
	E (0.0)
	Pass
	Credit
	No Credit

Respondents: 8
Class median: 3.4

31. In regard to your academic program, is this course best described as:

Percent	
88	In your major
	A distribution requirement
	An elective
	In your minor
12	A program requirement
	Other

Challenge and Engagement Index

CEI = 8 •••••••• (decile rank)

1. Percentages are based on the number of students who rated each item.

Batch UW 211-001476

SP07:04792 SURVEY ID

Respondents: 10
Enrollment: 30
Classes: 1

A Form Type

Mailbox: 355020
ChairCopy? Yes
printed: 7/2/2007

Instructional Assessment System

FISH
AQUATIC & FISHERY SCIENCES
OCEAN AND FISHERY SCIENCES
University of Washington

Steven Roberts
Assistant Professor
Autumn 2007

Department Copy

STUDENT EVALUATION OF INSTRUCTION

E=Excellent; VG=Very Good; G=Good; F=Fair; P=Poor; VP=Very Poor

PERCENTAGES [1]

	Respondents	E (5)	VG (4)	G (3)	F (2)	P (1)	VP (0)	MEDIAN	Adjusted Median
1. The course as a whole was:	13	15	31	31	23			3.4	3.4
2. The course content was:	13	31	23	31	15			3.7	3.7
3. The instructor's contribution to the course was:	13	31	23	38	8			3.7	3.7
4. The instructor's effectiveness in teaching the subj. matter was:	13	8	38	31	15	8		3.4	3.4
COMBINED ITEMS 1-4	52	21	29	33	15	2		3.5	3.6
									Relative Rank
5. Course organization was:	13	15	23	31	23	8		3.1	14
6. Clarity of instructor's voice was:	13	15	31	38	15			3.4	17
7. Explanations by instructor were:	13	8	31	54	8			3.3	16
8. Instr's ability to present alternative explan. when needed was:	13	15	38	31	15			3.6	11
9. Instructor's use of examples and illustrations was:	13	38	15	31	15			3.8	10
10. Quality of questions or problems raised by instructor was:	12	25	42	33				3.9	7
11. Student confidence in instructor's knowledge was:	13	46	46		8			4.4	6
12. Instructor's enthusiasm was:	13	15	46	15	23			3.8	15
13. Encouragement given students to express themselves was:	12	33	33	33				4.0	8
14. Answers to student questions were:	12	8	58	25	8			3.8	9
15. Availability of extra help when needed was:	12	33	50	8	8			4.2	4
16. Use of class time was:	13		46	23	31			3.3	13
17. Instructor's interest in whether students learned was:	12	50	33	8	8			4.5	1
18. Amount you learned in the course was:	13	46	15	23	15			4.3	2
19. Relevance and usefulness of course content were:	13	46	23	23	8			4.3	3
20. Evaluative and grading techniques (tests, papers, etc.) were:	13	8	31	54		8		3.3	12
21. Reasonableness of assigned work was:	13	15	54	23	8			3.9	5
22. Clarity of student responsibilities and requirements was:	13	8	23	31	23	15		2.9	18

Relative to other college courses you have taken:		Much Higher (7)	(6)	(5)	Average (4)	(3)	Much Lower (2)	(1)	
23. Do you expect your grade in this course to be:	12	8	25	17	50				4.5
24. The intellectual challenge presented was:	12	8	25	33	17	17			5.0
25. The amount of effort you put into this course was:	12	8	17	33	33	8			4.8
26. The amount of effort to succeed in this course was:	12		33	25	33	8			4.8
27. Your involvement in course (assignments, attendance, etc.) was:	12	17	8	50	25				5.0

28. On average, how many hours per week have you spent on this course?

Percent	
	Under 2
	2-3
17	4-5
33	6-7
33	8-9
17	10-11
	12-13
	14-15
	16-17
	18-19
	20-21
	22 or more

Respondents: 12
Class median: 7.5
Hours per credit: 2.50

29. From the total average hours spent, how many do you consider were valuable in advancing your education?

Percent	
8	Under 2
8	2-3
25	4-5
50	6-7
8	8-9
	10-11
	12-13
	14-15
	16-17
	18-19
	20-21
	22 or more

Respondents: 12
Class median: 5.8
Hours per credit: 1.94

30. What grade do you expect in this course?

Percent	
9	A (3.9-4.0)
64	A- (3.5-3.8)
18	B+ (3.2-3.4)
	B (2.9-3.1)
	B- (2.5-2.8)
	C+ (2.2-2.4)
	C (1.9-2.1)
	C- (1.5-2.1)
	D+ (1.2-1.4)
	D (0.9-1.1)
	D- (0.7-0.8)
	E (0.0)
9	Pass
	Credit
	No Credit

Respondents: 11
Class median: 3.6

31. In regard to your academic program, is this course best described as:

Percent	
27	In your major
	A distribution requirement
18	An elective
	In your minor
36	A program requirement
18	Other

Challenge and Engagement Index

CEI = 3 ••• (decile rank)

1. *Percentages are based on the number of students who rated each item.*

Batch UW 268-000911

AU07:03440 SURVEY ID

Respondents: 13
Enrollment: 14
Classes: 1

A Form Type

Mailbox: 355020
ChairCopy? Yes
printed: 1/22/2008

Instructional Assessment System

FISH & AQUA... & FISHERY SCIENCES
OCEAN AND FISHERY SCIENCES
University of Washington
Co-taught w/ Carolyn Friedman

Steven Roberts
Assistant Professor
Spring 2008

Department Copy

STUDENT EVALUATION OF INSTRUCTION

E=Excellent; VG=Very Good; G=Good; F=Fair; P=Poor; VP=Very Poor

	Respondents	PERCENTAGES [1] E (5)	VG (4)	G (3)	F (2)	P (1)	VP (0)	MEDIAN	Adjusted Median
1. The course as a whole was:	22	36	50	9	5			4.2	4.3
2. The course content was:	22	27	59	9	5			4.1	4.2
3. The instructor's contribution to the course was:	22	36	50	14				4.2	4.3
4. The instructor's effectiveness in teaching the subj. matter was:	22	32	45	18	5			4.1	4.2
COMBINED ITEMS 1-4	88	33	51	12	3			4.2	4.3
									Relative Rank
5. Course organization was:	22	27	50	18	5			4.0	3
6. Clarity of instructor's voice was:	22	14	68	14	5			4.0	18
7. Explanations by instructor were:	22	18	64	9	9			4.0	12
8. Instr's ability to present alternative explan. when needed was:	22	27	50	18	5			4.0	9
9. Instructor's use of examples and illustrations was:	22	45	36	14	5			4.4	2
10. Quality of questions or problems raised by instructor was:	22	18	64	9	9			4.0	13
11. Student confidence in instructor's knowledge was:	22	45	32	14	5	5		4.4	15
12. Instructor's enthusiasm was:	22	32	45	5	14	5		4.1	17
13. Encouragement given students to express themselves was:	22	36	41	14	5	5		4.2	11
14. Answers to student questions were:	22	18	68	9		5		4.0	10
15. Availability of extra help when needed was:	21	29	57	10	5			4.1	8
16. Use of class time was:	21	33	48	14		5		4.2	1
17. Instructor's interest in whether students learned was:	21	29	52	14		5		4.1	14
18. Amount you learned in the course was:	22	27	50	18			5	4.0	5
19. Relevance and usefulness of course content were:	22	23	55	18		5		4.0	16
20. Evaluative and grading techniques (tests, papers, etc.) were:	22	23	45	23		9		3.9	6
21. Reasonableness of assigned work was:	22	23	59	14		5		4.0	4
22. Clarity of student responsibilities and requirements was:	21	24	57	14			5	4.0	7

Relative to other college courses you have taken:		Much Higher (7)	(6)	(5)	Average (4)	(3)	Much Lower (2)	(1)	
23. Do you expect your grade in this course to be:	22	9	9	27	36	18			4.4
24. The intellectual challenge presented was:	22	5	41	45		9			5.4
25. The amount of effort you put into this course was:	22	5	45	27	18	5			5.5
26. The amount of effort to succeed in this course was:	22		50	32	9	9			5.5
27. Your involvement in course (assignments, attendance, etc.) was:	22	9	50	18	18	5			5.7

28. On average, how many hours per week have you spent on this course?

Percent
- Under 2
- 2-3
- 5 4-5
- 25 6-7
- 15 8-9
- 40 10-11
- 5 12-13
- 10 14-15
- 16-17
- 18-19
- 20-21
- 22 or more

Respondents: 20
Class median: 9.8
Hours per credit: 1.95

29. From the total average hours spent, how many do you consider were valuable in advancing your education?

Percent
- Under 2
- 2-3
- 5 4-5
- 40 6-7
- 15 8-9
- 30 10-11
- 5 12-13
- 5 14-15
- 16-17
- 18-19
- 20-21
- 22 or more

Respondents: 20
Class median: 8.2
Hours per credit: 1.63

30. What grade do you expect in this course?

Percent
- 19 A (3.9-4.0)
- 5 A- (3.5-3.8)
- 19 B+ (3.2-3.4)
- 24 B (2.9-3.1)
- 19 B- (2.5-2.8)
- 10 C+ (2.2-2.4)
- 5 C (1.9-2.1)
- C- (1.5-2.1)
- D+ (1.2-1.4)
- D (0.9-1.1)
- D- (0.7-0.8)
- E (0.0)
- Pass
- Credit
- No Credit

Respondents: 21
Class median: 3.0

31. In regard to your academic program, is this course best described as:

Percent
- 85 In your major
- A distribution requirement
- 5 An elective
- In your minor
- 5 A program requirement
- 5 Other

Challenge and Engagement Index

CEI = 7 ●●●●●●● (decile rank)

1. *Percentages are based on the number of students who rated each item.*

Batch UW 377-002196

SP08:02889 SURVEY ID

Respondents: **22**
Enrollment: **29**
Classes: **1**

A Form Type

Mailbox: **355020**
ChairCopy? **Yes**
printed: 7/17/2008

Instructional Assessment System

BIOL 533 D
BIOLOGY
ARTS & SCIENCES
University of Washington
w/ Harvell & Friedman

Steven Roberts
Assistant Professor
Summer 2010

Instructor Copy

STUDENT EVALUATION OF INSTRUCTION

E=Excellent; VG=Very Good; G=Good; F=Fair; P=Poor; VP=Very Poor

	Respondents	PERCENTAGES [1] E (5)	VG (4)	G (3)	F (2)	P (1)	VP (0)	MEDIAN	Adjusted Median
1. The course as a whole was:	11	55	36	9				4.6	4.5
2. The course content was:	10	40	50	10				4.3	4.2
3. The instructor's contribution to the course was:	11	45	45	9				4.4	4.4
4. The instructor's effectiveness in teaching the subj. matter was:	11	36	36	9	18			4.1	4.1
COMBINED ITEMS 1-4	43	44	42	9	5			4.4	4.3
									Relative Rank
5. Course organization was:	11	9	27	45	18			3.2	17
6. Clarity of instructor's voice was:	11	9	27	55	9			3.3	18
7. Explanations by instructor were:	11	55	18	18	9			4.6	9
8. Instr's ability to present alternative explan. when needed was:	11	45	36	18				4.4	13
9. Instructor's use of examples and illustrations was:	11	45	27	18	9			4.3	15
10. Quality of questions or problems raised by instructor was:	11	55	36	9				4.6	7
11. Student confidence in instructor's knowledge was:	11	91	9					5.0	6
12. Instructor's enthusiasm was:	11	73		27				4.8	10
13. Encouragement given students to express themselves was:	11	64	27	9				4.7	8
14. Answers to student questions were:	11	45	45	9				4.4	12
15. Availability of extra help when needed was:	11	73	27					4.8	2
16. Use of class time was:	11	36	36	18	9			4.1	14
17. Instructor's interest in whether students learned was:	11	55	18	27				4.6	11
18. Amount you learned in the course was:	11	55	36	9				4.6	5
19. Relevance and usefulness of course content were:	11	73	18	9				4.8	3
20. Evaluative and grading techniques (tests, papers, etc.) were:	10	50	30	20				4.5	4
21. Reasonableness of assigned work was:	11	64	18	9	9			4.7	1
22. Clarity of student responsibilities and requirements was:	11	27	36	27	9			3.9	16

Relative to other college courses you have taken:		Much Higher (7)	(6)	(5)	Average (4)	(3)	(2)	Much Lower (1)	MEDIAN
23. Do you expect your grade in this course to be:	11	9	27	9	55				4.4
24. The intellectual challenge presented was:	11	27	45	27					6.0
25. The amount of effort you put into this course was:	11	18	45	9	27				5.8
26. The amount of effort to succeed in this course was:	11	18	36	9	27	9			5.6
27. Your involvement in course (assignments, attendance, etc.) was:	11	36	27	18	18				6.0

28. On average, how many hours per week have you spent on this course?

Percent

	Under 2
	2-3
	4-5
	6-7
	8-9
	10-11
	12-13
	14-15
	16-17
	18-19
9	20-21
91	22 or more

Respondents: 11
Class median: 22.4
Hours per credit: 2.5

29. From the total average hours spent, how many do you consider were valuable in advancing your education?

Percent

	Under 2
	2-3
	4-5
	6-7
	8-9
18	10-11
	12-13
9	14-15
	16-17
	18-19
	20-21
73	22 or more

Respondents: 11
Class median: 22.1
Hours per credit: 2.5

30. What grade do you expect in this course?

Percent

82	A (3.9-4.0)
18	A- (3.5-3.8)
	B+ (3.2-3.4)
	B (2.9-3.1)
	B- (2.5-2.8)
	C+ (2.2-2.4)
	C (1.9-2.1)
	C- (1.5-2.1)
	D+ (1.2-1.4)
	D (0.9-1.1)
	D- (0.7-0.8)
	E (0.0)
	Pass
	Credit
	No Credit

Respondents: 11
Class median: 4.0

31. In regard to your academic program, is this course best described as:

Percent

55	In your major
	A distribution requirement
18	An elective
	In your minor
	A program requirement
27	Other

Challenge and Engagement Index

CEI = 6.0

(1=lowest; 7=highest)

1. Percentages are based on the number of students who rated each item.

Batch UW 733-000158

SU10:01773
SURVEY ID

Respondents: **11**
Enrollment: **11**
Classes: **1**

A
Form Type

Mailbox: **351812**
ChairCopy? **Yes**
printed: 9/15/2010

Instructional Assessment System

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OCEAN AND FISHERY SCIENCES
University of Washington

Steven Roberts
Assistant Professor
Autumn 2008

Department Copy

STUDENT EVALUATION OF INSTRUCTION

E=Excellent; VG=Very Good; G=Good; F=Fair; P=Poor; VP=Very Poor

	Respondents	PERCENTAGES [1] E (5)	VG (4)	G (3)	F (2)	P (1)	VP (0)	MEDIAN	Adjusted Median
1. The course as a whole was:	10	20	60	20				4.0	4.1
2. The course content was:	10	40	60					4.3	4.5
3. The instructor's contribution to the course was:	10	50	40	10				4.5	4.6
4. The instructor's effectiveness in teaching the subj. matter was:	10	20	70	10				4.1	4.2
COMBINED ITEMS 1-4	40	32	58	10				4.2	4.3
									Relative Rank
5. Course organization was:	10	60	30	10				4.7	1
6. Instructor's preparation for class was:	10	40	40	20				4.3	14
7. Instructor as a discussion leader was:	10	50	20	30				4.5	8
8. Instructor's contribution to discussion was:	10	40	50	10				4.3	13
9. Conduciveness of class atmosphere to student learning was:	10	50	30	20				4.5	5
10. Quality of questions or problems raised was:	10	30	60	10				4.2	12
11. Student confidence in instructor's knowledge was:	10	70	30					4.8	6
12. Instructor's enthusiasm was:	10	30	50	20				4.1	18
13. Encouragement given students to express themselves was:	10	30	50	20				4.1	16
14. Instructor's openness to student views was:	10	40	40	20				4.3	15
15. Interest level of class sessions was:	10	40	30	30				4.2	11
16. Use of class time was:	10	40	50	10				4.3	4
17. Instructor's interest in whether students learned was:	10	30	40	30				4.0	17
18. Amount you learned in the course was:	10	40	40	20				4.3	10
19. Relevance and usefulness of course content were:	10	50	30	20				4.5	7
20. Evaluative and grading techniques (tests, papers, etc.) were:	8	38	38	25				4.2	9
21. Reasonableness of assigned work was:	10	60	10	30				4.7	2
22. Clarity of student responsibilities and requirements was:	10	60	20	20				4.7	3

Relative to other college courses you have taken:		Much Higher (7)	(6)	(5)	Average (4)	(3)	(2)	Much Lower (1)	MEDIAN	
23. Do you expect your grade in this course to be:	7			14	86				4.1	
24. The intellectual challenge presented was:	9	11	33	22	22	11			5.3	
25. The amount of effort you put into this course was:	9			22	67		11		4.1	
26. The amount of effort to succeed in this course was:	9			11	78		11		4.0	
27. Your involvement in course (assignments, attendance, etc.) was:	9			11	22	67			4.3	

28. On average, how many hours per week have you spent on this course?

Percent

	Under 2
12	2-3
62	4-5
25	6-7
	8-9
	10-11
	12-13
	14-15
	16-17
	18-19
	20-21
	22 or more

Respondents: 8
Class median: 4.7
Hours per credit: 2.35

29. From the total average hours spent, how many do you consider were valuable in advancing your education?

Percent

	Under 2
11	2-3
67	4-5
22	6-7
	8-9
	10-11
	12-13
	14-15
	16-17
	18-19
	20-21
	22 or more

Respondents: 9
Class median: 4.7
Hours per credit: 2.33

30. What grade do you expect in this course?

Percent

12	A (3.9-4.0)
12	A- (3.5-3.8)
	B+ (3.2-3.4)
	B (2.9-3.1)
	B- (2.5-2.8)
	C+ (2.2-2.4)
	C (1.9-2.1)
	C- (1.5-2.1)
	D+ (1.2-1.4)
	D (0.9-1.1)
	D- (0.7-0.8)
	E (0.0)
12	Pass
62	Credit
	No Credit

Respondents: 8
Class median:

31. In regard to your academic program, is this course best described as:

Percent

20	In your major
	A distribution requirement
30	An elective
	In your minor
50	A program requirement
	Other

Challenge and Engagement Index

CEI = 1 • (decile rank)

1. *Percentages are based on the number of students who rated each item.*

Batch UW 457-002801

AU08:03931 SURVEY ID

Respondents: 10
Enrollment: 10
Classes: 1

C Form Type

Mailbox: 355020
ChairCopy? Yes
printed: 1/21/2009

Instructional Assessment System

FISH 5
AQUA[illegible] & FISHERY SCIENCES
OCEAN AND FISHERY SCIENCES
University of Washington

Steven Roberts
Assistant Professor
Winter 2009

Department Copy

STUDENT EVALUATION OF INSTRUCTION

E=Excellent; VG=Very Good; G=Good; F=Fair; P=Poor; VP=Very Poor

	Respondents	PERCENTAGES [1] E (5)	VG (4)	G (3)	F (2)	P (1)	VP (0)	MEDIAN	Adjusted Median
1. The course as a whole was:	7	57	43					4.6	4.4
2. The course content was:	7	57	43					4.6	4.4
3. The instructor's contribution to the course was:	7	43	57					4.4	4.3
4. The instructor's effectiveness in teaching the subj. matter was:	7	29	43	29				4.0	3.9
COMBINED ITEMS 1-4	28	46	46	7				4.4	4.3
									Relative Rank
5. Course organization was:	7	29	57	14				4.1	9
6. Clarity of instructor's voice was:	7	14	71	14				4.0	18
7. Explanations by instructor were:	7	14	71	14				4.0	12
8. Instr's ability to present alternative explan. when needed was:	7	43	43	14				4.3	8
9. Instructor's use of examples and illustrations was:	7	57	43					4.6	6
10. Quality of questions or problems raised by instructor was:	7	86	14					4.9	2
11. Student confidence in instructor's knowledge was:	7	43	57					4.4	13
12. Instructor's enthusiasm was:	7	29	57	14				4.1	17
13. Encouragement given students to express themselves was:	7	29	43	29				4.0	16
14. Answers to student questions were:	7	14	71	14				4.0	11
15. Availability of extra help when needed was:	7	71	29					4.8	3
16. Use of class time was:	7	29	71					4.2	7
17. Instructor's interest in whether students learned was:	7	29	43	29				4.0	15
18. Amount you learned in the course was:	7	86		14				4.9	1
19. Relevance and usefulness of course content were:	7	71	29					4.8	4
20. Evaluative and grading techniques (tests, papers, etc.) were:	7	43	43	14				4.3	5
21. Reasonableness of assigned work was:	7		86	14				3.9	10
22. Clarity of student responsibilities and requirements was:	7	14	57	14	14			3.9	14
Relative to other college courses you have taken:		Much Higher (7)	(6)	(5)	Average (4)	(3)	Much Lower (2)	(1)	
23. Do you expect your grade in this course to be:	7		14	43	29		14		4.7	
24. The intellectual challenge presented was:	7	14	43	14	29				5.7	
25. The amount of effort you put into this course was:	7		57	14	29				5.6	
26. The amount of effort to succeed in this course was:	7	14	29	29	29				5.3	
27. Your involvement in course (assignments, attendance, etc.) was:	7		57	29	14				5.6	

28. On average, how many hours per week have you spent on this course?

Percent

	Under 2
	2-3
	4-5
14	6-7
57	8-9
14	10-11
	12-13
14	14-15
	16-17
	18-19
	20-21
	22 or more

Respondents: 7
Class median: 8.8
Hours per credit: 2.92

29. From the total average hours spent, how many do you consider were valuable in advancing your education?

Percent

	Under 2
	2-3
14	4-5
14	6-7
43	8-9
14	10-11
	12-13
14	14-15
	16-17
	18-19
	20-21
	22 or more

Respondents: 7
Class median: 8.5
Hours per credit: 2.83

30. What grade do you expect in this course?

Percent

57	A (3.9-4.0)
29	A- (3.5-3.8)
	B+ (3.2-3.4)
	B (2.9-3.1)
	B- (2.5-2.8)
	C+ (2.2-2.4)
	C (1.9-2.1)
	C- (1.5-2.1)
	D+ (1.2-1.4)
14	D (0.9-1.1)
	D- (0.7-0.8)
	E (0.0)
	Pass
	Credit
	No Credit

Respondents: 7
Class median: 3.9

31. In regard to your academic program, is this course best described as:

Percent

67	In your major
	A distribution requirement
17	An elective
	In your minor
	A program requirement
17	Other

Challenge and Engagement Index

CEI = 7 •••••• (decile rank)

1. *Percentages are based on the number of students who rated each item.*

Batch UW 499-002515

WI09:04106 SURVEY ID

Respondents: 7
Enrollment: 8
Classes: 1

A Form Type

Mailbox: 355020
ChairCopy? Yes
printed: 4/13/2009

Instructional Assessment System

FISH ?
AQUA...C & FISHERY SCIENCES
OCEAN AND FISHERY SCIENCES
University of Washington
Co-taught w/ Carolyn Friedman

Steven Roberts
Assistant Professor
Spring 2009

Department Copy

STUDENT EVALUATION OF INSTRUCTION

E=Excellent; VG=Very Good; G=Good; F=Fair; P=Poor; VP=Very Poor

	Respondents	PERCENTAGES [1] E (5)	VG (4)	G (3)	F (2)	P (1)	VP (0)	MEDIAN	Adjusted Median
1. The course as a whole was:	18	39	33	28				4.2	4.1
2. The course content was:	18	28	56	11	6			4.1	4.0
3. The instructor's contribution to the course was:	18	22	33	39		6		3.7	3.6
4. The instructor's effectiveness in teaching the subj. matter was:	18	22	17	44	11		6	3.3	3.2
COMBINED ITEMS 1-4	72	28	35	31	4	1	1	3.9	3.8

	Respondents	E (5)	VG (4)	G (3)	F (2)	P (1)	VP (0)	MEDIAN	Relative Rank
5. Course organization was:	18	28	22	39	6	6		3.5	12
6. Clarity of instructor's voice was:	18	28	33	22	11	6		3.8	16
7. Explanations by instructor were:	18	28	17	39	6	6	6	3.4	17
8. Instr's ability to present alternative explan. when needed was:	18	28	22	39		6	6	3.5	15
9. Instructor's use of examples and illustrations was:	18	22	39	33			6	3.8	13
10. Quality of questions or problems raised by instructor was:	17	24	29	41			6	3.6	14
11. Student confidence in instructor's knowledge was:	18	44	33	22				4.3	9
12. Instructor's enthusiasm was:	18	17	39	17	22	6		3.6	18
13. Encouragement given students to express themselves was:	18	44	28	17	6		6	4.3	4
14. Answers to student questions were:	18	33	28	28	11			3.9	8
15. Availability of extra help when needed was:	18	39	44	12		6		4.2	3
16. Use of class time was:	18	28	33	22	6	11		3.8	6
17. Instructor's interest in whether students learned was:	17	29	41	18	6		6	4.0	10
18. Amount you learned in the course was:	18	22	61	17				4.0	5
19. Relevance and usefulness of course content were:	18	28	50	17	6			4.1	7
20. Evaluative and grading techniques (tests, papers, etc.) were:	18	28	44	17	6		6	4.0	1
21. Reasonableness of assigned work was:	18	28	44	22		6		4.0	2
22. Clarity of student responsibilities and requirements was:	18	17	44	33		6		3.8	11

Relative to other college courses you have taken:		Much Higher (7)	(6)	(5)	Average (4)	(3)	(2)	Much Lower (1)	MEDIAN	
23. Do you expect your grade in this course to be:	16	12	25	44	12	6			5.2	
24. The intellectual challenge presented was:	16	6	12	44	38				4.8	
25. The amount of effort you put into this course was:	16	6	25	31	25	12			4.9	
26. The amount of effort to succeed in this course was:	16	6	25	44	25				5.1	
27. Your involvement in course (assignments, attendance, etc.) was:	16	12	12	44	31				4.9	

28. On average, how many hours per week have you spent on this course?

Percent

	Under 2
	2-3
12	4-5
19	6-7
31	8-9
12	10-11
12	12-13
6	14-15
6	16-17
	18-19
	20-21
	22 or more

Respondents: 16
Class median: 8.7
Hours per credit: 1.74

29. From the total average hours spent, how many do you consider were valuable in advancing your education?

Percent

	Under 2
6	2-3
19	4-5
31	6-7
19	8-9
6	10-11
6	12-13
6	14-15
6	16-17
	18-19
	20-21
	22 or more

Respondents: 16
Class median: 7.1
Hours per credit: 1.42

30. What grade do you expect in this course?

Percent

6	A (3.9-4.0)
31	A- (3.5-3.8)
38	B+ (3.2-3.4)
19	B (2.9-3.1)
6	B- (2.5-2.8)
	C+ (2.2-2.4)
	C (1.9-2.1)
	C- (1.5-2.1)
	D+ (1.2-1.4)
	D (0.9-1.1)
	D- (0.7-0.8)
	E (0.0)
	Pass
	Credit
	No Credit

Respondents: 16
Class median: 3.4

31. In regard to your academic program, is this course best described as:

Percent

67	In your major
	A distribution requirement
	An elective
20	In your minor
13	A program requirement
	Other

Challenge and Engagement Index

CEI = 4 •••• (decile rank)

1. Percentages are based on the number of students who rated each item.

Batch UW 530-001352

SP09:03148
SURVEY ID

Respondents: **18**
Enrollment: **35**
Classes: **1**

A
Form Type

Mailbox: **355020**
ChairCopy? **Yes**
printed: 7/10/2009

Instructional Assessment System

FISH 44
AQUAT[illegible] & FISHERY SCIENCES
OCEAN AND FISHERY SCIENCES
University of Washington
Joint w/ FISH 541 A/B

Steven Roberts
Assistant Professor
Autumn 2009

ENV PHYSIOL.

Department Copy

STUDENT EVALUATION OF INSTRUCTION

E=Excellent; VG=Very Good; G=Good; F=Fair; P=Poor; VP=Very Poor

	Respondents	PERCENTAGES [1] E (5)	VG (4)	G (3)	F (2)	P (1)	VP (0)	MEDIAN	Adjusted Median
1. The course as a whole was:	14	29	57	14				4.1	4.1
2. The course content was:	14	29	57	14				4.1	4.1
3. The instructor's contribution to the course was:	14	50	43	7				4.5	4.5
4. The instructor's effectiveness in teaching the subj. matter was:	14	29	50	21				4.1	4.0
COMBINED ITEMS 1-4	56	34	52	14				4.2	4.1
									Relative Rank
5. Course organization was:	14	14	57	29				3.9	15
6. Clarity of instructor's voice was:	14	21	57	21				4.0	18
7. Explanations by instructor were:	14	43	50	7				4.4	9
8. Instr's ability to present alternative explan. when needed was:	14	29	57	14				4.1	13
9. Instructor's use of examples and illustrations was:	14	71	21	7				4.8	5
10. Quality of questions or problems raised by instructor was:	14	57	29	14				4.6	6
11. Student confidence in instructor's knowledge was:	14	86	7	7				4.9	7
12. Instructor's enthusiasm was:	14	36	43	21				4.2	17
13. Encouragement given students to express themselves was:	14	50	29	21				4.5	8
14. Answers to student questions were:	14	36	57	7				4.3	12
15. Availability of extra help when needed was:	13	38	31	31				4.1	16
16. Use of class time was:	14	29	57	14				4.1	11
17. Instructor's interest in whether students learned was:	14	36	50	14				4.2	14
18. Amount you learned in the course was:	14	64	29	7				4.7	3
19. Relevance and usefulness of course content were:	14	71	21	7				4.8	4
20. Evaluative and grading techniques (tests, papers, etc.) were:	14	71	21	7				4.8	1
21. Reasonableness of assigned work was:	14	64	29	7				4.7	2
22. Clarity of student responsibilities and requirements was:	14	43	29	21	7			4.3	10
Relative to other college courses you have taken:		Much Higher (7)	(6)	(5)	Average (4)	(3)	(2)	Much Lower (1)	
23. Do you expect your grade in this course to be:	14		29	29	43				4.8
24. The intellectual challenge presented was:	14		21	21	21	36			5.2
25. The amount of effort you put into this course was:	14		21	21	14	43			5.0
26. The amount of effort to succeed in this course was:	14		7	29	21	43			4.8
27. Your involvement in course (assignments, attendance, etc.) was:	14		21	29	14	36			5.5

28. On average, how many hours per week have you spent on this course?

Percent
- 7 Under 2
- 7 2-3
- 21 4-5
- 29 6-7
- 14 8-9
- 7 10-11
- 7 12-13
- 7 14-15
- 16-17
- 18-19
- 20-21
- 22 or more

Respondents: 14
Class median: 6.5
Hours per credit: 1.30

29. From the total average hours spent, how many do you consider were valuable in advancing your education?

Percent
- 7 Under 2
- 14 2-3
- 21 4-5
- 29 6-7
- 7 8-9
- 7 10-11
- 14 12-13
- 14-15
- 16-17
- 18-19
- 20-21
- 22 or more

Respondents: 14
Class median: 6.0
Hours per credit: 1.20

30. What grade do you expect in this course?

Percent
- 21 A (3.9-4.0)
- 50 A- (3.5-3.8)
- 29 B+ (3.2-3.4)
- B (2.9-3.1)
- B- (2.5-2.8)
- C+ (2.2-2.4)
- C (1.9-2.1)
- C- (1.5-2.1)
- D+ (1.2-1.4)
- D (0.9-1.1)
- D- (0.7-0.8)
- E (0.0)
- Pass
- Credit
- No Credit

Respondents: 14
Class median: 3.6

31. In regard to your academic program, is this course best described as:

Percent
- 64 In your major
- A distribution requirement
- 21 An elective
- 7 In your minor
- 7 A program requirement
- Other

Challenge and Engagement Index

CEI = 4 •••• (decile rank)

1. *Percentages are based on the number of students who rated each item.*

Batch UW 608-002191

AU09:03144 SURVEY ID

Respondents: 14
Enrollment: 20
Classes: 1

A Form Type

Mailbox: 355020
ChairCopy? Yes
printed: 1/26/2010

Instructional Assessment System

FISH 5(
AQUA[illegible] & FISHERY SCIENCES
OCEAN AND FISHERY SCIENCES
University of Washington

Steven Roberts
Assistant Professor
Winter 2010

Department Copy

STUDENT EVALUATION OF INSTRUCTION

E=Excellent; VG=Very Good; G=Good; F=Fair; P=Poor; VP=Very Poor

	Respondents	PERCENTAGES [1] E (5)	VG (4)	G (3)	F (2)	P (1)	VP (0)	MEDIAN	Adjusted Median
1. The course as a whole was:	7	14	29	43	14			3.3	3.3
2. The course content was:	7	14	14	57	14			3.1	3.1
3. The instructor's contribution to the course was:	7	14	29	43	14			3.3	3.4
4. The instructor's effectiveness in teaching the subj. matter was:	7		14	57	29			2.9	2.9
COMBINED ITEMS 1-4	28	11	21	50	18			3.1	3.1

									Relative Rank
5. Course organization was:	7		14	57	29			2.9	17
6. Clarity of instructor's voice was:	7	29	29	43				3.8	15
7. Explanations by instructor were:	7	14	43	29		14		3.7	13
8. Instr's ability to present alternative explan. when needed was:	7	29	43	29				4.0	8
9. Instructor's use of examples and illustrations was:	7	29	43	14	14			4.0	11
10. Quality of questions or problems raised by instructor was:	7	43	43		14			4.3	4
11. Student confidence in instructor's knowledge was:	7	71	29					4.8	3
12. Instructor's enthusiasm was:	7	14	71	14				4.0	14
13. Encouragement given students to express themselves was:	7	43	29		29			4.3	7
14. Answers to student questions were:	7	43	29	14	14			4.3	5
15. Availability of extra help when needed was:	7	57	29			14		4.6	1
16. Use of class time was:	7	14	14	29	43			2.8	18
17. Instructor's interest in whether students learned was:	7	57		43				4.6	2
18. Amount you learned in the course was:	7	14	29	43	14			3.3	16
19. Relevance and usefulness of course content were:	7	43	29	14		14		4.3	6
20. Evaluative and grading techniques (tests, papers, etc.) were:	7	29	29	43				3.8	9
21. Reasonableness of assigned work was:	7	29	29	43				3.8	10
22. Clarity of student responsibilities and requirements was:	7	14	43	14	29			3.7	12

Relative to other college courses you have taken:		Much Higher (7)	(6)	(5)	Average (4)	(3)	Much Lower (2)	(1)		
23. Do you expect your grade in this course to be:	7			14	86				4.1	
24. The intellectual challenge presented was:	7		57	29	14				5.6	
25. The amount of effort you put into this course was:	7			14	43	29	14		3.7	
26. The amount of effort to succeed in this course was:	7		14	14	57	14			4.1	
27. Your involvement in course (assignments, attendance, etc.) was:	7	14		14	71				4.2	

28. On average, how many hours per week have you spent on this course?

Percent

	Under 2
	2-3
29	4-5
71	6-7
	8-9
	10-11
	12-13
	14-15
	16-17
	18-19
	20-21
	22 or more

Respondents: 7
Class median: 6.1
Hours per credit: 2.0

29. From the total average hours spent, how many do you consider were valuable in advancing your education?

Percent

14	Under 2
14	2-3
57	4-5
14	6-7
	8-9
	10-11
	12-13
	14-15
	16-17
	18-19
	20-21
	22 or more

Respondents: 7
Class median: 4.3
Hours per credit: 1.4

30. What grade do you expect in this course?

Percent

14	A (3.9-4.0)
71	A- (3.5-3.8)
14	B+ (3.2-3.4)
	B (2.9-3.1)
	B- (2.5-2.8)
	C+ (2.2-2.4)
	C (1.9-2.1)
	C- (1.5-2.1)
	D+ (1.2-1.4)
	D (0.9-1.1)
	D- (0.7-0.8)
	E (0.0)
	Pass
	Credit
	No Credit

Respondents: 7
Class median: 3.6

31. In regard to your academic program, is this course best described as:

Percent

43	In your major
	A distribution requirement
57	An elective
	In your minor
	A program requirement
	Other

Challenge and Engagement Index

CEI = 3.9

(1=lowest; 7=highest)

1. Percentages are based on the number of students who rated each item.

Batch UW 646-000001

WI10:03566
SURVEY ID

Respondents: 7
Enrollment: 8
Classes: 1

A
Form Type

Mailbox: 355020
ChairCopy? Yes
printed: 4/14/2010

Instructional Assessment System

FISH 3?
AQUAT. & FISHERY SCIENCES
OCEAN AND FISHERY SCIENCES
University of Washington

Steven Roberts
Assistant Professor
Spring 2010

Department Copy

STUDENT EVALUATION OF INSTRUCTION

E=Excellent; VG=Very Good; G=Good; F=Fair; P=Poor; VP=Very Poor

	Respondents	PERCENTAGES [1] E (5)	VG (4)	G (3)	F (2)	P (1)	VP (0)	MEDIAN	Adjusted Median
1. The course as a whole was:	18	33	50	6	11			4.2	4.1
2. The course content was:	18	39	39	17	6			4.2	4.1
3. The instructor's contribution to the course was:	18	39	28	22	11			4.1	4.0
4. The instructor's effectiveness in teaching the subj. matter was:	18	39	33	17	6	6		4.2	4.1
COMBINED ITEMS 1-4	72	38	38	15	8	1		4.2	4.1
									Relative Rank
5. Course organization was:	18	33	56	11				4.2	6
6. Clarity of instructor's voice was:	18	33	28	28	11			3.9	17
7. Explanations by instructor were:	18	28	39	22	11			3.9	15
8. Instr's ability to present alternative explan. when needed was:	18	39	44	11	6			4.3	9
9. Instructor's use of examples and illustrations was:	18	44	22	17	11	6		4.3	10
10. Quality of questions or problems raised by instructor was:	18	39	28	28	6			4.1	13
11. Student confidence in instructor's knowledge was:	18	61	22	11	6			4.7	8
12. Instructor's enthusiasm was:	18	28	17	17	33	6		3.2	18
13. Encouragement given students to express themselves was:	18	39	33	17	11			4.2	14
14. Answers to student questions were:	18	22	44	33				3.9	16
15. Availability of extra help when needed was:	18	39	33	28				4.2	11
16. Use of class time was:	18	39	39	17	6			4.2	7
17. Instructor's interest in whether students learned was:	18	39	39	22				4.2	12
18. Amount you learned in the course was:	18	61	22	11	6			4.7	1
19. Relevance and usefulness of course content were:	18	61	33		6			4.7	3
20. Evaluative and grading techniques (tests, papers, etc.) were:	18	39	50	11				4.3	4
21. Reasonableness of assigned work was:	18	39	56	6				4.3	5
22. Clarity of student responsibilities and requirements was:	18	61	28	11				4.7	2

Relative to other college courses you have taken:		Much Higher (7)	(6)	(5)	Average (4)	(3)	Much Lower (2)	(1)	MEDIAN
23. Do you expect your grade in this course to be:	18	6	28	50	11	6			5.2
24. The intellectual challenge presented was:	18	11	22	17	44	6			4.5
25. The amount of effort you put into this course was:	18	17	11	39	33				4.9
26. The amount of effort to succeed in this course was:	18	17	22	11	50				4.5
27. Your involvement in course (assignments, attendance, etc.) was:	18	22	17	11	50				4.5

28. On average, how many hours per week have you spent on this course?

Percent	
	Under 2
6	2-3
6	4-5
28	6-7
33	8-9
11	10-11
	12-13
11	14-15
	16-17
	18-19
	20-21
6	22 or more

Respondents: 18
Class median: 8.2
Hours per credit: 1.6

29. From the total average hours spent, how many do you consider were valuable in advancing your education?

Percent	
	Under 2
17	2-3
6	4-5
33	6-7
17	8-9
17	10-11
6	12-13
	14-15
	16-17
	18-19
	20-21
6	22 or more

Respondents: 18
Class median: 7.2
Hours per credit: 1.4

30. What grade do you expect in this course?

Percent	
22	A (3.9-4.0)
22	A- (3.5-3.8)
11	B+ (3.2-3.4)
33	B (2.9-3.1)
11	B- (2.5-2.8)
	C+ (2.2-2.4)
	C (1.9-2.1)
	C- (1.5-2.1)
	D+ (1.2-1.4)
	D (0.9-1.1)
	D- (0.7-0.8)
	E (0.0)
	Pass
	Credit
	No Credit

Respondents: 18
Class median: 3.3

31. In regard to your academic program, is this course best described as:

Percent	
100	In your major
	A distribution requirement
	An elective
	In your minor
	A program requirement
	Other

Challenge and Engagement Index

CEI = 4.3

(1=lowest; 7=highest)

1. *Percentages are based on the number of students who rated each item.*

Batch UW 701-001114

SP10:04046
SURVEY ID

Respondents: 18
Enrollment: 36
Classes: 1

A
Form Type

Mailbox: 355020
ChairCopy? Yes
printed: 7/13/2010

Instructional Assessment System

FISH 44
SCH. OF AQUATIC & FISHERY SCIENCES
COLLEGE OF THE ENVIRONMENT
University of Washington

Steven Roberts
Assistant Professor
Autumn 2010

Department Copy

STUDENT EVALUATION OF INSTRUCTION

E=Excellent; VG=Very Good; G=Good; F=Fair; P=Poor; VP=Very Poor

	Respondents	PERCENTAGES [1] E (5)	VG (4)	G (3)	F (2)	P (1)	VP (0)	MEDIAN	Adjusted Median
1. The course as a whole was:	5	60	40					4.7	4.2
2. The course content was:	5	80	20					4.9	4.4
3. The instructor's contribution to the course was:	5	60	40					4.7	4.3
4. The instructor's effectiveness in teaching the subj. matter was:	5	40	60					4.3	3.9
COMBINED ITEMS 1-4	20	60	40					4.7	4.2
									Relative Rank
5. Course organization was:	5	40		60				3.3	18
6. Clarity of instructor's voice was:	5	60	20	20				4.7	15
7. Explanations by instructor were:	5	60	40					4.7	13
8. Instr's ability to present alternative explan. when needed was:	5	60	40					4.7	11
9. Instructor's use of examples and illustrations was:	5	100						5.0	4
10. Quality of questions or problems raised by instructor was:	5	80	20					4.9	3
11. Student confidence in instructor's knowledge was:	5	100						5.0	10
12. Instructor's enthusiasm was:	5	40	20	40				4.0	17
13. Encouragement given students to express themselves was:	5	80	20					4.9	8
14. Answers to student questions were:	5	60	40					4.7	9
15. Availability of extra help when needed was:	5	60	20	20				4.7	12
16. Use of class time was:	5	40	40	20				4.3	16
17. Instructor's interest in whether students learned was:	5	60	40					4.7	14
18. Amount you learned in the course was:	5	60	40					4.7	6
19. Relevance and usefulness of course content were:	5	100						5.0	2
20. Evaluative and grading techniques (tests, papers, etc.) were:	5	80	20					4.9	1
21. Reasonableness of assigned work was:	5	60	40					4.7	5
22. Clarity of student responsibilities and requirements was:	5	60	40					4.7	7
		Much Higher (7)	(6)	(5)	Average (4)	(3)	Much Lower (2)	(1)	
Relative to other college courses you have taken:									
23. Do you expect your grade in this course to be:	5		60	20		20			5.7
24. The intellectual challenge presented was:	5	40	60						6.3
25. The amount of effort you put into this course was:	5	60		20		20			6.7
26. The amount of effort to succeed in this course was:	5	60	20	20					6.7
27. Your involvement in course (assignments, attendance, etc.) was:	5	40	40		20				6.3

28. On average, how many hours per week have you spent on this course?

Percent

	Under 2
	2-3
20	4-5
20	6-7
20	8-9
20	10-11
	12-13
20	14-15
	16-17
	18-19
	20-21
	22 or more

Respondents: 5
Class median: 8.5
Hours per credit: 1.7

29. From the total average hours spent, how many do you consider were valuable in advancing your education?

Percent

	Under 2
	2-3
20	4-5
20	6-7
40	8-9
	10-11
	12-13
20	14-15
	16-17
	18-19
	20-21
	22 or more

Respondents: 5
Class median: 8.0
Hours per credit: 1.6

30. What grade do you expect in this course?

Percent

40	A (3.9-4.0)
40	A- (3.5-3.8)
	B+ (3.2-3.4)
	B (2.9-3.1)
	B- (2.5-2.8)
	C+ (2.2-2.4)
	C (1.9-2.1)
20	C- (1.5-2.1)
	D+ (1.2-1.4)
	D (0.9-1.1)
	D- (0.7-0.8)
	E (0.0)
	Pass
	Credit
	No Credit

Respondents: 5
Class median: 3.7

31. In regard to your academic program, is this course best described as:

Percent

100	In your major
	A distribution requirement
	An elective
	In your minor
	A program requirement
	Other

Challenge and Engagement Index

CEI = 6.0

(1=lowest; 7=highest)

1. *Percentages are based on the number of students who rated each item.*

Batch UW 755-002602

AU10:02532
SURVEY ID

Respondents: 5
Enrollment: 8
Classes: 1

A
Form Type

Mailbox: 355020
ChairCopy? Yes
printed: 1/19/2011

Instructional Assessment System

FISH 5
SCH. OF AQUATIC & FISHERY SCIENCES
COLLEGE OF THE ENVIRONMENT
University of Washington

Steven Roberts
Assistant Professor
Autumn 2010

Department Copy

STUDENT EVALUATION OF INSTRUCTION

E=Excellent; VG=Very Good; G=Good; F=Fair; P=Poor; VP=Very Poor

	Respondents	PERCENTAGES [1] E (5)	VG (4)	G (3)	F (2)	P (1)	VP (0)	MEDIAN	Adjusted Median
1. The course as a whole was:	4	25	50	25				4.0	3.6
2. The course content was:	4		75	25				3.8	3.5
3. The instructor's contribution to the course was:	4		75	25				3.8	3.6
4. The instructor's effectiveness in teaching the subj. matter was:	4		75	25				3.8	3.5
COMBINED ITEMS 1-4	16	6	69	25				3.9	3.5
									Relative Rank
5. Course organization was:	4	25	25	50				3.5	15
6. Clarity of instructor's voice was:	4	25	75					4.2	11
7. Explanations by instructor were:	4		75	25				3.8	14
8. Instr's ability to present alternative explan. when needed was:	4		50	50				3.5	17
9. Instructor's use of examples and illustrations was:	4	50	25	25				4.5	8
10. Quality of questions or problems raised by instructor was:	4	50	25	25				4.5	5
11. Student confidence in instructor's knowledge was:	4	75	25					4.8	6
12. Instructor's enthusiasm was:	4	25		75				3.2	18
13. Encouragement given students to express themselves was:	4	50		50				4.0	13
14. Answers to student questions were:	4		100					4.0	10
15. Availability of extra help when needed was:	4	75	25					4.8	1
16. Use of class time was:	4		50	50				3.5	16
17. Instructor's interest in whether students learned was:	3	33	33	33				4.0	12
18. Amount you learned in the course was:	4	25	50		25			4.0	9
19. Relevance and usefulness of course content were:	4	50	50					4.5	7
20. Evaluative and grading techniques (tests, papers, etc.) were:	4	50	25		25			4.5	2
21. Reasonableness of assigned work was:	4	50	50					4.5	3
22. Clarity of student responsibilities and requirements was:	4	50	25	25				4.5	4

Relative to other college courses you have taken:		Much Higher (7)	(6)	(5)	Average (4)	(3)	Much Lower (2)	(1)	MEDIAN
23. Do you expect your grade in this course to be:	4	25	25		50				5.0
24. The intellectual challenge presented was:	4	25	25	25			25		5.5
25. The amount of effort you put into this course was:	4	50	25	25					6.5
26. The amount of effort to succeed in this course was:	4	25	25	25	25				5.5
27. Your involvement in course (assignments, attendance, etc.) was:	4	25	50		25				6.0

28. On average, how many hours per week have you spent on this course?

Percent
- Under 2
- 2-3
- 4-5
- 25 6-7
- 8-9
- 10-11
- 50 12-13
- 25 14-15
- 16-17
- 18-19
- 20-21
- 22 or more

Respondents: 4
Class median: 12.5
Hours per credit: 2.5

29. From the total average hours spent, how many do you consider were valuable in advancing your education?

Percent
- Under 2
- 25 2-3
- 4-5
- 25 6-7
- 8-9
- 10-11
- 25 12-13
- 25 14-15
- 16-17
- 18-19
- 20-21
- 22 or more

Respondents: 4
Class median: 9.5
Hours per credit: 1.9

30. What grade do you expect in this course?

Percent
- 50 A (3.9-4.0)
- 50 A- (3.5-3.8)
- B+ (3.2-3.4)
- B (2.9-3.1)
- B- (2.5-2.8)
- C+ (2.2-2.4)
- C (1.9-2.1)
- C- (1.5-2.1)
- D+ (1.2-1.4)
- D (0.9-1.1)
- D- (0.7-0.8)
- E (0.0)
- Pass
- Credit
- No Credit

Respondents: 4
Class median: 3.8

31. In regard to your academic program, is this course best described as:

Percent
- 33 In your major
- A distribution requirement
- 67 An elective
- In your minor
- A program requirement
- Other

Challenge and Engagement Index

CEI = 5.3

(1=lowest; 7=highest)

1. *Percentages are based on the number of students who rated each item.*

Batch UW 755-002608

AU10:02546
SURVEY ID

Respondents: 4
Enrollment: 5
Classes: 1

A
Form Type

Mailbox: 355020
ChairCopy? Yes
printed: 1/19/2011

Instructional Assessment System

FISH 51
SCH. OF AQUATIC & FISHERY SCIENCES
COLLEGE OF THE ENVIRONMENT
University of Washington

Steven Roberts
Assistant Professor
Winter 2011

Department Copy

STUDENT EVALUATION OF INSTRUCTION

E=Excellent; VG=Very Good; G=Good; F=Fair; P=Poor; VP=Very Poor

	Respondents	PERCENTAGES [1] E (5)	VG (4)	G (3)	F (2)	P (1)	VP (0)	MEDIAN	Adjusted Median
1. The course as a whole was:	14	21	57	21				4.0	4.1
2. The course content was:	14	21	57	21				4.0	4.1
3. The instructor's contribution to the course was:	14	29	43	29				4.0	4.1
4. The instructor's effectiveness in teaching the subj. matter was:	13	38	46	15				4.3	4.3
COMBINED ITEMS 1-4	55	27	51	22				4.1	4.1
									Relative Rank
5. Course organization was:	14	21	57	21				4.0	12
6. Instructor's preparation for class was:	13	38	54	8				4.3	13
7. Instructor as a discussion leader was:	14	21	64	14				4.1	16
8. Instructor's contribution to discussion was:	13	31	46	23				4.1	17
9. Conduciveness of class atmosphere to student learning was:	13	46	31	23				4.4	9
10. Quality of questions or problems raised was:	14	50	29	21				4.5	5
11. Student confidence in instructor's knowledge was:	13	69	23	8				4.8	8
12. Instructor's enthusiasm was:	14	36	43	21				4.2	18
13. Encouragement given students to express themselves was:	13	54	31	15				4.6	10
14. Instructor's openness to student views was:	13	38	54	8				4.3	15
15. Interest level of class sessions was:	13	46	46	8				4.4	4
16. Use of class time was:	13	46	46	8				4.4	2
17. Instructor's interest in whether students learned was:	13	38	38	23				4.2	14
18. Amount you learned in the course was:	13	31	54	15				4.1	11
19. Relevance and usefulness of course content were:	14	57	36	7				4.6	3
20. Evaluative and grading techniques (tests, papers, etc.) were:	10	40	50	10				4.3	7
21. Reasonableness of assigned work was:	12	50	42	8				4.5	1
22. Clarity of student responsibilities and requirements was:	13	46	46	8				4.4	6

Relative to other college courses you have taken:		Much Higher (7)	(6)	(5)	Average (4)	(3)	Much Lower (2)	(1)	MEDIAN
23. Do you expect your grade in this course to be:	12	17		25	58				4.4
24. The intellectual challenge presented was:	12	17	25	33	25				5.3
25. The amount of effort you put into this course was:	12	8		8	33	50			3.5
26. The amount of effort to succeed in this course was:	12	8		8	33	50			3.5
27. Your involvement in course (assignments, attendance, etc.) was:	11	9		9	55	27			3.9

28. On average, how many hours per week have you spent on this course?

Percent
- 8 Under 2
- 42 2-3
- 42 4-5
- 6-7
- 8-9
- 8 10-11
- 12-13
- 14-15
- 16-17
- 18-19
- 20-21
- 22 or more

Respondents: 12
Class median: 3.5
Hours per credit: 1.8

29. From the total average hours spent, how many do you consider were valuable in advancing your education?

Percent
- 8 Under 2
- 50 2-3
- 33 4-5
- 6-7
- 8-9
- 8 10-11
- 12-13
- 14-15
- 16-17
- 18-19
- 20-21
- 22 or more

Respondents: 12
Class median: 3.2
Hours per credit: 1.6

30. What grade do you expect in this course?

Percent
- 25 A (3.9-4.0)
- A- (3.5-3.8)
- B+ (3.2-3.4)
- B (2.9-3.1)
- B- (2.5-2.8)
- C+ (2.2-2.4)
- C (1.9-2.1)
- C- (1.5-2.1)
- D+ (1.2-1.4)
- D (0.9-1.1)
- D- (0.7-0.8)
- E (0.0)
- 17 Pass
- 58 Credit
- No Credit

Respondents: 12
Class median:

31. In regard to your academic program, is this course best described as:

Percent
- 46 In your major
- A distribution requirement
- 31 An elective
- In your minor
- 23 A program requirement
- Other

Challenge and Engagement Index

CEI = 3.6

(1=lowest; 7=highest)

1. *Percentages are based on the number of students who rated each item.*

Batch UW 811-001880

WI11:03176
SURVEY ID

Respondents: **14**
Enrollment: **16**
Classes: **1**

C
Form Type

Mailbox: **355020**
ChairCopy? **Yes**
printed: 4/11/2011

Instructional Assessment System

FISH 31
Sch. of Aquatic & Fishery Sciences
College of the Environment
University of Washington

Steven Roberts
Assistant Professor
Spring 2011

Department Copy

STUDENT EVALUATION OF INSTRUCTION

E=Excellent; VG=Very Good; G=Good; F=Fair; P=Poor; VP=Very Poor

	Respondents	PERCENTAGES [1] E (5)	VG (4)	G (3)	F (2)	P (1)	VP (0)	MEDIAN	Adjusted Median
1. The course as a whole was:	30	30	47	20	3			4.1	4.1
2. The course content was:	30	20	57	20	3			4.0	3.9
3. The instructor's contribution to the course was:	30	23	40	27	7	3		3.8	3.8
4. The instructor's effectiveness in teaching the subj. matter was:	30	30	40	20	7	3		4.0	4.0
COMBINED ITEMS 1-4	120	26	46	22	5	2		4.0	4.0
									Relative Rank
5. Course organization was:	30	33	47	13	3	3		4.1	8
6. Clarity of instructor's voice was:	29	34	34	24		3	3	4.1	16
7. Explanations by instructor were:	30	37	33	23	3	3		4.1	10
8. Instr's ability to present alternative explan. when needed was:	29	28	41	14	14	3		4.0	12
9. Instructor's use of examples and illustrations was:	28	43	43	7	4	4		4.3	5
10. Quality of questions or problems raised by instructor was:	30	37	40	20			3	4.2	7
11. Student confidence in instructor's knowledge was:	29	34	38	17	7		3	4.1	17
12. Instructor's enthusiasm was:	30	23	30	17	17	10	3	3.6	18
13. Encouragement given students to express themselves was:	29	38	38	10	10		3	4.2	11
14. Answers to student questions were:	30	27	40	27	3		3	3.9	14
15. Availability of extra help when needed was:	26	31	35	27	4		4	3.9	15
16. Use of class time was:	30	23	50	13	10		3	4.0	8
17. Instructor's interest in whether students learned was:	28	39	39	7	11		4	4.2	9
18. Amount you learned in the course was:	30	43	40	7	7		3	4.3	1
19. Relevance and usefulness of course content were:	28	29	46	11	14			4.0	13
20. Evaluative and grading techniques (tests, papers, etc.) were:	29	38	38	17	7			4.2	2
21. Reasonableness of assigned work was:	29	34	45	14	7			4.2	4
22. Clarity of student responsibilities and requirements was:	29	38	31	24	7			4.1	6

Relative to other college courses you have taken:		Much Higher (7)	(6)	(5)	Average (4)	(3)	Much Lower (2)	(1)	MEDIAN
23. Do you expect your grade in this course to be:	29	10	28	17	34	7	3		4.8
24. The intellectual challenge presented was:	29	7	28	31	28	7			5.0
25. The amount of effort you put into this course was:	29	7	28	24	24	3	10	3	4.9
26. The amount of effort to succeed in this course was:	29	7	17	28	34	3	7	3	4.6
27. Your involvement in course (assignments, attendance, etc.) was:	28	14	18	21	36	4	4	4	4.7

28. On average, how many hours per week have you spent on this course?

Percent	
7	Under 2
10	2-3
14	4-5
14	6-7
28	8-9
10	10-11
3	12-13
3	14-15
7	16-17
	18-19
3	20-21
	22 or more

Respondents: 29
Class median: 7.9
Hours per credit: 1.6

29. From the total average hours spent, how many do you consider were valuable in advancing your education?

Percent	
10	Under 2
17	2-3
28	4-5
14	6-7
10	8-9
7	10-11
3	12-13
	14-15
7	16-17
3	18-19
	20-21
	22 or more

Respondents: 29
Class median: 5.1
Hours per credit: 1.0

30. What grade do you expect in this course?

Percent	
17	A (3.9-4.0)
30	A- (3.5-3.8)
30	B+ (3.2-3.4)
13	B (2.9-3.1)
	B- (2.5-2.8)
7	C+ (2.2-2.4)
	C (1.9-2.1)
3	C- (1.5-2.1)
	D+ (1.2-1.4)
	D (0.9-1.1)
	D- (0.7-0.8)
	E (0.0)
	Pass
	Credit
	No Credit

Respondents: 30
Class median: 3.4

31. In regard to your academic program, is this course best described as:

Percent	
82	In your major
4	A distribution requirement
4	An elective
7	In your minor
	A program requirement
4	Other

Challenge and Engagement Index

CEI = 4.4

(1=lowest; 7=highest)

1. Percentages are based on the number of students who rated each item.

Batch UW 833-000334

SP11:02699
SURVEY ID

Respondents: **30**
Enrollment: **33**
Classes: **1**

A
Form Type

Mailbox: **355020**
ChairCopy? **Yes**
printed: 7/7/2011

Instructional Assessment System

FISH 44[illegible]
Sch. of Aquatic & Fishery Sciences
College of the Environment
University of Washington

Steven Roberts
Assistant Professor
Autumn 2011

Department Copy

STUDENT EVALUATION OF INSTRUCTION

E=Excellent; VG=Very Good; G=Good; F=Fair; P=Poor; VP=Very Poor

	Respondents	PERCENTAGES [1] E (5)	VG (4)	G (3)	F (2)	P (1)	VP (0)	MEDIAN	Adjusted Median
1. The course as a whole was:	9	100						5.0	4.5
2. The course content was:	9	67	33					4.8	4.3
3. The instructor's contribution to the course was:	9	89	11					4.9	4.6
4. The instructor's effectiveness in teaching the subj. matter was:	9	78	22					4.9	4.4
COMBINED ITEMS 1-4	36	83	17					4.9	4.5
									Relative Rank
5. Course organization was:	9	44	44	11				4.4	15
6. Clarity of instructor's voice was:	9	67	33					4.8	17
7. Explanations by instructor were:	9	67	33					4.8	9
8. Instr's ability to present alternative explan. when needed was:	9	67	33					4.8	7
9. Instructor's use of examples and illustrations was:	9	67	33					4.8	11
10. Quality of questions or problems raised by instructor was:	9	56	33	11				4.6	13
11. Student confidence in instructor's knowledge was:	9	89	11					4.9	12
12. Instructor's enthusiasm was:	9	67	22	11				4.8	18
13. Encouragement given students to express themselves was:	9	67	33					4.8	14
14. Answers to student questions were:	9	78	22					4.9	4
15. Availability of extra help when needed was:	9	67	22	11				4.8	8
16. Use of class time was:	9	44	44	11				4.4	16
17. Instructor's interest in whether students learned was:	9	67	33					4.8	10
18. Amount you learned in the course was:	9	78	22					4.9	2
19. Relevance and usefulness of course content were:	9	89	11					4.9	6
20. Evaluative and grading techniques (tests, papers, etc.) were:	9	78	11	11				4.9	1
21. Reasonableness of assigned work was:	9	67	33					4.8	5
22. Clarity of student responsibilities and requirements was:	9	78	11	11				4.9	3

Relative to other college courses you have taken:		Much Higher (7)	(6)	(5)	Average (4)	(3)	Much Lower (2)	(1)	MEDIAN
23. Do you expect your grade in this course to be:	9		67	11	22				6.8
24. The intellectual challenge presented was:	9	11	33	22	33				5.3
25. The amount of effort you put into this course was:	9		33	33	22	11			6.0
26. The amount of effort to succeed in this course was:	9		44	22	33				5.3
27. Your involvement in course (assignments, attendance, etc.) was:	9		44	22	33				5.3

28. On average, how many hours per week have you spent on this course?

Percent
- 11 Under 2
- 11 2-3
- 11 4-5
- 44 6-7
- 11 8-9
- 11 10-11
- 12-13
- 14-15
- 11 16-17
- 18-19
- 20-21
- 22 or more

Respondents: 9
Class median: 6.8
Hours per credit: 1.4

29. From the total average hours spent, how many do you consider were valuable in advancing your education?

Percent
- 11 Under 2
- 11 2-3
- 22 4-5
- 33 6-7
- 11 8-9
- 11 10-11
- 12-13
- 14-15
- 11 16-17
- 18-19
- 20-21
- 22 or more

Respondents: 9
Class median: 6.5
Hours per credit: 1.3

30. What grade do you expect in this course?

Percent
- 11 A (3.9-4.0)
- 78 A- (3.5-3.8)
- B+ (3.2-3.4)
- B (2.9-3.1)
- B- (2.5-2.8)
- C+ (2.2-2.4)
- 11 C (1.9-2.1)
- C- (1.5-1.8)
- D+ (1.2-1.4)
- D (0.9-1.1)
- D- (0.7-0.8)
- E (0.0)
- Pass
- Credit
- No Credit

Respondents: 9
Class median: 3.6

31. In regard to your academic program, is this course best described as:

Percent
- 100 In your major
- A distribution requirement
- An elective
- In your minor
- A program requirement
- Other

Challenge and Engagement Index

CEI = 4.6

(1=lowest; 7=highest)

1. Percentages are based on the number of students who rated each item.

Batch UW 919-002101

AU11:01413
SURVEY ID

Respondents: 9
Enrollment: 11
Classes: 1

A
Form Type

Mailbox: 355020
ChairCopy? Yes
printed: 1/23/2012

Instructional Assessment System

FISH 54
Sch. of Aquatic & Fishery Sciences
College of the Environment
University of Washington

Steven Roberts
Assistant Professor
Autumn 2011

Department Copy

STUDENT EVALUATION OF INSTRUCTION

E=Excellent; VG=Very Good; G=Good; F=Fair; P=Poor; VP=Very Poor

	Respondents	PERCENTAGES [1] E (5)	VG (4)	G (3)	F (2)	P (1)	VP (0)	MEDIAN	Adjusted Median
1. The course as a whole was:	3	100						5.0	4.4
2. The course content was:	3	67	33					4.8	4.2
3. The instructor's contribution to the course was:	3	67	33					4.8	4.2
4. The instructor's effectiveness in teaching the subj. matter was:	3	100						5.0	4.4
COMBINED ITEMS 1-4	12	83	17					4.9	4.3
									Relative Rank
5. Course organization was:	3		100					4.0	15
6. Clarity of instructor's voice was:	3	67	33					4.8	10
7. Explanations by instructor were:	3	67	33					4.8	7
8. Instr's ability to present alternative explan. when needed was:	3	67	33					4.8	6
9. Instructor's use of examples and illustrations was:	3	100						5.0	4
10. Quality of questions or problems raised by instructor was:	3	33	67					4.3	13
11. Student confidence in instructor's knowledge was:	3	100						5.0	8
12. Instructor's enthusiasm was:	3	33	67					4.3	18
13. Encouragement given students to express themselves was:	3	33	67					4.3	16
14. Answers to student questions were:	3		100					4.0	17
15. Availability of extra help when needed was:	3	33	67					4.3	14
16. Use of class time was:	3	33	67					4.3	11
17. Instructor's interest in whether students learned was:	3	67	33					4.8	9
18. Amount you learned in the course was:	3	67	33					4.8	5
19. Relevance and usefulness of course content were:	3	100						5.0	3
20. Evaluative and grading techniques (tests, papers, etc.) were:	3	100						5.0	2
21. Reasonableness of assigned work was:	3	100						5.0	1
22. Clarity of student responsibilities and requirements was:	3	33	67					4.3	12

Relative to other college courses you have taken:		Much Higher (7)	(6)	(5)	Average (4)	(3)	Much Lower (2)	(1)	MEDIAN
23. Do you expect your grade in this course to be:	3	33	67						6.3
24. The intellectual challenge presented was:	3		100						6.0
25. The amount of effort you put into this course was:	3	33	67						6.3
26. The amount of effort to succeed in this course was:	3		100						6.0
27. Your involvement in course (assignments, attendance, etc.) was:	3	33	33		33				6.0

28. On average, how many hours per week have you spent on this course?

Percent	
	Under 2
	2-3
33	4-5
33	6-7
33	8-9
	10-11
	12-13
	14-15
	16-17
	18-19
	20-21
	22 or more

Respondents: 3
Class median: 6.5
Hours per credit: 1.3

29. From the total average hours spent, how many do you consider were valuable in advancing your education?

Percent	
	Under 2
	2-3
100	4-5
	6-7
	8-9
	10-11
	12-13
	14-15
	16-17
	18-19
	20-21
	22 or more

Respondents: 3
Class median: 4.5
Hours per credit: 0.9

30. What grade do you expect in this course?

Percent	
67	A (3.9-4.0)
33	A- (3.5-3.8)
	B+ (3.2-3.4)
	B (2.9-3.1)
	B- (2.5-2.8)
	C+ (2.2-2.4)
	C (1.9-2.1)
	C- (1.5-1.8)
	D+ (1.2-1.4)
	D (0.9-1.1)
	D- (0.7-0.8)
	E (0.0)
	Pass
	Credit
	No Credit

Respondents: 3
Class median: 3.9

31. In regard to your academic program, is this course best described as:

Percent	
33	In your major
33	A distribution requirement
33	An elective
	In your minor
	A program requirement
	Other

Challenge and Engagement Index

CEI = 5.9

(1=lowest; 7=highest)

1. *Percentages are based on the number of students who rated each item.*

Batch UW 919-002091

AU11:01429
SURVEY ID

Respondents: 3
Enrollment: 5
Classes: 1

A
Form Type

Mailbox: 355020
ChairCopy? Yes
printed: 1/23/2012

IASystem
The Course Evaluation Standard

FISH 546 A
Sch. of Aquatic & Fishery Sciences
College of the Environment
University of Washington

Steven Roberts
Assistant Professor
Winter 2012

Department Copy

STUDENT EVALUATION OF INSTRUCTION

E=Excellent; VG=Very Good; G=Good; F=Fair; P=Poor; VP=Very Poor

	Respondents	PERCENTAGES [1] E (5)	VG (4)	G (3)	F (2)	P (1)	VP (0)	MEDIAN	Adjusted Median
1. The course as a whole was:	10	30	50	20				4.1	4.0
2. The course content was:	10	10	80	10				4.0	3.9
3. The instructor's contribution to the course was:	10	20	50	30				3.9	3.9
4. The instructor's effectiveness in teaching the subj. matter was:	10	10	60	20	10			3.8	3.8
COMBINED ITEMS 1-4	40	18	60	20	2			4.0	3.9

									Relative Rank
5. Course organization was:	10		30	30	30		10	2.8	17
6. Clarity of instructor's voice was:	10	30	50	20				4.1	15
7. Explanations by instructor were:	10	20	50	20		10		3.9	13
8. Instr's ability to present alternative explan. when needed was:	10	30	60		10			4.2	8
9. Instructor's use of examples and illustrations was:	10	60	30	10				4.7	3
10. Quality of questions or problems raised by instructor was:	10	40	50	10				4.3	6
11. Student confidence in instructor's knowledge was:	10	60	30	10				4.7	5
12. Instructor's enthusiasm was:	10	40	60					4.3	11
13. Encouragement given students to express themselves was:	10	70	20	10				4.8	2
14. Answers to student questions were:	10	20	70	10				4.1	9
15. Availability of extra help when needed was:	10	60	30		10			4.7	1
16. Use of class time was:	10		30	30	30	10		2.8	18
17. Instructor's interest in whether students learned was:	10	30	40	30				4.0	14
18. Amount you learned in the course was:	10	20	50	30				3.9	10
19. Relevance and usefulness of course content were:	10	50	50					4.5	4
20. Evaluative and grading techniques (tests, papers, etc.) were:	8	12	38	50				3.5	16
21. Reasonableness of assigned work was:	10	30	40	30				4.0	7
22. Clarity of student responsibilities and requirements was:	10	30	30	20	10	10		3.8	12

Relative to other college courses you have taken:		Much Higher (7)	(6)	(5)	Average (4)	(3)	Much Lower (2)	(1)		
23. Do you expect your grade in this course to be:	9		22	22	56				4.4	
24. The intellectual challenge presented was:	9	11	44	11	33				5.6	
25. The amount of effort you put into this course was:	10	10	20	30	40				4.8	
26. The amount of effort to succeed in this course was:	10	10	10	50	30				4.9	
27. Your involvement in course (assignments, attendance, etc.) was:	10	10	30	20	40				5.0	

28. On average, how many hours per week have you spent on this course?

Percent

	Under 2
	2-3
30	4-5
10	6-7
	8-9
30	10-11
10	12-13
20	14-15
	16-17
	18-19
	20-21
	22 or more

Respondents: 10
Class median: 10.2
Hours per credit: 3.4

29. From the total average hours spent, how many do you consider were valuable in advancing your education?

Percent

	Under 2
	2-3
56	4-5
	6-7
	8-9
11	10-11
22	12-13
11	14-15
	16-17
	18-19
	20-21
	22 or more

Respondents: 9
Class median: 5.3
Hours per credit: 1.8

30. What grade do you expect in this course?

Percent

38	A (3.9-4.0)
38	A- (3.5-3.8)
25	B+ (3.2-3.4)
	B (2.9-3.1)
	B- (2.5-2.8)
	C+ (2.2-2.4)
	C (1.9-2.1)
	C- (1.5-1.8)
	D+ (1.2-1.4)
	D (0.9-1.1)
	D- (0.7-0.8)
	E (0.0)
	Pass
	Credit
	No Credit

Respondents: 8
Class median: 3.7

31. In regard to your academic program, is this course best described as:

Percent

83	In your major
	A distribution requirement
17	An elective
	In your minor
	A program requirement
	Other

Challenge and Engagement Index

CEI = 4.6

(1=lowest; 7=highest)

1. *Percentages are based on the number of students who rated each item.*

Batch UW 986-002232

WI12:02591
SURVEY ID

Respondents: 10
Enrollment: 10
Classes: 1

A
Form Type

Mailbox: 355020
ChairCopy? Yes
printed: 4/10/2012

IASystem
The Course Evaluation Standard

FISH 310 A
Sch. of Aquatic & Fishery Sciences
College of the Environment
University of Washington

Steven Roberts
Assistant Professor
Spring 2012

Instructor Copy

STUDENT EVALUATION OF INSTRUCTION

E=Excellent; VG=Very Good; G=Good; F=Fair; P=Poor; VP=Very Poor

	Respondents	PERCENTAGES [1] E (5)	VG (4)	G (3)	F (2)	P (1)	VP (0)	MEDIAN	Adjusted Median
1. The course as a whole was:	38	21	42	32	5			3.8	3.8
2. The course content was:	38	24	53	18	5			4.0	4.0
3. The instructor's contribution to the course was:	38	26	37	24	13			3.9	3.9
4. The instructor's effectiveness in teaching the subj. matter was:	38	18	32	37	8	5		3.5	3.5
COMBINED ITEMS 1-4	152	22	41	28	8	1		3.8	3.8
									Relative Rank
5. Course organization was:	38	32	18	32	18			3.5	14
6. Clarity of instructor's voice was:	38	37	24	26	8	3	3	3.9	15
7. Explanations by instructor were:	38	24	37	32	8			3.8	13
8. Instr's ability to present alternative explan. when needed was:	38	26	37	24	11	3		3.9	11
9. Instructor's use of examples and illustrations was:	38	42	32	21	5			4.3	2
10. Quality of questions or problems raised by instructor was:	38	32	29	32	8			3.9	12
11. Student confidence in instructor's knowledge was:	38	61	16	21	3			4.7	1
12. Instructor's enthusiasm was:	38	18	18	29	29	5		3.0	18
13. Encouragement given students to express themselves was:	38	37	37	24	3			4.1	8
14. Answers to student questions were:	38	29	39	26	3	3		4.0	9
15. Availability of extra help when needed was:	38	24	26	45	3		3	3.5	17
16. Use of class time was:	38	21	37	29	11	3		3.7	10
17. Instructor's interest in whether students learned was:	38	26	29	32	13			3.7	16
18. Amount you learned in the course was:	38	32	32	29	8			3.9	7
19. Relevance and usefulness of course content were:	38	32	47	18	3			4.1	6
20. Evaluative and grading techniques (tests, papers, etc.) were:	38	32	29	32	8			3.9	4
21. Reasonableness of assigned work was:	38	32	34	26	8			4.0	3
22. Clarity of student responsibilities and requirements was:	38	32	34	24	8		3	4.0	5

Relative to other college courses you have taken:		Much Higher (7)	(6)	(5)	Average (4)	(3)	Much Lower (2)	(1)	MEDIAN
23. Do you expect your grade in this course to be:	37	8	27	30	30	3	3		5.0
24. The intellectual challenge presented was:	37	3	43	35	16	3			5.4
25. The amount of effort you put into this course was:	37	5	38	35	22				5.3
26. The amount of effort to succeed in this course was:	37	11	35	35	19				5.4
27. Your involvement in course (assignments, attendance, etc.) was:	37	8	43	27	22				5.5

28. On average, how many hours per week have you spent on this course?

Percent	
	Under 2
	2-3
25	4-5
11	6-7
22	8-9
14	10-11
14	12-13
11	14-15
	16-17
	18-19
3	20-21
	22 or more

Respondents: 36
Class median: 8.8
Hours per credit: 1.8

29. From the total average hours spent, how many do you consider were valuable in advancing your education?

Percent	
	Under 2
14	2-3
32	4-5
14	6-7
5	8-9
14	10-11
8	12-13
14	14-15
	16-17
	18-19
	20-21
	22 or more

Respondents: 37
Class median: 6.1
Hours per credit: 1.2

30. What grade do you expect in this course?

Percent	
11	A (3.9-4.0)
25	A- (3.5-3.8)
36	B+ (3.2-3.4)
14	B (2.9-3.1)
8	B- (2.5-2.8)
3	C+ (2.2-2.4)
3	C (1.9-2.1)
	C- (1.5-1.8)
	D+ (1.2-1.4)
	D (0.9-1.1)
	D- (0.7-0.8)
	E (0.0)
	Pass
	Credit
	No Credit

Respondents: 36
Class median: 3.3

31. In regard to your academic program, is this course best described as:

Percent	
88	In your major
	A distribution requirement
	An elective
6	In your minor
6	A program requirement
	Other

Challenge and Engagement Index

CEI = 5.0

(1=lowest; 7=highest)

1. *Percentages are based on the number of students who rated each item.*

Batch UW 138-000277

SP12:03906
SURVEY ID

Respondents: **38**
Enrollment: **44**
Classes: **1**

A
Form Type

Mailbox: **355020**
ChairCopy? **Yes**
printed: 6/19/2012

IASystem
The Course Evaluation Standard

BIOL 533 B
Biology
College of Arts and Sciences
University of Washington
Ecology; Harvell, Friedman

Steven Roberts
Assistant Professor
Summer 2012

Instructor Copy

STUDENT EVALUATION OF INSTRUCTION

E=Excellent; VG=Very Good; G=Good; F=Fair; P=Poor; VP=Very Poor

	Respondents	PERCENTAGES [1] E (5)	VG (4)	G (3)	F (2)	P (1)	VP (0)	MEDIAN	Adjusted Median
1. The course as a whole was:	11	82	18					4.9	4.5
2. The course content was:	11	64	27	9				4.7	4.3
3. The instructor's contribution to the course was:	11	55	27	18				4.6	4.3
4. The instructor's effectiveness in teaching the subj. matter was:	11	45	9	45				4.0	3.7
COMBINED ITEMS 1-4	44	61	20	18				4.7	4.3
									Relative Rank
5. Course organization was:	11	36	45	18				4.2	11
6. Clarity of instructor's voice was:	11	27	18	18	36			3.3	18
7. Explanations by instructor were:	10	40	20	40				4.0	16
8. Instr's ability to present alternative explan. when needed was:	10	40	40	20				4.3	14
9. Instructor's use of examples and illustrations was:	11	45	45		9			4.4	12
10. Quality of questions or problems raised by instructor was:	11	73	18	9				4.8	3
11. Student confidence in instructor's knowledge was:	11	91	9					5.0	8
12. Instructor's enthusiasm was:	11	36	45	18				4.2	17
13. Encouragement given students to express themselves was:	11	73	18	9				4.8	7
14. Answers to student questions were:	11	36	55	9				4.3	13
15. Availability of extra help when needed was:	11	100						5.0	1
16. Use of class time was:	11	64	18	18				4.7	2
17. Instructor's interest in whether students learned was:	11	64	18	18				4.7	9
18. Amount you learned in the course was:	11	64	27	9				4.7	4
19. Relevance and usefulness of course content were:	11	73	27					4.8	5
20. Evaluative and grading techniques (tests, papers, etc.) were:	11	55	45					4.6	6
21. Reasonableness of assigned work was:	11	36	64					4.3	10
22. Clarity of student responsibilities and requirements was:	11	36	27	18	18			4.0	15

Relative to other college courses you have taken:		Much Higher (7)	(6)	(5)	Average (4)	(3)	(2)	Much Lower (1)	MEDIAN
23. Do you expect your grade in this course to be:	10		50	30	20				5.5
24. The intellectual challenge presented was:	10	50	20	20	10				6.5
25. The amount of effort you put into this course was:	10	50	30	10	10				6.5
26. The amount of effort to succeed in this course was:	10	30	40	20	10				6.0
27. Your involvement in course (assignments, attendance, etc.) was:	10	50	20	20	10				6.5

28. On average, how many hours per week have you spent on this course?

Percent
- Under 2
- 2-3
- 4-5
- 6-7
- 8-9
- 10-11
- 12-13
- 14-15
- 16-17
- 18-19
- 20-21
- 100 22 or more

Respondents: 11
Class median: 22.5
Hours per credit: 2.5

29. From the total average hours spent, how many do you consider were valuable in advancing your education?

Percent
- Under 2
- 2-3
- 4-5
- 6-7
- 8-9
- 10-11
- 12-13
- 14-15
- 16-17
- 18-19
- 20-21
- 100 22 or more

Respondents: 10
Class median: 22.5
Hours per credit: 2.5

30. What grade do you expect in this course?

Percent
- 50 A (3.9-4.0)
- 40 A- (3.5-3.8)
- 10 B+ (3.2-3.4)
- B (2.9-3.1)
- B- (2.5-2.8)
- C+ (2.2-2.4)
- C (1.9-2.1)
- C- (1.5-1.8)
- D+ (1.2-1.4)
- D (0.9-1.1)
- D- (0.7-0.8)
- E (0.0)
- Pass
- Credit
- No Credit

Respondents: 10
Class median: 3.8

31. In regard to your academic program, is this course best described as:

Percent
- 55 In your major
- A distribution requirement
- 18 An elective
- In your minor
- A program requirement
- 27 Other

Challenge and Engagement Index

CEI = 6.6

(1=lowest; 7=highest)

1. *Percentages are based on the number of students who rated each item*

Batch UW 172-000037

SU12:01170
SURVEY ID

Respondents: **11**
Enrollment: **11**
Classes: **1**

A
Form Type

Mailbox: **351812**
ChairCopy? **Yes**
printed: 9/12/2012

Peer Evaluation of Instruction

Instructional peer review

Instructor:	Dr. Steven Roberts, Assistant Professor School of Aquatic and Fishery Sciences College of Ocean and Fishery Sciences University of Washington, Seattle
Course:	Fish 310: Shellfish Biology Spring term, 2007
Time & date attended:	All Lectures (50% of the course and labs, 2x/week)
Reviewer:	Dr. Carolyn Friedman, Associate Professor School of Aquatic and Fishery Sciences College of Ocean and Fishery Sciences University of Washington, Seattle

Observations and comments:

Steven and I co-taught Fish 310 Spring 2007. Given my schedule, I asked Steven to take the lead and he accepted this request without reservations, despite that this was a new course for Steven (and his first quarter-long class). He immediately updated the website and included an interactive webpage for students, TAs and instructors. I was constantly impressed with his zeal and computer/web abilities. Steven gave approximately half of the lectures in Fish 310 with a focus on physiology, feeding and locomotion, especially of the crustacea. He gave an amazing first lecture (really top notch!!). The second lecture was difficult to follow as, in trying to ensure student participation, Steven on gave only a portion of the slides for handouts making it a bit hard to follow and take notes. After a lecture or two, based on requested feedback from students and TAs, Steven changed his format to include all power point slides. This made note taking much easier for the students. Steven experimented with presentation styles and responded to requests from the students regarding his instruction style. As a result of this interaction, his lectures improved throughout the course and were quite enjoyable. My only suggestion is to practice saying taxonomic names prior to the class as some can be a challenge to deliver for the first time.

Steven used animation to help illustrate animal behavior very effectively throughout the course and the students enjoyed and learned from these animations and movies. Steven tied the lecture to the laboratory session when possible, but this could occur more frequently to provide a better link between lecture and lab and further instill the importance and application of the material being taught.

Students paid attention to the class material and were engaged as evidenced by asking questions and taking notes to clarify or add to the class notes downloaded off the course web site.

Steven used humor to aid in teaching but I think it took the students a few weeks to understand his dry sense of humor. Lectures given on time and finished within the allotted class period.

Steven attended most of the lab session (more than I did!) and allowed the TAs to teach the lab portion of the course but was available to help when needed. He initiated a Friday instructor-TA meeting to prepare for the labs the following week. However, difficulty in coordination with Greg Jensen, who collected the invertebrates, did not allow us to run through the lab exercises prior to class, as planned. This will be changed for the next year so that all manipulative experiments can be done the week prior to the lab sessions.

Steven encouraged the TAs to participate in the course and requested input from them throughout the course.

My overall assessment is that Steven is a good teacher with great potential to become a great teacher. He is eager to improve and applies student responses to this end. Steven is a great asset to our department.

Instructional peer review

Instructor:	Dr. Steven Roberts, Assistant Professor School of Aquatic and Fishery Sciences College of Ocean and Fishery Sciences University of Washington, Seattle
Course:	Fish 310: Biology of Shellfish Spring term, 2008
Time & date attended:	All lectures, 11:30 am – 12:20 pm
Reviewer:	Dr. Carolyn Friedman, Associate Professor School of Aquatic and Fishery Sciences College of Ocean and Fishery Sciences University of Washington, Seattle

Observations and comments:

I co-taught Fish 310 with Dr. Roberts for the past two years. Both years I was impressed with his creativity and abilities with creating and using web sites for courses. This was especially true for Fish 310 this past year during which students were provided with copies of lecture slides and an audio recording for all lectures via the web site. Students found this very helpful and used this recourse during the course. Students were able to download lecture notes, if desired, as well as providing students with high quality slides for study.

There are approximately 30 students enrolled in the class. Steven's teaching/lecture ability improved over the course of the initial quarter of teaching. However, this past year I was absolutely amazed at the transformation of Steven from a shy lecturer to a completely engaged, funny and instructive lecturer. Steven was well liked by the students and he held them captivated during the lectures. He was creative, not only in his presentation style, but also in his methods. Steven included video, class participation activities and class competitions into teaching. Students were encouraged to talk among themselves to answer questions posed, broken into groups to play 'games' in which students had to recall or synthesize information from the class, and even act out behavioral methods used by various species. Aside from being enjoyable these activities were educational and also helped develop a community within this course.

My overall assessment is that Steven is an excellent teacher and clearly has the skills to instruct students on shellfish biology and any other topic he undertakes.

Please feel free to contact me with any questions.

Regards,

Carolyn S. Friedman, Ph.D.
Associate Professor

SCHOOL OF AQUATIC and FISHERY SCIENCES

UNIVERSITY OF WASHINGTON

May 11, 2009

Dear David

Evaluation of teaching: Steven Roberts

Steven asked me to attend one of his lectures (today) in FISH 210 (Biology of Shellfish) as a peer reviewer of his teaching efforts. Prior to that, Steven gave me access to the class website.

"Stroll through the phyla" courses are never easy to teach: they tend to be heavy on some fairly dry information, and it takes some skill to engage students in lectures. Thus, I was interested to see how Stephen tackled these challenges in the third year of teaching this course. Steven co-teaches with Carolyn Friedman, with the lecture load split approximately 50:50. The course enrolment for 2009 is in the mid-thirties.

I heard Steven lecture on the suborder Malacostraca. Steven has a quietly engaging presence – he is both approachable and authoritative, and uses his dry sense of humor well. Overall, what immediately impressed me most about his lecturing style is that he makes the lecture inclusive by frequently asking questions and, when answers aren't immediately forthcoming, he encourages students to connect the lecture material with the lab material, and this normally elicited a response. The students appear to be very comfortable with him, with a good number of unsolicited questions coming up during the course of the lecture. He answers 'off the wall' questions graciously. He also continually reinforced the key terminology he expects the students to know. He seems to be entirely at ease in front of his audience. The classroom is FISH 102, a large space for a relatively small class. My only suggestion to Steven at the end of the lecture was that he repeats student questions/answers since it is difficult to hear students when seated towards the back of the room. However, there were few of us hiding in the back of the room which may say something about students being engaged with the instructor.

Steven uses the usual Powerpoint format but he mixes in animations and movies and this helps to keep students engaged in the taxonomic material. The animations and movies serve a clear purpose rather than being for amusement or to give a change of pace. The speed through which Steven worked through the lecture material was appropriate. One feature of his lecture that impressed me was when he asked all the students to stand up and perform the "crustacean dance" – this seems to have been introduced several lectures back. Using hand and arm movements, every student participated (and seemed to enjoy) in miming the different diagnostic features of the orders! I wish I'd had a videorecorder……

Overall, then, Steven has quickly developed a style of lecturing that is engaging and effective, if the evidence of a single lecture is a good measure.

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Regarding the support resources for this course, the Catalyst-based course website is impressive and makes full use of Catalyst. Sections for the schedule, lecture and laboratory slide downloads, lab worksheets and reports are available. Although I didn't discuss this with Steven, it appears that all students are expected to design a "species profile" webpage. Other resources available are "question sets", reading lists, videos, and fun stuff. Of particular note is that the lectures are available as podcasts. I asked Steven if he was able to track the number of podcast downloads but this is not a feature of Catalyst. Anecdotally, he told me that it was a popular feature, especially appreciated by students with English as a second language. Finally, the course website hosts a very well used discussion board. Since this is a team-taught course, it is not clear who the driving force has been in making the course website resource-rich, but I have seen similar features on other course websites of Steven's. I know from my own attempts at using Catalyst that the development of all the features on the FISH 310 website must have required considerable time and effort.

In summary, observing Steven in teaching mode was a positive and informative 50 minutes. It was also educational to see how the potential of Catalyst can be used effectively for this kind of course. I have no concerns about Steven's performance in lecturing mode – quite the opposite, in fact. Kudos to Steven and Carolyn for putting in the effort to make full use of Catalyst a dynamic and interactive teaching resource. Overall, Steven impresses me as an effective teacher who has quickly developed his own teaching style that students respond positively to.

Sincerely

Graham Young

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SCHOOL OF AQUATIC and FISHERY SCIENCES

UNIVERSITY OF WASHINGTON

February 27, 2010

Dear David

Peer Evaluation of Teaching: Steven Roberts

I sat in today on a lecture that Steven gave to my FISH 324 (Aquatic Animal Physiology and Reproduction) course. I previously gave a report on a lecture of Steven's that I attended that he gave in FISH 210 (Biology of Shellfish) last year. I thought that seeing Steven's interaction with a class of students I am familiar with would give me added insights into his development as a teacher. In my previous report, I gave Steven kudos for his ability to engage students, and for his use of web-based resources in teaching. The latter comment is based on a review of websites and teaching material of all the courses that Steven currently teaches.

FISH 324 has approximately 50 enrolled students and several others auditing. The class this year is fairly quiet in terms of students' readiness to engage in a back and forth with me. They do so, and do well when they do, but they always seem to need a little encouragement. I was interested to see how Steven coped with situations of asking questions and not necessarily having an immediate response from the students.

Steven gave a lecture on "Defense": a basic introduction to immune responses in invertebrates and vertebrates, with emphasis on the "innate" immune system – the ancient, non-antibody-based system found in invertebrates. He gave a nicely structured lecture with a range of examples. As I have noted previously, Steven is an engaging teacher – he is both approachable and authoritative, and uses his dry sense of humor well. He engaged the students from the outset, dealt nicely with silences to his questions, and really drew the students out in terms of responses to his questions and their willingness to ask him questions. He continually asked the students to view the material from an evolutionary and environmental perspective. The pace of the lecture was appropriate, and his slide format was good – students had been provided with a pdf handout before the lecture. He dealt with "off the wall" questions and answers graciously and positively. Steven also did a nice job of introducing students to ongoing research in SAFS – particularly his work and Carolyn Friedman's and Glen VanBlaricom's work on abalone disease.

In summary, observing Steven this year simply confirms that he has matured as a teacher who makes uses of a range of pedagogical approaches to engage the class. I have absolutely no concerns about his effectiveness as a teacher

Sincerely

Graham Young

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School of Aquatic and Fishery Sciences **Collegial Teaching Evaluation**

Classroom Teaching Observation

Faculty Observed:	Steven Roberts	Rank:	Assistant Professor
Date Observed:	5/27/2011	Course Observed:	FISH 310 (Biology of Shellfish)
Observed by:	Graham Young	Rank	Professor

CONTENT

Main ideas are clear, specific, and accurate **5/5**
Main ideas tied to previous and upcoming class topics **5/5**
Lecture incorporates required readings **3/5**
Incorporation of primary research material **4/5**

INSTRUCTIONAL METHODS

Effective speaking skills (eye contact, clear vocal delivery, rate appropriate) **4/5**
Visual aids/handouts clear **5/5**
Effective use of in-class technology (e.g. computers, clickers)
In-class activities promote active learning **5/5**

INTERACTION & ENGAGEMENT

Effective at encouraging student participation/questions **5/5**
The majority of students are engaged **5/5**
Incorporates student responses into the lecture **4/5**
Intellectual challenge appropriate for course level (100, 200. 300, 400) **5/5**

NOTES:

What were the instructor's strengths as demonstrated in this classroom observation?

FISH 310, with a focus on shellfish but with a survey of other major invertebrate groups could potentially be a very dry "stroll through the phyla" course, and the main challenge is to engage students actively. The lecture I attended was on Hemichordates. Steven immediately engaged in a two-way exchange with students at the start of the lecture, provided a brief review based on a phylogenetic tree, and used the tree to ask questions about material previously covered. The students were clearly used to, and comfortable with responding to Steven's questions.

Steven also took several opportunities to update students on current thoughts on phylogenetic relationships based on recent molecular data that are unlikely to be in the course text book. He showed some primary research work from a paper of Billy Swalla's (Biology) on phylogeny, and took some time to describe why *Ciona intestinalis* had become a model organism for phylogenetic and genomics research. He regularly asked question of the students to connect the material presented in this lecture to previous ones, and was impressive in encouraging the students to think from an evolutionary and environmental perspective.

As I have noted in previous evaluations, Steven is an engaging teacher who seems at ease in the classroom – he is both approachable and quietly authoritative, and uses his dry sense of humor well. He engaged the students from the outset, dealt nicely with silences to his questions, and really drew the students out in terms of responses to his questions and their willingness to ask him questions. The pace of the lecture was appropriate, the lecture was well organized, and Steven was clearly comfortable with the material. His slide format was for the most part good – students had been provided with a pdf handout before the lecture. Illustrations were appropriate and the use of small movie clips enhanced the material. He dealt with "off the wall" questions and answers graciously and positively.

Overall, what was most impressive was that all the students seemed to be awake, engaged, and actually gave the impression of enjoying the lecture. The back and forth between teacher and students was not based on just a few students being prepared to respond to questions. Out of a class of about 30 students today, I estimate that close to half of the class made comments and/or responded to questions. In conversation with Steven after the lecture, I asked him how he ended up with such a responsive class – it seems he starts a dialog from the outset, and weathers the periods of muted or lack of response early on. Although I did not observe in class activities today, he stated that he does regularly conduct small group activities – one that sounds very effective is to have a group select a taxa and come up with a mnemonic device that the rest of the class discusses and adds to. Others have groups of student pantomiming key features of a particular taxon.

The encouragement of dialog is also apparent within the excellent course website. As well as containing all the course and supplementary material, students are expected to participate in on-line quizzes weekly, and also have to produce a species profile: either Powerpoint, a web page, or, in three cases, videos that are posted on Youtube. This one is definitely worth looking at – shipworms, the musical (http://www.youtube.com/watch?v=bGsKfEKVLdI).

The course has a Twitter site that Steven states is not particularly well used. It also has a discussion board that is very heavily used – I counted at least 120 separate topics that had been posted, and in most cases, each of these had numerous comments from others in the class – that's impressive.

In summary, observing Steven this year confirms my previous impressions that he has matured as a teacher who makes uses of a range of pedagogical approaches to engage the class. For the first two years, Steven co-taught this class with a very experienced and highly rated teacher but has taught it solo for the past three years. Steven mentioned that his early challenge when the course was co-taught was to find his own style rather than try to emulate the other instructor's style. In my opinion, he has risen to this challenge in a very effective fashion.

I have absolutely no concerns about Steven's effectiveness as a teacher, and have had to try very hard to come up with anything I see as "room for improvement". Steven receives very good student evaluations for this course, with an overall score in the low 4s.

School of Aquatic and Fishery Sciences **Collegial Teaching Evaluation**

What suggestions do you have for improvement of this instructor's pedagogical style?

My suggestions are minor. Steven has a fairly quiet voice and on a couple of occasions, I struggled to make out what he has said – this may be my old ears rather than a criticism of Steven, since it did not seem to bother the students. Regarding slides, they were with only a couple of exceptions where the font or size of figure could have been bigger.

Signatures:

Reviewer Reviewee

Date of Debriefing Session: ___5/27/2011_____

Additional comments from debriefing session between observer and observed faculty:

Steven and I talked about many specific and general aspects of pedagogy, confirming my suspicion that Steven actively reflects on his performance in the classroom. Both reviewer and reviewee agreed that the above is a fair assessment.