

**ATTACHMENT B. Annual Project Report Form (Revised 11.21.19)**

**1. Project Number:**

19170115

**2. Project Title:**

Genomic mechanisms that underlie lack of recovery of Prince William Sound herring following the 1990s collapse

**3. Principal Investigator(s) Names:**

Andrew Whitehead, University of California Davis

**4. Time Period Covered by the Report:**

February 1, 2019-January 31, 2020

**5. Date of Report:**

February 2020

**6. Project Website (if applicable):**

<https://pwssc.org/herring/>

**7. Summary of Work Performed:**

Overview: The causes of the collapse of the Prince William Sound (PWS) Pacific herring stock are controversial, and the reasons for the lack of recovery remain a mystery. In this series of projects we interrogate the genome structure and genome function of PWS fish to test hypotheses about the causes and consequences of the collapse, by revealing ecological, evolutionary, and genetic mechanisms governing the demographic trajectory of PWS fish over the past ~30 years.

Conspicuous events that coincided with the dramatic PWS collapse include the *Exxon Valdez* oil spill four years previous, and the emergence of disease. We test hypotheses concerning the effects of oil exposure, the effects of disease challenge, and the potential interactive effects of oil exposure and disease challenge, on herring health and fitness. Physiological measurements and patterns of genome-wide gene expression will serve to reveal similarities and differences in mechanisms of response to these stressors between PWS and reference population fish. These studies should provide novel insights into the causes and consequences of recent dramatic demographic changes in PWS fish, potentially inform novel intervention strategies, and provide modern genomic resources for management and conservation of Pacific herring. We have performed work on three aspects of the project during this third year of the research program.

- 1) Animal exposure experiments: This year we elaborated upon the multi-population exposure experiments that were conducted in the previous reporting period. Those exposure experiments included three populations of Pacific herring that were exposed to low concentrations of mechanically dispersed oil during embryogenesis. Herring were hatched in clean water, raised until juvenile stage, and subsequently challenged with viral hemorrhagic septicemia virus (VHSV). We conducted one virus challenge as reported in the previous reporting period. Since then, we performed an additional virus challenge. This was necessary because temperatures in the first virus challenge were too warm to trigger disease. We repeated the virus challenge when temperatures were colder during March 2019. We also used the larvae from the embryonic oil challenge to perform a starvation experiment, in which we tested whether fish exposed to oil as embryos suffered greater starvation impacts on growth than those fish that were exposed to control conditions during embryogenesis. We also exposed starved and un-starved fish to VHSV to test whether starvation affected susceptibility to disease. A final set of animal experiments again used fish exposed to oil and control conditions during embryogenesis and tested later-life (juvenile) susceptibility to bacterial pathogen (*Ichthyophonus*). These three challenge experiments were conducted during this reporting period, and data are currently being analyzed and summarized. Briefly, we have so far found that embryonic exposure to very low doses of oil does not significantly affect later-life (juvenile) survival following VHSV challenge. It is possible that our dose range was too low to elicit an oil exposure impact on immune system function or development. Alternatively, our oil exposure may not have targeted the most important life stage. For example, oil exposure may perturb immune system function during the larval stage when the immune system is maturing, and not affect very early stages (embryonic) of immune system development. We are initiating a new set of experiments to test this hypothesis, where newly hatched herring will be exposed to oil during larval development, then transferred to clean water to grow out to the juvenile stage and then challenged with VHSV. Analysis of results from starvation and bacterial challenge experiments are ongoing.
- 2) Genome sequencing and assembly: We had made much progress in genome assembly last year, but ran into unexpected difficulties during the long-range assembly that was using Hi-C reads to connect scaffolds into chromosomes. All of the quality metrics indicated that we had made good libraries, and sequence data looked robust. But when we attempted long-range assembly this failed to produce adequate results. Hi-C data were new to us, so we figured we were doing something wrong. After much troubleshooting, the lead software engineer at Phase Genomics (which developed Hi-C) offered to help. Even he could not figure out the problem, and we made the decision to abandon the data. We went back and made some new long-range reads using Pacific Biosciences (PacBio) technology and have recruited collaborator Dr. Wes Warren to make new Hi-C libraries. We have completed the new PacBio sequencing, and Dr. Warren is in the midst of Hi-C data collection. We anticipate that Hi-C will be completed within the next month, at which time the full genome assembly can be completed. Though we are now behind schedule for completion of the reference genome, this has not affected our overall progress. The reference genome is an important mapping reference for the population genomics data. However, this past year colleagues at Uppsala University in Sweden completed a high-quality reference assembly for Atlantic herring. We tried mapping our Pacific herring population re-sequencing

reads to that genome, which returned nearly 99% mapping success, which is excellent. So, the data collection for population genomics (next section) has been proceeding while we finish the reference genome assembly.

- 3) Population genomics: We have made progress on the population genomics portion of the work, but it has been delayed for two reasons. The first is that calling variants was much more computationally intensive than we had anticipated. We have used the same methods to call variants in other data sets, but those data sets were not nearly as big as this Pacific herring data set. We have discovered that computational time for variant calling increases exponentially (not linearly) with more samples. We found that variant calling would be running on our supercomputer for close to one month before a node failed or the job timed out. At which point we would have to re-start. We finally decided to change variant calling methods to one that was new to us but was supposed to be more computationally efficient. Variant calling is now completed, but it took several months longer than anticipated. The second reason that we are behind schedule is that Postdoctoral scholar Dr. Elias Oziolor, who was recruited for the population genomics analysis, accepted a job with Pfizer and left University of California Davis. This is wonderful for Elias' career, but suspended progress in the population genomics analysis. I am currently in the final stages of recruiting a new postdoc who will re-start this analysis.

## **8. Coordination/Collaboration:**

### **A. Long-term Monitoring and Research Program Projects**

#### **1. Within the Program**

This project is a formal collaboration with my research group at University of California Davis and that of Dr. Paul Hershberger at the U.S. Geological Survey, Marrowstone Marine Field Station. Animal experiments are being conducted by Dr. Hershberger's group (Project 18120111-E) at the Marrowstone facility. A Ph.D. student and technician from my research group travelled to Marrowstone to participate in animal experiments in 2019. In the past year I have travelled to the Seattle area for face-to-face logistics and project coordination meetings with Dr. Hershberger and his group. My Ph.D. student Tony Gill and Ph.D. student Madison Armstrong (I serve on her guidance committee) also joined us for those meetings.

#### **2. Across Programs**

##### **a. Gulf Watch Alaska**

N/A

##### **b. Data Management**

We have nothing yet to report, as no data have yet been made public. We plan to make our data publicly available once quality control is completed. The reference genome sequence will be uploaded to the National Center for Biotechnology Information (NCBI). Population genomics data have been uploaded to NCBI for long-term archival but have not yet been made public – we will make these data public once analyses are completed. We are finishing data curation for RNA-seq data and plan to upload to NCBI soon. We will then create a project page at EBI BioStudies that will include links to raw data at NCBI and will also

house variant call files for the population genomics data and matrices of read counts for the RNA-seq data. The EBI site will also house data from animal challenge experiments. All custom bioinformatics scripts will be archived at GitHub and will be linked through the EBI BioStudies project site. So far, we have started archiving all of our scripting for population genomics work on a dedicated GitHub page: [https://github.com/eozior/phpopgen\\_home](https://github.com/eozior/phpopgen_home). This is a durable archive and it is publicly available. We will expand on descriptions and annotations as the project matures. Publications will also eventually be linked through the BioStudies project site. The databases described above are designed to accommodate the types of data that we need to make public, and they are durable, and they are standard practice for our research field. Once these data archives become public, we will create links to them through the Gulf of Alaska Data Portal.

## **B. Individual Projects**

N/A

## **C. With Trustee or Management Agencies**

This project is a formal collaboration with my research group at University of California Davis and that of Dr. Paul Hershberger at the U.S. Geological Survey, Marrowstone Marine Field Station. Animal experiments are being conducted by Dr. Hershberger's group (Project 18120111-E) at the Marrowstone facility. A Ph.D. student and technician from my research group travelled to Marrowstone to participate in animal experiments in 2019. In the past year I have travelled to the Seattle area for face-to-face logistics and project coordination meetings with Dr. Hershberger and his group. My Ph.D. student also joined us for those meetings. National Oceanic and Atmospheric Administration (NOAA) Fisheries scientists John Incardona and Nathaniel Scholz (NOAA Northwest Fisheries Science Center, Seattle) are collaborating in animal exposure experiments, since they have research goals that include exposure impacts on growth and development. We have coordinated our activities with the NOAA group so that we could add a starvation challenge to our experimental design. The NOAA group is also advising on our design for larval exposure experiments planned for early 2020.

# **9. Information and Data Transfer:**

## **A. Publications Produced During the Reporting Period**

### **1. Peer-reviewed Publications**

Oziolor, E.M., N.M. Reid, S. Yair, K.M. Lee, S. Guberman VerPloeg, P.C. Bruns, J.R. Shaw, A. Whitehead\*, and C.W. Matson\*. 2019. Adaptive introgression enables evolutionary rescue from extreme environmental pollution. *Science*. 364:455-457. (\* co-corresponding authors)

Note: though research reported in the above publication does not include Pacific herring data, it did use methods and analyses that our group has developed over the past two years for our *Exxon Valdez* Oil Spill Trustee Council (EVOSTC) funded Pacific herring research. We therefore cite the EVOSTC funding in the acknowledgements for this paper.

## 2. Reports

Whitehead, A. 2019. Genomic mechanisms that underlie lack of recovery of Prince William Sound herring following the 1990s collapse. FY18 annual report to the *Exxon Valdez* Oil Spill Trustee Council, project 18170115. *Exxon Valdez* Oil Spill Trustee Council, Anchorage, AK.

## 3. Popular articles

Ph.D. student Tony Gill, with me and Paul Hershberger, wrote an article describing our research projects for the Delta Sound Connections periodical published by the Prince William Sound Science Center. It should be in print soon.

## B. Dates and Locations of any Conference or Workshop Presentations where EVOSTC-funded Work was Presented

### 1. Conferences and Workshops

Gill, T., T. Linbo, P. Hershberger, J. Incardona, and A. Whitehead. 2019. Interactions between oil exposure and immune function relevant for Pacific herring population collapse. Annual Meeting of the Society of Environmental Toxicology and Chemistry. Toronto, ON, Canada. November.

### 2. Public presentations

None yet.

## C. Data and/or Information Products Developed During the Reporting Period, if Applicable

Population genomics data collection is complete. Raw sequence read data has been uploaded for long-term archive at NCBI, as per common practice in our field. Data have not yet been publicly released – we will keep data under embargo until our analyses are complete, at which time we will provide links to the data to be posted on the Gulf of Alaska Data Portal. We have started archiving our population genomics bioinformatics analysis scripts in GitHub ([https://github.com/eozior/phpopgen\\_home](https://github.com/eozior/phpopgen_home)). When script archives are completed following our analysis, we will provide links through the Gulf of Alaska Data Portal.

## D. Data Sets and Associated Metadata that have been Uploaded to the Program’s Data Portal

None yet

## 10. Response to EVOSTC Review, Recommendations and Comments:

**EVOSTC Science Panel Comment.** The Science Panel was very pleased with the project and its development and the extent to which the PI has trouble-shot various approaches and arrived a rigorous way to address questions without being able to simultaneously access samples from all populations. The reallocation of funds by the PI to purchase the oil dosing system had the unanimous support of the science panel, and we look forward to seeing further results. The PI has made rapid use of technological advances in genomic analysis leading to what we considered “great bang for the buck”. The Science Panel and Science Coordinator remained impressed with this work and the broad application these results will have to other fisheries globally. We are excited to see comparisons

made with data from Puget Sound. We continue to be enthusiastic for your project and appreciate your hard work and efforts.

**PI Response.** Thank you for your comments.

**11. Budget:**

Budget Category:	Proposed	Proposed FY 18	Proposed FY 19	Proposed FY 20	Proposed FY 21	TOTAL PROPOSED	ACTUAL CUMULATIVE
Personnel			180.2	175.2	127.4	\$483	\$ 379.9
Travel			2.6	2.6	2.6	\$8	\$ 19.7
Contractual			0	0.0	0.0	\$0	
Commodities			71.5	18.3	20.2	\$110	\$ 158.8
Equipment			50.3	0.0	0.0	\$50	
Indirect Costs ( <i>will vary by proposer</i> )			133.9	99.9	72.6	\$306	\$ 306.8
<b>SUBTOTAL</b>	200	452	\$438.5	\$296.0	\$222.8	\$957	\$ 865.2
General Administration (9% of	\$18.0	\$40.7	\$39.5	\$26.6	\$20.1	\$145	N/A
<b>PROJECT TOTAL</b>	\$218.0	\$492.7	\$478.0	\$322.7	\$242.9	\$1,754.2	
Other Resources (Cost Share Funds)	\$0.0	\$0.0	\$0.0	\$0.0	\$0.0	\$0.0	