EVOSTC FY17-FY21 INVITATION FOR PROPOSALS
FY18 LINGERING OIL CONTINUING PROJECT PROPOSAL SUMMARY PAGE

Proposals requesting FY18 funding are due to shiway.wang@alaska.gov and elise.hsieh@alaska.gov by August 23, 2017. Please note that the information in your proposal and budget form will be used for funding review. Late proposals, revisions or corrections may not be accepted.

Project Number and Title

18170115 Genomic mechanisms that underlie lack of recovery of Prince William Sound herring following the 1990s collapse

Primary Investigator(s) and Affiliation(s)

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Date Proposal Submitted

August 23, 2016

Project Abstract

The long-term health of fisheries is of crucial importance for the economic health of our coastal communities and for the food security of our nation. Therefore, the causes and consequences of changes in stock abundance merit careful scientific evaluation. 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 the research proposed here 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 (EVOS) four years previous, and the emergence of disease. We test hypotheses concerning the effects of oil exposure, the effects of disease challenge, and their potential interactive effects, on herring health and fitness. We will test predictions and hypotheses by reconstructing genome-wide genetic change through time (over the past 30 years) in PWS fish, and compare this to population genetic change through time in two reference site populations. Furthermore, a series of laboratory-based experiments will test for population differences in their response to oil exposure in early life and subsequent resilience to pathogen exposures. 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.

**The abstract should provide a brief overview of the overall goals and hypotheses of the project and provide sufficient information for a summary review as this is the text that will be used in the public work plan and may be relied upon by the PAC and other parties.*

EVOSTC Funding Requested* *(must include 9% GA)*

FY17	FY18	FY19	FY20	FY21	TOTAL
$224,703.5	$492,750.4	$420,259.3	$319,845.2	$240,070.3	$1,697,628.7

Non-EVOSTC Funds to be used, please include source and amount per source:

FY17	FY18	FY19	FY20	FY21	TOTAL

**If the amount requested here does not match the amount on the budget form, the request on the budget form will considered to be correct.*

1. EXECUTIVE SUMMARY

Please provide a summary of the project including key hypotheses and overall goals, as submitted in your original proposal. If there are highlights that you would like to include from your FY17 work, please include them here. Also, please list any publications that have been submitted and/or accepted since you submitted your last proposal.

GOALS and HYPOTHESES:

Genetic attributes unique to the PWS population, that either pre-existed or emerged in the years following the EVOS, may help explain the lack of recovery in the PWS stock following the 1993 collapse, and may also illuminate the causes of the collapse. Diseases are key variables that help explain the population dynamics of PWS herring since the 1990s decline (Marty et al. 2010). The contribution of the EVOS to the PWS decline is more controversial (Pearson et al. 1999; Carls et al. 2002). However, recent studies have shown that herring embryos are sensitive to fitness impacts at very low concentrations of oil (Incardona et al. 2015), these low-level exposures can affect fitness in the field (Heintz et al. 2000), and natural selection from pollutants can quickly drive complex genetic change in PAH-exposed populations (Reid et al. in review-b). Our **overarching question** is: *Are there functional connections that link the PWS herring collapse and lack of recovery with disease impacts and the EVOS?*

Our hypotheses are:

H1: Natural selection following EVOS exposure came at the cost of compromised immune function.

H1-alternate: No evidence for oil-induced selection, but population collapse resulted in erosion of genetic diversity, especially in immune system genes, which impairs protective innate and adaptive immune responses.

H2: Exposure to oil during development compromises the ability in later life to mount an effective immune response to pathogen exposure.

To test our hypotheses, we propose the following **approaches**: 1) Retrospective population genomics; 2) Experimental/comparative physiology; 3) Experimental/comparative functional genomics.

Expected outcomes:

1. A reference genome sequence and assembly for Pacific herring will enable 21st century genetics/genomics research for this ecologically and economically important species.
2. Genetic variation is the raw material that sustains populations over time. Our careful evaluation of how genetic variation differs between populations, and how it changes through time, should serve several purposes. Erosion of genetic variation may provide an early warning signal of stock decline or collapse, allowing for intervention and prevention measures to be quickly enacted. Furthermore, stock identification is crucial for fisheries management, for which genetic data are one of the most important tools in the kit. However genetic stock identification is sometimes difficult because of lack of resolution, and because genetic change through time can complicate assessments. Genome scale data offer the highest level of resolution for stock identification, so data from our studies will be useful for managers. Furthermore, our characterization of genetic change though time will identify regions of the genome that are not only diagnostic of stock, but

also that are stable through time and thereby provide reliable diagnostic markers of stock identity.
3. These experiments should offer insight into the mechanisms whereby disease and oil exposures may affect fish health, and offer insights into the sustainability of fish stocks through time.

HIGHLIGHTS from FY17 WORK:

I am happy to report that the project is off to a very strong start, thanks to the enthusiastic involvement of a number of new colleagues (listed in section 2). Activities proposed for FY17 are under weigh, including sequencing of the reference genome, and early life development and pathogen challenge experiments. We have developed new protocols to successfully extract and purify extremely high molecular weight DNA from fish tissues appropriate for sequencing and assembling our reference genome using the latest technologies (10X Genomics). We are also extracting very high molecular weight RNA from the same individual as used for our reference genome for transcriptome sequencing and assembly for genome annotation. We anticipate this work to be completed ahead of schedule in September 2017. Animal experiments were initiated in partnership with collaborators at USGS Marrowstone (Paul Hershberger and his group) and NOAA (John Incardona and Nat Scholz and their group) in April 2017. Pathogen challenge experiments are on schedule for September 2017. Some activities proposed for FY18 are already under weigh and ahead of schedule. For example, because of a very rapid and helpful response from Alaska DFG tissue collections folks, we received tissue samples ahead of schedule. New high-throughput sample preparation techniques in our lab has allowed us to finish DNA extraction from nearly 1,000 tissues. Sequencing for population genomics is being conducted right now, which is 1 year ahead of schedule. As such, it is advantageous to hire the post-doctoral research associate ahead of schedule. We therefore request a re-distribution of funds across years to keep pace with our accelerated work schedule.

2. COORDINATION AND COLLABORATION

A. Within an EVOTC-Funded Program

Provide a list and clearly describe the functional and operational relationships with other EVOSTC-funded program projects. This includes any coordination that has taken or will take place and what form the coordination will take (shared field sites or researchers, research platforms, sample collection, data management, equipment purchases, etc.).

B. With Other EVOSTC-funded Projects

Indicate how your proposed project relates to, complements or includes collaborative efforts with other proposed or existing projects funded by the EVOSTC that are not part of a EVOSTC-funded program.

C. With Trustee or Management Agencies

Please discuss if there are any areas which may support EVOSTC trust or other agency work or which have received EVOSTC trust or other agency feedback or direction, including the contact name of the agency staff. Please include specific information as to how the subject area may assist EVOSTC trust or other agency work. If the proposed project requires or includes collaboration with other agencies, organizations or scientists to accomplish the work, such arrangements should be fully explained and the names of agency or organization representatives involved in the project should be provided. If your proposal is in conflict with another project, note this and explain why.

2.A. This project is a formal collaboration with my research group at UC Davis and that of Dr. Paul Hershberger at USGS Marrowstone. Animal experiments are being conducted by his group at the Marrowstone facility.

Furthermore, a Ph.D. student from my research group will travel to Marrowstone to participate in pathogen challenge experiments in September 2017. Members of Dr. Hershberger's group also collected fresh tissues from Sitka Sound and Prince William Sound in March 2017 for reference genome sequencing and population genomics sequencing.

2.B. N/A

2.C. I am happy to report that a number of Alaska State Agency scientists, herring fishery industry personnel, and NOAA Fisheries scientists, have enthusiastically embraced this project, and have already contributed in important ways. 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. These measurements are easily added to tasks associated with our (Whitehead and Hershberger) planned and ongoing exposure experiments, so it made sense to join forces and leverage the collective experience, personnel, and equipment of our groups – the most important of these being the outstanding aquatics systems for rearing live herring at Marrowstone. A crucial source of historic tissue samples is the frozen tissue collection maintained by the Alaska Department of Fish and Game. Collections director Chris Habicht and staff members Judy Berger and Heather Hoyt were extremely helpful in facilitating the selection and transfer of hundreds of tissues from their collection to my lab at UC Davis. Also at Alaska DFG, Sherri Dressel is the Statewide Herring Fisheries Scientist. She and her group, including Katie Sechrist in particular, were instrumental in arranging for fresh tissue samples from 100 herring to be sent to my lab from the Bering Sea (Togiak Bay) with the enthusiastic participation of Ben Cain at Silver Bay Seafoods (Bristol Bay/Naknek plant). Outside of Alaska, other groups are graciously contributing samples for genetics analysis, including personnel from the Department of Fisheries and Oceans Canada (Kristen Daniel and Jaclyn Cleary) and California Department of Fish and Wildlife (Kathy Hieb). I have also been in touch with herring geneticist Sharon Wildes (NOAA Federal, Alaska Fisheries Science Center). She is enthusiastic about the project, and consulted on our choice of populations for genetics analysis.

3. PROJECT DESIGN – PLAN FOR FY18

A. Objectives for FY18

Identify the primary objectives for your project for FY18 as submitted in your original proposal.

B. Changes to Project Design

If the project design has changed from your original proposal, please identify any substantive changes and the reason for the changes. Include any information on problems encountered with the research or methods, if any. This may include logistic or weather challenges, budget problems, personnel issues, etc. Please also include information as to how any problem has been or will be resolved. This may also include new insights or hypotheses that develop and prompt adjustment to the project.

3.A. Population genomics data collection is ahead of schedule, such that population genetics analysis will begin in FY18. Our first set of exposure experiments will be completed in the Fall of 2017, such that gene expression data will be collected and analyzed in FY18. Our second set of animal exposures experiments, which includes additional populations, will be conducted in FY18. We anticipate completing reference genome sequencing in FY17 such that we anticipate drafting a genome release manuscript in FY18.

3.B. The core objectives of our project, and our experimental design, have not changed. However, two features of our original proposed work have changed. First, because of the enthusiastic participation of colleagues (see section 2.C.), and because of new high-throughput methods we have developed in our lab for the genetics data collection, we are already one year ahead of schedule for the population genomics work. Therefore, we have needed to accelerate our plan for analysis and hiring of personnel. This has resulted in our requested shift of some funds from later years to earlier years (but with no change to total requested funds). Second, recent method development in our lab has made the population genetics data collection much less expensive. We can therefore

include a third Alaska population in our time-course analysis (Togiak Bay). Coupled with our time-course analysis of Prince William Sound and Sitka Sound populations this will improve our scope for inferring the causes and consequences of genetic change through time in Alaskan herring populations. The data that we produce will not only be important for testing our hypotheses and advancing our stated goals, the genetics data will be a huge resource for studying genetic change in this important species into the future. Because we see products of this project as an incredible resource for future study, we considered some additional sampling that we could currently do at low cost to maximize the future utility of this resource. One thing we considered was expanding our sampling to span the entire North American range of the species, including regions outside of Alaska. Accordingly, we have coordinated with the Department of Fisheries and Oceans Canada, NOAA Fisheries in Seattle, and California Department of Fish and Wildlife to secure contemporary (2017) tissue samples for possible future use.

4. SCHEDULE

A. Program Milestones for FY18

For each project objective listed, specify when critical project tasks will be completed, as submitted in your original proposal. Please identify any substantive changes and the reason for the changes.

B. Measurable Project Tasks for FY18

Specify, by each quarter of each fiscal year, when critical project tasks (for example, sample collection, data analysis, manuscript submittal, etc.) will be completed, as submitted in your original proposal. Please identify any substantive changes and the reason for the changes.

4.A.

We should complete our second set of animal experiments by mid-Fall 2018. RNA-seq data collection for the current FY17 experiments should be completed in spring 2018, and preliminary data analysis should be completed soon thereafter. Genome assembly and annotation should be completed, and publically released, in spring 2018. Population genomics data collected should be completed before the beginning of FY18, read mapping and variant calling should be completed by spring 2018, and population genomics analysis should be well underway by the end of FY18

4.B.

Multi-population live animal experiments: start in April 2018 and complete by end of September 2018.
Genome assembly and annotation: completed March 2018
Genome release manuscript: submitted for peer review December 2018
Population genomics read mapping and variant calling: Completed May 2018
RNA-seq data collection from FY17 experiments, including preliminary data analysis: completed March 2018

5. PROJECT PERSONNEL – CHANGES AND UPDATES

If there are any staffing changes to Primary Investigators or other senior personnel please provide CV's for any new personnel and describe their role on the project.

There are no staffing changes to senior personnel.

6. Budget

A. Budget Forms (Attached)

Provide completed budget forms.

B. Changes from Original Proposal

If your FY18 funding request differs from your original proposal, provide a detailed list of the changes and discuss the reason for each change.

C. Sources of Additional Funding

Identify non-EVOSTC funds or in-kind contributions used as cost-share for the work in this proposal. List the amount of funds, the source of funds, and the purpose for which the funds will be used. Do not include funds that are not directly and specifically related to the work being proposed in this proposal.

6.A. Attached

6.B. The population genomics portion of the project has progressed much more quickly than expected. To keep pace, we need to advance support for human resources. We therefore request that funds from later years be shifted to earlier years, including FY18. We specifically request that funds for post-doc be shifted to FY18, and that funds for the Staff Research Associate be boosted to 75% time for FY18.