AGRICULTURAL RESEARCH FOUNDATION
INTERIM REPORT
FUNDING CYCLE 2021– 2023

TITLE: Effects of Climate Change on Dungeness Crab

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COOPERATORS:

SUMMARY/ABSTRACT:
The Dungeness crab (*Cancer magister*) is one of the most economically valuable shellfish to the West coast of the United States. The 2019-2020 commercial harvest produced $72.27 million in Oregon alone (ODFW, 2020). However, ocean acidification has become a rising threat to the Dungeness crab fishery. Ocean acidification occurs when carbon dioxide is absorbed by seawater and consequentially lowers the pH. The uptake of anthropogenic carbon dioxide has heavily increased along the U.S West Coast, intensifying the process (Chavez et al., 2017; Feely et al., 2016). Additionally, the U.S. West Coast is specifically susceptible to acidification due to the seasonal upwelling of the California current (Bednarsek et al., 2017). Such extreme changes to seawater composition can lead to adverse changes in Dungeness crab physiology, disrupting abundance and distributions, leading to lower economic yields for the commercial industry (Fabry et al., 2008).
Studies have previously shown that a low pH environment can delay pre-larval development, cause carapace dissolution, and reduce survival of Dungeness crabs (Miller et al., 2016; Bednarsek et al., 2020), yet we know little about changes caused at the molecular level. Like other crustaceans, Dungeness crabs house a hepatopancreas organ. The hepatopancreas is known primarily as a digestive gland, responsible for absorption and storage of nutrients, synthesis of digestive enzymes, and energy production (Wang et al., 2014). However, the Hepatopancreas also plays significant roles in the immune system and reproduction. Not only is it the primary site for the synthesis and excretion of immune molecules, but it also produces vitellogenin and various sex hormones (Li et al., 2013; Wang et al., 2014). This project will evaluate how ocean acidification conditions alter gene expression in Dungeness crab and whether a good diet can alleviate some of these changes.

OBJECTIVES:
Objective 1: Sequence the genome of the Dungeness crab.
Objective 2: Evaluate how Dungeness crab physiology is altered by ocean acidification using transcriptomics.
The purpose of this study is to evaluate how ocean acidification influences the differential expression of immune, metabolism, and endocrine genes of juvenile Dungeness crabs. Currently, there is no available genome for any crab species. Lack of reference genome has created a significant bottleneck in the genomic research of Dungeness crabs. However, high-throughput RNA-sequencing offers the ability to evaluate gene expression levels, even without

a published reference genome (Kukurba & Montgomery, 2015). Advances in technology and bioinformatics allow the construction of a de novo reference transcriptome where we can map sequence reads back to (Ozsolak & Milos, 2011). The present study will use Illumina sequencing and bioinformatics to analyze differential gene expression.

PROCEDURES:
Dungeness crabs were spawned and raised in the lab up to the juvenile stage. Forty-six juveniles were divided into four treatment groups: ambient pH + a high-quality diet, ambient pH + low-quality diet, lowered pH + high-quality diet, and lowered pH + low-quality diet. The high-quality diet included bivalve meat with homogenized freeze-dried clam powder as an additional nutritional source. The low-quality diet contained the same ingredients but only included ¼ of the freeze-dried clam powder as the high-quality diet. We added a cellulose filler to the low-quality diet to offset differences in food consistency between treatments. We housed individual crabs in a 2L tank receiving UV sterilized and 5um filtered seawater. Low pH treatments received seawater altered with controlled carbon dioxide input via mixing tanks. The Galloway lab at the Oregon Institute of Marine Biology in Charleston, Oregon, performed all experimental conditions, which lasted six weeks. At the end of treatment, each crab was collected and humanely euthanized by storage in a -80° C freezer. We cut individual crabs in halves, one half collected into 10mL of RNAlater and stored at -80° C. Then, we extracted RNA from four crabs of each treatment group using the Direct-zol RNA Microprep kit (Zymo). Quality was assessed and deemed high integrity (RIN > 8) using a Bioanalyzer. The concentration of extracted RNA from each sample was identified using a Qubit. Library prep of each sample was completed using the NEB Next Ultra II RNA Library Prep Kit for Illumina (New England Biolabs). Completed library preps were sent to the Center for Qualitative Life Sciences at Oregon State University for sequencing.

SIGNIFICANT ACCOMPLISHMENTS TO DATE:
The crabs were prepared and the sequencing library constructed (currently in the sequencing process)

ADDITIONAL FUNDING RECEIVED DURING PROJECT TERM:
N/A

FUTURE FUNDING POSSIBILITIES:
NOAA, USDA, NSF

References:

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