

### **Effect of low pH and low O<sub>2</sub> conditions on developmental gene expression and hatching of *Doryteuthis opalescens* embryos**

Eastern boundary upwelling systems (EBUS) are productive ecosystems that support as much as one-fifth of global fish harvest and are highly vulnerable to climate change. In the California Current upwelling system, long-term trends in ocean warming, stratification, deoxygenation, and ocean acidification are already being observed, including a shoaling of the hypoxic boundary and a decrease in calcium carbonate saturation state. These observations suggest that low pH and low O<sub>2</sub> (“low pH<sub>Ox</sub>”) conditions will become more common and intense in the future as a result of climate change. Organisms adapted to the dynamic pH, oxygen, and temperature conditions in the California Current may be more resilient to changes in these environmental factors driven by anthropogenic climate change. Studying the mechanisms that tolerant organisms use to cope with low oxygen and low pH may be highly informative for understanding and predicting future responses to climate change in the ocean.

For my dissertation research, I focused on the California market squid, *Doryteuthis opalescens*, a primary forage species for fish, sharks, mammals, and seabirds in the California Current, and California’s largest fishery. While adults are highly mobile, developing *D. opalescens* embryos are attached to the substrate and must be able to survive fluctuations in environmental conditions such as pH, oxygen and temperature over the course of their development. Modulation of gene expression is an important mechanism for cellular adaptation to environmental stress, and expression-related developmental plasticity has been shown to improve resilience, alter adult phenotype, and may facilitate the evolution of adaptive traits. This dissertation is focused on investigating changes in gene expression that allow for developmental resilience in the face of variable environmental conditions.

In species with no reference genome, genome-wide gene expression (RNA-Seq) projects must initially involve assembling a high-quality, comprehensive set of expressed transcripts (‘transcriptome’) to form the basis for all downstream analyses. The lack of a standardized, open access, biologist-friendly pipelines has resulted in a patchwork of published transcriptomes varying in accuracy and completeness. As a result, Chapter 1 focused on developing an open-source, automated, best-practices transcriptome analysis pipeline to conduct transcriptome assembly, quality assessment, annotation, and expression analyses in a single step (available at <https://github.com/bluegenes/MakeMyTranscriptome>).

In Chapter 2, I investigated the effects of low pH and low oxygen on squid development, using the transcriptome built in Ch1. Embryos were harvested in La Jolla, CA and reared in replicate flow-through seawater tanks of ambient (control; 240  $\mu\text{mol/kg}$  O<sub>2</sub>, pH 7.95) and low-pH, low-O<sub>2</sub> (low pH<sub>Ox</sub>; 90  $\mu\text{mol/kg}$  O<sub>2</sub>, pH 7.55) until hatching. These conditions represent the limits of pH<sub>Ox</sub> observed in squid spawning habitat. Physiology and hatching analyses showed that embryos exposed to low pH<sub>Ox</sub> conditions experienced a 7.6% increase in development time and a 4% decrease in hatching success. Embryos exposed to low pH<sub>Ox</sub> conditions upregulated stress-response and oxygen-sensitive genes, and altered expression of genes associated with cell-cycle regulation, development and lipid utilization. These results suggest that exposure to chronic low pH<sub>Ox</sub>, even within ranges typically found in the environment, represents a physiological stress for *D. opalescens* embryos. To further investigate the impacts of low pH<sub>Ox</sub>, ongoing analysis for Chapter 3 is focused on analyzing the developmental transcriptome of *D. opalescens* at six time points over development at normal and low pH<sub>Ox</sub> conditions.

Assessing global gene expression is now a widespread technique for investigating the physiological response of organisms to environmental variables. However, few studies have combined gene expression analysis with studies of ecologically relevant measurements such as hatching survival to understand the impact of environmental stressors at the population and ecosystem levels. This dissertation combines RNA-Seq with hatching survival to assess the physiological response of *D. opalescens* embryos to low pH and low oxygen conditions and investigate potential effects of these conditions on recruitment into the population. As these embryos already experience strong changes in environmental conditions during development, this species may be pre-adapted to changes in pH and oxygen and represents an ideal opportunity to investigate mechanisms of resilience to climate change in highly dynamic environments such as the California Current.