Project Report
Applying cutting-edge technology for reproductive control in emerging bivalve species
(NOAA Award NA18NMF4720007) for the period **05/1/21 – 10/31/21**

A. Project summary

To increase the productivity and sustainability of the shellfish aquaculture sector, while at the same time enabling hatchery responsiveness to both environmental challenges and market demands through breeding and maturation control, a time-efficient, practical, and cost-effective means to produce sterile shellfish is critically needed. The overarching goal of the proposed project is to develop a novel tool for conferring sterility on farmed shellfish that mitigates some of the shortcomings of ploidy manipulation. An attractive alternative to ploidy manipulation is the induction of sterility by inactivation of genes essential for germ cell formation.

One of the major roadblocks to the development of this technology is the lack of knowledge of these genes in bivalves. Single-cell RNA-Seq (scRNA-Seq) has emerged as a technology that will enable the identification of genes involved in germ cell differentiation via transcriptional profiling of single embryonic cells.

The primary milestone associated with our project will be a temporal atlas of gene expression in developing embryos at the single cell level. This outcome will not only have tremendous impact on the understanding of bivalve developmental biology, but importantly for our purposes, will provide gene targets for generating shellfish stocks that offer ecological security and optimal food production efficiency.

B. Summary of progress and results

- Applied optimized cell dissociation protocols (developed for geoduck in April and May of 2020) to prepare scRNA-Seq libraries of C. gigas gastrula samples. Performed bioinformatic analysis to validate candidate genes and identify new candidate genes associated with primordial germ cell (PGC) specification in bivalves.

- Continued lab work to perform whole mount *in situ* hybridizations (ISH) to visualize the spatial and temporal expression of genes identified as candidates for PGC specification identified from the scRNA-Seq data, including: primer design and PCR to generate ISH probes and fixing of multiple stages of bivalve embryos.

C. Challenges

The COVID-19 pandemic has resulted in minimal staffing allowed at the University of Washington and the Jamestown Point Whitney Shellfish Laboratory. We have been able to obtain and work with geoduck broodstock from both Taylor Shellfish & Jamestown S'Klallam hatcheries, but we are still facing logistical and staff training challenges due to restrictions stemming from the pandemic.

Pilot experiments performed in April-May of 2021 prepared us to perform an scRNA-Seq library of geoduck embryos in June of 2021. However, despite multiple spawning attempts (at two commercial hatcheries) we were unable to obtain the high-quality gametes necessary to perform this work. The reasons for this likely include the unusually high temperatures at the end of June coupled with the fact that the broodstock were nearing the end of their spawning season. As of November 2021, geoduck broodstock conditioning has been initiated at the hatcheries and we expect to be able to obtain geoduck embryos in the next few months