Project Report
Applying cutting-edge technology for reproductive control in emerging bivalve species
11/1/20 – 4/30/21

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

- Bioinformatic analysis and manuscript preparation of 47Gb of scRNA-Seq data to identify genes associated with primordial germ cell (PGC) specification in bivalves.

- Initiated whole mount *in situ* hybridizations to visualize the spatial and temporal expression of genes identified as candidates for PGC specification identified from the scRNA-Seq data.

- Performed pilot experiments with geoduck broodstock in preparation for scRNA-Seq library preparation of geoduck embryos (planned for June 2021) including: testing fertilization success of strip spawned versus volitionally spawned individuals, documenting developmental timing of geoduck to the blastula and gastrula stages under experimental conditions, testing cell dissociation protocols to ensure a single-cell suspension of viable cells from early geoduck 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.