Project Report
Applying cutting-edge technology for reproductive control in emerging bivalve species
(NOAA Award NA18NMF4720007) for the period **11/1/22 – 4/31/23**

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.

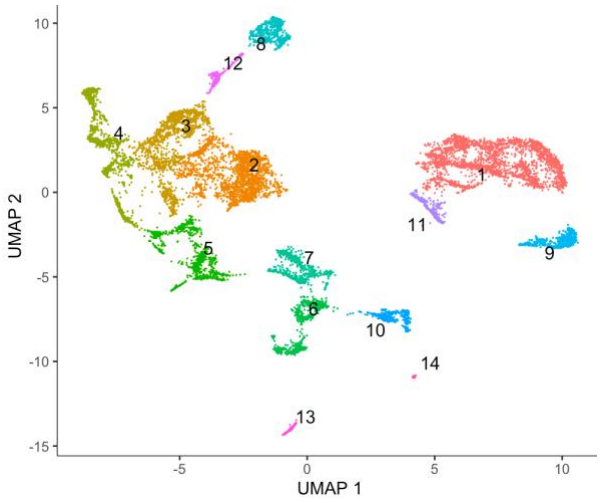
One of the major roadblocks to the development of this technology is the lack of knowledge of these genes in bivalves. Single-cell RNA-Seq (scRNA-Seq) has emerged as a technology that will enable the identification of genes involved in germ cell differentiation via transcriptional profiling of single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

- Sequencing and analysis of single-cell sequencing for geoduck early embryo (17 hour post fertilization) libraries. Top marker genes for cell groups have been annotated

Visualization of 14 identifiable cell groups in geoduck embryo library sequenced:



Single-cell sequencing has been carried out on 17 hpf embryos with distinct cell clusters identified based on expression (above). Cell group 4 patterns suggest association with gonad development. Top marker genes for group 4 are listed and corresponding expression in tissue libraries (below).

gene_id	gene_ID	ctenidia	heart	gonad
PGEN_.00g199430	G2/mitotic-specific cyclin-B	28	0	56
PGEN_.00g070070	inner centromere protein	3	0	2
PGEN_.00g089980	G2/mitotic-specific cyclin-A	6	0	9
PGEN_.00g259100	histone-lysine N-methyltransferase NSD2	7	4	10
PGEN_.00g054930	sperm-specific protein PHI-2B/PHI-3	0	0	50
PGEN_.00g068740	dentin sialophosphoprotein-like	2	21	3
PGEN_.00g078980	homeobox protein CDX-1	0	0	3
PGEN_.00g330580	lymphocyte-specific helicase	2	0	2
PGEN_.00g120520	putative UDP-GlcNAc:betaGal beta-1,3-N-acetylglucosaminyltransferase LOC100288842	2	0	2
PGEN_.00g281640	metal-response element-binding transcription factor 2-like	3	1	1

C. Challenges

No new challenges during this reporting period

PSMFC Job #	Project Title	Progress and Results	Challenges	Outreach/ Publications
1126G1.19	EMERGING BIVALVE SPECIES REPRODUCTIVE CONTROL TECH	Sequencing and analysis of single-cell sequencing for geoduck early embryo libraries.	None	Poster presented at National Shellfisheries Associated Meeting in Baltimore MD.