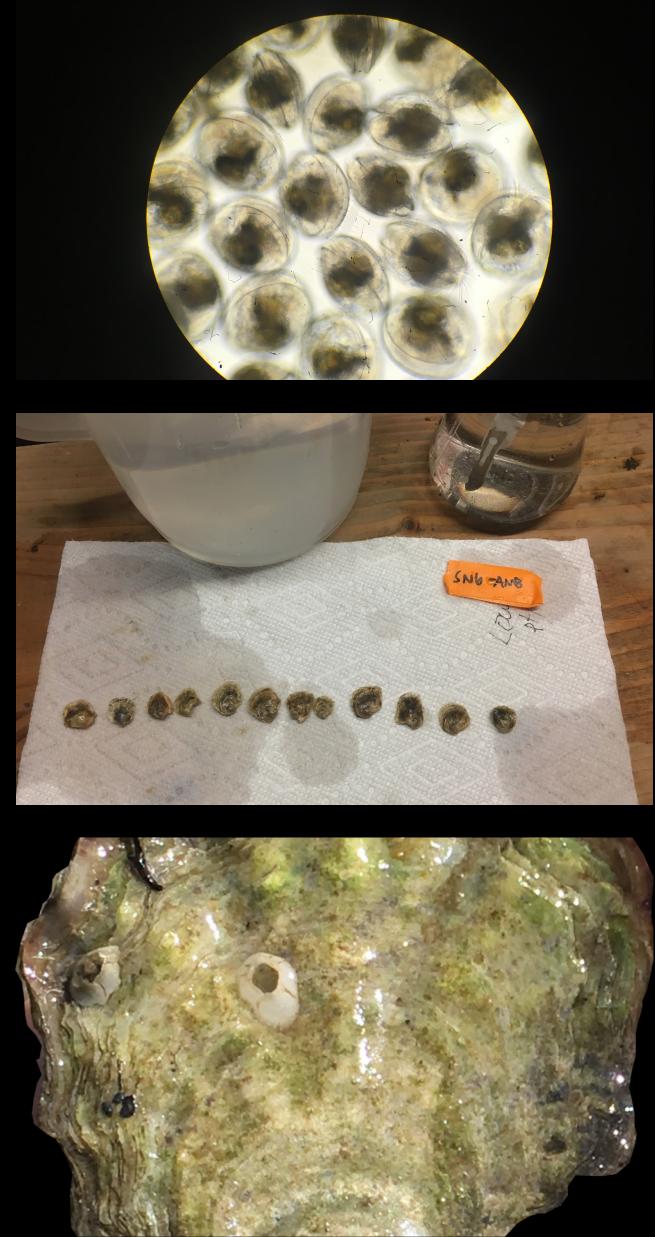


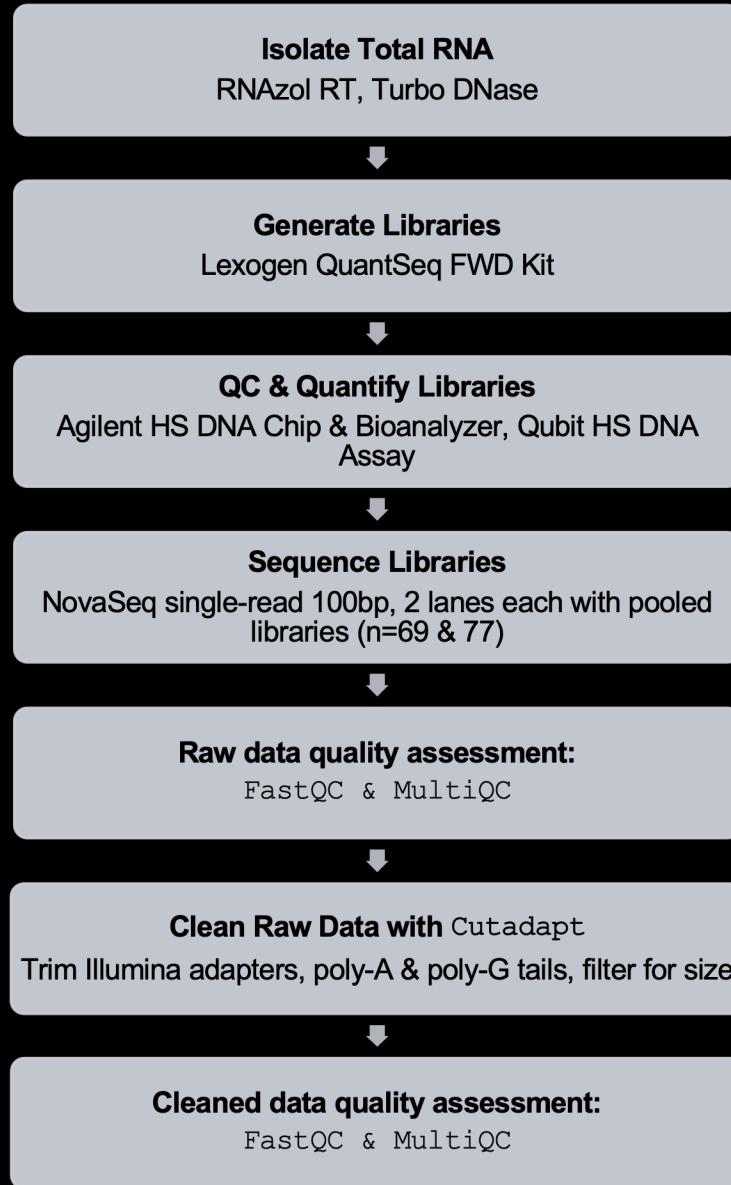
# PHYSIOLOGICAL CARRYOVER EFFECTS OF HIGH pCO<sub>2</sub> EXPOSURE

## QUANTSEQ 2020 PROJECT

- Does/how does high pCO<sub>2</sub> exposure affect adult gill tissue gene expression?
- Does parental low pH exposure affect offspring gene expression
  - ~2wk-old larvae upon release
  - 15month-old juveniles at end of field trial in Port Gamble Bay, WA
- → Important: offspring never exposed to different pCO<sub>2</sub> conditions



# Goal: generate high quality gene expression data (RNASeq)



Adult ctenidia  
After pCO<sub>2</sub> exposure

Larvae, whole body, pooled by “pulse”  
Upon release from brood

Juvenile, whole body  
~15 month old, after 3-month field trial

My bioinformatics workflow

Part 1: Raw read processing

# Goal: generate accurate gene count matrices

## Option A:

Align reads to genome & count reads/gene

Align with Bowtie2 or STAR

Generate Bowtie or Salmon index from genome

Generate *counts per gene* with featureCounts, genome feature files

My bioinformatics workflow

Part 2: translating reads to gene counts

## Option B:

Generate counts without aligning

Salmon in mapping-based mode with selective-alignment

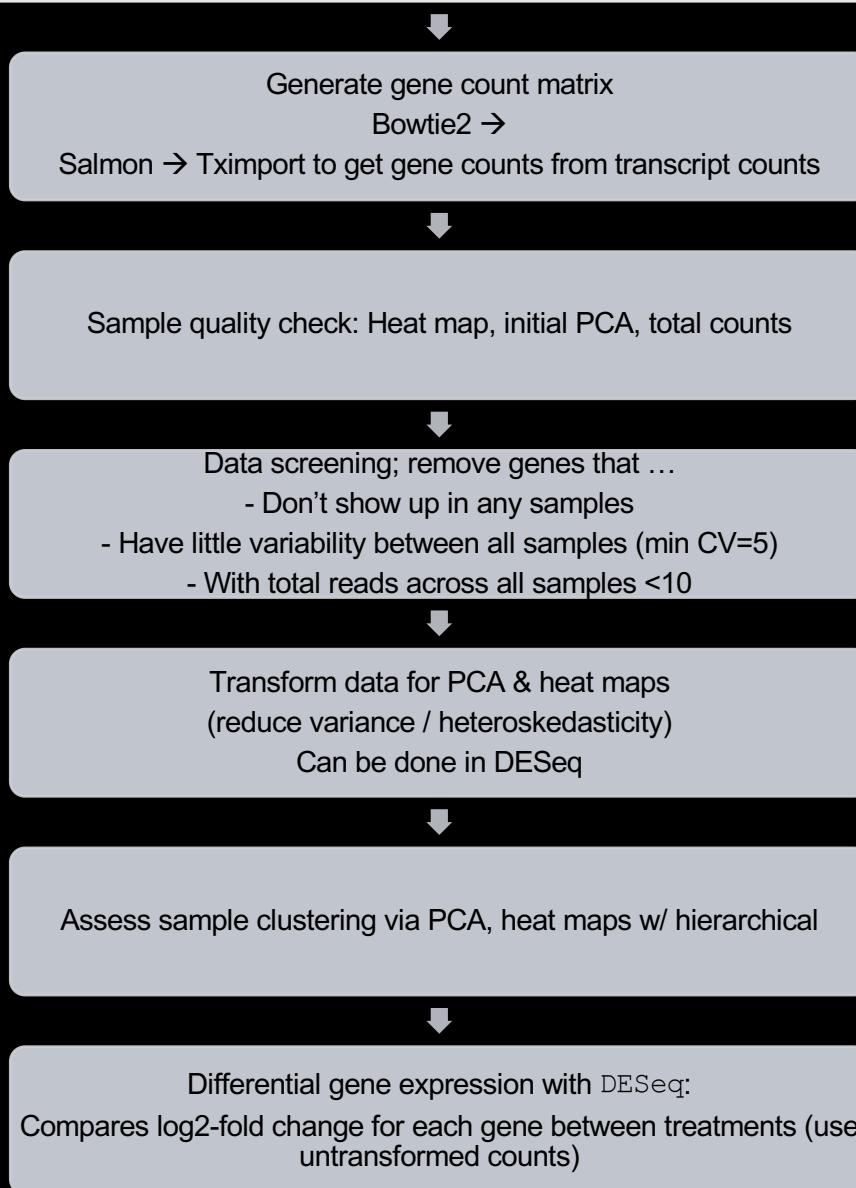
Generate Salmon index from mega-transcriptome. Use genome to create list of “decoy” sequences

Generate *counts per transcript* with Salmon, decoy-aware mega-transcriptome index

Annotate mega-transcriptome (denovo, v3) via Blast & Uniprot/Swissprot db

Summarize *counts per gene* via Tximport, annotated transcriptome

# Goal: Identify differentially expressed genes among parental treatments, cohorts



My bioinformatics workflow

Part 3: Comparing gene expression among treatments

# QuantSeq / TagSeq Library Prep

