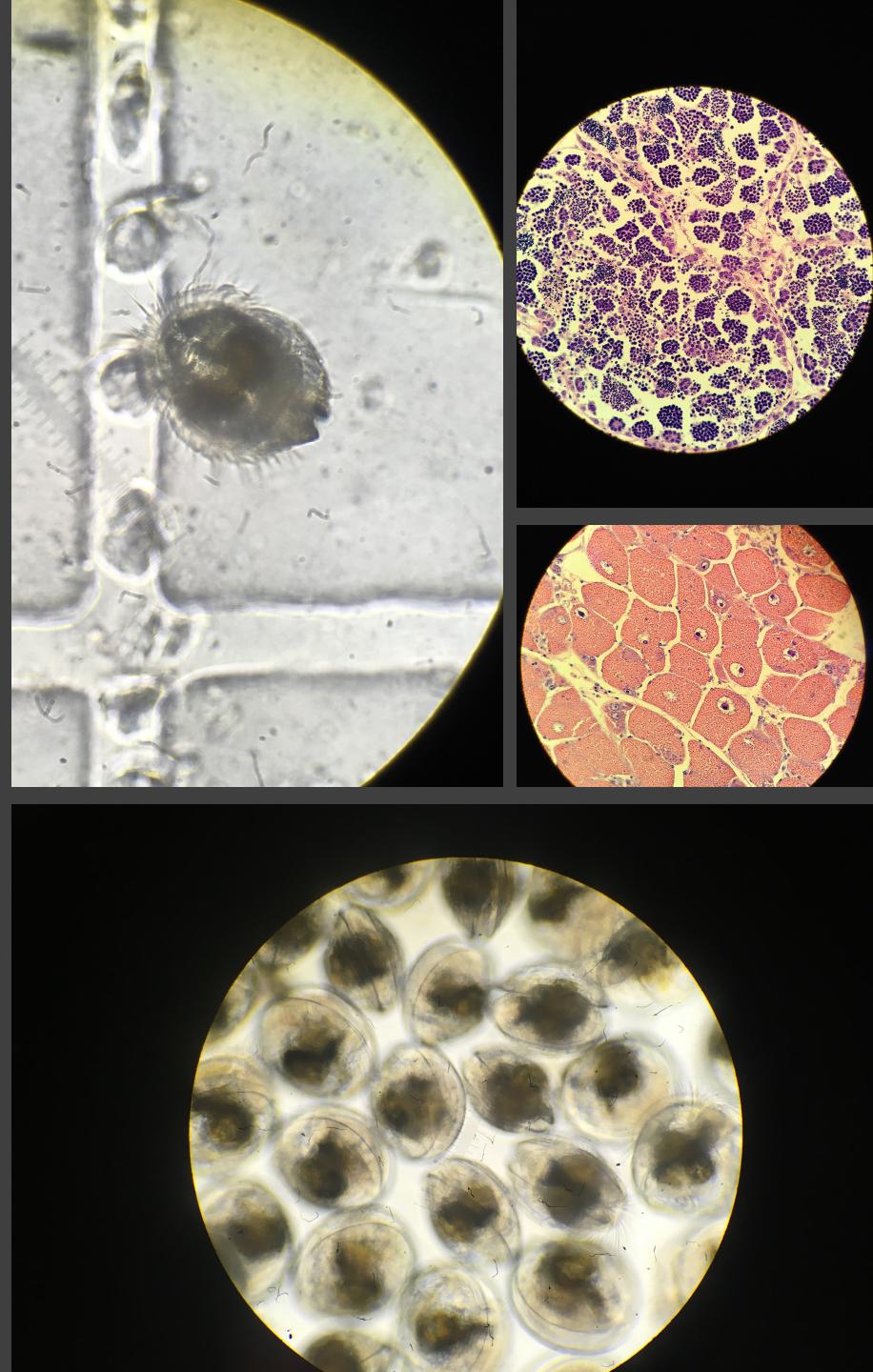


OLYMPIA OYSTER QUESTIONS

1. Does/how does low pH exposure affect gonad tissue gene expression?
2. Does parental low pH exposure affect offspring gene expression (larvae)
→ Important: larvae not exposed to different pH or environments



Goal: denovo assembled reference transcriptome

Goal: count matrix from RNA, 24 samples

RNASeq data from gonad tissue
4 individuals pooled

QuantSeq data from gonad tissue and larvae (whole body)

Quality check: FastQC

Clean data: FASTX Toolkit
Trim adaptors, deduplicate, quality filter (90% bases >20 score)

Trinity Assembly on Mox
Includes Trimmomatic

Derive pseudo-counts: Kallisto
(used script within Trinity)

Quality check: Transrate

Annotate: blast

Create collapsed transcriptome to remove isoforms: cd-hit

**My
bioinformatics
workflow**

Goal: Differential Gene Expression analysis

Data analysis workflow

Count matrix from Kallisto
DESeq in RStudio/Markdown

Sample quality check: Heat map,
initial PCA, total counts

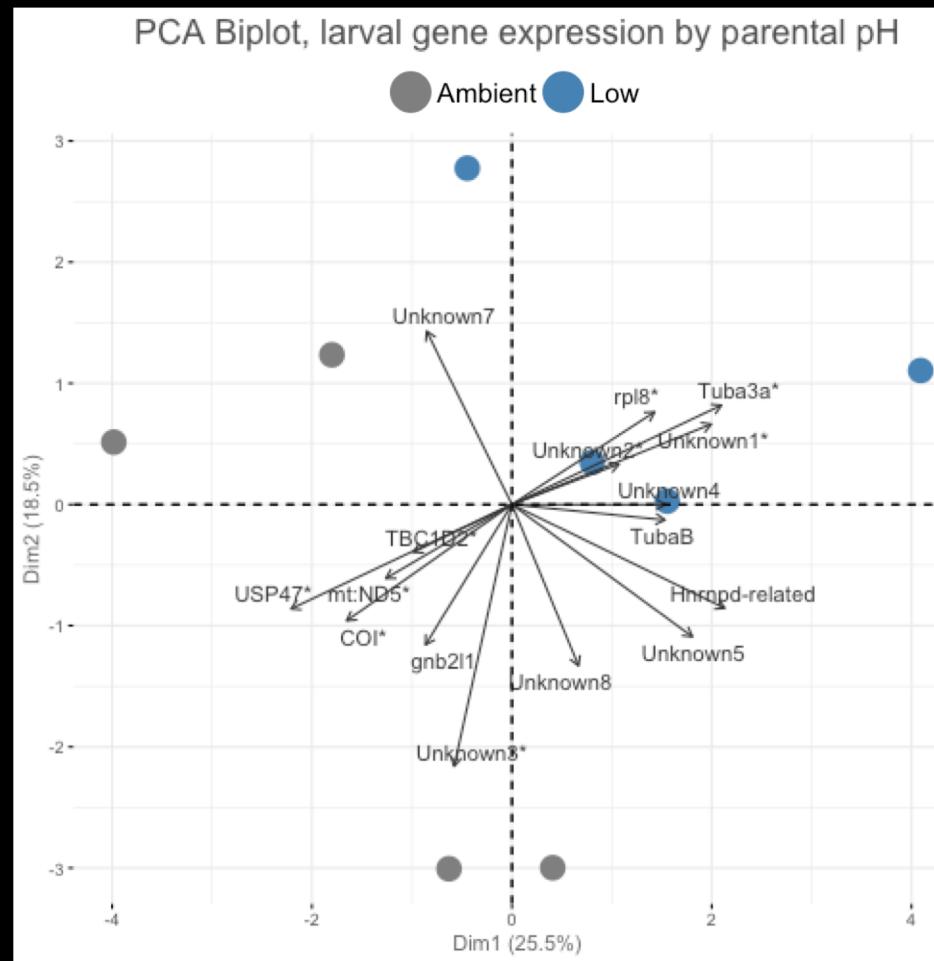
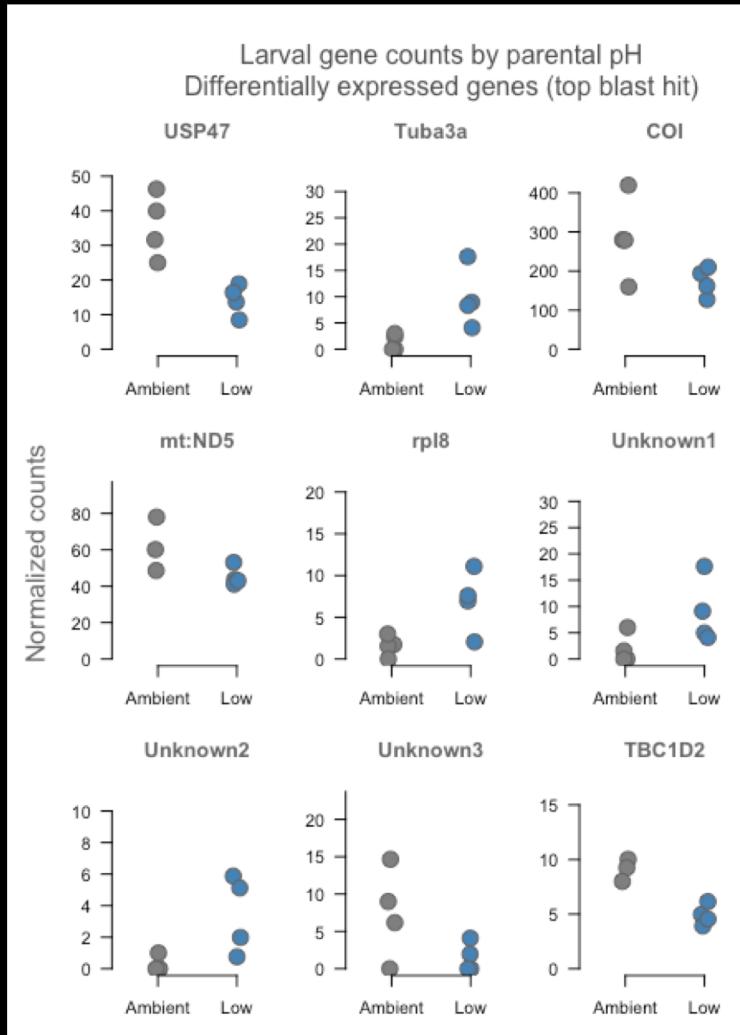
DESeq:
Compares log2-fold change for
each gene between treatments

Transform data for PCA
(reduce heteroskedasticity)

PCA analysis, visualize

Gonad and
larval samples
run in parallel

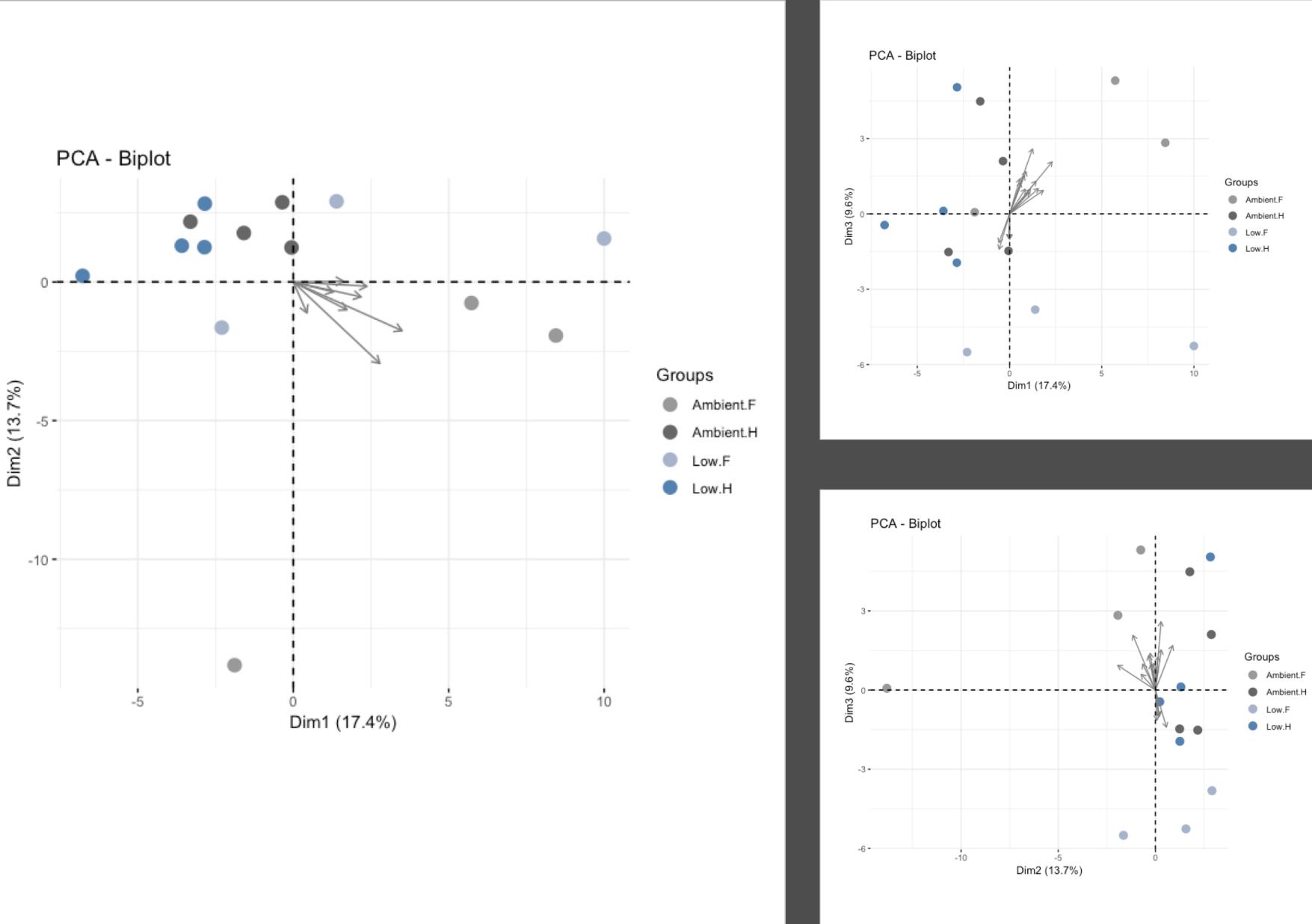
LARVAL SAMPLE RESULTS



COI: Cytochrome c oxidase, aerobic respiration
mt:ND5: NADH-ubiquinone oxidoreductase, mitochondrial electron transport

USP47: Ubiquitin component, DNA repair
TBC1D2: protein transport, GTPase activity

Tuba3a: Tubulin, cytoskeleton
Rpl8: ribosomal protein, cytoplasmic translation



QuantSeq / TagSeq Library Prep

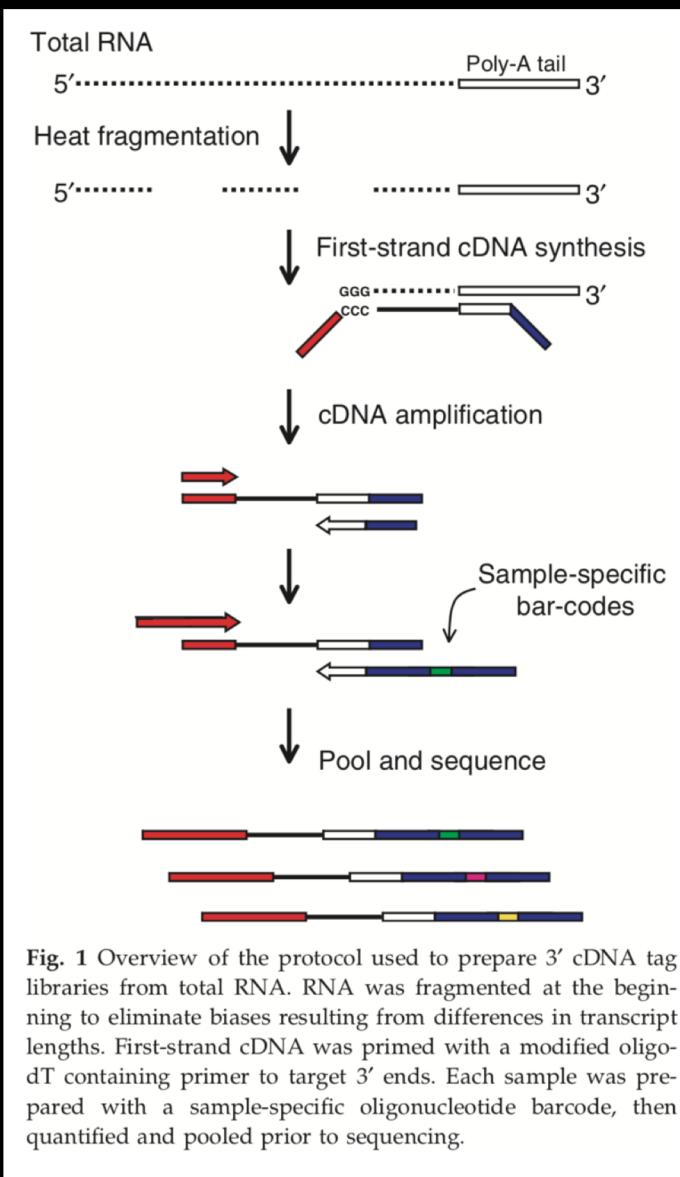


Fig. 1 Overview of the protocol used to prepare 3' cDNA tag libraries from total RNA. RNA was fragmented at the beginning to eliminate biases resulting from differences in transcript lengths. First-strand cDNA was primed with a modified oligo-dT containing primer to target 3' ends. Each sample was prepared with a sample-specific oligonucleotide barcode, then quantified and pooled prior to sequencing.