

GEODUCK AS INDICATORS OF ENVIRONMENTAL CHANGE

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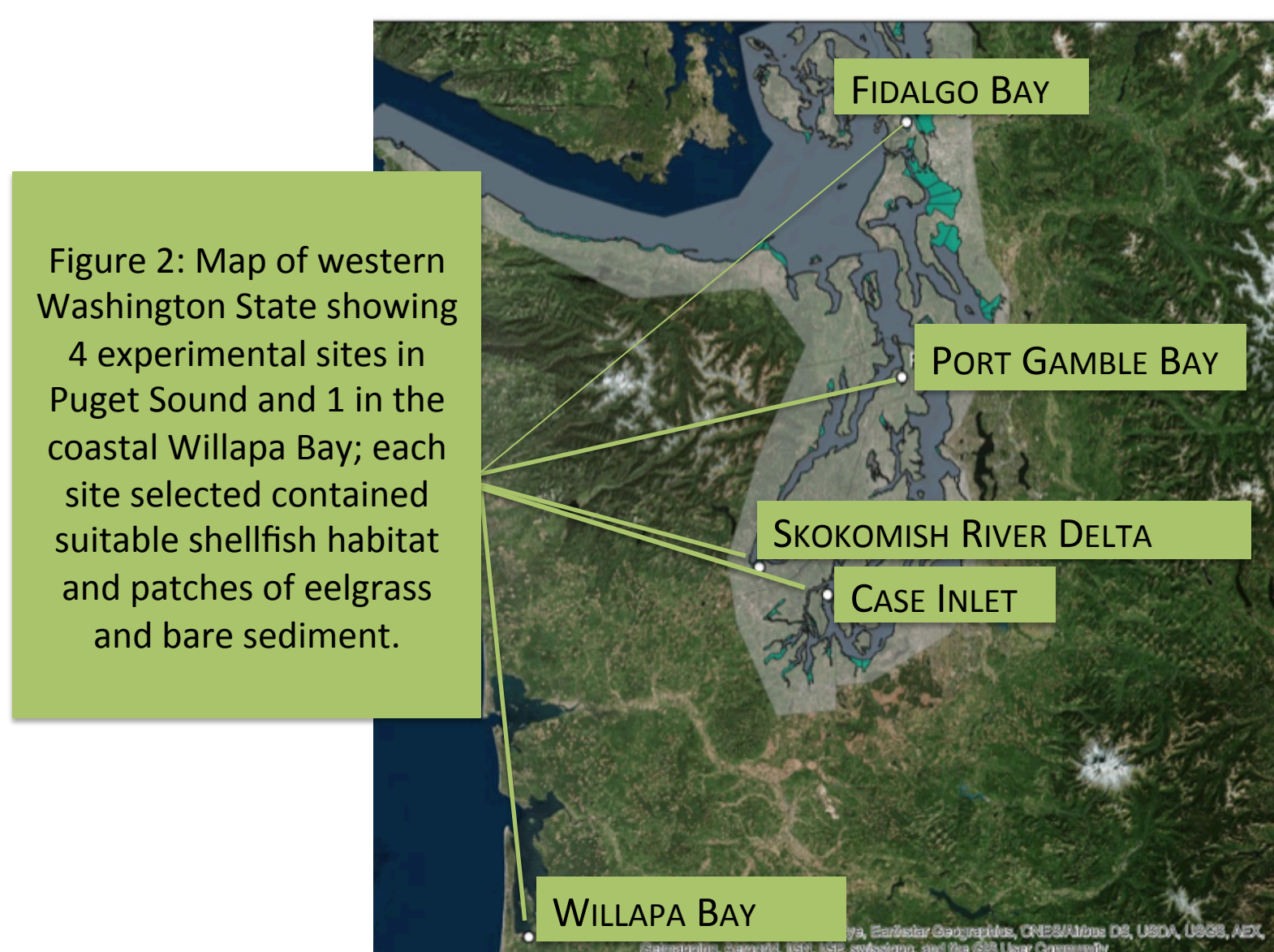
THE ANIMAL

Pacific geoduck (*Panopea generosa*)

- Largest clam native to the Pacific Northwest
- Important commercial fishery (wild & farmed)
- Sedentary, calcifying, sub-tidal
- Likely to be impacted by rising ocean temperature and pCO₂

THE TRIALS

- 5 sites throughout western Washington
- 2 treatments per site: eelgrass bed, bare sediment
- 4 replicates per treatment
- 2 month-long trials in June and August 2016
- Continuous water quality measurements: pH, temperature, salinity, depth, chlorophyll
- Gill tissue sampled for protein extraction



THE QUESTIONS

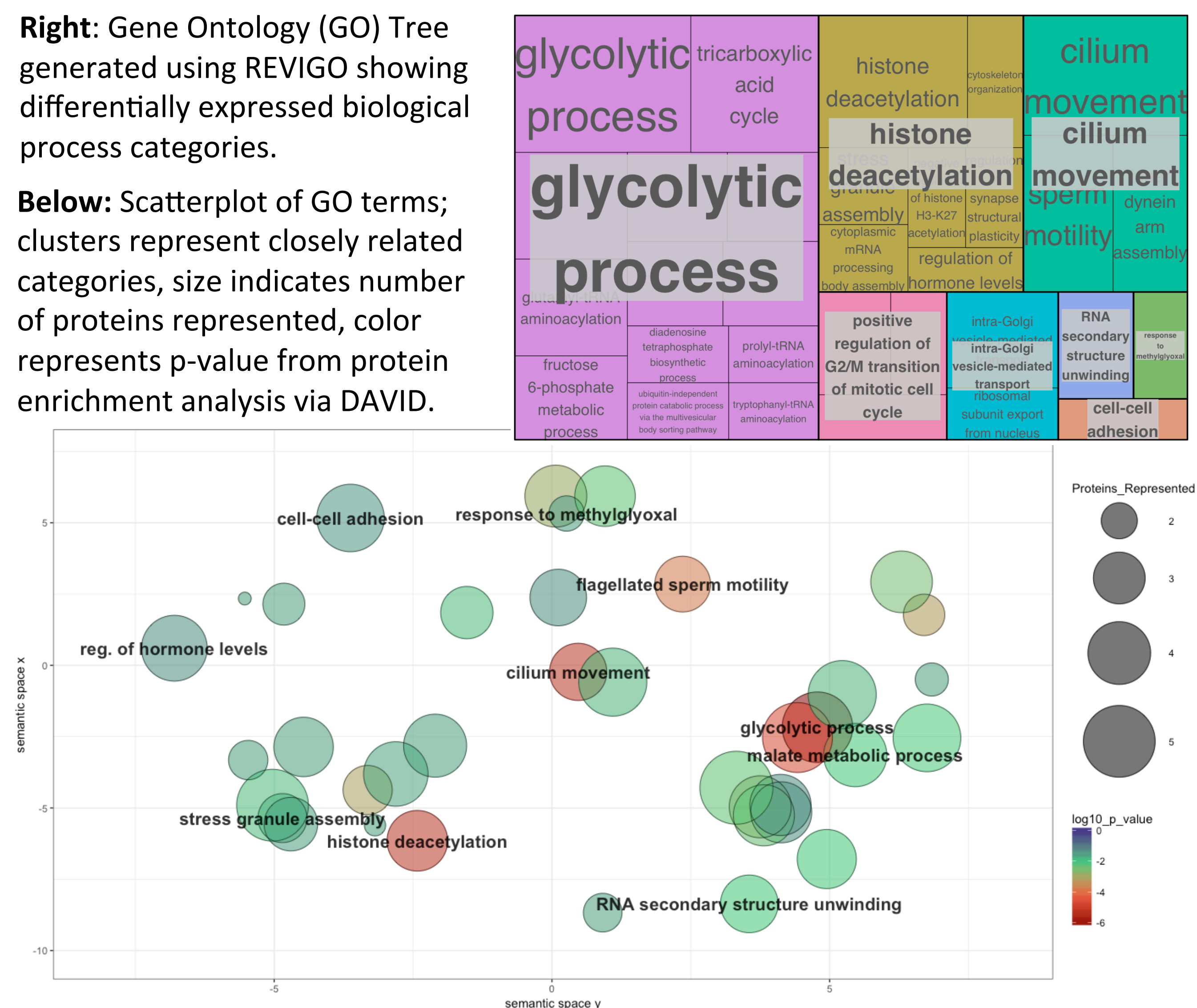
- Can we detect an **environmental signature** via differences in **protein expression**?
- Does treatment (eelgrass, no eelgrass) or geographical site correlate with expression of stress-related proteins?
- What is the overall variability in gill tissue protein expression?

THE PRELIMINARY RESULTS

Shotgun proteomics reveals protein groups differentially expressed in eelgrass beds

Right: Gene Ontology (GO) Tree generated using REVIGO showing differentially expressed biological process categories.

Below: Scatterplot of GO terms; clusters represent closely related categories, size indicates number of proteins represented, color represents p-value from protein enrichment analysis via DAVID.



THE METHODS

Shotgun proteomics for discovery analysis

EXTRACT PROTEINS & FRAGMENT

Mass Spec Sample Prep

- Sonication and cell lyses
- Protein quantification
- Mini-Trypsin digestion
- Desalting and peptide isolation
- Add standards

IDENTIFY AND QUANTIFY PEPTIDES

Data-Independent Tandem Mass Spectrometry

- Orbitrap Fusion Lumos machine
- Does not require pre-selected precursor ions
- Captures all peptides within designated mass/charge ratio

IDENTIFY PROTEINS IN SAMPLES

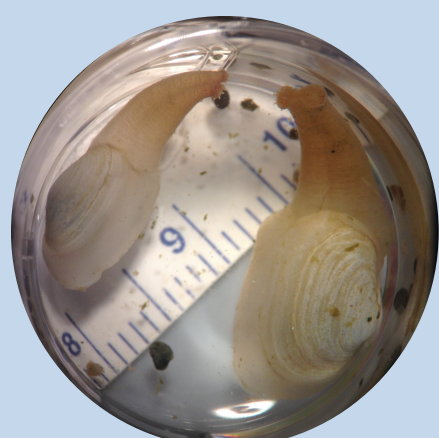
PECAN

- Survey peptides against annotated background proteome
- Generate summary file for direct use in Skyline

QUANTIFY RELATIVE PROTEIN ABUNDANCE

Skyline

- View summary statistics for peptide abundance
- Export statistics for processing and visualization



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