

# EXPLORING PROTEOMIC VARIATION IN PACIFIC OYSTERS (*CRASSOSTREA GIGAS*)



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## BACKGROUND



- *Crassostrea gigas* provides ecosystem services, basis for aquaculture operations in Washington<sup>1</sup>
- Susceptible to ocean acidification and ocean warming<sup>2,3</sup>
- Limited set of experiments examining oysters' response to multiple stressors that reflect wild conditions

**OBJECTIVE:** Understand how different environments drive differential protein expression in response to stress.

## EXPERIMENTAL DESIGN

Figure 1. Map of study sites in and around Puget Sound, WA. Inset. Oysters placed in both eelgrass and bare patches at each site to assess the impact of larger-scale ecological interactions on stress response.



- **Five experimental sites:** Case Inlet, Fidalgo Bay, Port Gamble Bay, Skokomish River Delta, and Willapa Bay
- Outplanted **150 adult sibling *C. gigas***, 30 per site
- Eelgrass presence: assess effect of **large-scale ecological interactions**
- **Continuous environmental chemistry monitoring**
- Gill tissue collected after one month
- **Data-independent mass spectrometry** and **shotgun proteomic methods** used to generate peptide spectra for samples<sup>4,5</sup>
- **Protein abundance data based on oyster seed spectra**<sup>4,5</sup>

## DATA-INDEPENDENT PROTEOMICS

**RESULTS:** Matched peptide spectra in ten oyster samples with stress-related proteins present in the *C. gigas* proteome<sup>4,5</sup>. We identified 6,688 proteins across five sites and two eelgrass conditions, of which 43 were stress-related. Additionally, the carbohydrate metabolism pathway was overrepresented amongst all samples.

Figure 2. Overrepresented gene ontology terms for stress-related proteins subsetted from all proteins found in *C. gigas* samples. Size of bubble is representative of frequency in data.

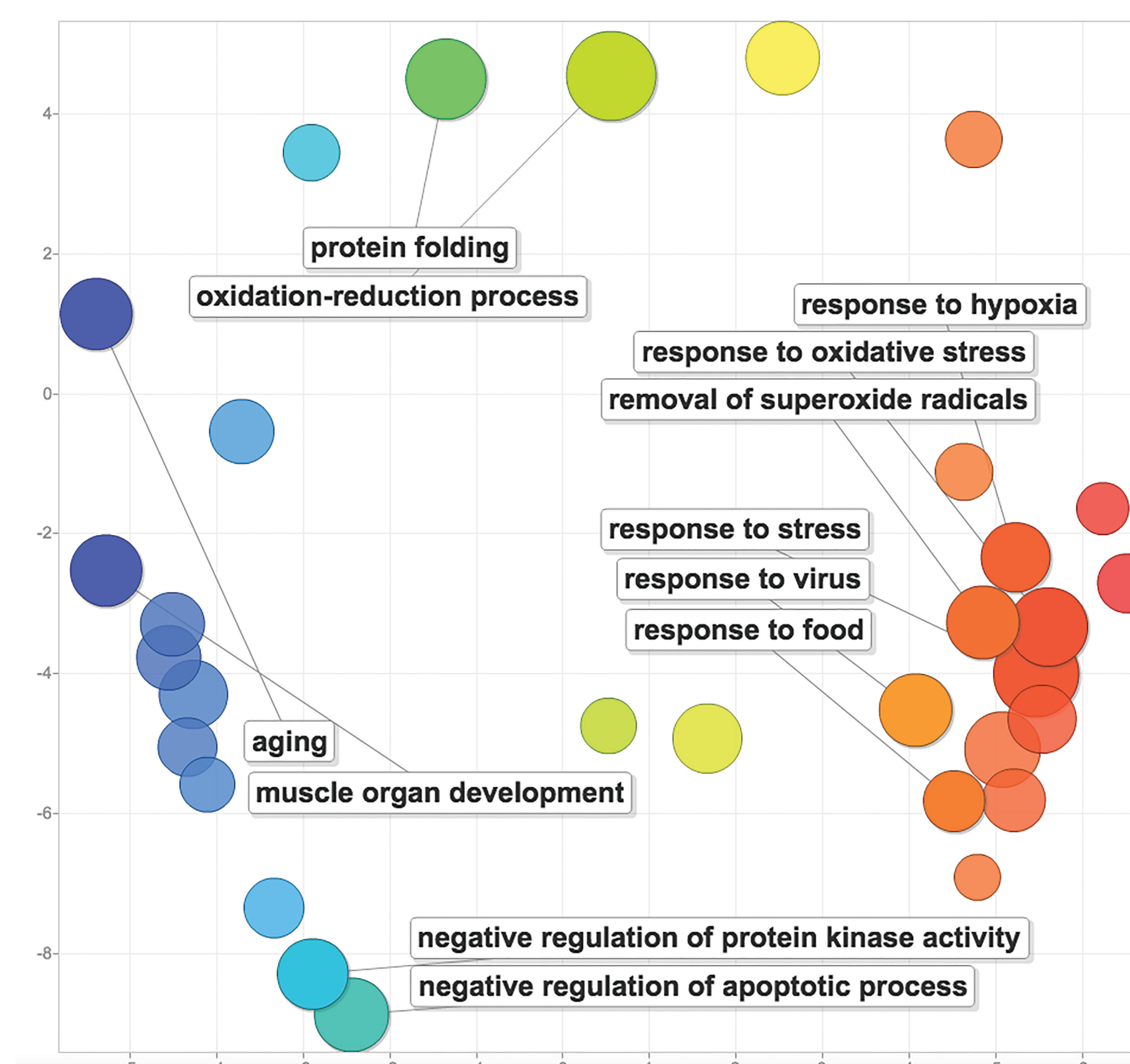
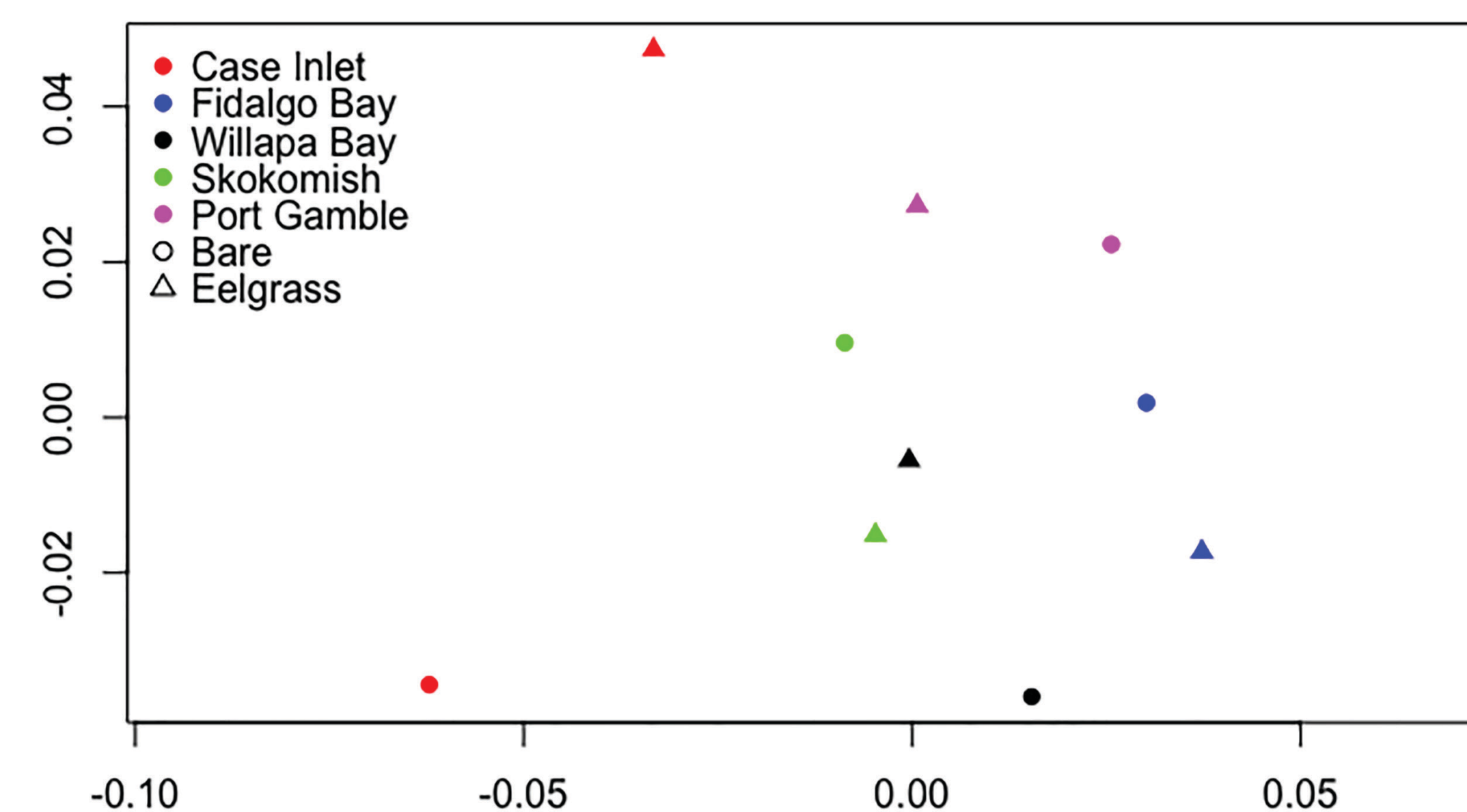


Figure 3. NMDS cluster analysis of protein abundance across sites and eelgrass conditions.



PROTEIN	VARIATION
ATP-binding cassette sub-family A member 1	1.2533
Apoptosis-inducing factor	0.9215
Peroxiredoxin-5	0.9894
Metabotropic glutamate receptor 7	2.6745
Catalase	0.4586
Heat shock protein beta-1	1.1964
DnaJ homolog subfamily C member 3	1.0597
Heat shock protein beta-1	1.1687
Heat shock protein beta-1	1.3033
Glucose-6-phosphate 1-dehydrogenase	1.1410
Glycogen synthase kinase-3 beta	0.8576
Peroxidase	0.9291
Heat shock 70 kDa protein 4L	0.8001
Heat shock protein 83	1.2868
Heat shock protein HSP 90-alpha 1	1.2881
Hypoxia up-regulated protein 1	1.8691
Superoxide dismutase [Cu-Zn]	1.3620
Peroxiredoxin	0.7054
Bcl-2-like protein 1	1.4524
Histone deacetylase 4	1.8109
Insulin-like growth factor 2 mRNA-binding protein 1	0.6434
Universal stress protein YxiE	1.9225
Universal stress protein Sli1388	1.4023
Putative universal stress protein SAOUHSC_01819	2.0495
Universal stress protein MSMEG_3950/MSMEI_3859	2.6966
Universal stress protein in QAH/OAS sulfhydrylase 3'region	1.8572
Putative universal stress protein SAOUHSC_01819	2.0495
Universal stress protein A-like protein	1.0244
Stress response protein NhaX	0.9036
Heat shock protein beta-1	1.0513
Cytochrome P450 1A1	1.4766
DnaJ homolog subfamily A member 1	1.4455
Heat shock protein beta-1	0.9842
Hepatocyte growth factor receptor	1.8164
Multidrug resistance-associated protein 1	0.8982
Heat shock protein beta-1	1.7581
Heat shock protein HSP 90-beta	1.2277
Heat shock protein beta-1	1.3033
Superoxide dismutase [Mn], mitochondrial	1.2061
Stress-induced phosphoprotein 1	1.9065
Heat shock protein 75 kDa, mitochondrial	0.5907
Universal stress protein in QAH/OAS sulfhydrylase 3'region	1.5555
Extracellular superoxide dismutase [Cu-Zn]	0.5662

Table 1. Coefficients of variation for abundance of stress-related proteins across sites and eelgrass conditions. The values indicate a level of variation in stress-related proteins between samples.

**IMPORTANCE:** Differences in protein abundance across sites and eelgrass conditions suggests site-specific environmental variables influence *C. gigas* physiology (i.e. stress response and energy metabolism).

## FUTURE DIRECTIONS

- Preliminary insight demonstrates how **stress-response** is impacted by different **environmental conditions**
- Use **data-specific peptide spectra**
- Repeat proteomic analyses with **increased sample size**
- Develop **targeted assay** for stress-related proteins

## ACKNOWLEDGMENTS

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