

EXPLORING PROTEOMIC VARIATION IN PACIFIC OYSTERS



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BACKGROUND



- Pacific oysters (*Crassostrea gigas*) provides ecosystem services and are a significant aquaculture product¹
- Susceptible to ocean acidification and ocean warming^{2,3}
- Limited set of experiments examining oysters' response to multiple stressors that reflect wild conditions

OBJECTIVE: Understand how environment drives differential protein expression to better predict ecosystem health.

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^{4,5}
- **Protein abundance data based on oyster seed spectra**^{4,5}

DATA-INDEPENDENT PROTEOMICS

RESULTS: Expression of 6,688 proteins was characterized in oysters from five sites in Puget Sound using shotgun proteomic approaches. Numerous biological processes were identified as impacted based on environmental conditions and will provide basis for future work.

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.

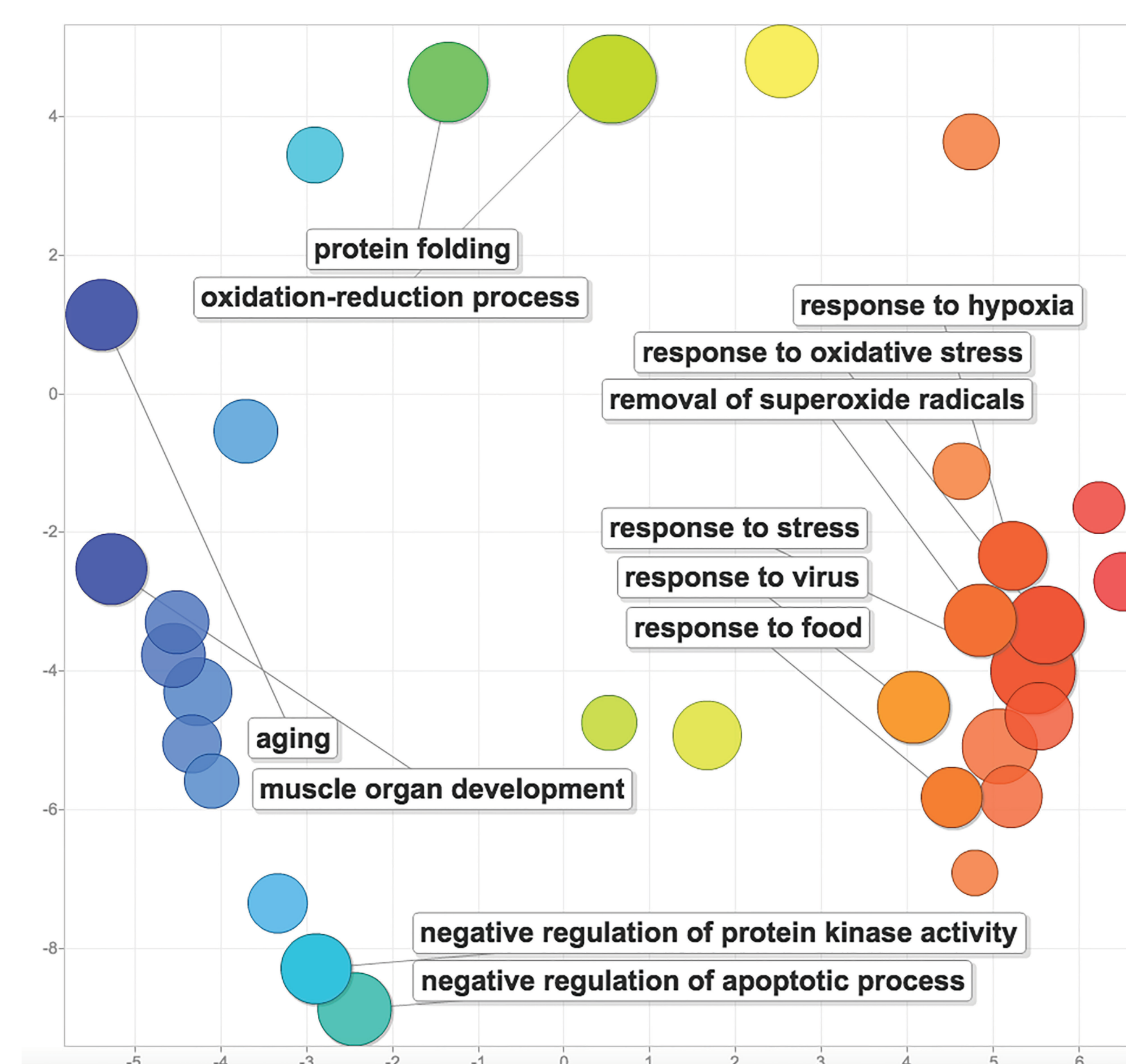
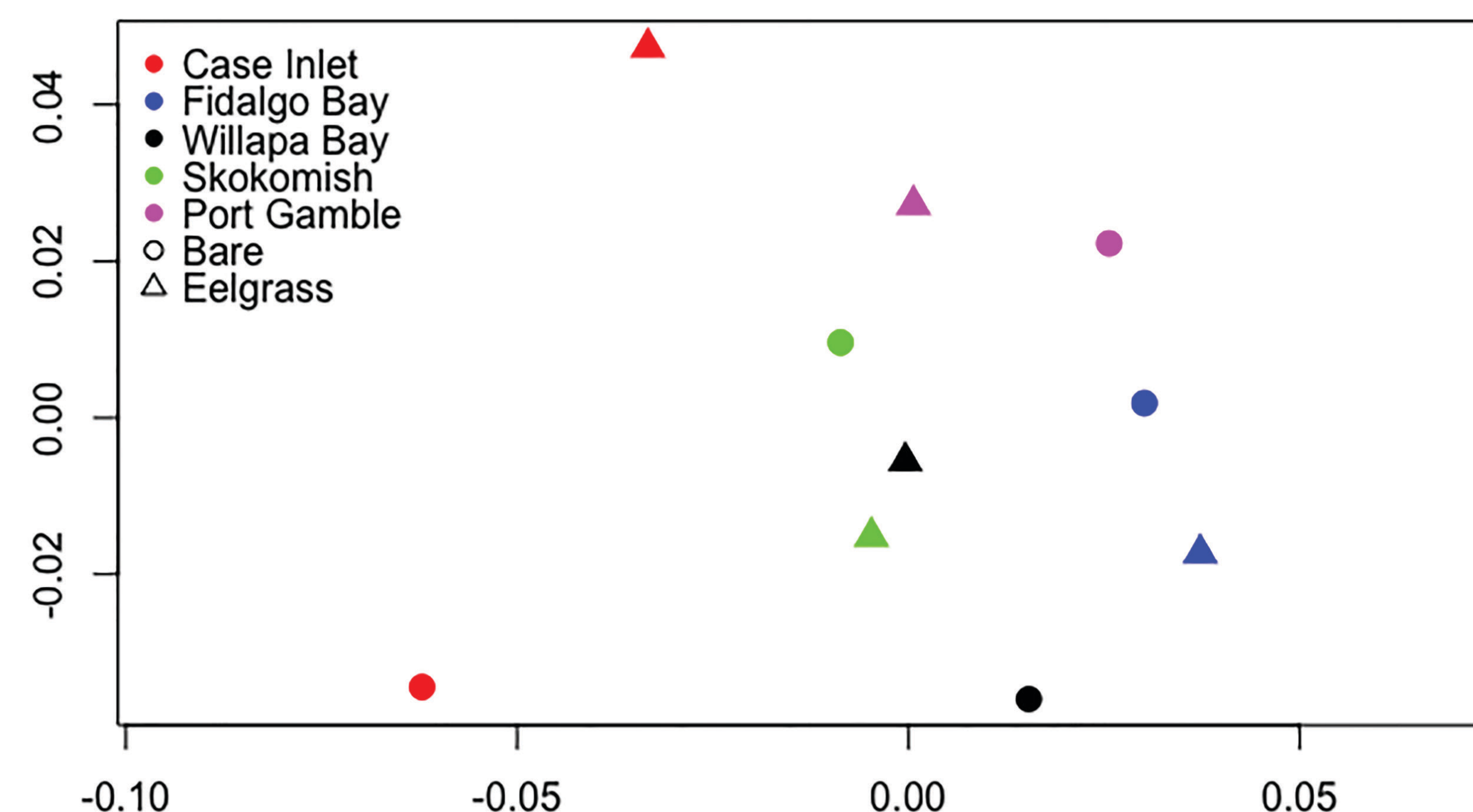


Figure 3. NMDS cluster analysis of protein abundance across sites and eelgrass conditions.



PROTEIN	VARIATION
Metabotropic glutamate receptor 7	2.6745
Putative universal stress protein SAOUHSC_01819	2.0495
Stress-induced-phosphoprotein 1	1.9065
Hypoxia up-regulated protein 1	1.8691
Hepatocyte growth factor receptor	1.8164
Histone deacetylase 4	1.8109
Cytochrome P450 1A1	1.4766
Bcl-2-like protein 1	1.4524
DnaJ homolog subfamily A member 1	1.4455
Superoxide dismutase [Cu-Zn]	1.362
Heat shock protein beta-1	1.3033
Heat shock protein HSP 90-alpha 1	1.2881
Heat shock protein 83	1.2868
ATP-binding cassette sub-family A member 1	1.2533
Heat shock protein HSP 90-beta	1.2277
Superoxide dismutase [Mn], mitochondrial	1.2061
Glucose-6-phosphate 1-dehydrogenase	1.141
Universal stress protein A-like protein	1.0244
Heat shock protein 75 kDa, mitochondrial	0.9907
Peroxioredoxin-5	0.9894
Peroxiidasin	0.9291
Apoptosis-inducing factor	0.9215
Stress response protein NhxX	0.9036
Multidrug resistance-associated protein 1	0.8982
Glycogen synthase kinase-3 beta	0.8576
Heat shock 70 kDa protein 4L	0.8001
Peroxioredoxin	0.7054
Insulin-like growth factor 2 mRNA-binding protein 1	0.6434
Extracellular superoxide dismutase [Cu-Zn]	0.5662
Catalase	0.4586

Table 1. Coefficients of variation for abundance of select proteins across sites and eelgrass conditions. The values indicate a level of variation in protein expression across samples.

IMPORTANCE: These data could be used to develop assays to examine oyster physiology and ecosystem health.

FUTURE DIRECTIONS

- Preliminary insight demonstrates how **select proteins** are 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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