

EXPLORING PRELIMINARY PROTEOMIC VARIATION FOR STRESS RESPONSE IN PACIFIC OYSTERS (*CRASSOSTREA GIGAS*)



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BACKGROUND



- *C. gigas* provides ecosystem services, basis for aquaculture operations in Washington¹
- 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 different environments and drive differential protein expression in response to stress

EXPERIMENTAL DESIGN

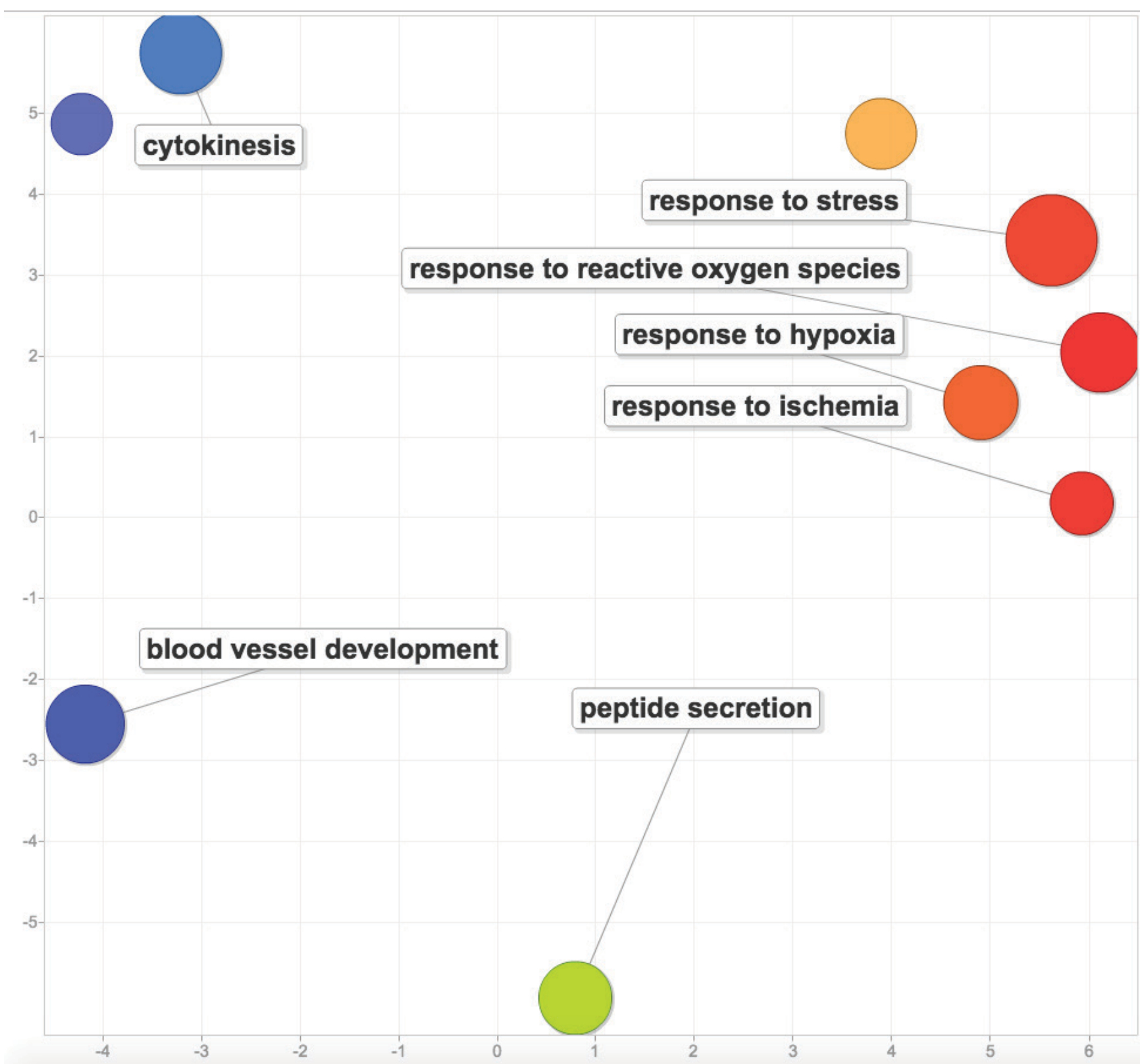
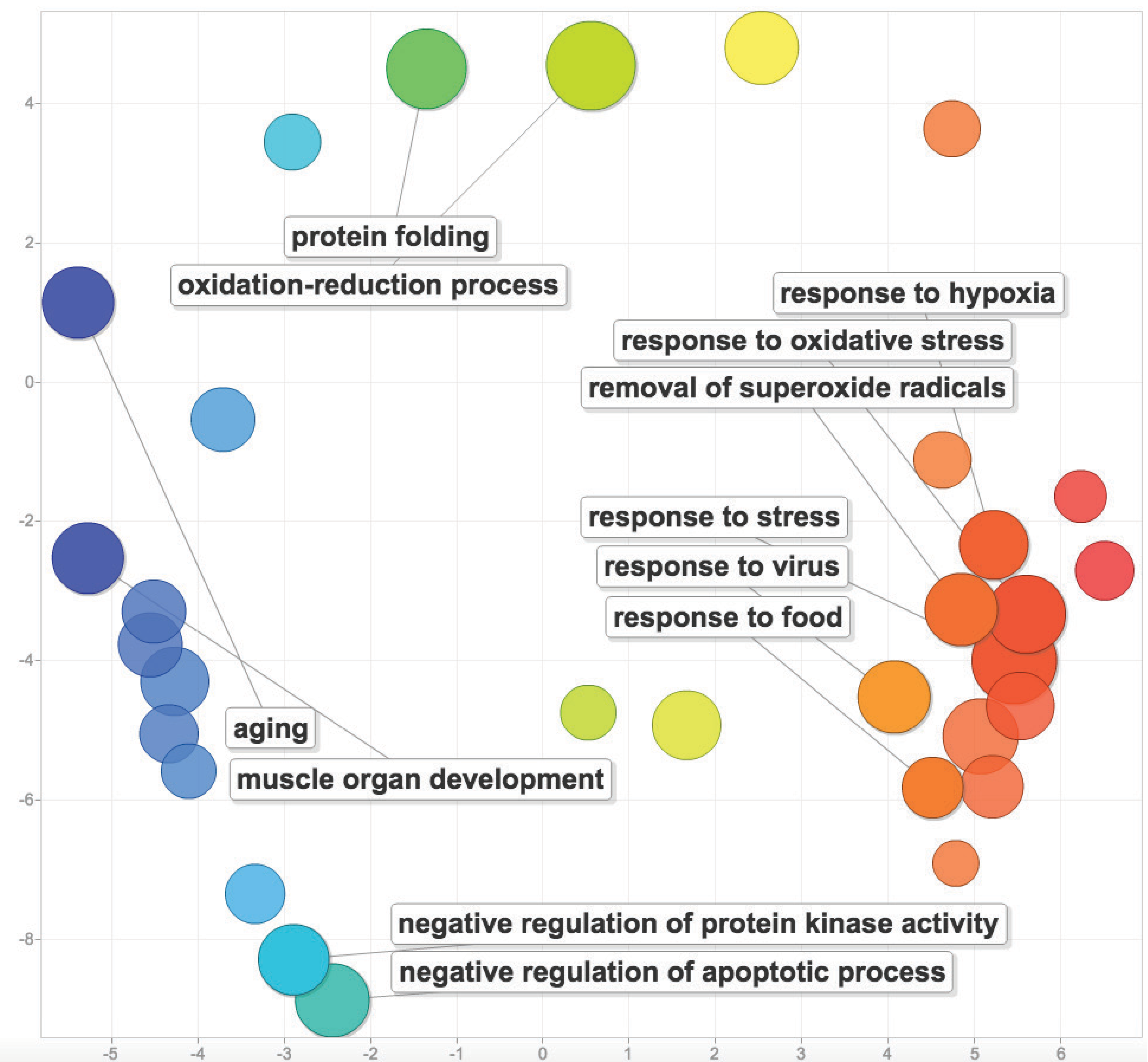
Figure 1. Map of study sites in and around Puget Sound, WA. Inset. Oysters placed in both eelgrass and bare patches at each site to assess the impact of larger-scale ecological interactions on stress response.



- **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 for proteomic analyses

DATA-INDEPENDENT PROTEOMICS

OBJECTIVE: Match peptide spectra in ten oyster samples with stress-related proteins present in the *C. gigas* proteome^{4,5}



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

Figure 3. Coefficients of variation for stress-related proteins. Size of bubbles corresponds to coefficient value.

FINDING: Evidence for variation in stress response across five sites and eelgrass conditions

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**

¹ "Washington Shellfish Initiative." n.d. http://www.westcoast.fisheries.noaa.gov/publications/aquaculture/wa_shellfishinitiative_20111209.pdf.
² Lennig G, Eilers S, Pörtner HO, Sokolova IM, Back C. Impact of ocean acidification on energy metabolism of oyster, *Crassostrea gigas*—changes in metabolic pathways and thermal response. *Mar drugs* 2010; 8(8):2318–2339. doi:10.3390/md8082318.
³ Timmins-Schiffman et al.: Shotgun proteomics reveals physiological response to ocean acidification in *Crassostrea gigas*. *BMC Genomics* 2014 15:951.
⁴ Ting, Ying S., Jarrett D. Egerton, Samuel H. Payne, Sangtae Kim, Brendan MacLean, Lukas Käll, Ruedi Aebersold, Richard D. Smith, William Stafford Noble, and Michael J. MacCoss. 2015. "Peptide-Centric Proteome Analysis: An Alternative Strategy for the Analysis of Tandem Mass Spectrometry Data." *Molecular & Cellular Proteomics*. MCP 14 (9): 2301–7.
⁵ Timmins-Schiffman, Emma B., Grace A. Crandall, Brent Vadopalas, Michael E. Riffle, Brook L. Nunn, and Steven B. Roberts. 2016. "Integrating Discovery-Driven Proteomics and Selected Reaction Monitoring to Develop a Non-Invasive Assay for Geoduck Reproductive Maturation." *bioRxiv*. doi:10.1101/084615.