Using Proteomics to Understand Ocean Acidification Stress in the Pacific Oyster

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Crassostrea gigas

- Important aquaculture species in the PNW and worldwide
- Ecological services: water filtration, habitat, food
Ocean Acidification and Bivalves

- Acidosis and shell dissolution
- \( \text{CO}_3^{2-} \) availability

Energy/resource use

Growth & calcification
- Acidosis and shell dissolution
- \( \text{CO}_3^{2-} \) availability

\( p\text{CO}_2 \)
Characterize how ocean acidification affects the oyster’s response to a second stressor
Experimental Design

$pCO_2$ (μatm)

400 600 800 1000 1200 2800

1 month exposure

$\Delta t_0$: shell weight

No additional stress

Mechanical stress

- Shell weight
- Gill tissue:
  - Transcriptomics
  - Proteomics
- Whole body: fatty acids

Heat shock
- 2 sublethal temperatures: 42 & 43°C
- 1 lethal temperature: 44°C
Experimental Design

\[ \text{pCO}_2 \text{ (\text{\textmu}atm)} \]

\begin{align*}
400 & \quad 600 & \quad 800 & \quad 1000 & \quad 1200 & \quad 2800 \\
\end{align*}

1 month exposure

No additional stress

- Shell weight
- Gill tissue:
  - Transcriptomics
  - Proteomics
- Whole body: fatty acids

Mechanical stress
Experimental Design

- Mechanical stress as a proxy for environmental stress
- MS promotes a catecholmine stress response in C. gigas (Lacoste et al. 2001)
Methods: Proteomics

- Expression of genes and proteins change in response to the environment
- Shotgun techniques provide non-biased surveys of molecular physiological changes
Shotgun sequencing using LC-MS/MS
Protein fragments (peptides) are sequenced
Sequences are identified using a database of proteins
Results: Proteomics

- 2,677 proteins were identified
- Coverage of entire C. gigas proteome: 9.5%
Results: Proteomics

Transcriptional processes are the most significantly enriched process.
pCO$_2$ has an effect on the proteome
$pCO_2$ significantly affects shell hardness.
There is less of a proteomic response to a second stressor at high pCO₂
Mechanical Stress

High MS

1029

Venny, Oliveros 2007
Mechanical Stress

High MS

Low MS

537

492

382
pCO$_2$ and Mechanical Stress

- **Stress Response**
  - Heat shock protein 70
  - Caspase

- **pH Homeostasis**
  - V-type proton ATPase

- **Immune Response**
  - Cathepsin B
  - MAP kinase kinase
Proteomics can provide insight into the physiological response to ocean acidification.
Exposure to multiple stressors can impact an organism’s ability to mount a successful response to either stressor.
Implications

We should continue to consider multiple stressors when assessing responses to environmental change.
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