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

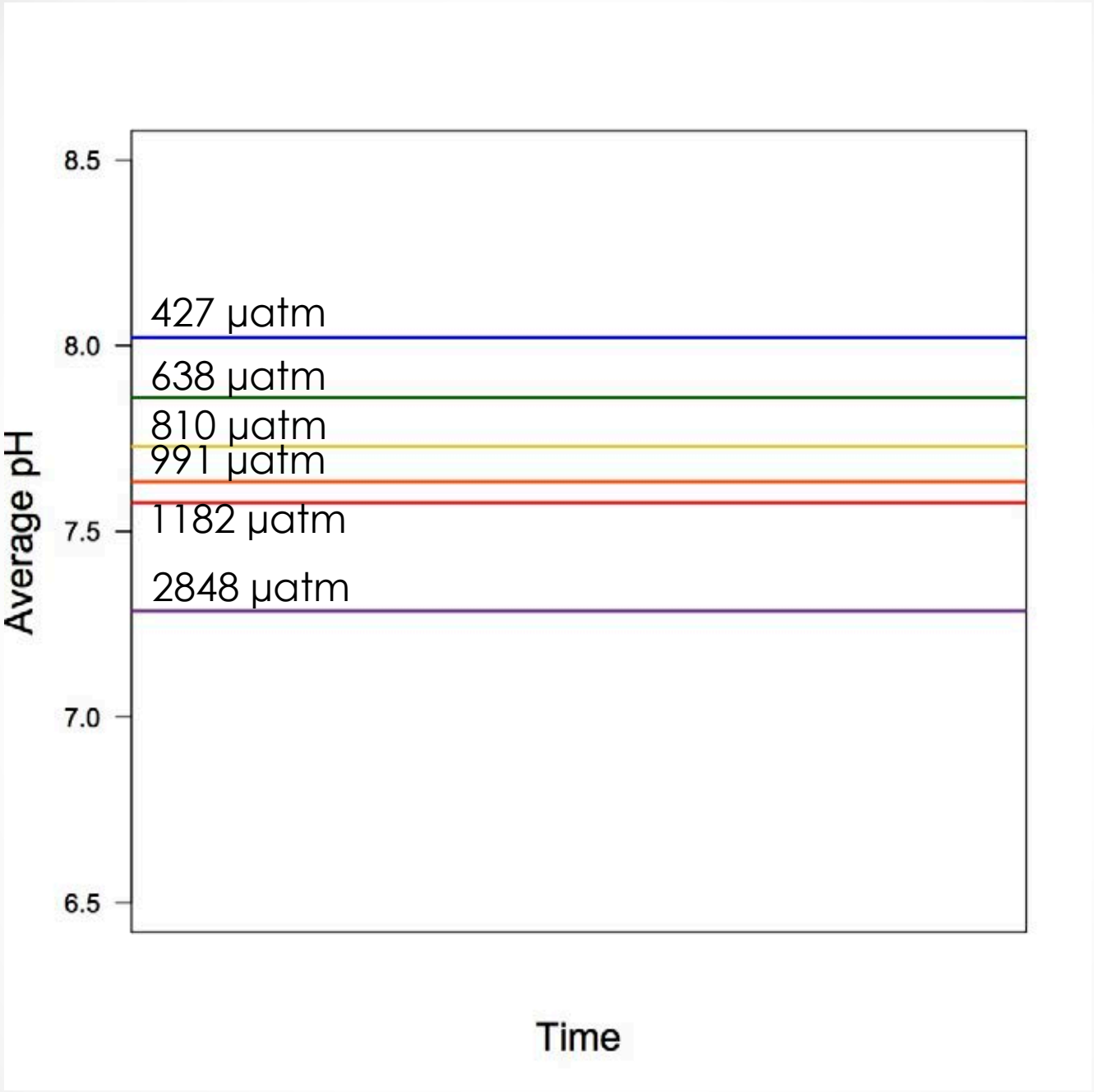
Ocean Acidification and Bivalves

pCO_2

Energy/resource use

Growth & calcification
- Acidosis and shell dissolution
- CO_3^{2-} availability

Characterize how ocean acidification affects the oyster's response to a second stressor


8.5
8.0
7.5
7.0
6.5
Average pH
427 µatm
638 µatm
810 µatm
991 µatm
1182 µatm
2848 µatm
Time

Experimental Design

pCO_2 (µatm)

400 600 800 1000 1200 2800

t_0: shell weight

1 month exposure

No additional stress

Mechanical stress

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

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

Experimental Design

pCO_2 (µatm)

400 600 800 1000 1200 2800

1 month exposure

No additional stress

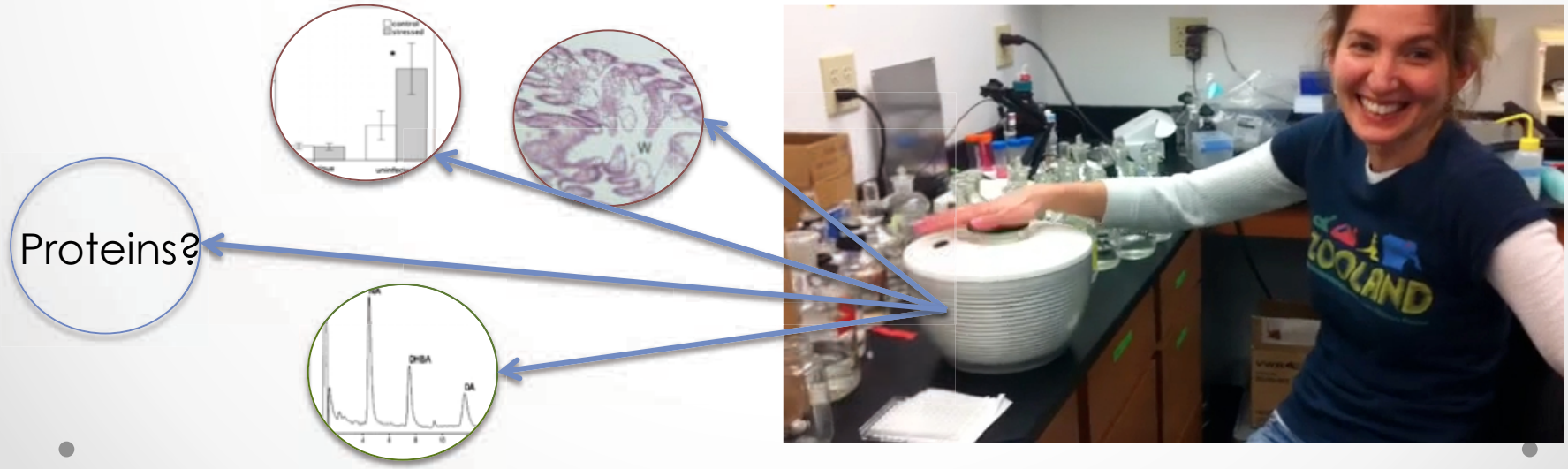
Mechanical stress

- Shell weight
- Gill tissue:
  - Transcriptomics
  - Proteomics

- Whole body: fatty acids

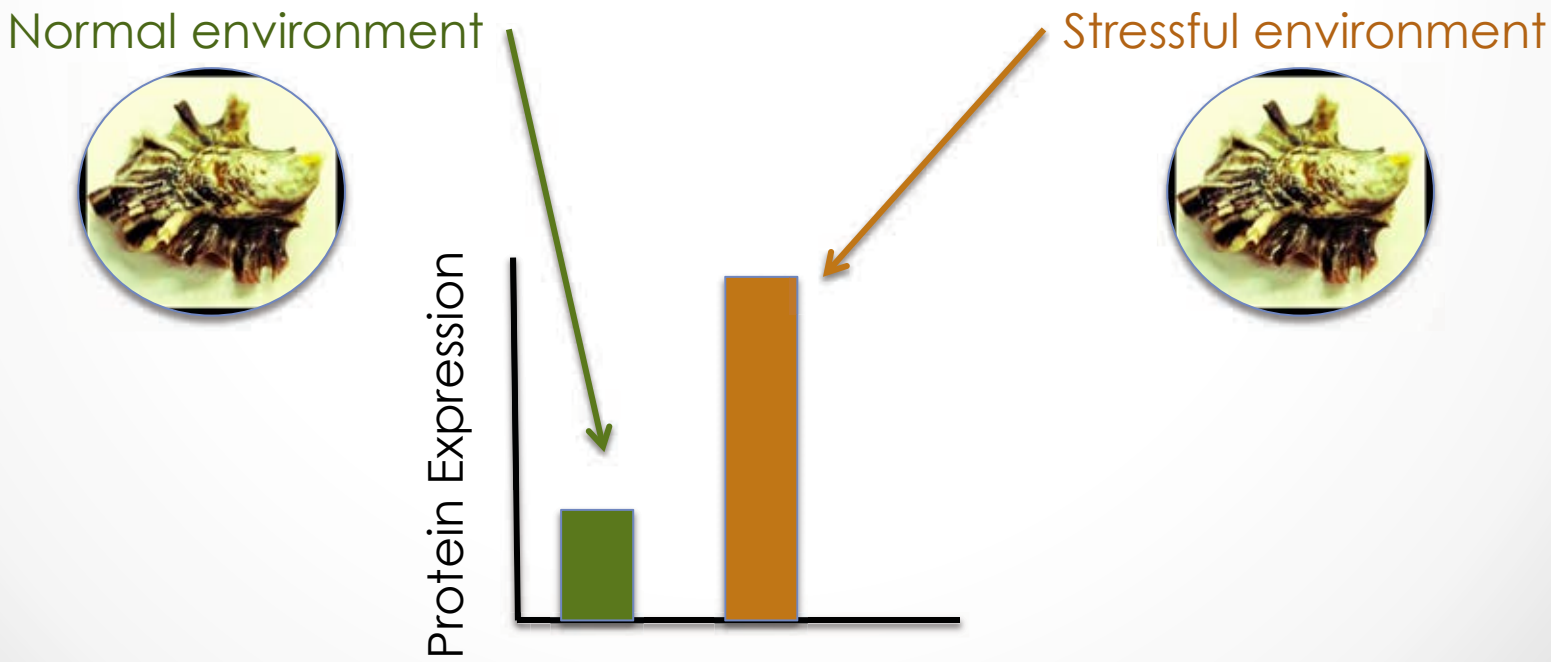
Experimental Design

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



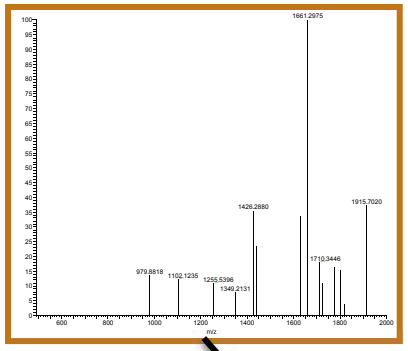
Methods: Proteomics

- Expression of genes and proteins change in response to the environment
- Shotgun techniques provide non-biased surveys of molecular physiological changes



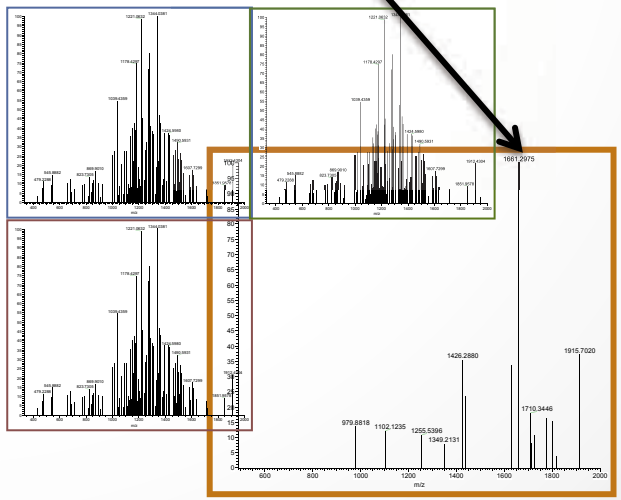
Methods: Proteomics

- Shotgun sequencing using LC-MS/MS
- Protein fragments (peptides) are sequenced
- Sequences are identified using a database of proteins

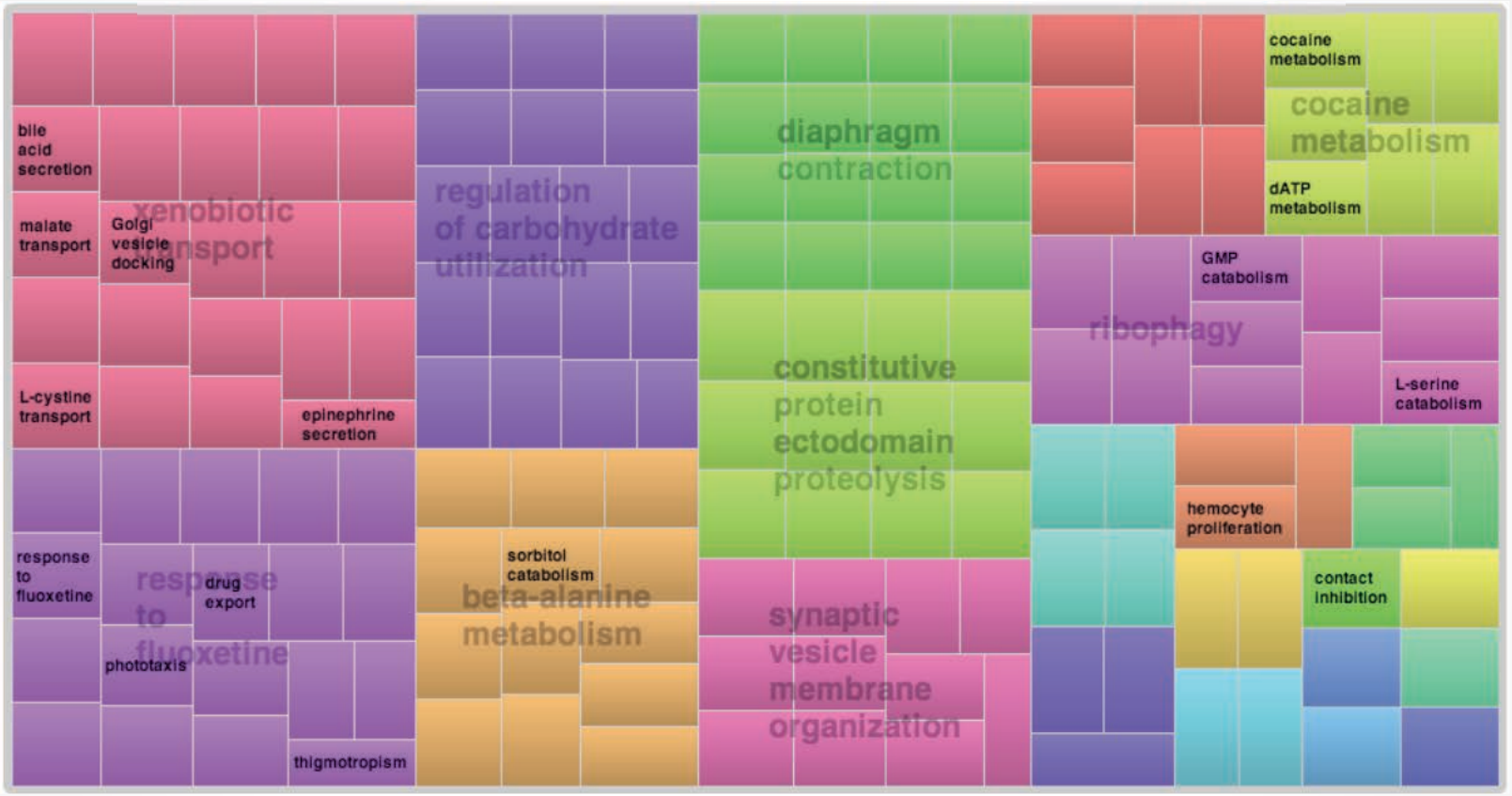


Sequenced spectrum

Matched to "spectrum" in database

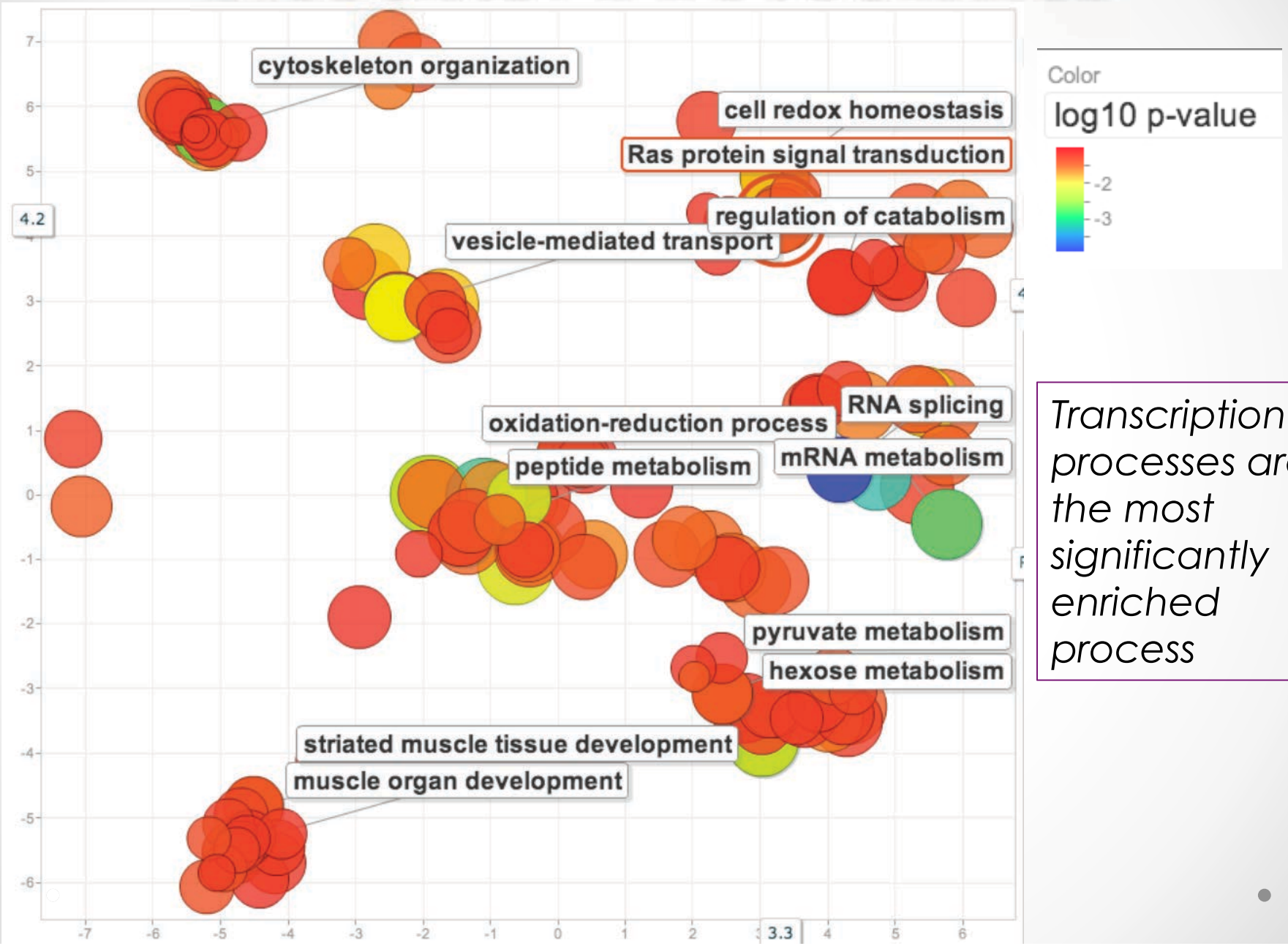


Results: Proteomics



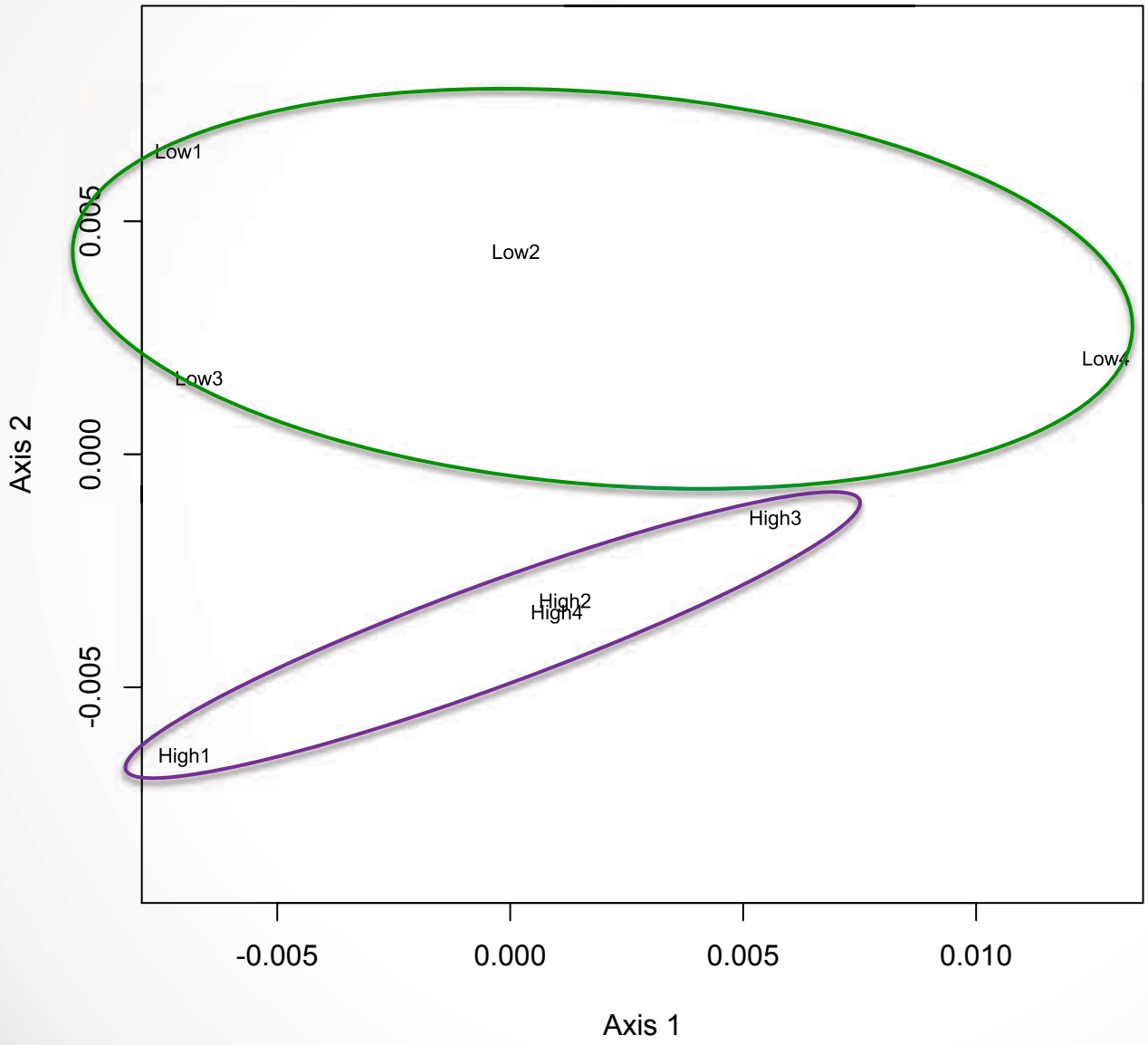
- 2,677 proteins were identified
- Coverage of entire *C. gigas* proteome: 9.5%

Results: Proteomics



Transcriptional processes are the most significantly enriched process

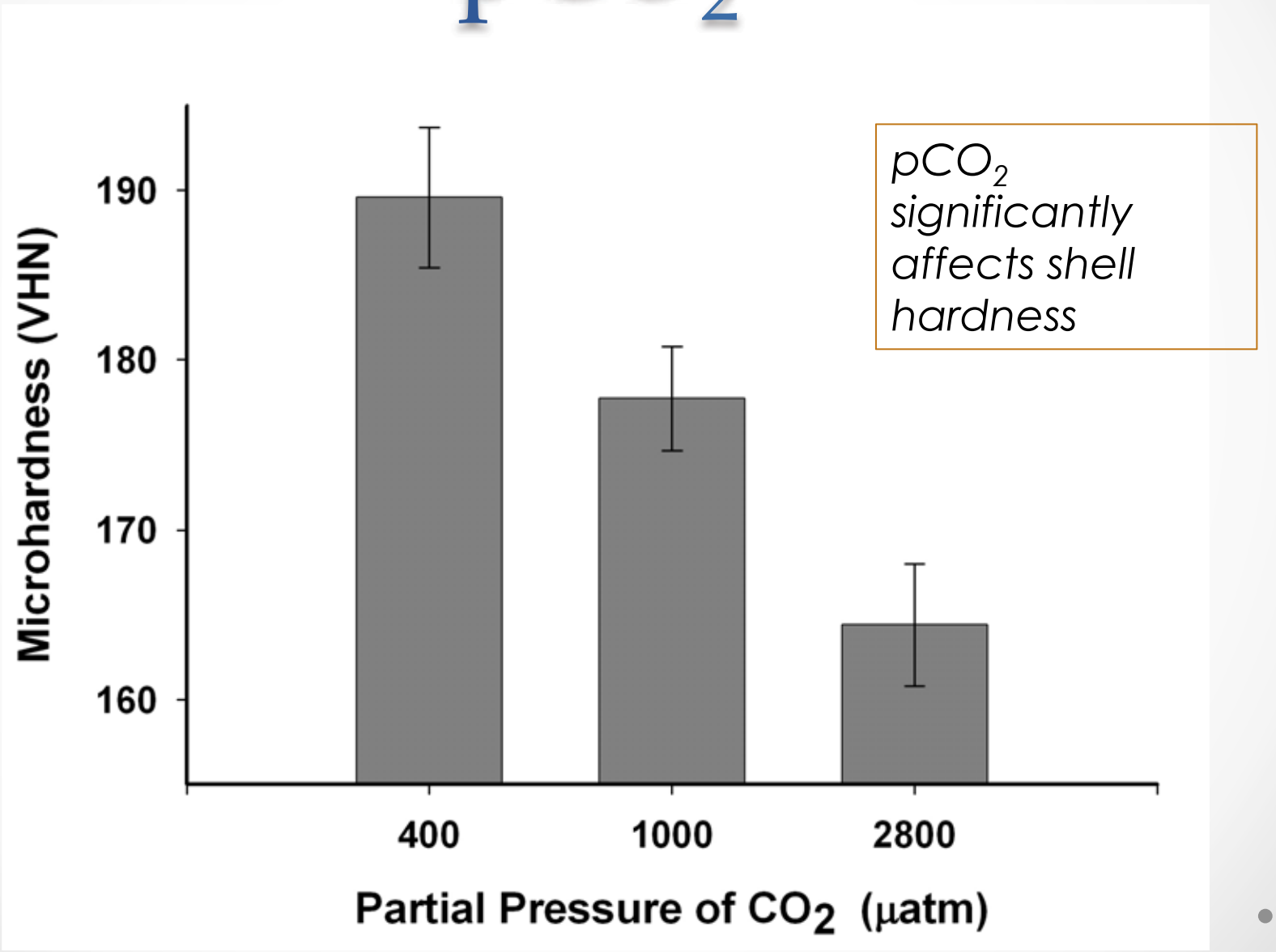
pCO_2



pCO_2 has an effect on the proteome

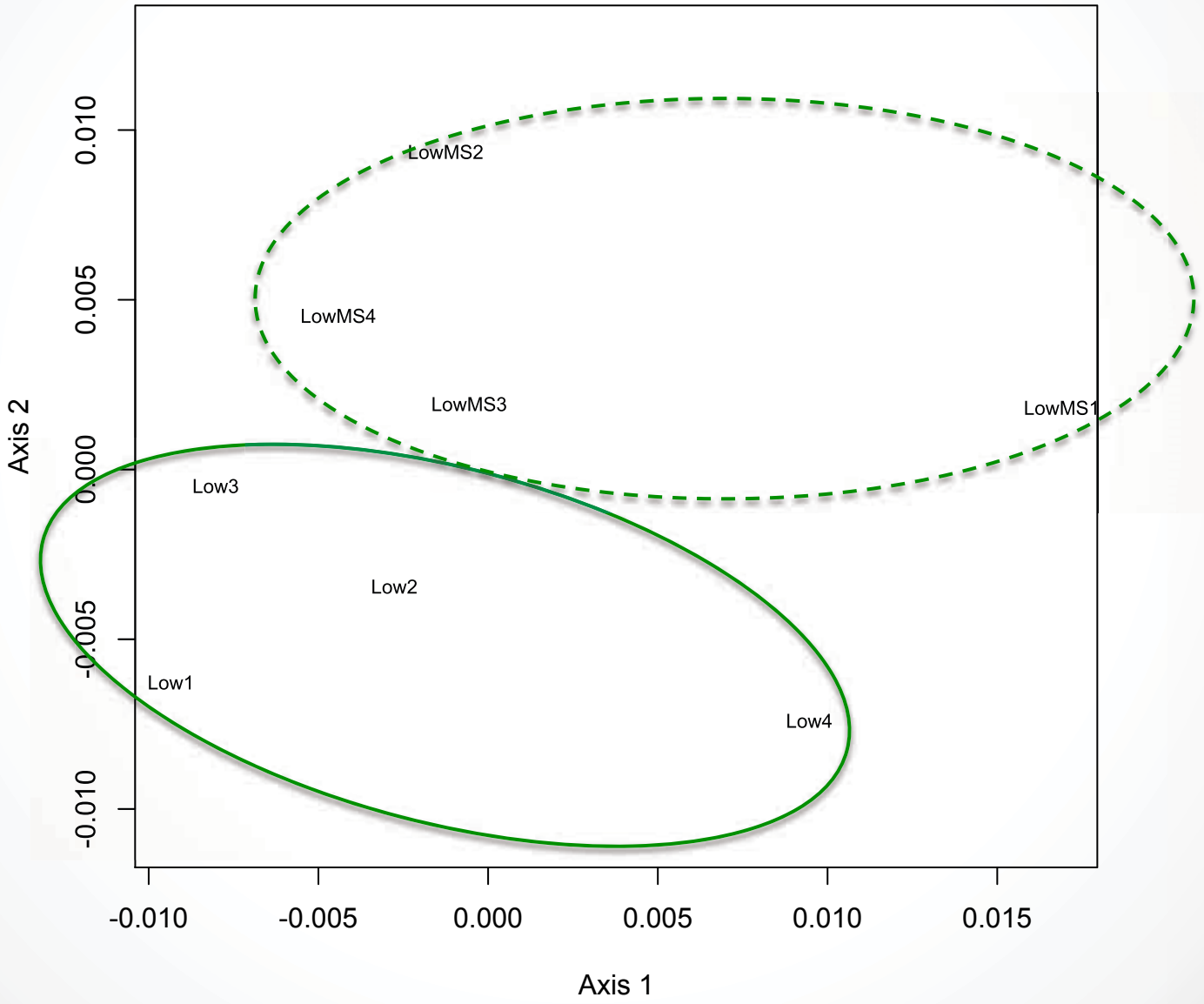
translational elongation
translation
modification-dependent protein catabolism
posttranscriptional gene silencing by RNA
macromolecule catabolism
carbohydrate catabolism
glucose catabolism
protein transport
ribonucleoprotein complex biogenesis
5
4
3
2
1
0
-1
-2
-3
-4
-5
-5
-4
-3
-2
-1
0
1
2
3
4
5
6

pCO_2

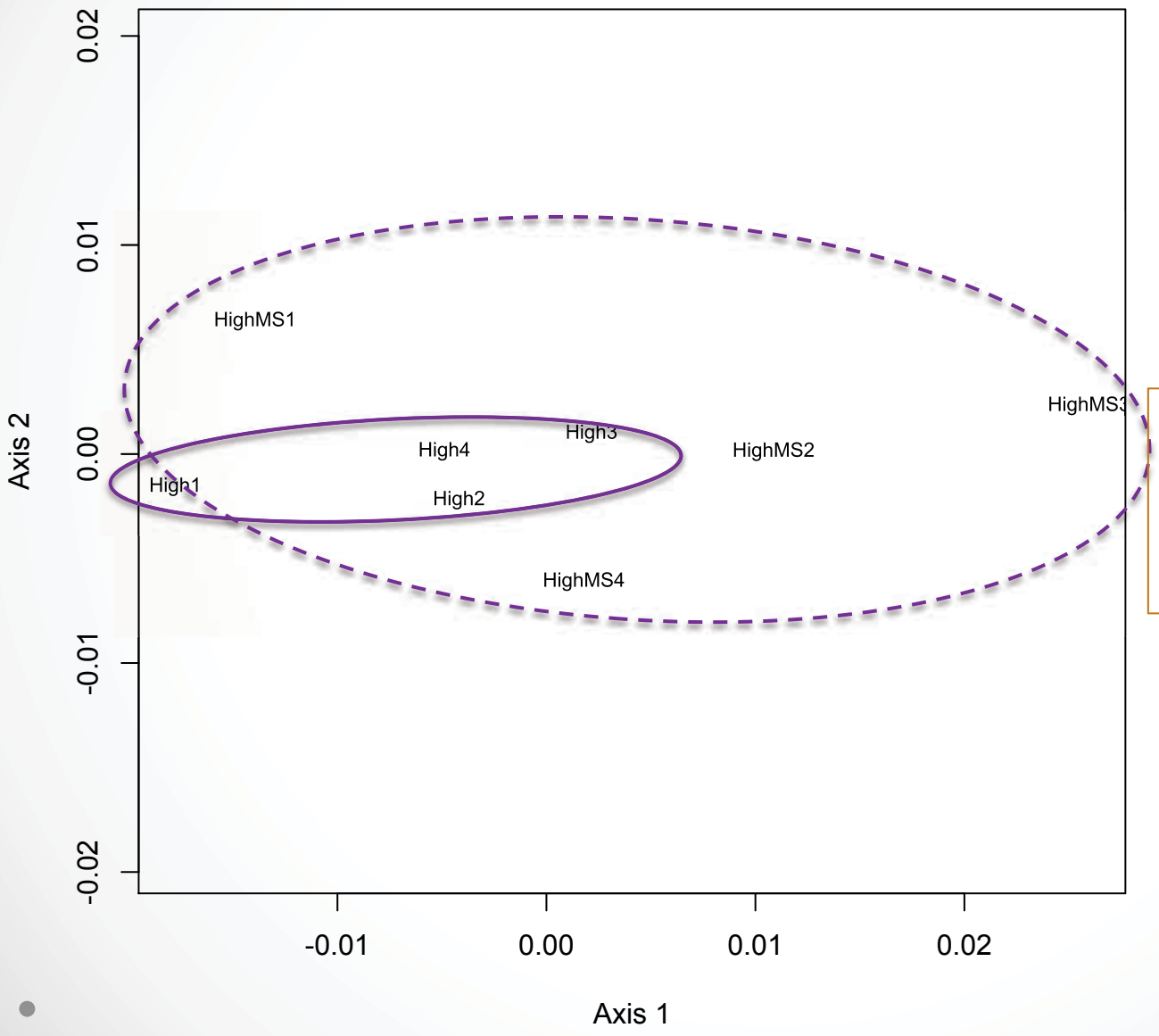


pCO_2 significantly affects shell hardness

Mechanical Stress

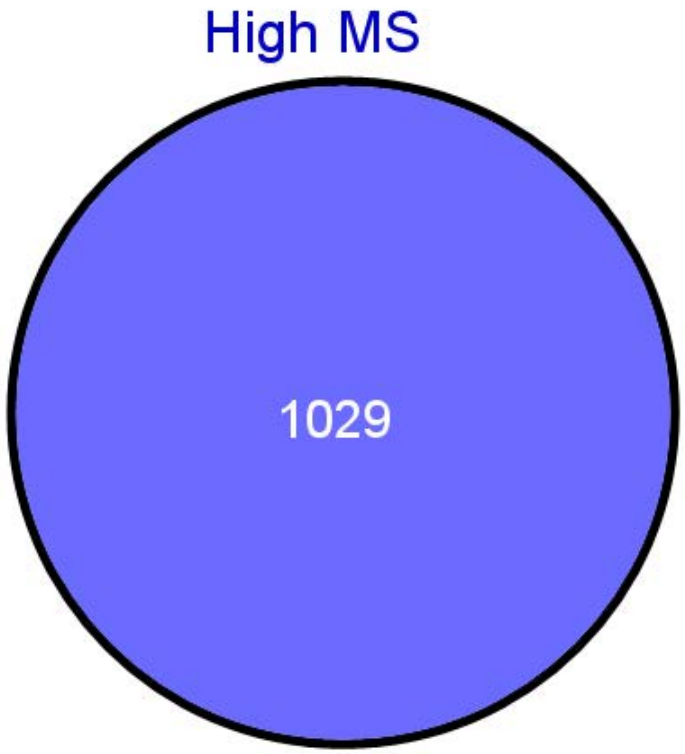


Mechanical Stress



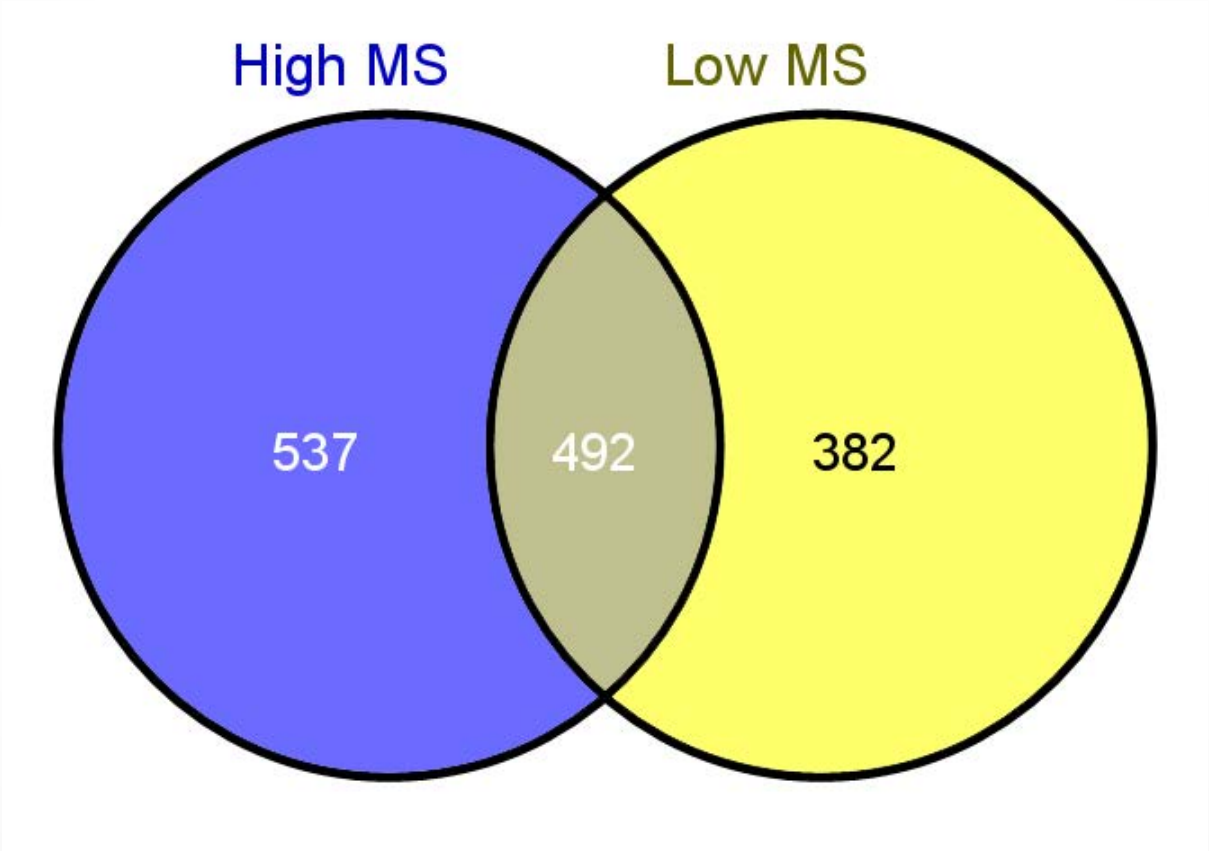
There is less of a proteomic response to a second stressor at high pCO_2

Mechanical Stress



Venny, Oliveros 2007

Mechanical Stress



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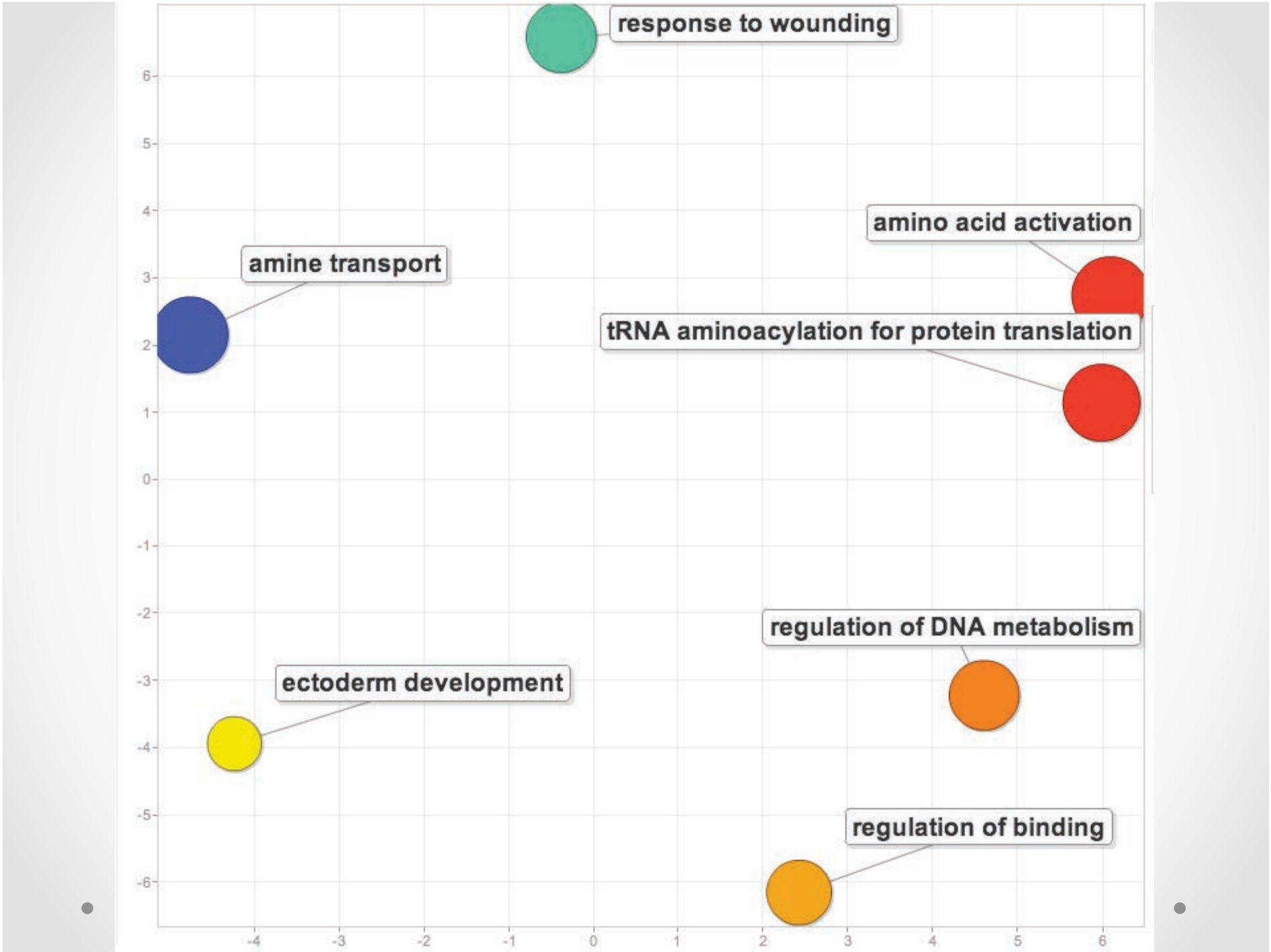
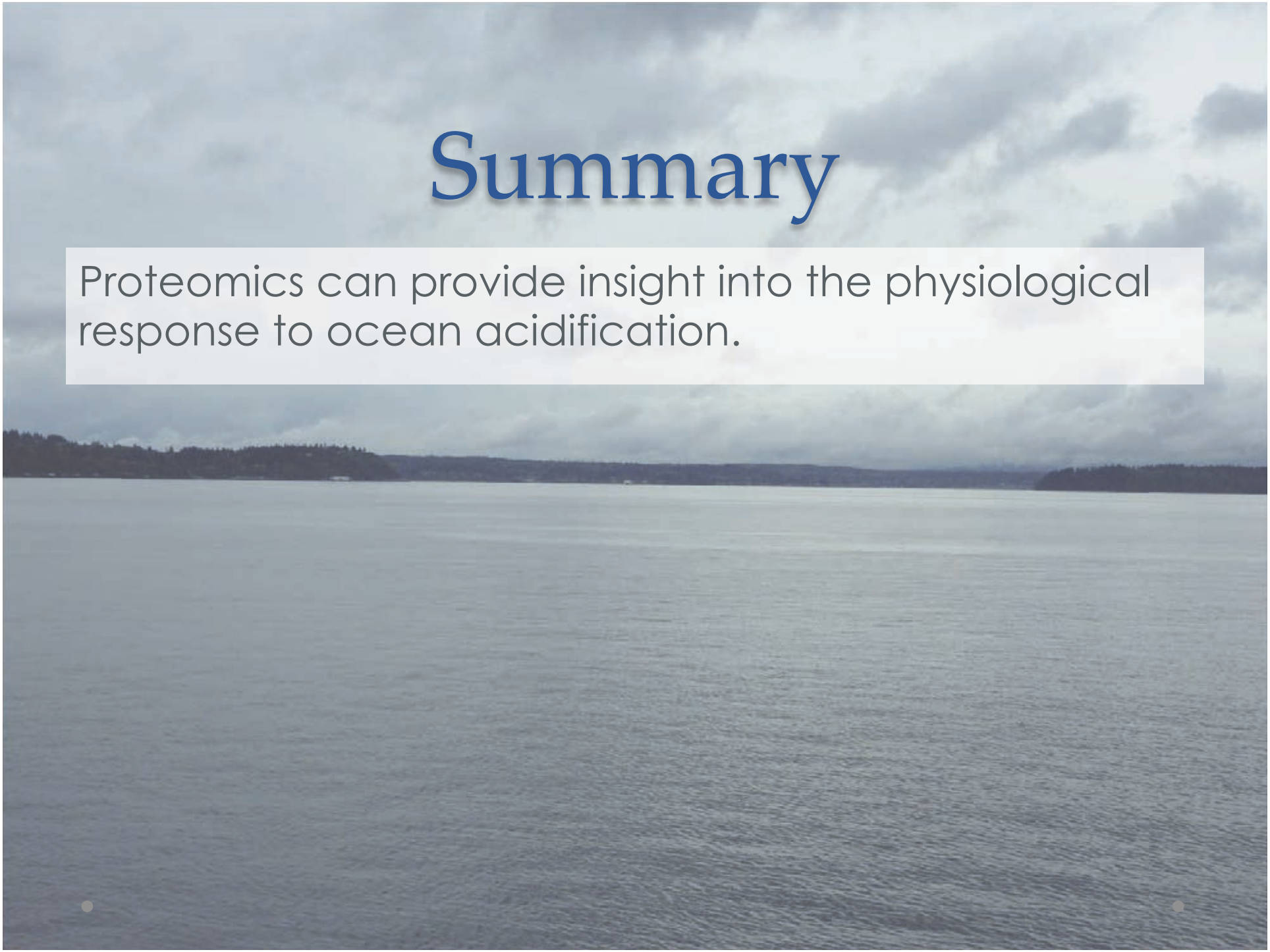

response to wounding
amino acid activation
amine transport
tRNA aminoacylation for protein translation
regulation of DNA metabolism
ectoderm development
regulation of binding
6
5
4
3
2
1
0
-1
-2
-3
-4
-5
-6
-4
-3
-2
-1
0
1
2
3
4
5
6

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



Summary

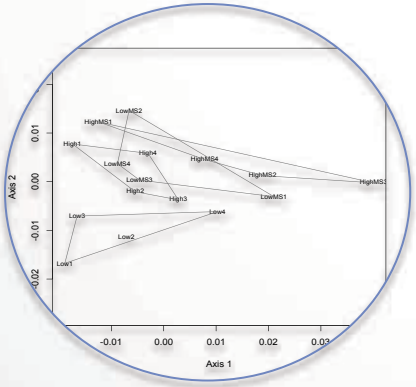
Proteomics can provide insight into the physiological response to ocean acidification.

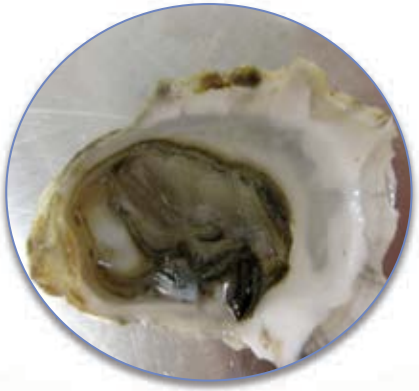
Summary

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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