



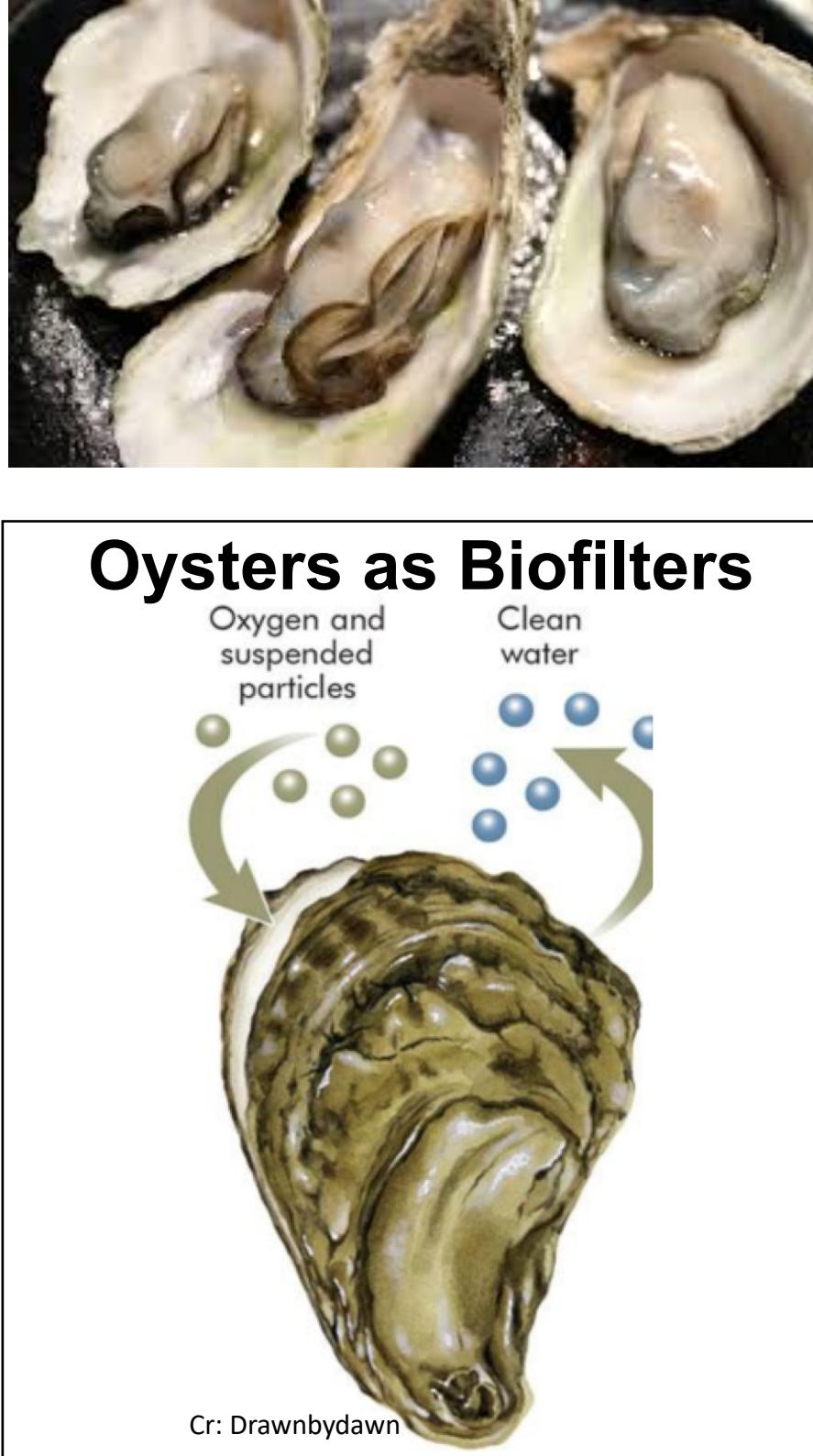
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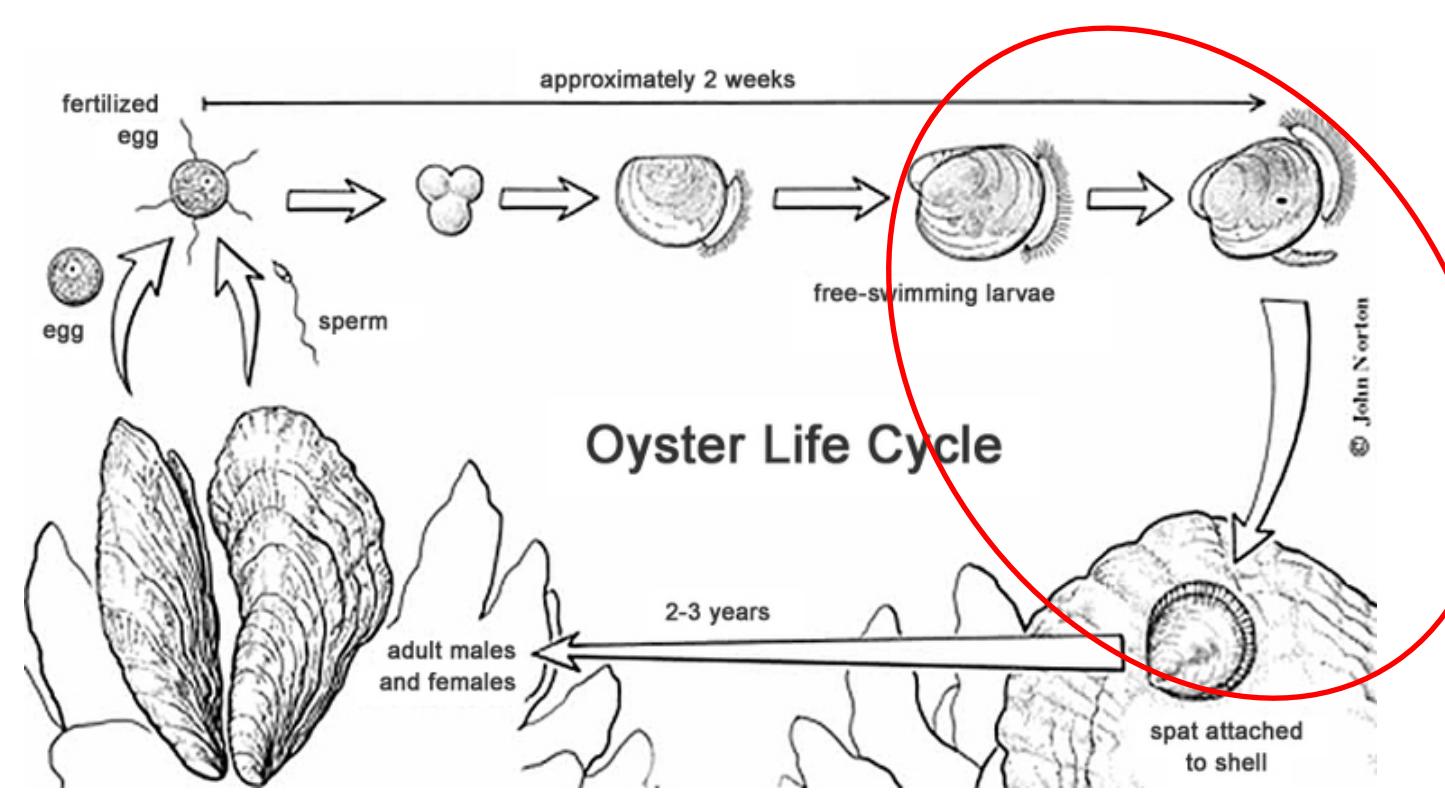
<sup>3</sup>Department of Genome Sciences, University of Washington, Seattle, Washington, USA

## BACKGROUND

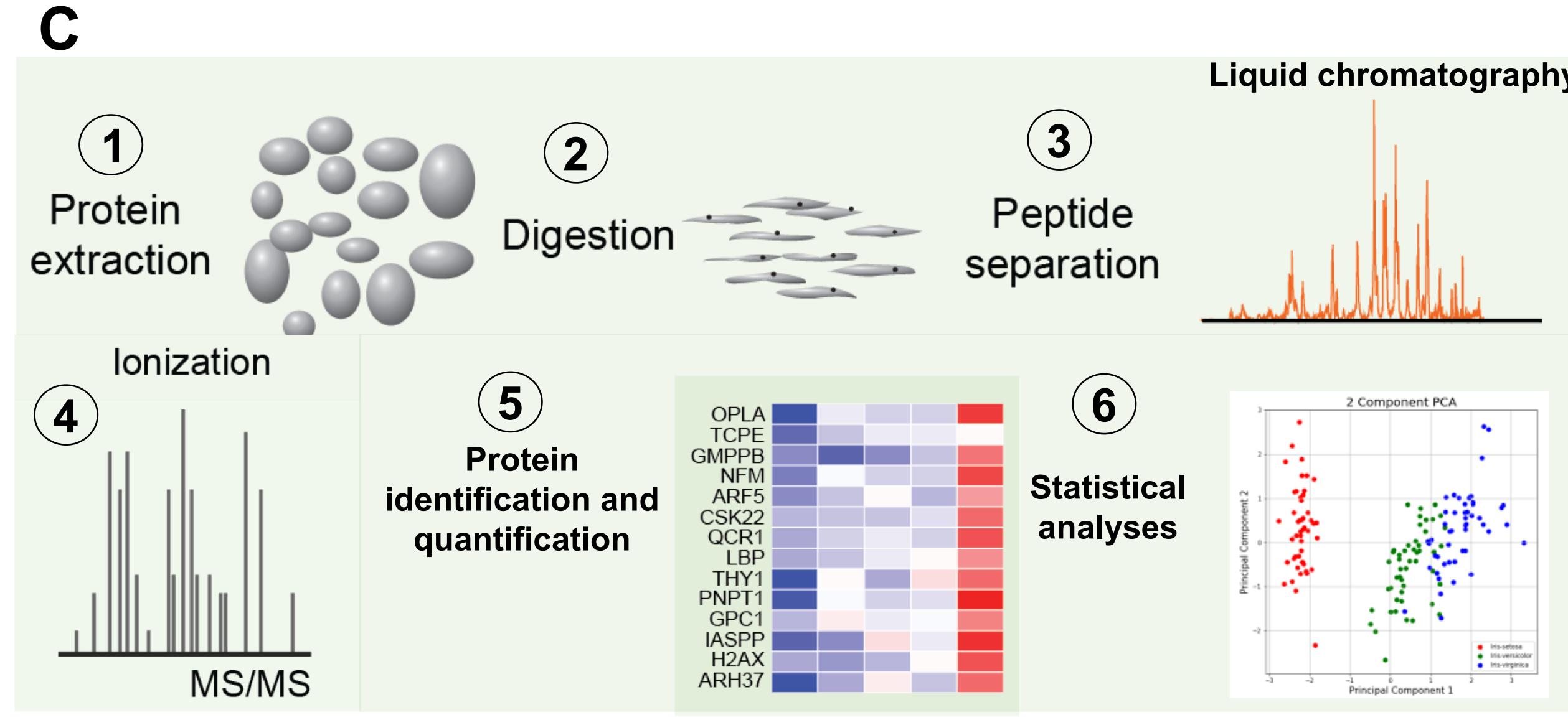
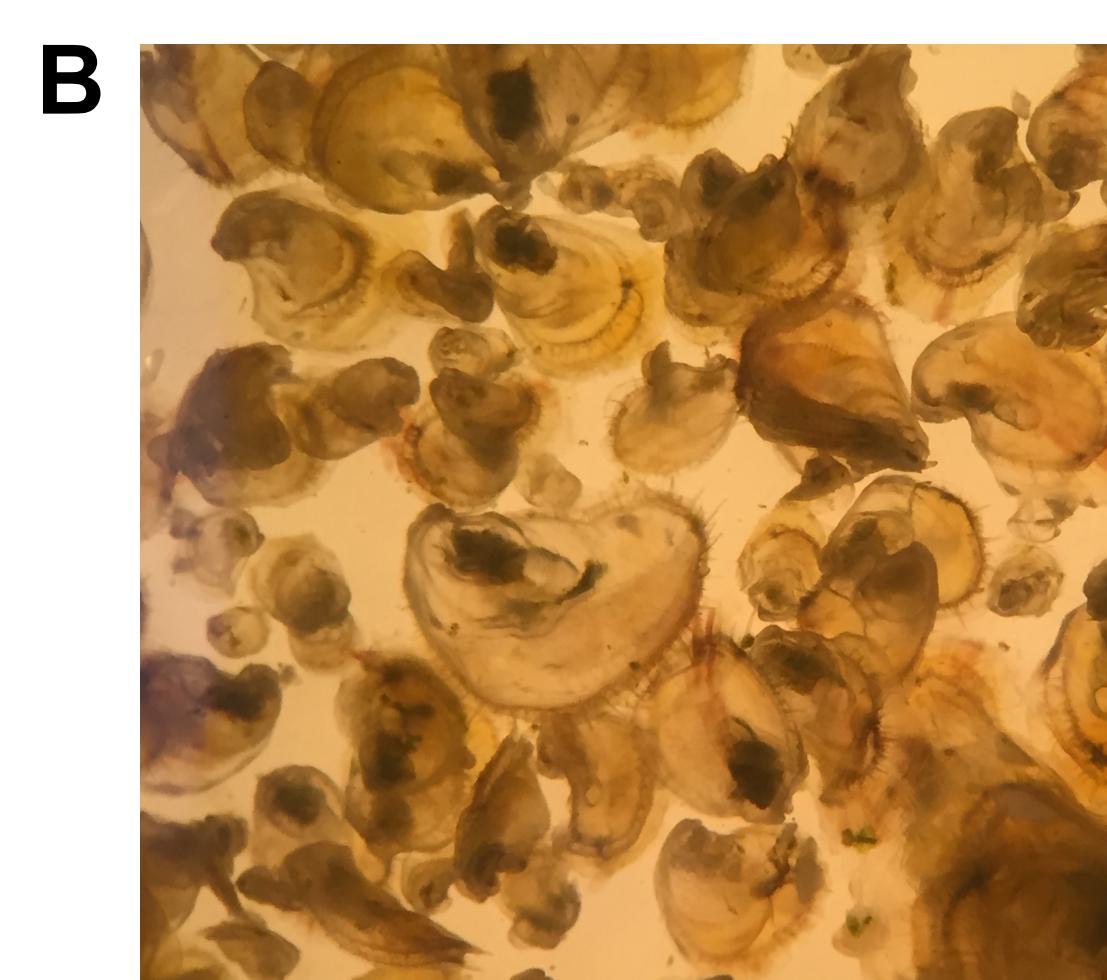
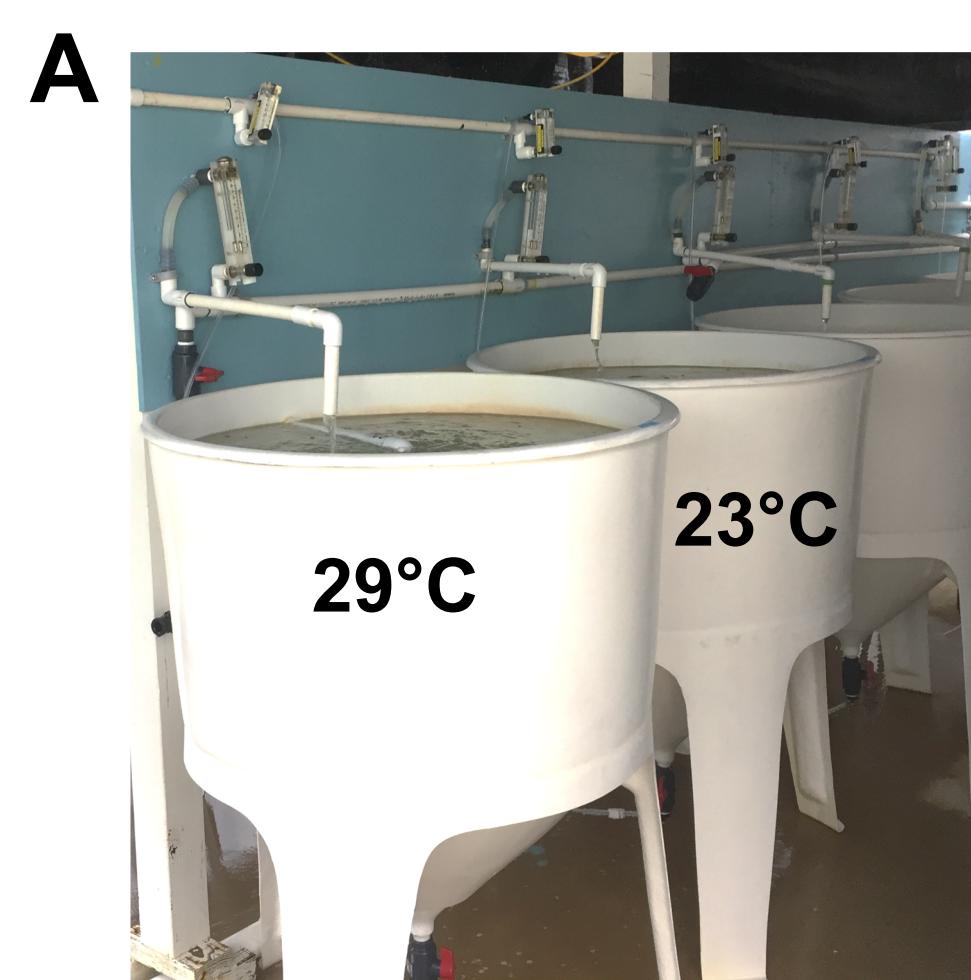
- Pacific oyster is an economically and ecologically important species distributed along the North American Pacific Coast.
- Larval sensitivity to environmental conditions can lead to mass mortality, and negative impacts on industry and ecosystems
- Past experiments have shown reduced mass mortality in larvae reared in 27°C-29°C compared to 23°C seawater<sup>1,2</sup>, but the biological processes underlying this are not clear.
- It is not known how the landscape protein expression changes during larval development or how temperature affects it.
- Proteomics can be used to characterize biological processes potentially affected by temperature<sup>4,5</sup>.



## EXPERIMENTAL METHODS



**Summary of experimental procedures.** A) 1 M larvae from Puget Sound were reared in a hatchery setting in 200-L tanks with flowing filtered seawater at either 23°C or 29°C for 13 days. B) Pooled samples (~25 larvae/pool) were collected every other day for a total of 6 time points and flash frozen at -80°C for proteomics analysis. C) Proteomics workflow; LC-MS/MS data-dependent acquisition was performed at the University of Washington Proteomics Resource.



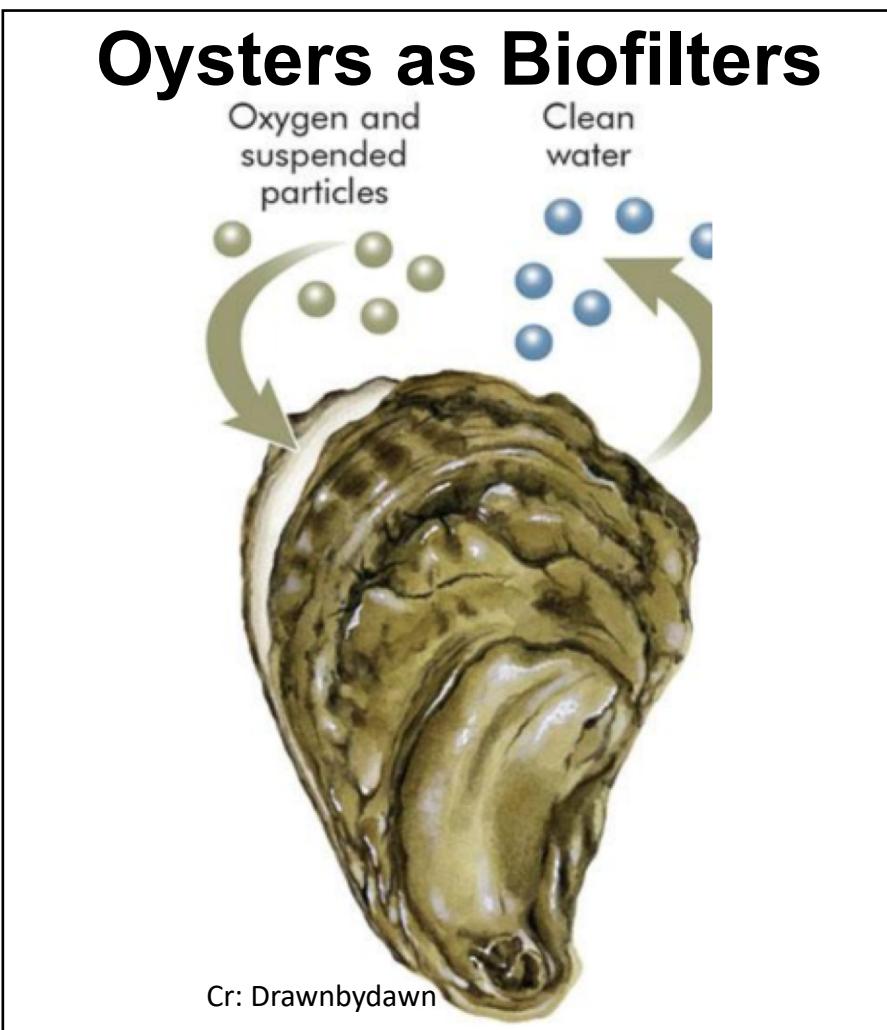
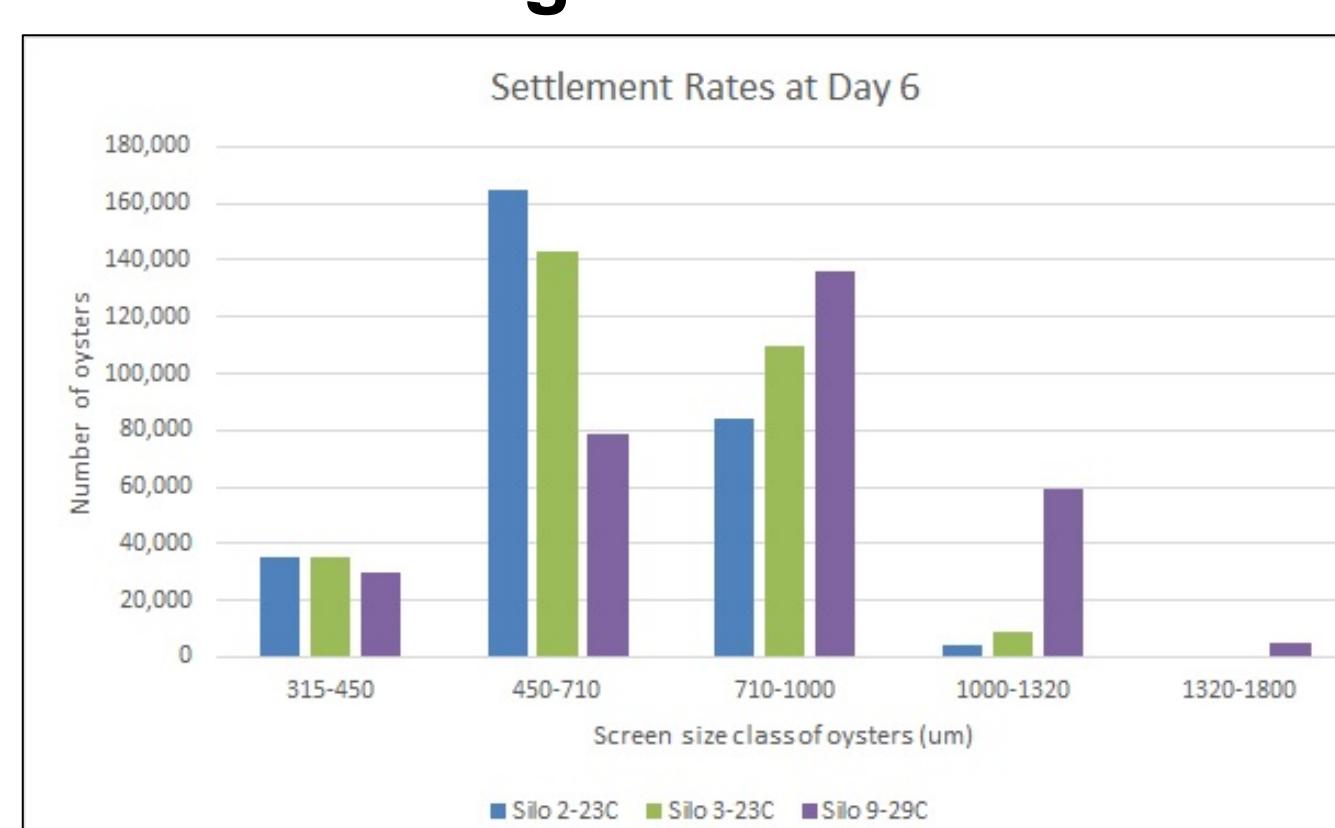
## ACKNOWLEDGEMENTS AND REFERENCES

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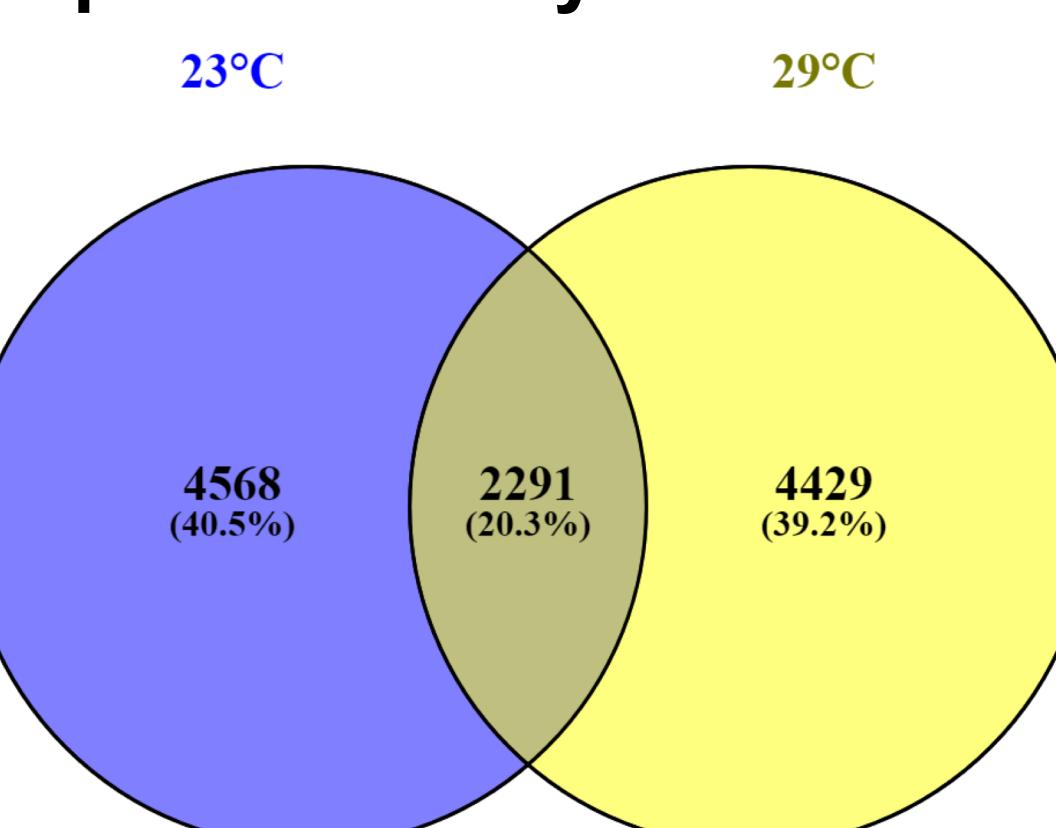
1. Fisheries of the United States 2017. National Marine Fisheries Service, National Oceanic and Atmospheric Administration (2018).
2. Kheder and Robert, *Aquaculture* **309**, 286-289 (2010).
3. Rico Villa B., Pouvreau S., and Rene R., *Aquaculture* **287**, 395-401 (2009).
4. Lopez, CE et al. *Biology Open* **6**, 943-955 (2017).
5. Timmins-Schiffman E, Nunn BL, Goodlett DR, and Roberts SB. *Conservation phys.* (2013).

## RESULTS

### Higher temperature gives rise to larger animals



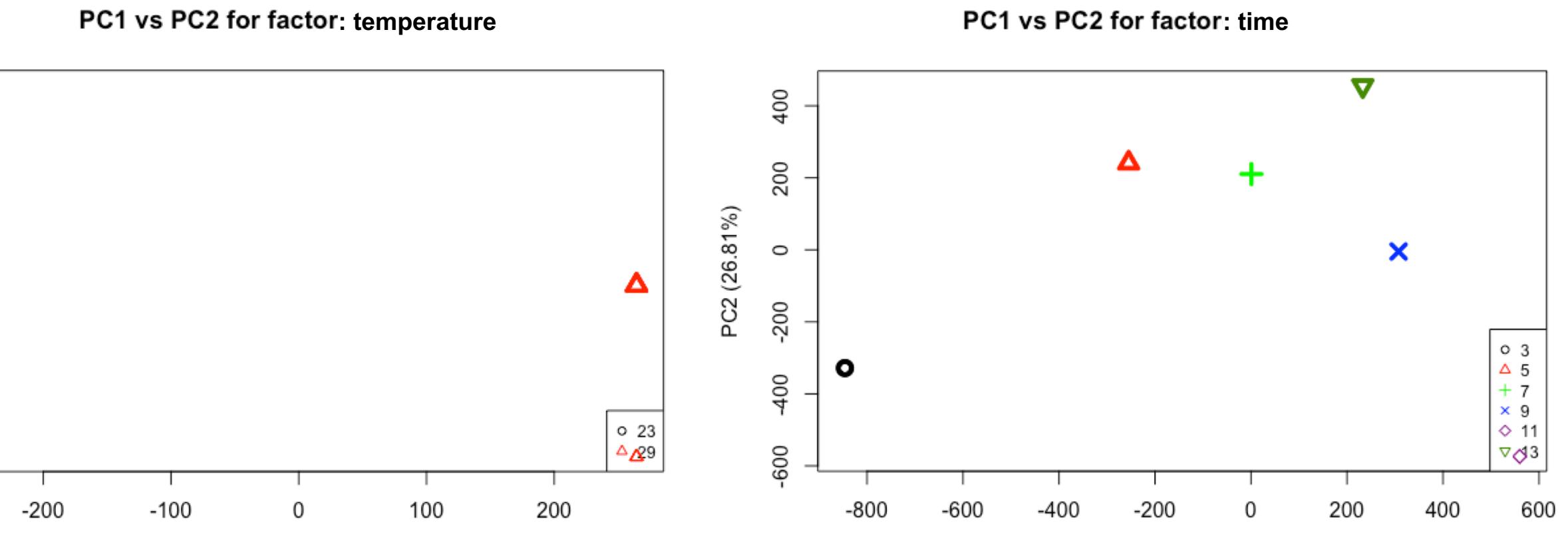
### Proteomics detects 11288 proteins in oyster larvae



After 6 days of temperature conditioning, there were no differences in survival between 23°C and 29°C, but animals reared at 29°C measured larger than those reared at 23°C.

Across all time points, 6859 and 6720 proteins were detected in 23°C and 29°C treated larvae pool samples, respectively.

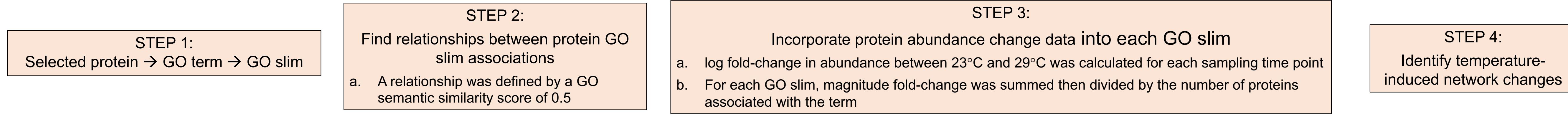
### PCAs show separation of temperature conditions and developmental time points



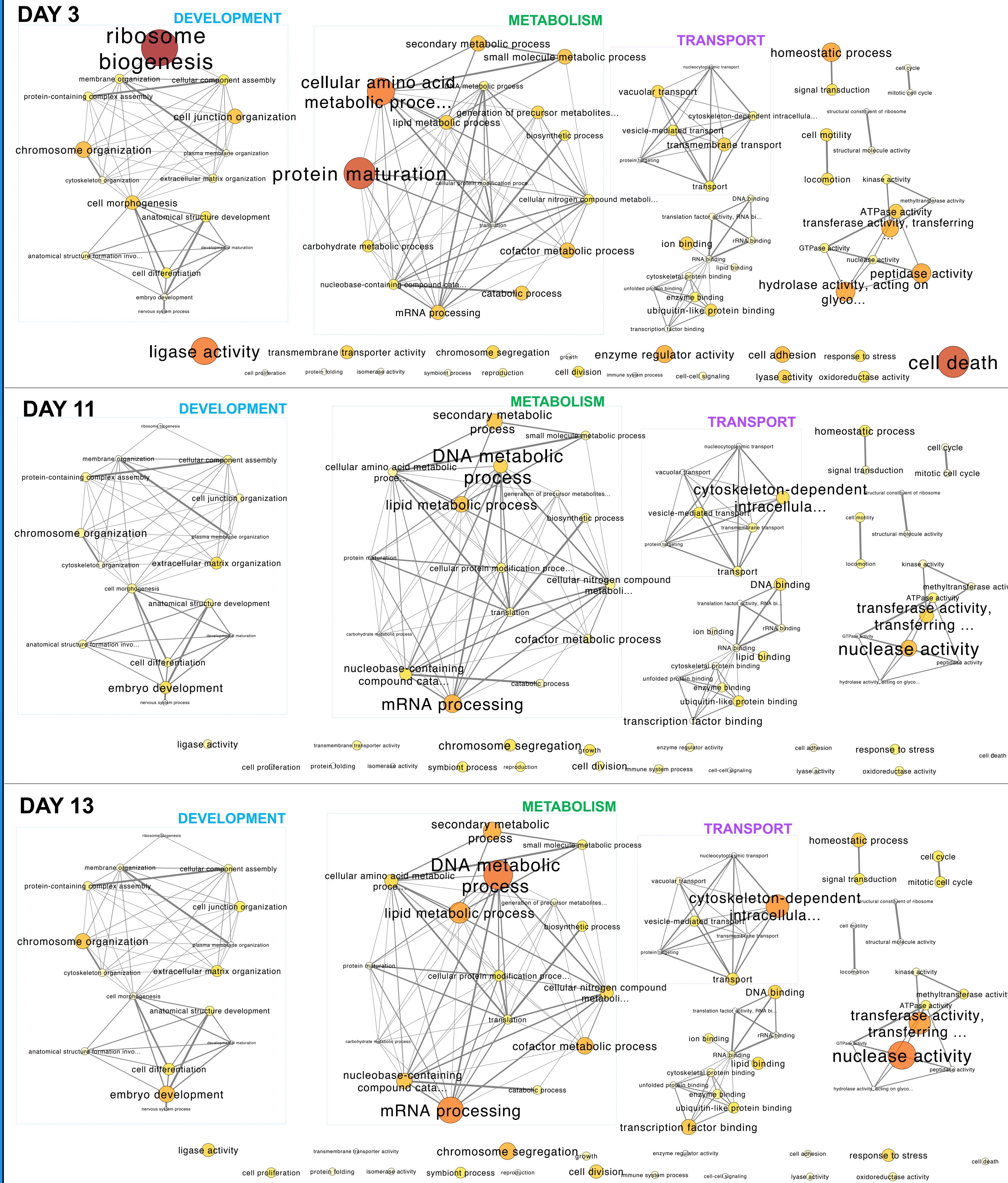
PCAs derived from ANOVA-simultaneous component analysis show that samples separate into groups for temperature and time submodels. 100% variation can be explained by PC1 in the temperature sub-model, so PC1 loadings values were used to assign initial ranks to proteins.

153 proteins showed a significant difference (FDR-corrected  $P$  value  $< 0.1$ ) in abundance between temperatures at given time point by a Chi-square proportions test. Collectively, ASCA ranked proteins (PC1 loadings value cutoff  $> 0.025$ ) and proportions test identified 263 proteins of which 132 mapped to Uniprot accessions at an e-value cutoff of 10e-10. Because proteins mapped to multiple species, we looked to GO annotations from Uniprot to explore relationships among proteins rather than physical protein interactions.

### Pipeline for exploring protein relationships through shared GO terms



### Temperature-influenced network differences



## DISCUSSION

In oyster larvae reared at higher temperature:

- alterations in ribosome biogenesis, protein maturation, amino acid metabolism, and decreased cell death associated protein abundance occur in early development
- alterations in DNA and lipid metabolic processes, mRNA processing, nuclease and transerase activity, and cytoskeleton-dependent transport occur in late development
- increases in larvae size can be attributed the alterations of these processes during specific developmental time points

While no difference in survival was observed in animals conditioned for 6 days at 23°C and 29°C:

- biological processes molecular functions altered in larvae reared at a higher temperature may render the animals less susceptible to mass-mortality events.
- altered processes under higher rearing temperature may override and/or prevent other processes that get tripped off in normal rearing conditions