Quantitative analysis of oyster larval proteome provides new insights into the effects of multiple climate change stressors
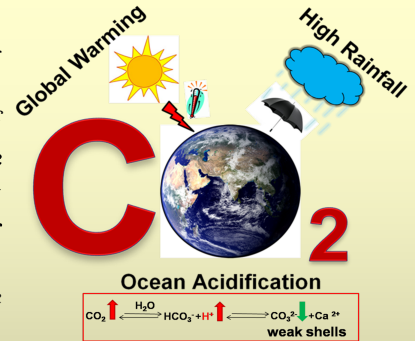
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Outline

The **metamorphosis** of planktonic larvae of the **Pacific oyster (*Crassostrea gigas*)** underpins their complex life-history strategy by switching on the molecular machinery required for sessile life and building calcite shells. The use of **quantitative proteomics** in conjunction with the annotated genome of the *C. gigas* has allowed us to construct a detailed proteomic profile of oyster larvae under **multiple climate change stressors: decreased pH (pH 7.4), increased temperature (30°C),** and **reduced salinity (15 psu)**, which enabled us to make predictions about the differential susceptibility of **molecular pathways** and their importance in determining an individual's physiological response to stress. In this study, we investigated multiple stressors on the **Pacific oyster (*C. gigas*) as a model** to address the following questions.

How does the larval proteome respond to multiple stressors?

Do multiple stressors act independently or synergistically on the proteome?

Global Warming · High Rainfall

Ocean Acidification

$CO_2 \uparrow \xrightarrow{H_2O} HCO_3^- + H^+ \uparrow \rightleftharpoons CO_3^{2-} \downarrow + Ca^{2+}$ weak shells
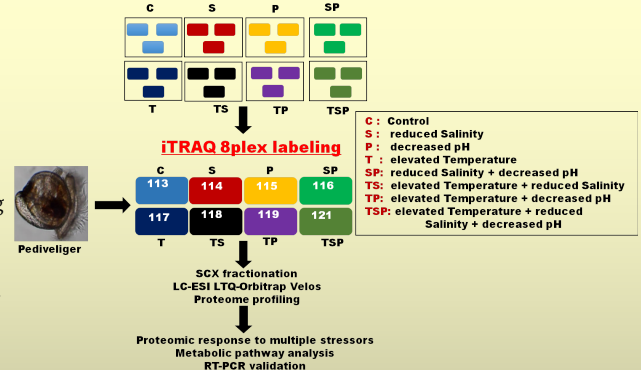
Methods

Experimental Design: The fertilized embryos were raised under different climate change stressor conditions in 50 L plastic culture tanks with biological replicates

iTRAQ labeling: Proteomics samples were collected from the pediveliger larvae from each treatment were digested, desalted prior to 8-plex iTRAQ labeling

LC MS/MS analysis and protein identification: The SCX fractionated peptides were analyzed using LTQ_Orbitrap Velos. Protein identification was carried out using Sequest HT search algorithm against 27900 *Cgigas* annotated protein sequences
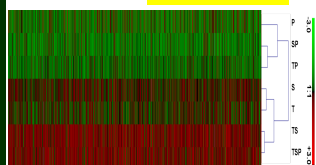
Metabolic pathway analysis: The differentially expressed proteins were subjected to protein- protein interaction analysis using Cytoscape 3.0 ClueGO to identify pathways contributing to oyster stress and resilience
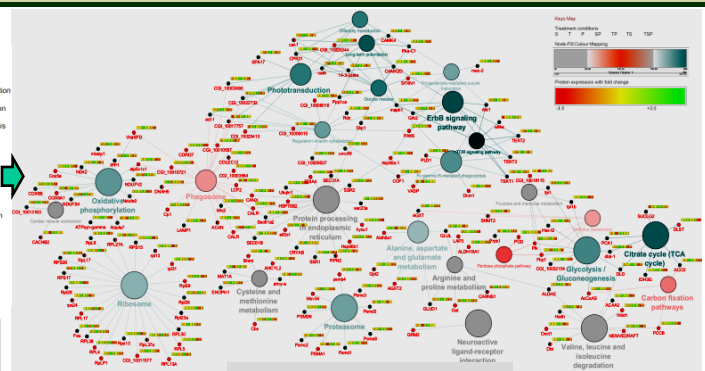
Multiple climate change stressors

C   S   P   SP
T   TS   TP   TSP

iTRAQ 8plex labeling

| C 113 | S 114 | P 115 | SP 116 |
| T 117 | TS 118 | TP 119 | TSP 121 |

Pediveliger

C : Control
S : reduced Salinity
P : decreased pH
T : elevated Temperature
SP : reduced Salinity + decreased pH
TS : elevated Temperature + reduced Salinity
TP : elevated Temperature + decreased pH
TSP : elevated Temperature + reduced Salinity + decreased pH

SCX fractionation
LC-ESI LTQ-Orbitrap Velos
Proteome profiling

Proteomic response to multiple stressors
Metabolic pathway analysis
RT-PCR validation

Results

1300 proteins

Hierarchical clustering shows **down-regulation** of P, SP and TP and **up-regulation** of TS and TSP treatments

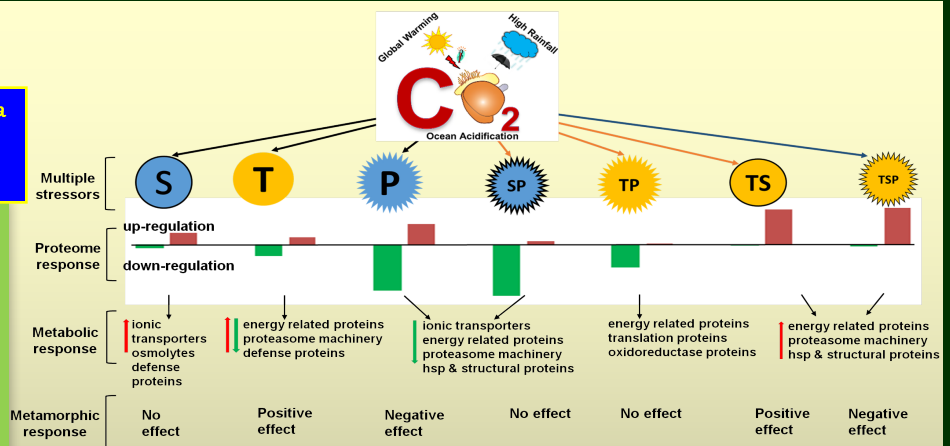Decreased pH **severely** affected cellular and metabolic processes

Metabolic pathway response

Conclusion

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Ocean Acidification

The proteome of *C. gigas* larvae from Yellow Sea show phenotypic plasticity to survive multiple climate stressors, but with physiological costs

Proteomics cum bioinformatics analysis revealed that multiple stress impairs protein processing, cell signaling, energy production, growth and development and induces amino acid, fatty acid and nucleotide metabolism as an alternative energy sources to compensate the energy requirements during multiple stresses

	S	T	P	SP	TP	TS	TSP
Multiple stressors	S	T	P	SP	TP	TS	TSP
Proteome response	up-regulation / down-regulation						
Metabolic response	ionic transporters osmolytes defense proteins	energy related proteins proteasome machinery defense proteins	ionic transporters energy related proteins proteasome machinery hsp & structural proteins		energy related proteins translation proteins oxidoreductase proteins		energy related proteins proteasome machinery hsp & structural proteins
Metamorphic response	No effect	Positive effect	Negative effect	No effect	No effect	Positive effect	Negative effect