The Effects of Ocean Acidification on Multiple Life History Stages of the Pacific Oyster, Crassostrea gigas: Implications for Physiological Trade-offs

A dissertation defense by Emma Timmins-Schiffman
February 5, 2014

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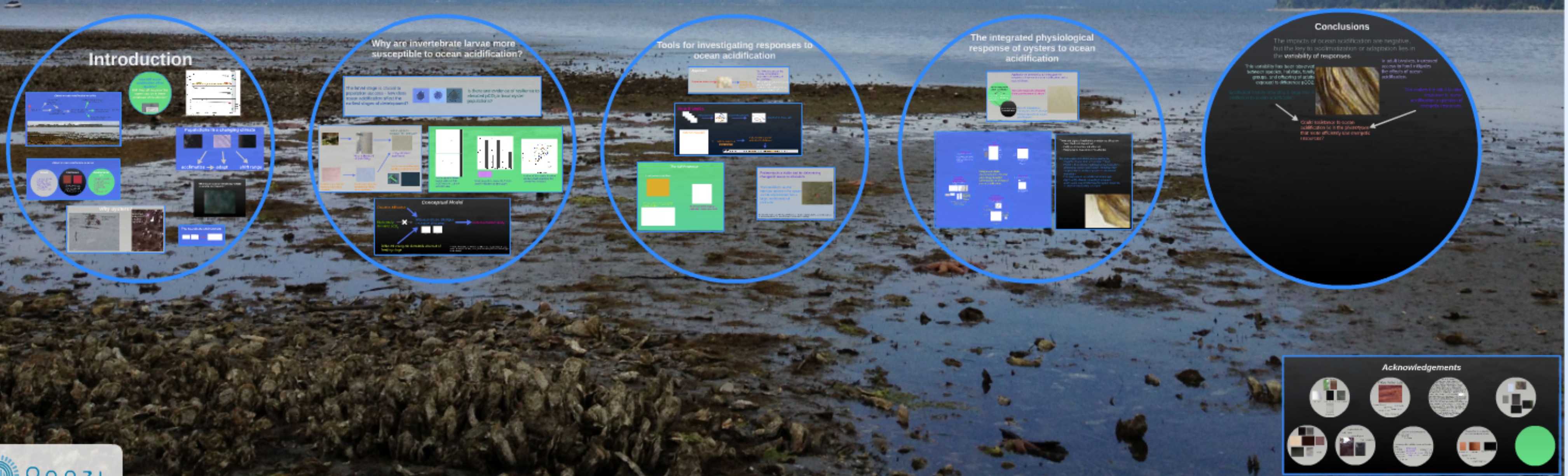
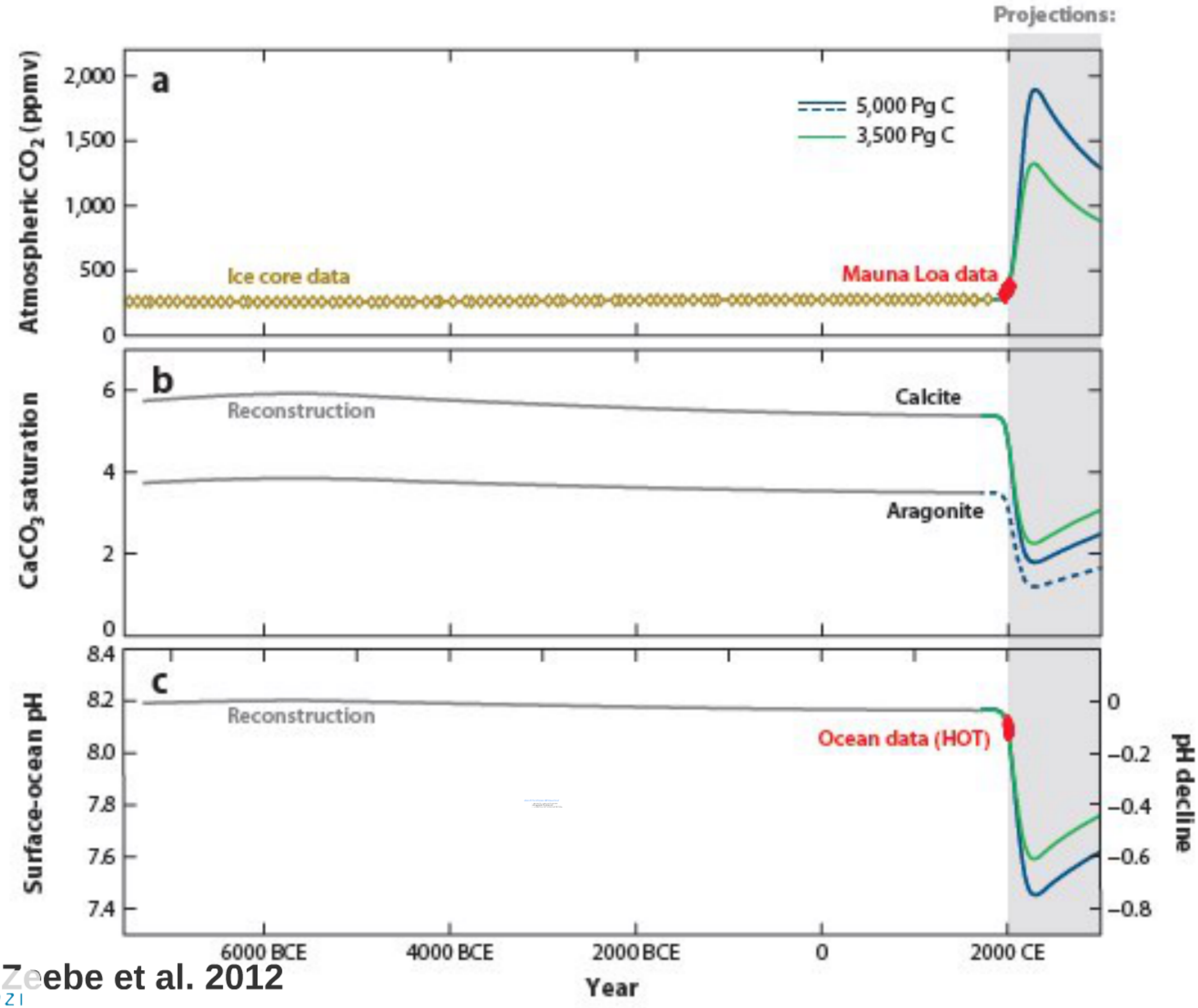



Introduction
How will ocean acidification affect oysters?
Will they all respond the same way or is there evidence of resilience?
Effects of ocean acidification on adults
CO_2 & H^+
decrease pH of body fluids
e.g. Thomsen et al. 2013
calcification more difficult
e.g. Michaelidis et al. 2005
change allocation of energy resources
depleted energy storage
e.g. Dickinson et al. 2012
shift in which resources used
e.g. Lannig et al. 2010
increased cellular stress
e.g. Tomanek et al. 2011
Zeebe et al. 2012
Populations in a changing climate
acclimatize
adapt
shift range
Effects of ocean acidification on larvae
Growth
Invertebrate larvae at low pH are frequently smaller than ambient pH counterparts.
e.g. Kurihara et al. 2007
Calcification
Calcifying compartment may be difficult to isolate or may require relatively more carbonate
e.g. Waldbusser et al. 2013
Developmental rate
Energetic constraints from ocean acidification stress may lead to developmental delay.
e.g. Stumpp et al. 2011
What about species that already inhabit a variable environment?
Why oysters?
Ecology:
• sessile
• filter feeders
• bioaccumulate
• many molecular tools
• improve water quality
Economics: molluscs = 75.5% of marine aquaculture
The nearshore environment
Prezi

How will ocean acidification affect oysters?

Will they all respond the same way or is there evidence of resilience?

- Background on ocean acidification
- Why are invertebrate larvae more susceptible to ocean acidification?
- Tools for investigating responses to ocean acidification
- The integrated physiological response of oysters to ocean acidification



Zeebe et al. 2012

Ocean Chemistry Changes with Increased pCO2

- Hydrogen ion concentration goes up
- pH goes down ("acidification")
- Saturation state of carbonate ion decreases

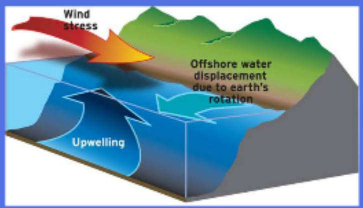
Populations in a changing climate

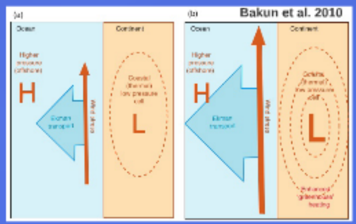
acclimatize → adapt

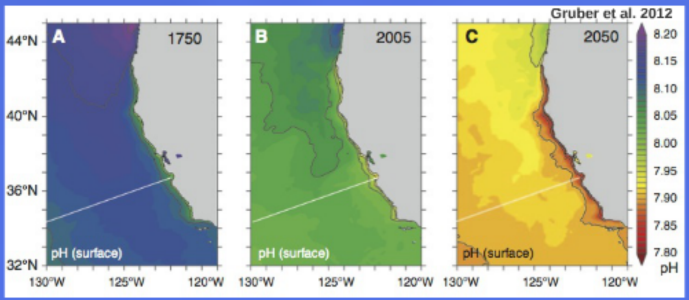
shift range

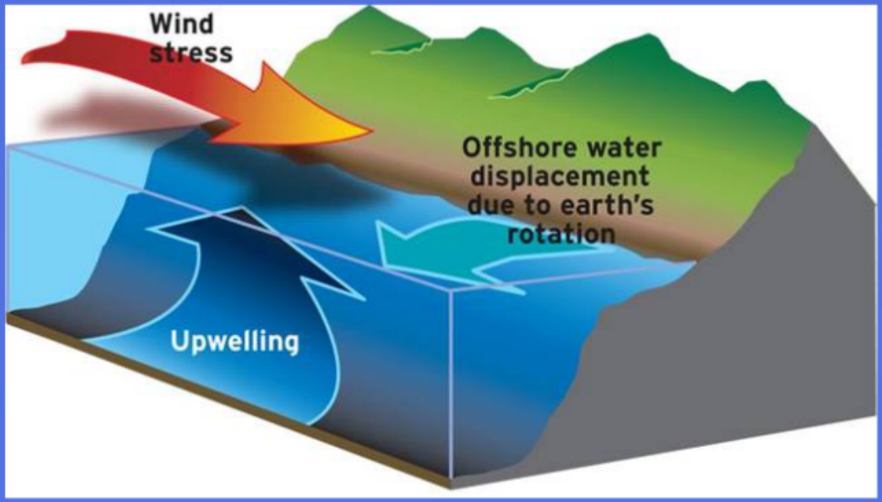
What about species that already inhabit a variable environment?

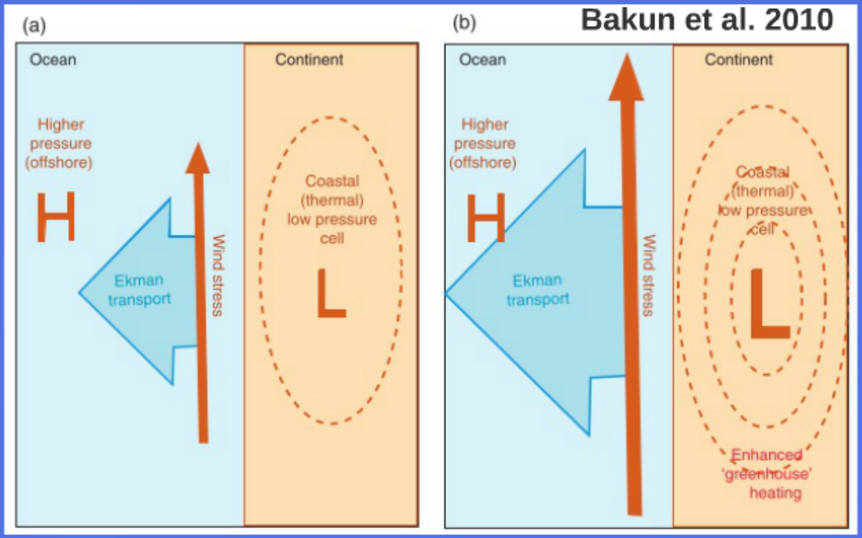
The nearshore environment

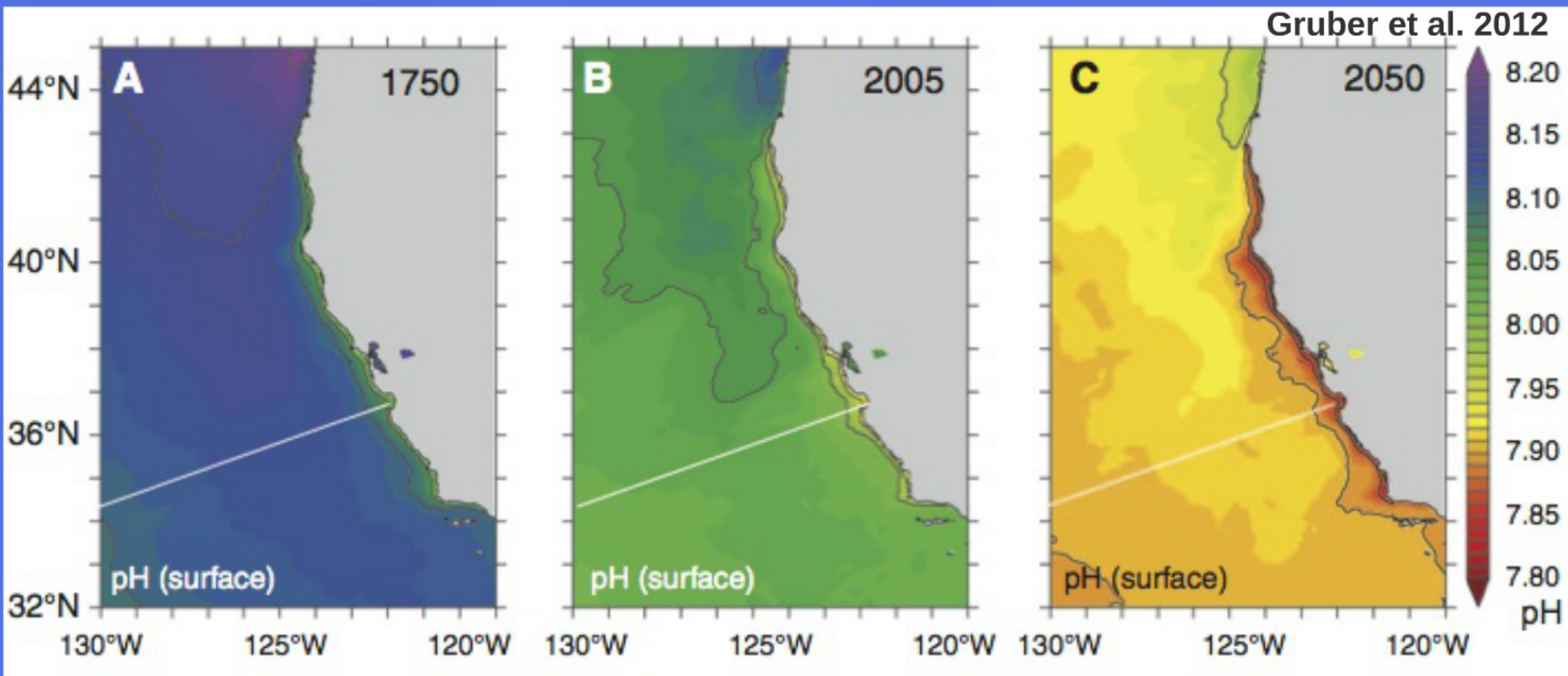







Wind stress
Offshore water displacement due to earth's rotation
Upwelling


Bakun et al. 2010
(a)
Ocean
Continent
Higher pressure (offshore)
H
Ekman transport
Wind stress
Coastal (thermal) low pressure cell
L
(b)
Ocean
Continent
Higher pressure (offshore)
H
Ekman transport
Wind stress
Coastal (thermal) low pressure cell
L
Enhanced 'greenhouse' heating



Gruber et al. 2012
A
1750
B
2005
C
2050
44°N
40°N
36°N
32°N
130°W
125°W
120°W
pH (surface)
8.20
8.15
8.10
8.05
8.00
7.95
7.90
7.85
7.80
pH

Why oysters?


Ecology:
• sessile
• filter feeders
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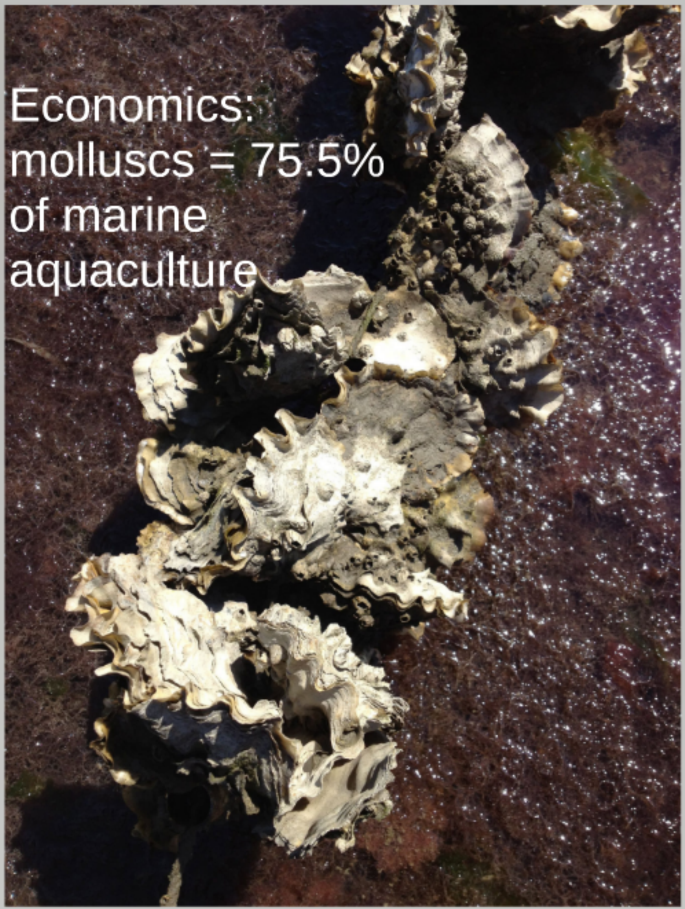
• many molecular tools
• improve water quality


Economics:
molluscs = 75.5% of marine aquaculture

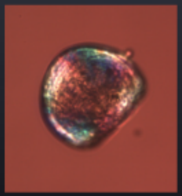
Effects of ocean acidification on larvae

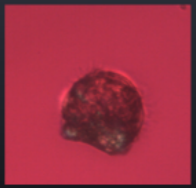
Growth

Invertebrate larvae at low pH are frequently smaller than ambient pH counterparts.

e.g. Kurihara et al. 2007

Calcification





Calcifying compartment may be difficult to isolate or may require relatively more carbonate

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Developmental rate

Energetic constraints from ocean acidification stress may lead to developmental delay.

e.g. Stumpp et al. 2011

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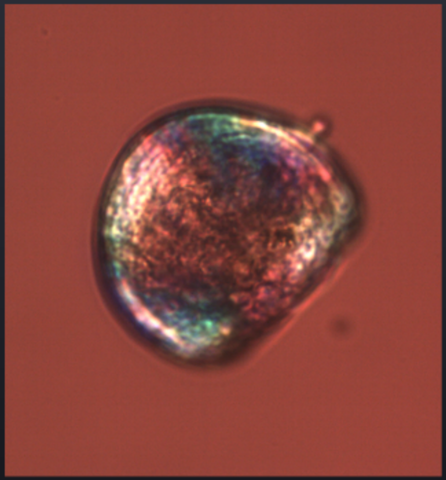

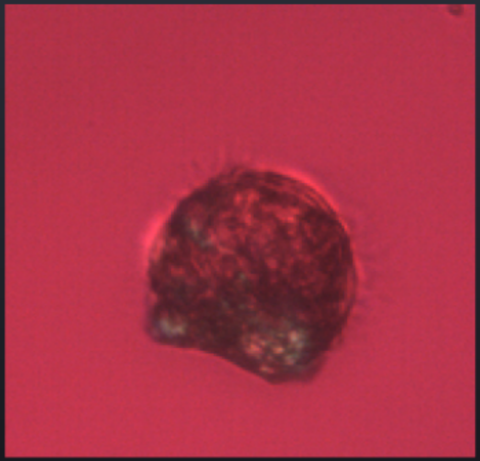

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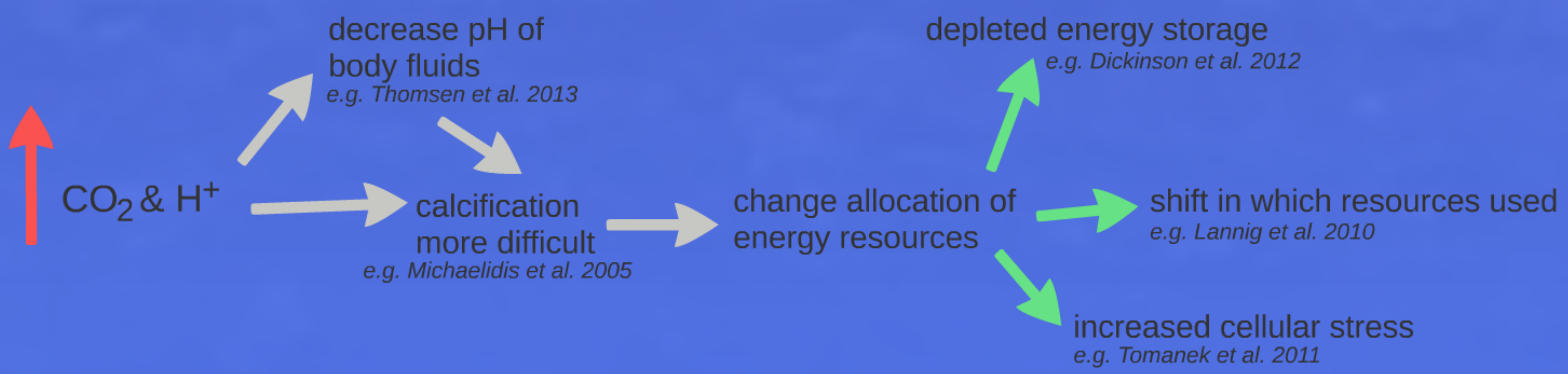
Effects of ocean acidification on adults



Why are invertebrate larvae more susceptible to ocean acidification?
The larval stage is crucial to population success - how does ocean acidification affect the earliest stages of development?
Is there any evidence of resilience to elevated pCO_2 in local oyster populations?
Oysters are collected from nearby natural population
Sperm and eggs are combined to mimic mass spawning
Water is filtered and stripped of CO_2
control: 400 µatm
elevated: 700, 1000 µatm
pCO_2 controlled in each cooler
Larvae are measured and calcified material is scored
Larvae grew between days 1 and 3 at 400 and 700 µatm, but not at 1000 µatm.
Shell deposition slowed by 3 days post-fertilization at 1000 µatm.
Larvae at 1000 µatm followed similar growth trajectory but growth was delayed.
Conceptual Model
Ocean acidification
Moderately elevated pCO_2
Imposes stress, changes resource allocation
Developmental delay
Different energetic demands at onset of feeding stage
For more information, see Timmins-Schiffman et al. (2012) Elevated pCO2 causes developmental delay in early larval Pacific oysters, Crassostrea gigas. Marine Biology.
Prezi

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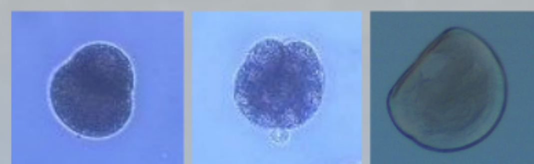

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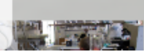

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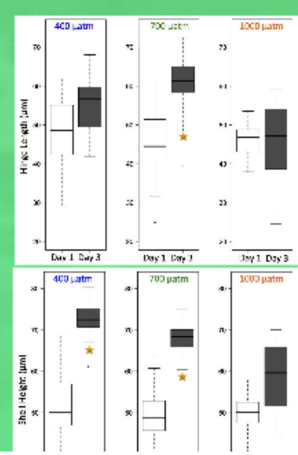

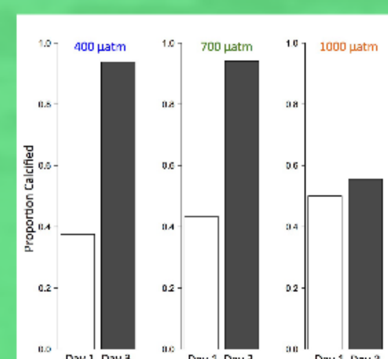

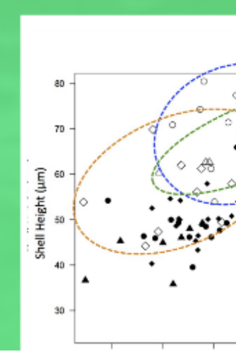

Oysters are collected from nearby natural population

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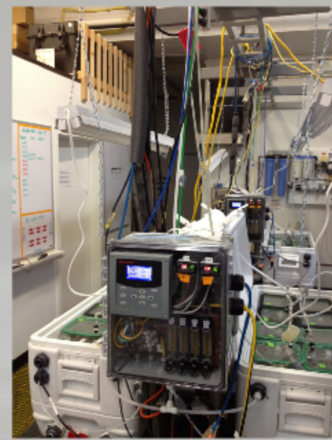

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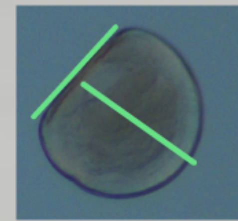

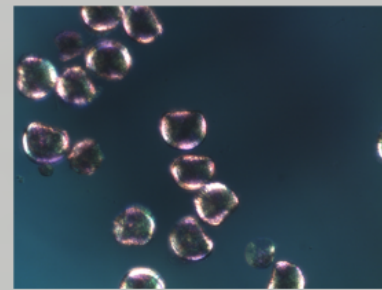

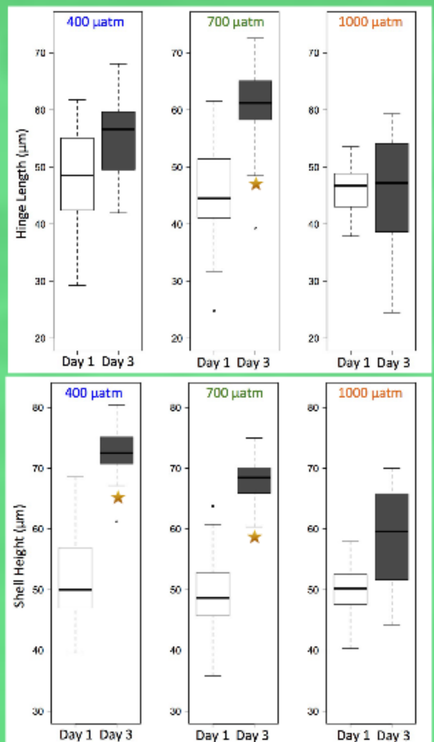

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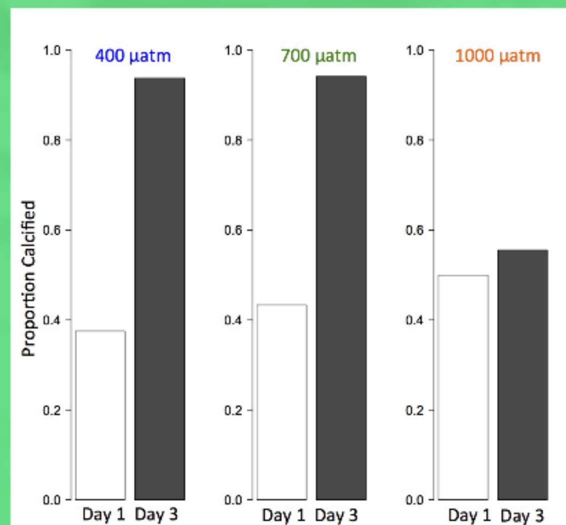
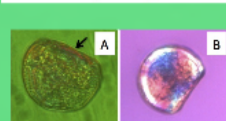
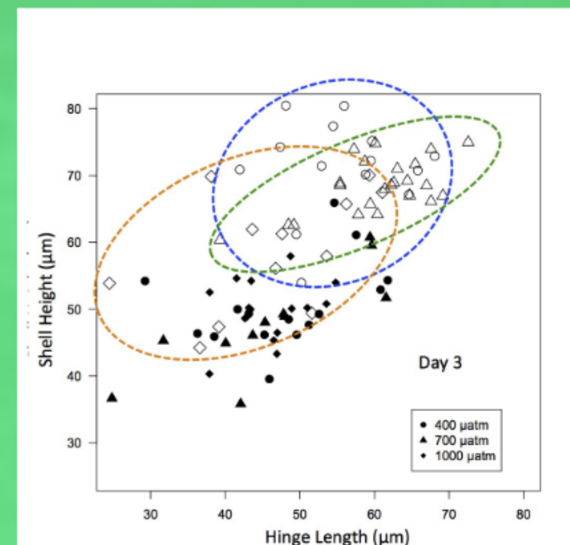




Shell deposition slowed by 3 days post-fertilization at 1000 µatm.



Larvae at 1000 µatm followed similar growth trajectory but growth was delayed.

nceptual Model

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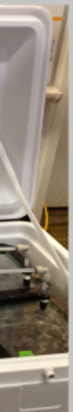
0 µatm



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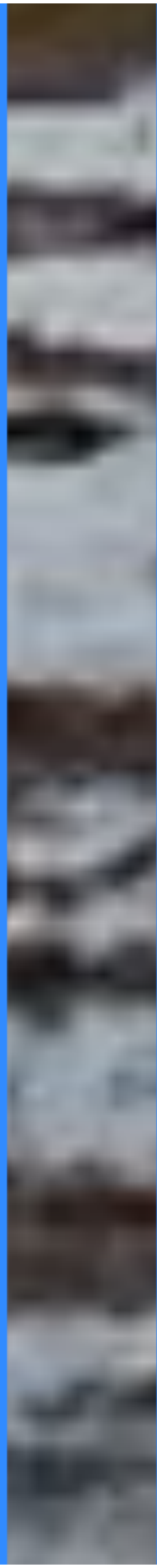
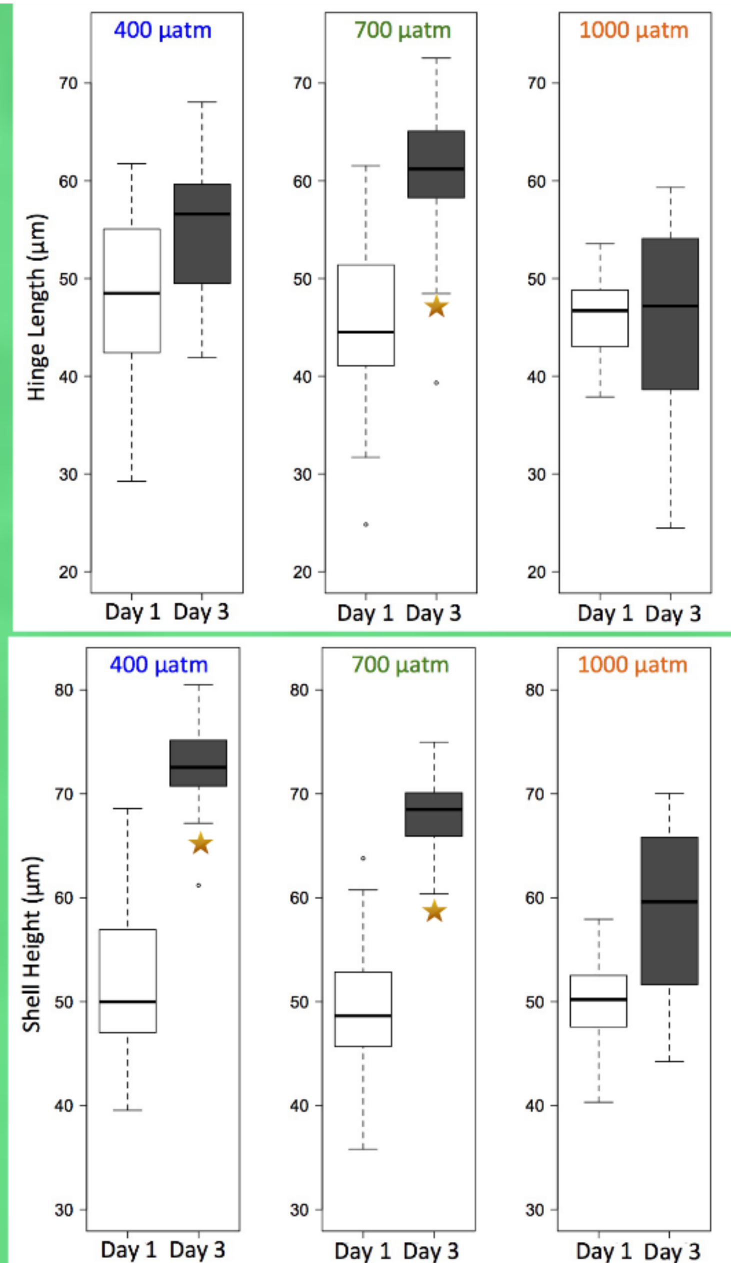
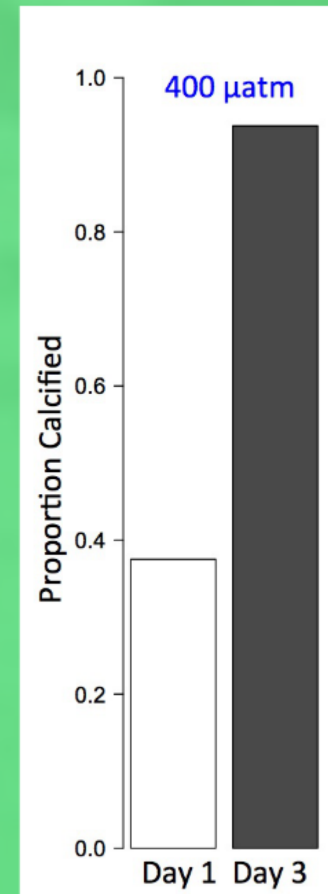
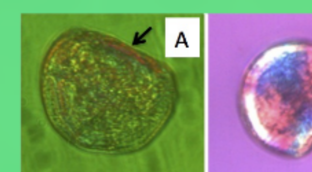


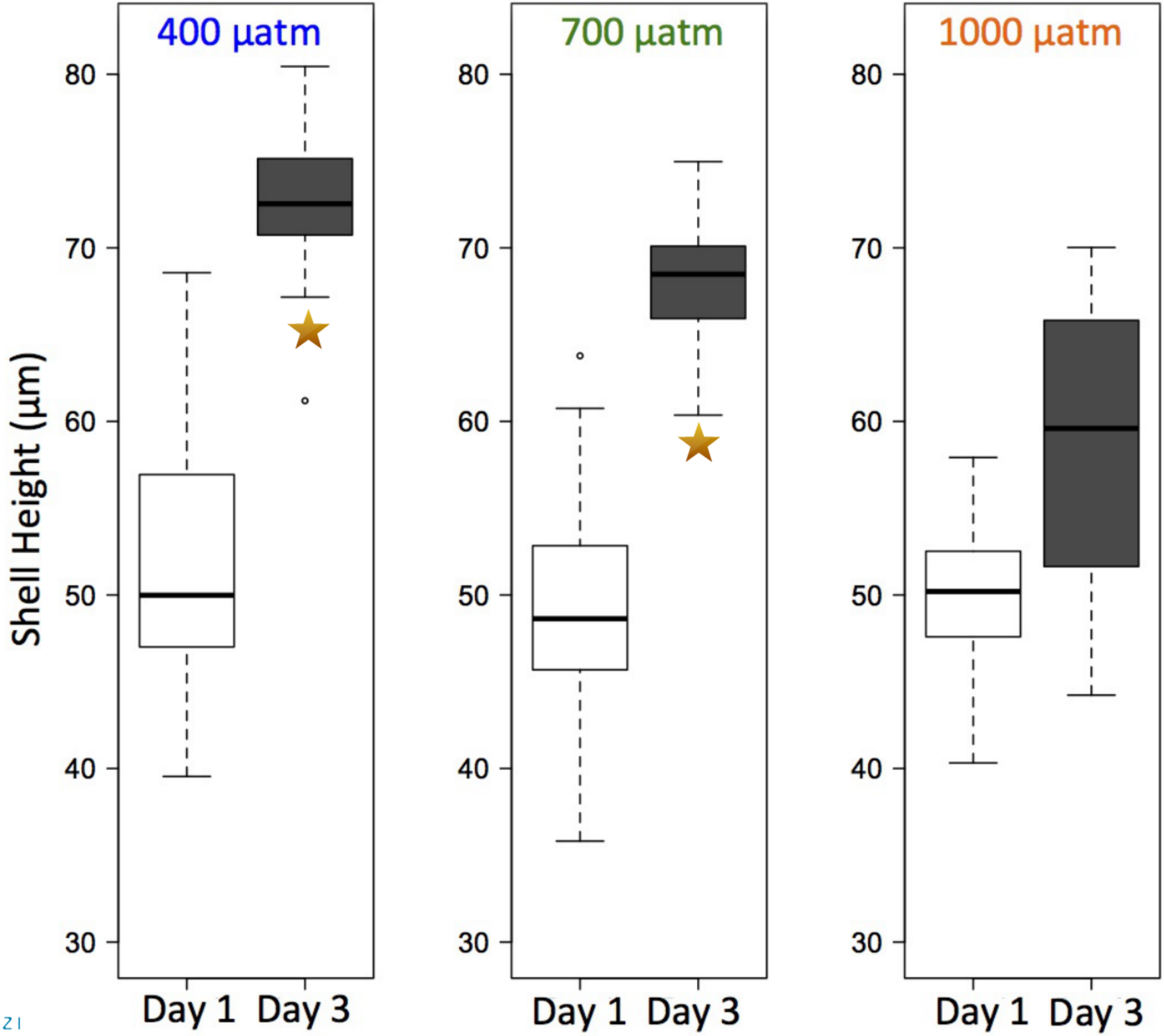


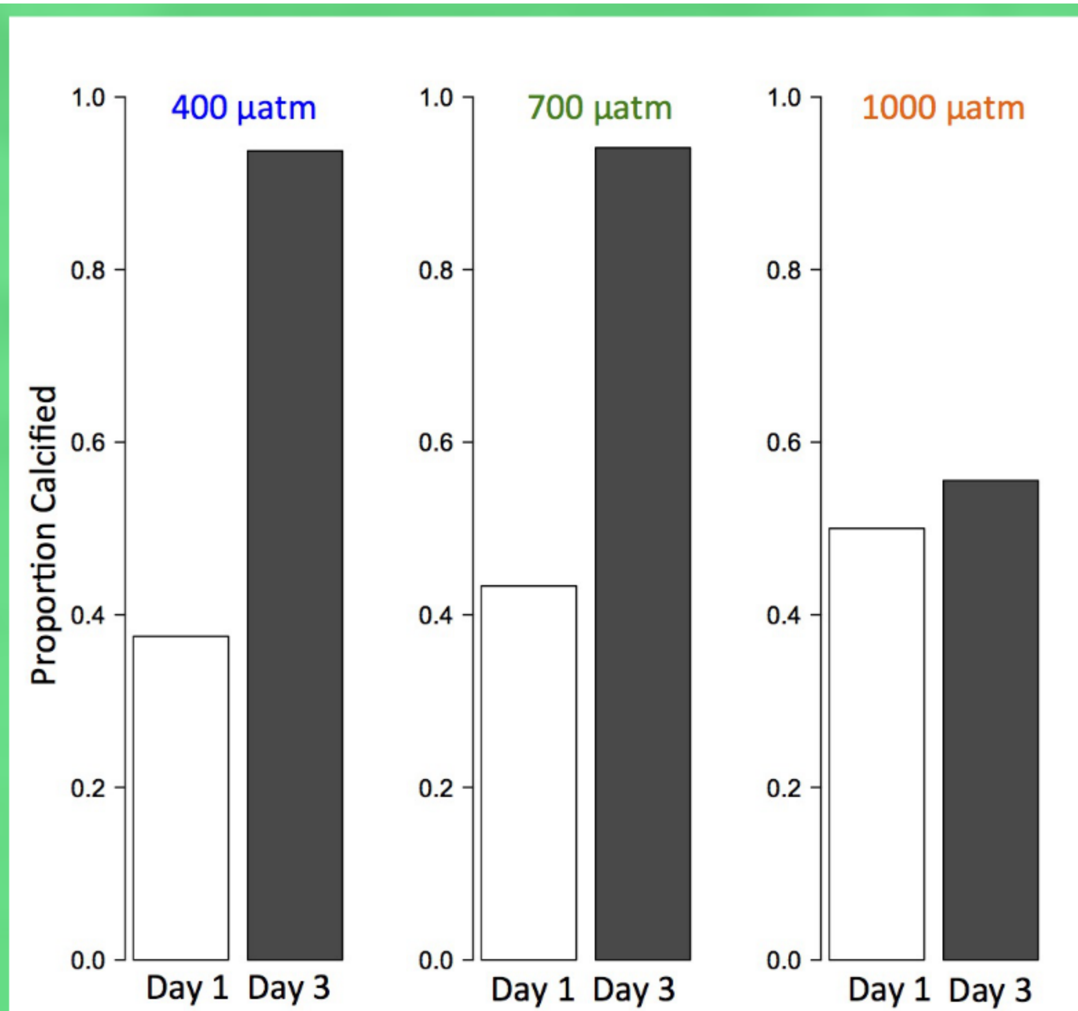
Larvae grew between days 1 and 3 at 400 and 700 µatm, but not at 1000 µatm.

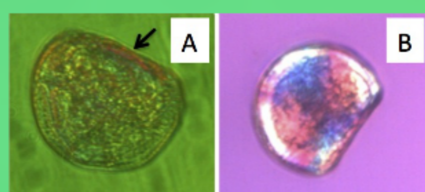


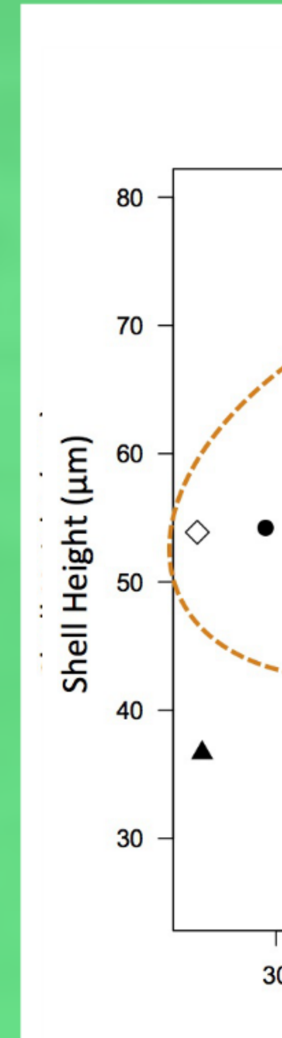


Shell deposit
post-fertilizat


400 µatm
700 µatm
1000 µatm
Shell Height (µm)
80
70
60
50
40
30
Day 1
Day 3



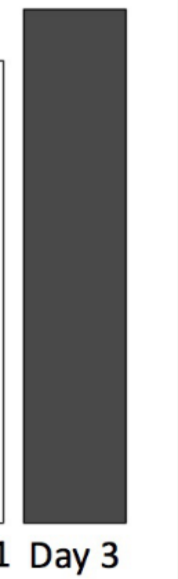


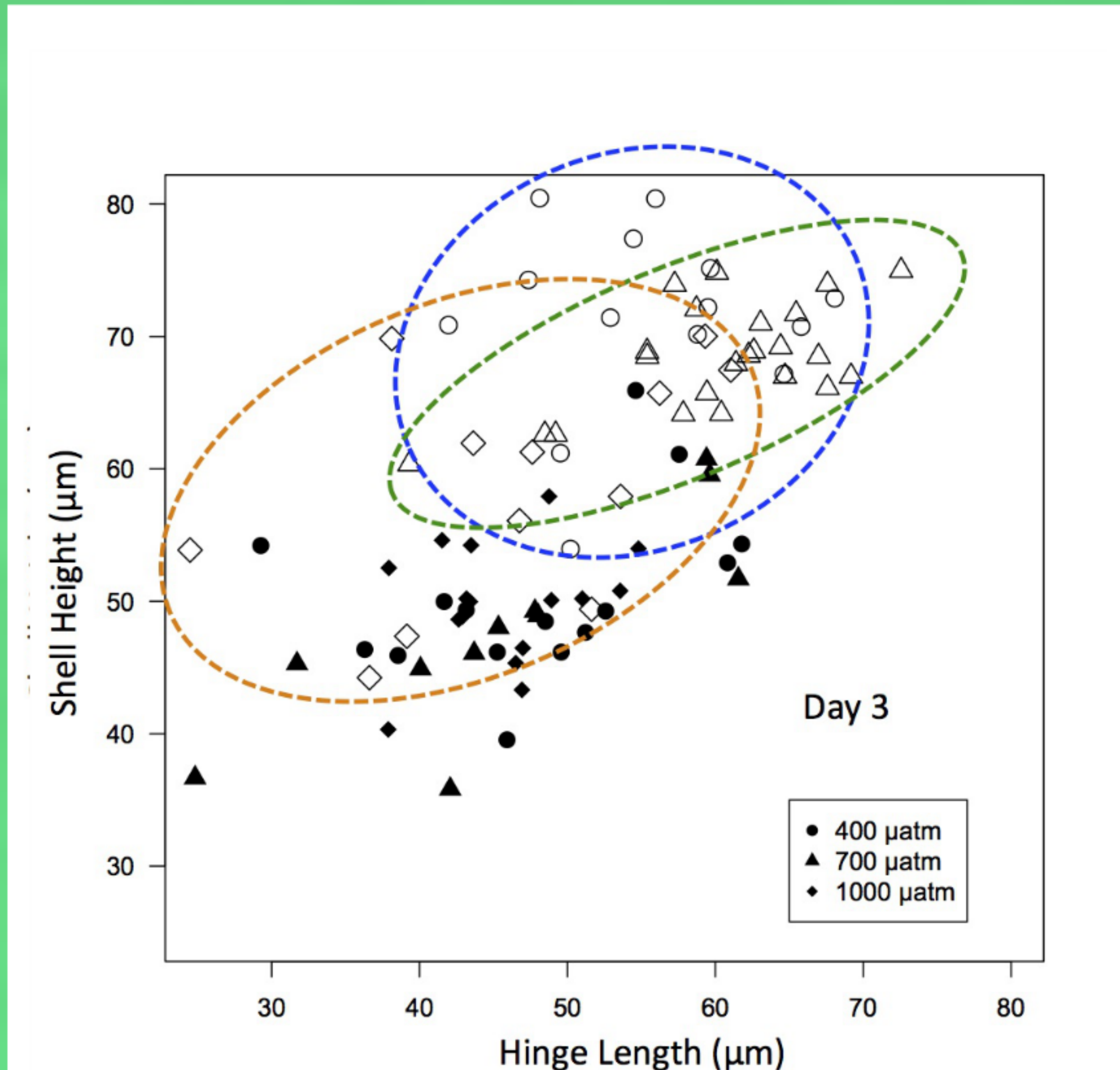


Larva
simila
growt

Shell deposition slowed by 3 days post-fertilization at 1000 µatm.

0 µatm





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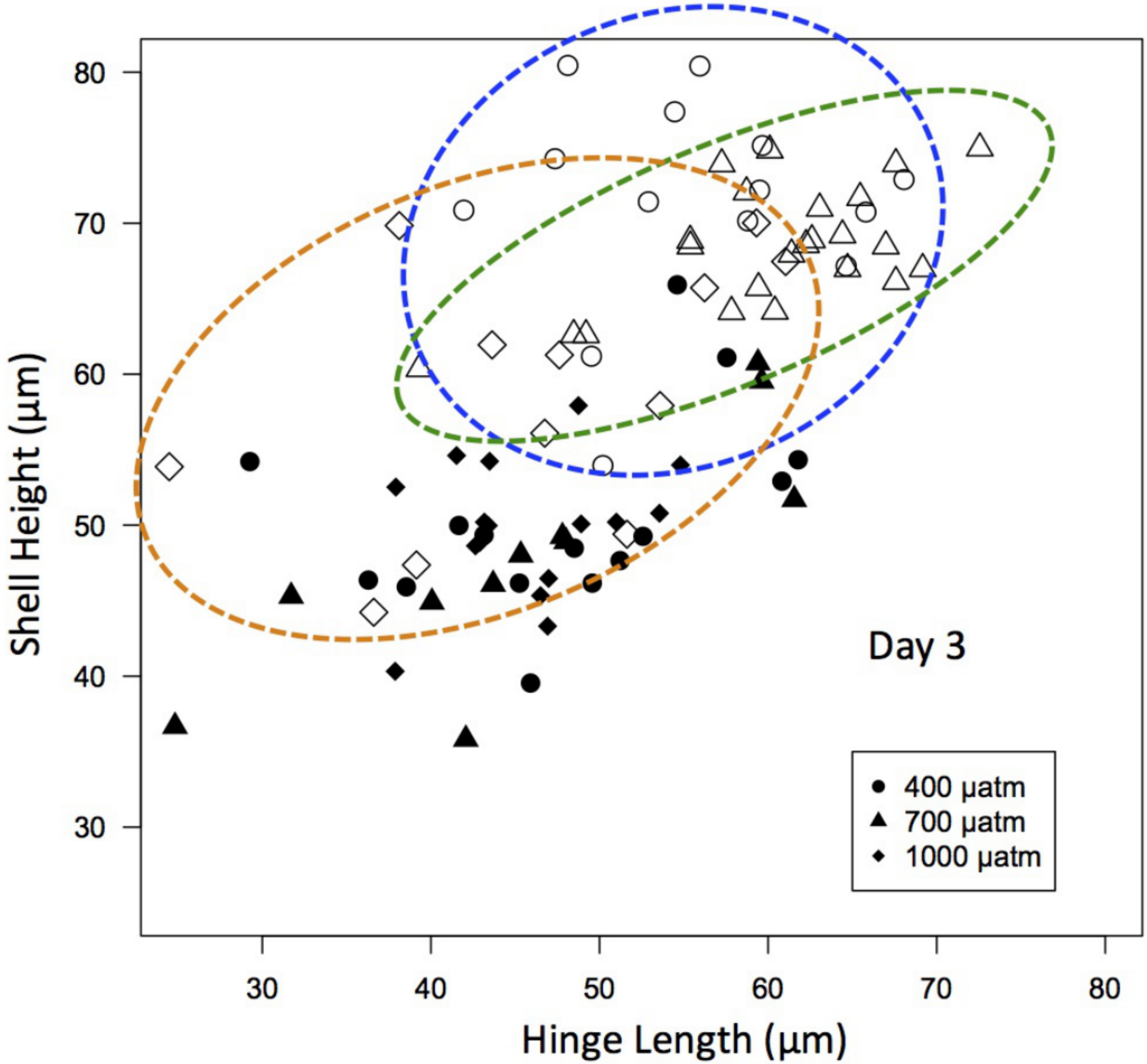
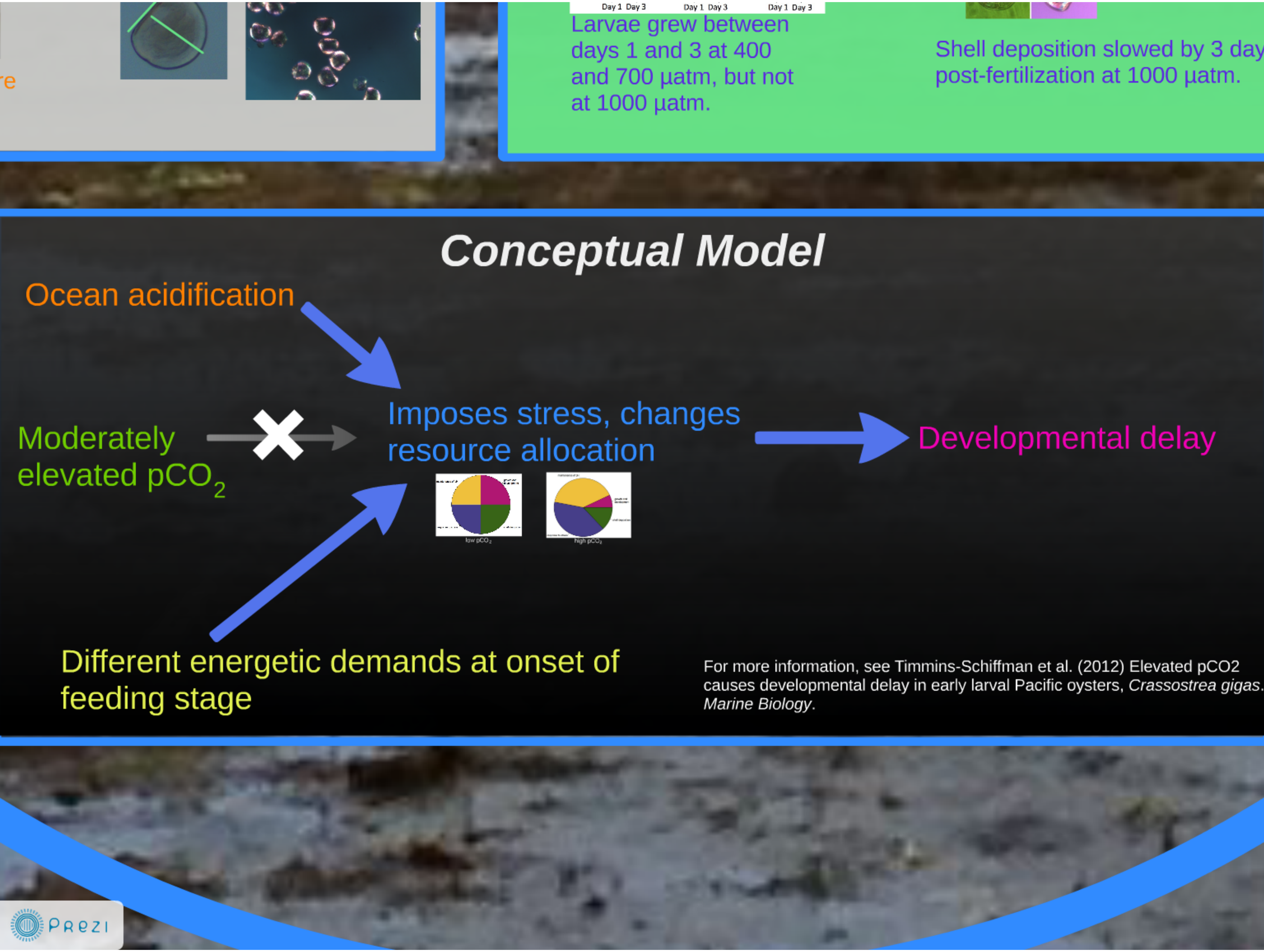


Day 3
400 µatm
700 µatm
1000 µatm
Shell Height (µm)
Hinge Length (µm)
30
40
50
60
70
80


Day 1 Day 3
Day 1 Day 3
Day 1 Day 3
Larvae grew between days 1 and 3 at 400 and 700 µatm, but not at 1000 µatm.
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Conceptual Model
Ocean acidification
Moderately elevated pCO_2
Imposes stress, changes resource allocation
Developmental delay
Different energetic demands at onset of feeding stage
For more information, see Timmins-Schiffman et al. (2012) Elevated pCO2 causes developmental delay in early larval Pacific oysters, *Crassostrea gigas*. *Marine Biology*.
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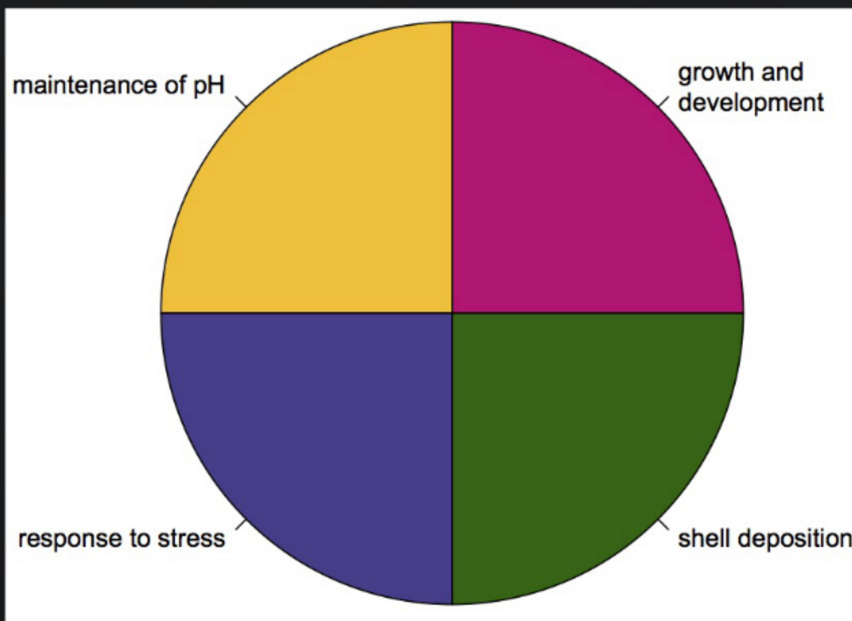
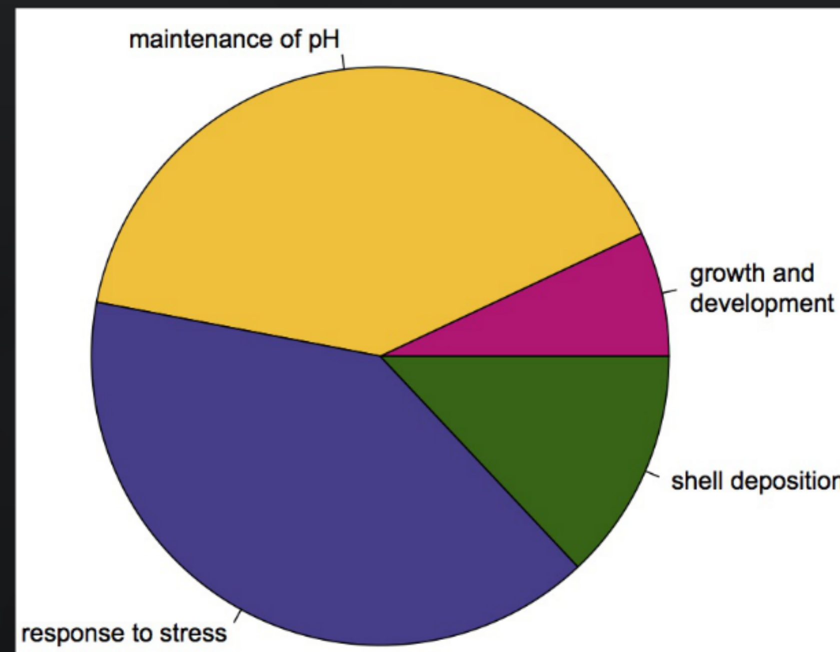
source allocatio

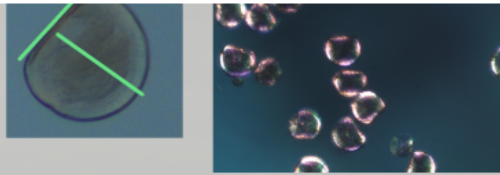


low pCO_2



high pCO_2

re
c



Day 1 Day 3 Day 1 Day 3 Day 1 Day 3

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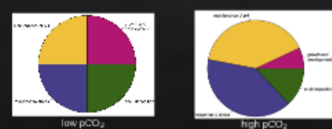
Conceptual Model

Ocean acidification

Moderately elevated pCO_2

Imposes stress, changes resource allocation



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Tools for investigating responses to ocean acidification
Approach
Environmental change
Proteomics
collaboration with Brook Nunn and Dave Goodlett
Main functions of ctenidia include ion regulation, respiration, and sorting of food particles.
Which physiological processes are changing?
How are interactions among processes changing?
How it works
extract proteins
cleave into peptides
sequence on mass spec
Data from mass spec
=
peptide sequence
match to entire protein sequence in database
The Gill Proteome
1,043 proteins identified
Technical and biological replication were consistent.
Proteomics is a viable tool for determining changes in resource allocation.
The ctenidium, as the interface between the oyster and its environment has a large, multifunctional proteome.
For more information, see Timmins-Schiffman et al. (2013) Shotgun proteomics as a viable approach for biological discovery in the Pacific oyster. Conservation Physiology.
Prezi

or investigating respons
ocean acidification

Approach

Environmental change

Proteomics

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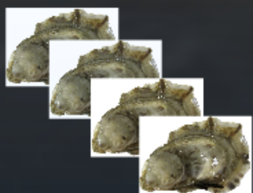
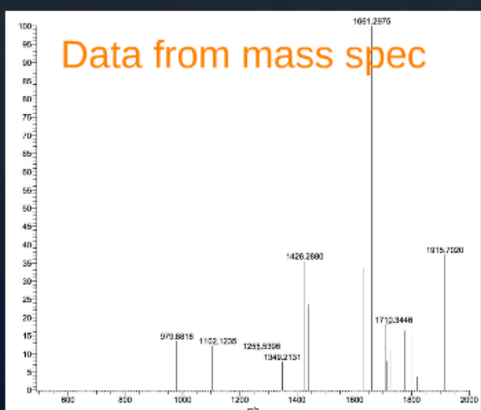
How it works



extract proteins

cleave into peptides

sequence on mass spec



= peptide sequence

YQKSTE LLIRKLPFQR

match to entire protein sequence in database

>CGI_10000055

MARTKQTARK STGGKAPRKQ LATKAARKSA PSTGGVKKPH RYRPGTVALR EIRRYQKSTE LLIRKLPFQR LVREIAQDFK TDLRFQSAAI GALQEASEAY LVGLFEDTNL CAIHAKRVTI MPKDIQLARR IRGERA

MONO MW: 15319, pI: 11.27

...e Gill Proteome

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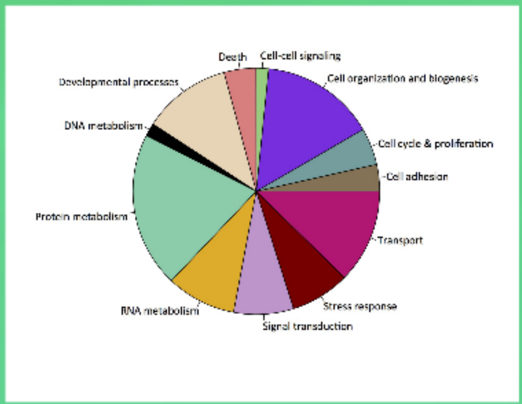
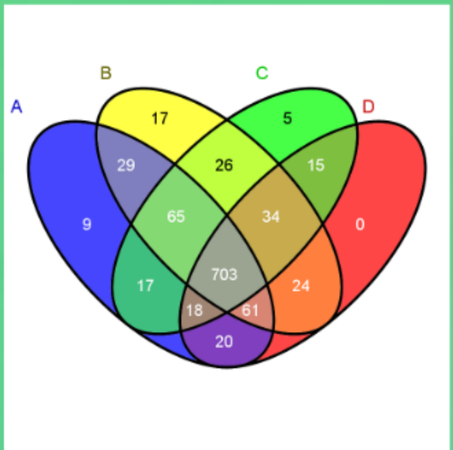
changes in resource allocatio...

The Gill Proteome

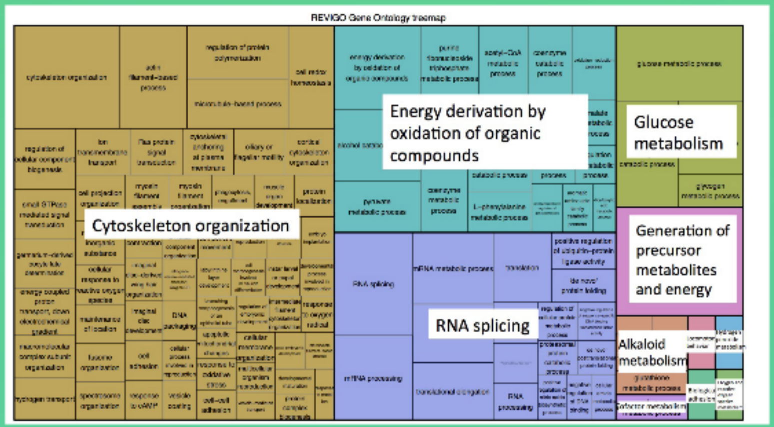
1,043 proteins identified





Technical and biological replication were consistent.

The gill tissue proteome has an over-representation of some biological processes, providing insight into tissue-specific functions.



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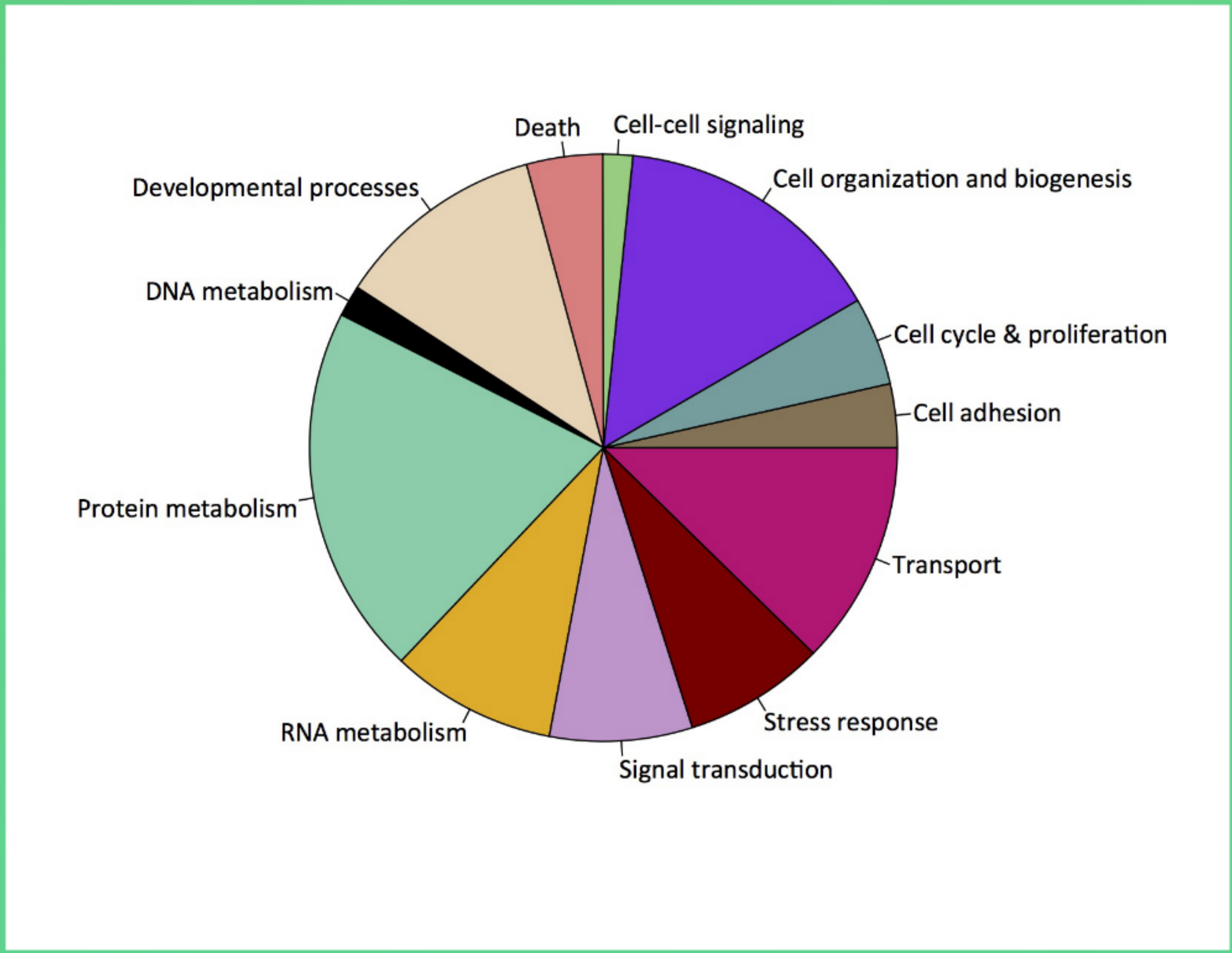

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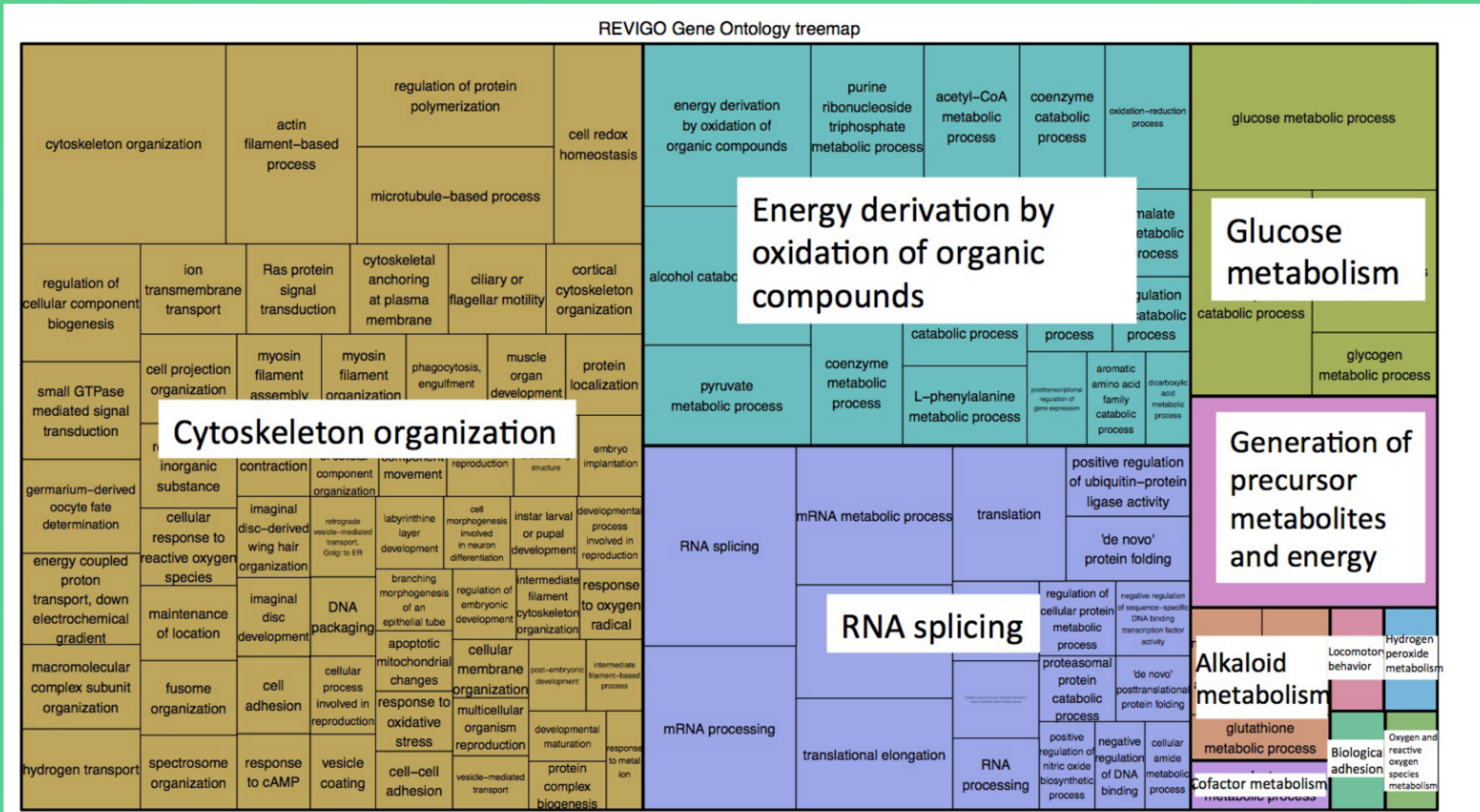
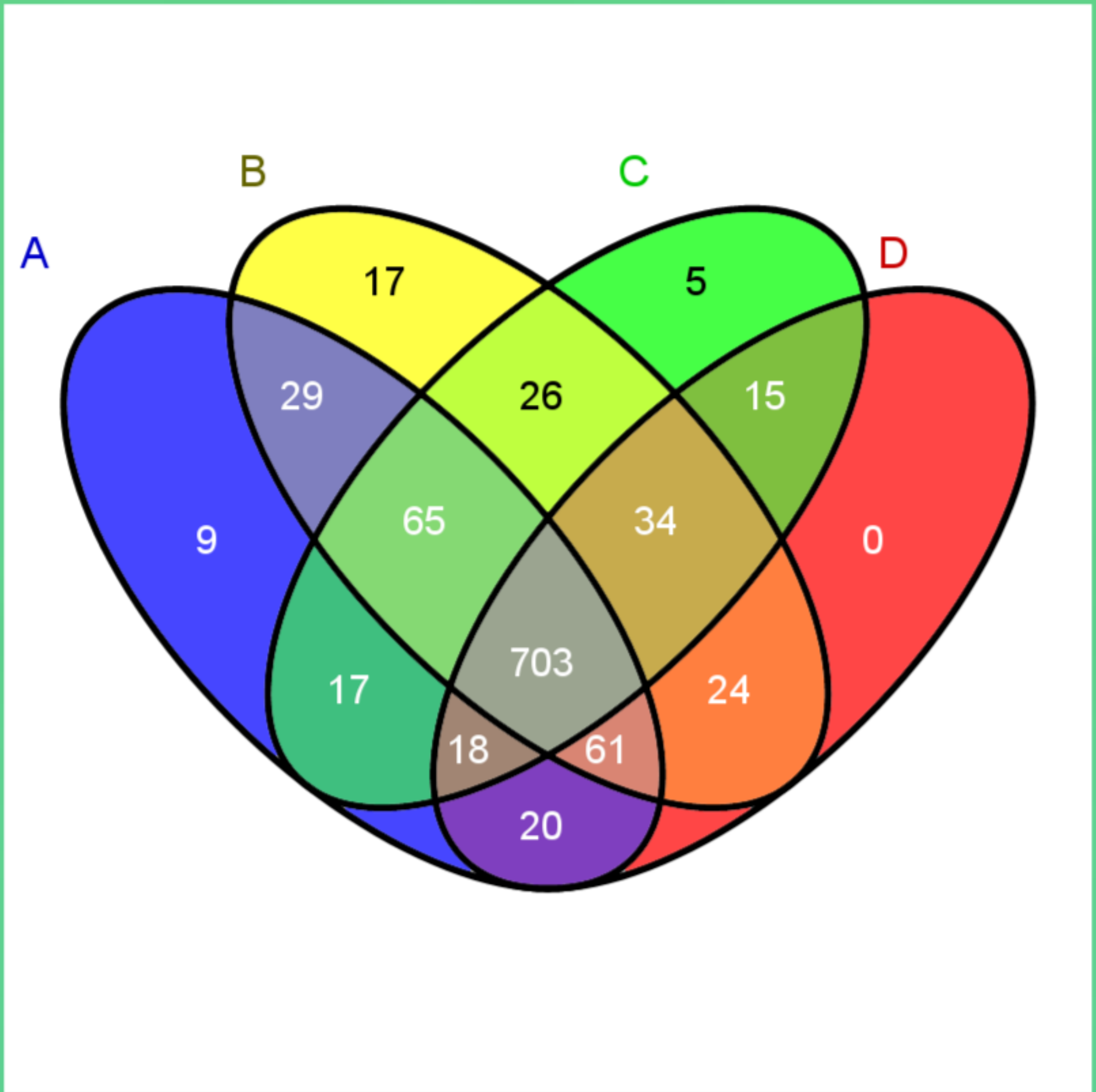


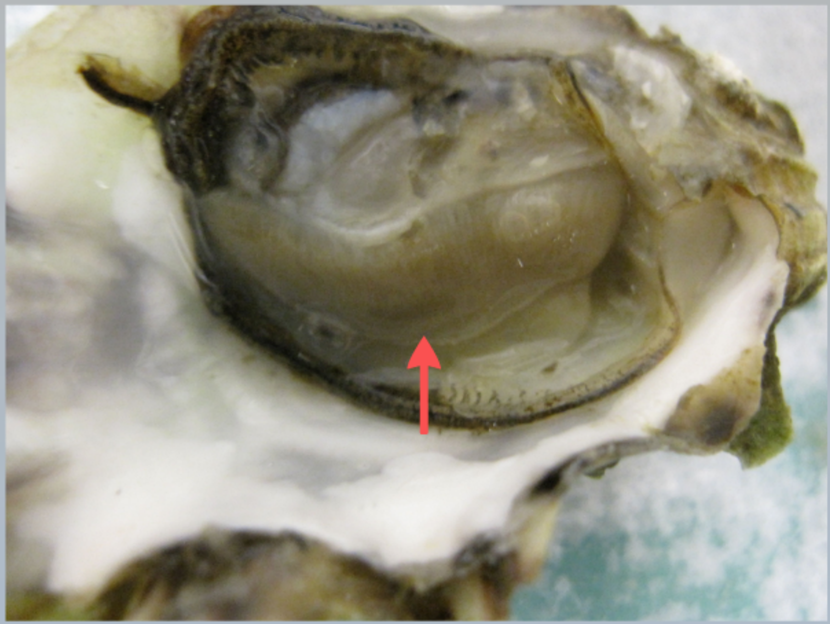
Prezi



Technical and biological replication were consistent.

Proteomics is a viable tool for determining changes in resource allocation.

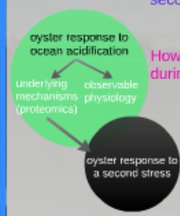
The ctenidium, as the interface between the oyster and its environment has a large, multifunctional proteome.



For more information, see Timmins-Schiffman et al. (2013) Shotgun proteomics as a viable approach for biological discovery in the Pacific oyster. *Conservation Physiology.*

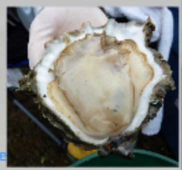
The integrated physiological response of oysters to ocean acidification

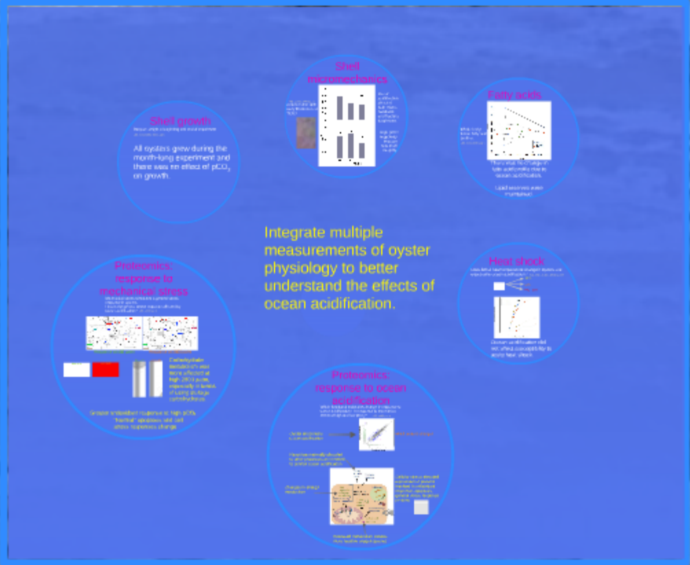
Application of proteomics to investigate the response of oysters to ocean acidification and a second stress.



How are resources allocated during environmental stress?

How do interactions among processes change during exposure to ocean acidification?





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- New shell was deposited
- Fatty acid reserves not affected
- Response to heat shock not affected

But there were limitations to this resilience:

- Integrity of new shell was compromised
- Proteins from diverse pathways/processes were affected by ocean acidification, implying wide-ranging effects on the organism-environment interaction
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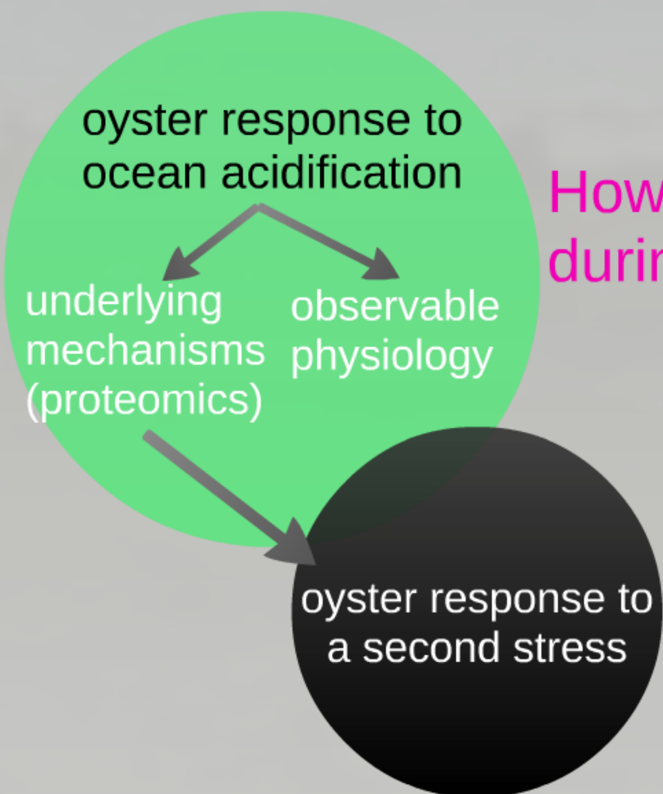


How are resources allocated during environmental stress?

How do interactions among processes change during exposure to ocean acidification?

Shell growth

Buoyant weight at beginning and end of experiment

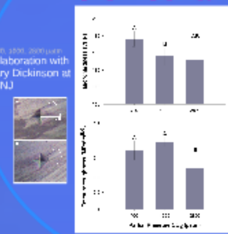
400, 600, 1000, 2800 µatm

All oysters grew during the month-long experiment and there was no effect of pCO_2 on growth.

Shell micromechanics

400, 1000, 2800 µatm

collaboration with Gary Dickinson at TCNJ



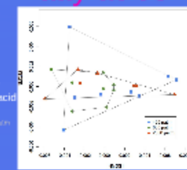
Ocean acidification affected both micro-hardness and fracture toughness.

High pCO_2 negatively impacts new shell integrity.

Fatty acids

Whole body tissue fatty acid profiles.

400, 800, 2800 µatm



There was no change in fatty acid profile due to ocean acidification.

Lipid reserves were maintained.

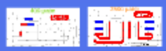
Integrate multiple measurements of oyster physiology to better understand the effects of ocean acidification.

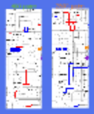
Proteomics: response to mechanical stress

Mechanical stress stimulates a general stress response in oysters.
How is the general stress response affected by ocean acidification? 400, 2800 µatm

Response at 400 µatm

Response at 2800 µatm





Carbohydrate metabolism was more affected at high 2800 µatm, especially in terms of using storage carbohydrates.

Greater antioxidant response at high pCO_2

"Normal" apoptosis and cell stress responses change

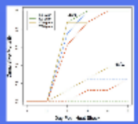
Heat shock

Does lethal heat temperature change if oysters are exposed to ocean acidification? 400, 800, 1000, 2800 µatm

42°C

43°C

44°C - LHT

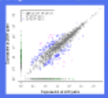


Ocean acidification did not affect susceptibility to acute heat shock.

Proteomics: response to ocean acidification

Which functional molecules change in response to ocean acidification? In response to mechanical stress at high and low pCO_2? 400, 2800 µatm

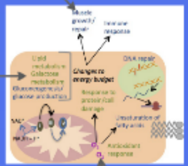
Oyster encounters ocean acidification



which proteins change?

Resources normally allocated to other processes are needed to combat ocean acidification

Changes in energy metabolism



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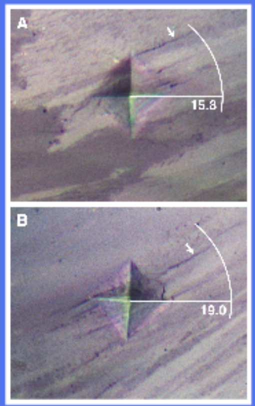

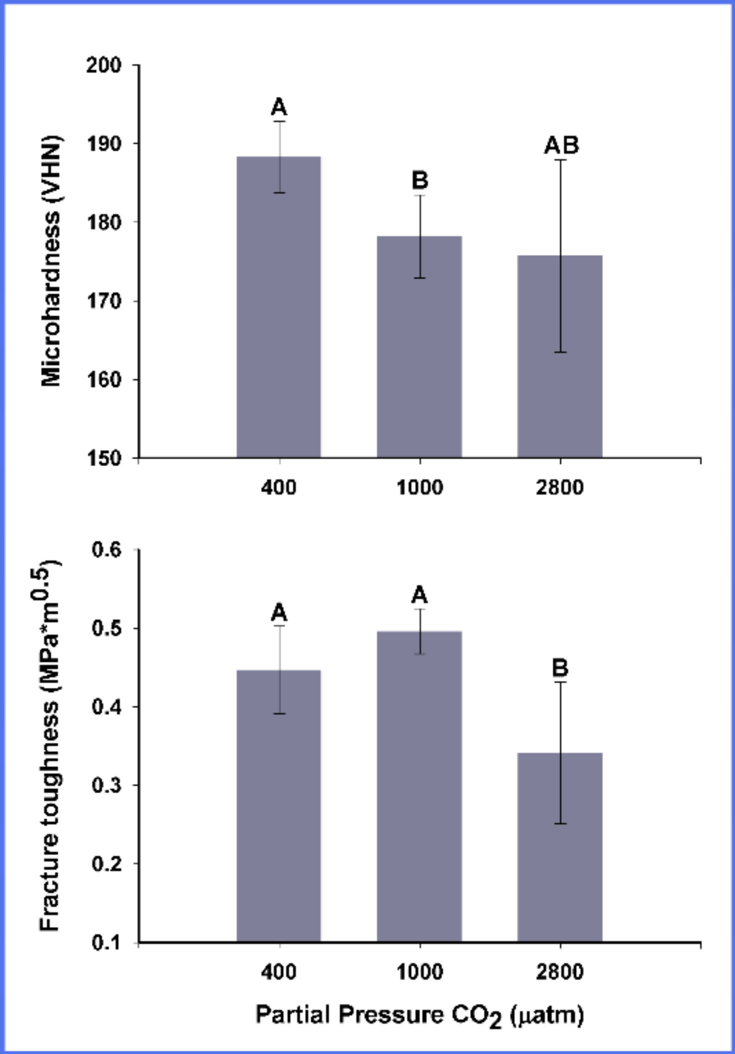

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Prezi

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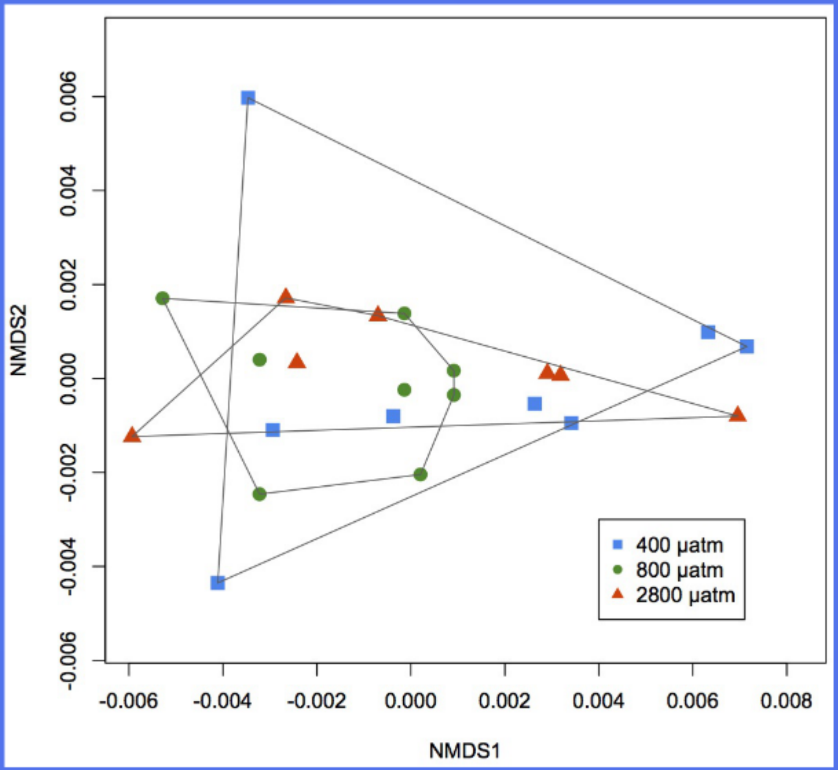

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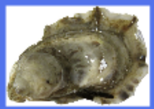

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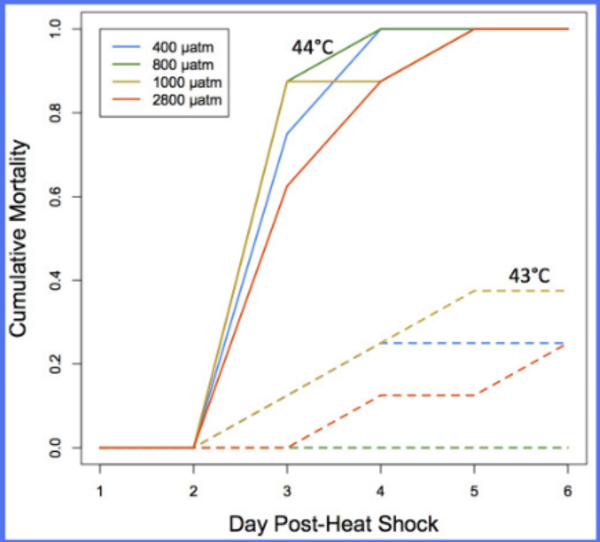

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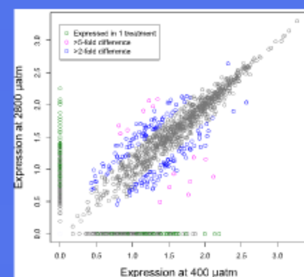

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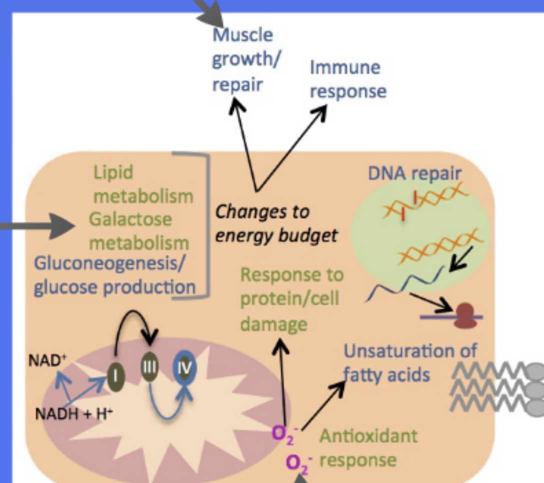
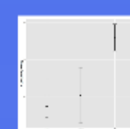
Resources normally allocated to other processes are needed to combat ocean acidification

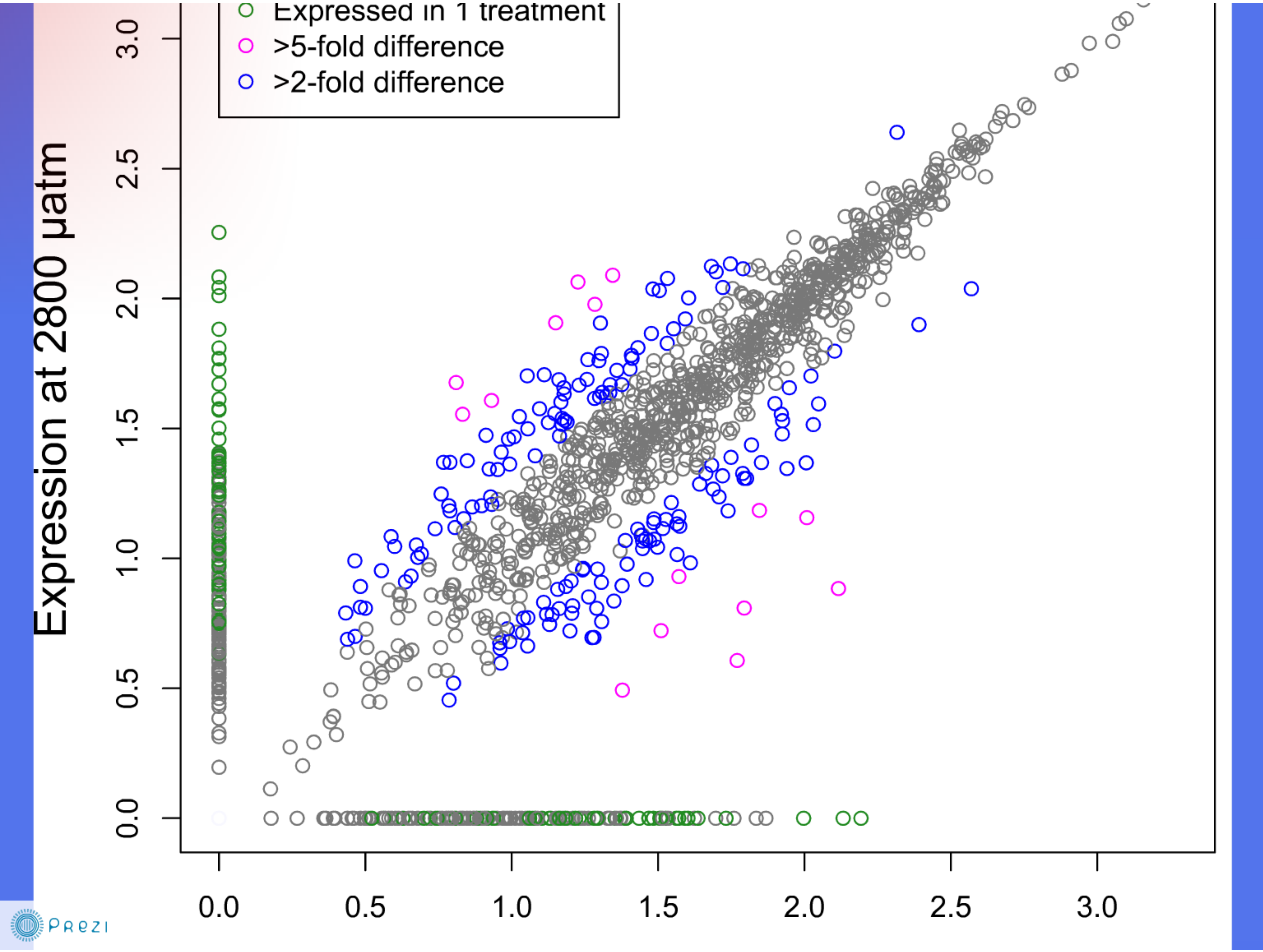
Changes in energy metabolism



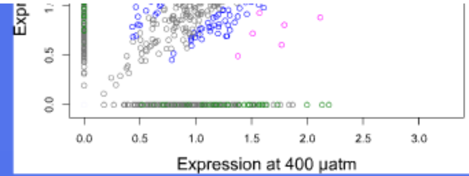
Cellular stress: elevated expression of proteins involved in antioxidant response, apoptosis, general stress response proteins



Increased metabolism creates more reactive oxygen species


Expressed in 1 treatment
>5-fold difference
>2-fold difference
Expression at 2800 µatm
0.0
0.5
1.0
1.5
2.0
2.5
3.0
Prezi

ocean acidification



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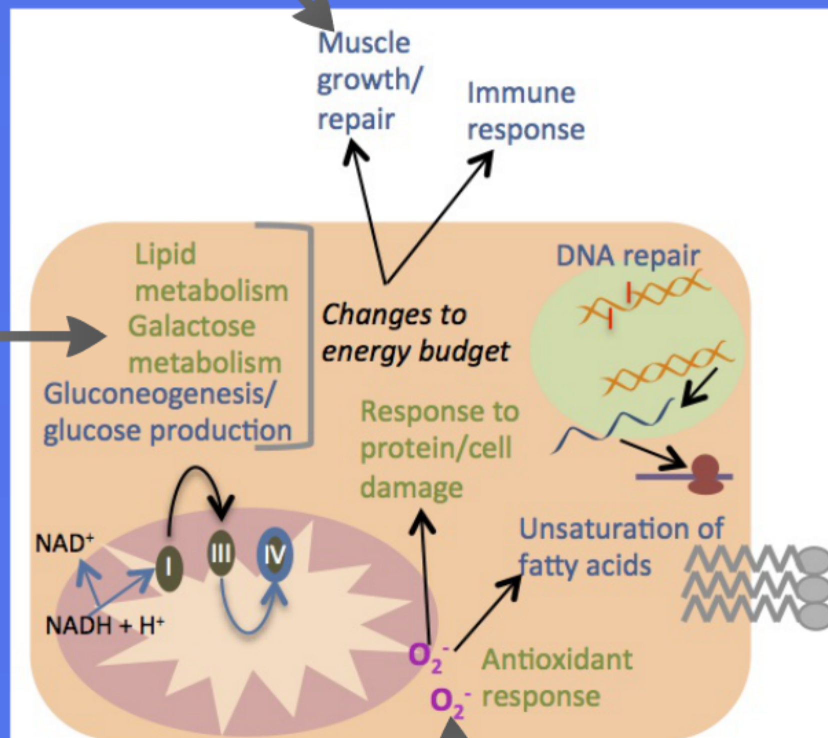

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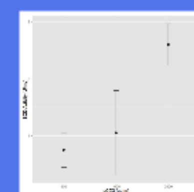

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Prezi

to combat ocean acidification

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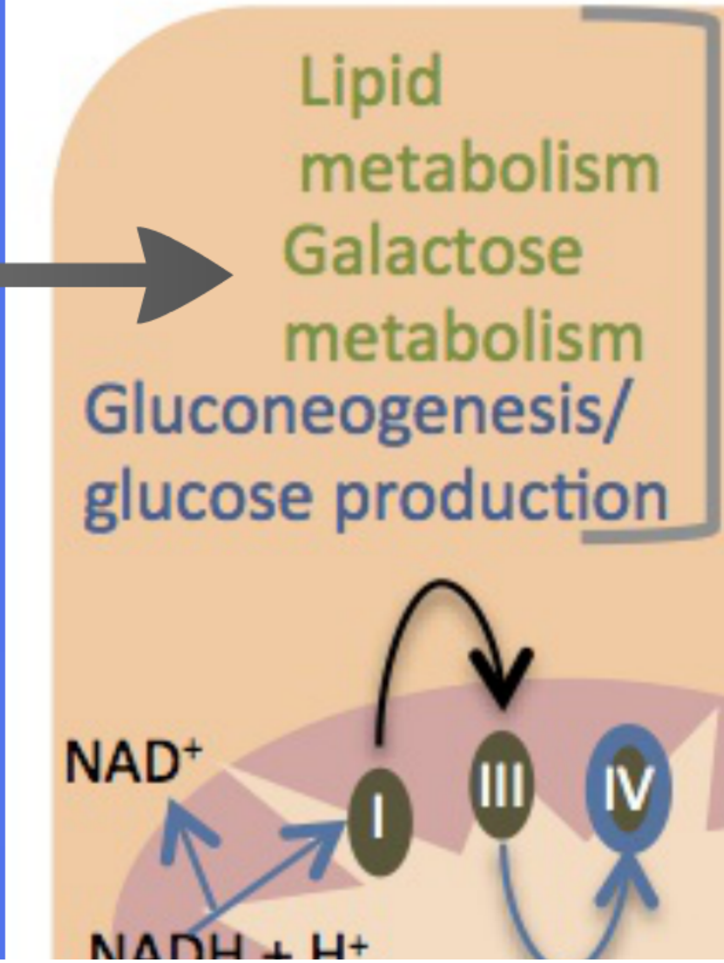

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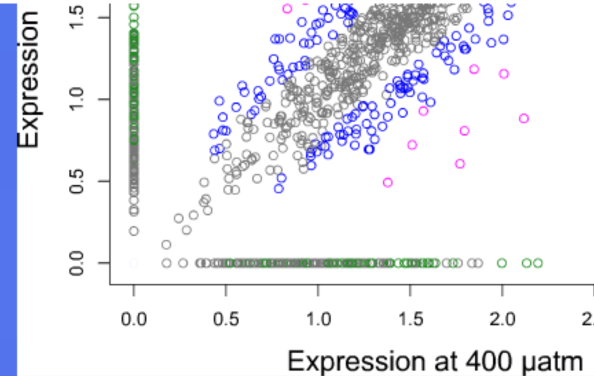

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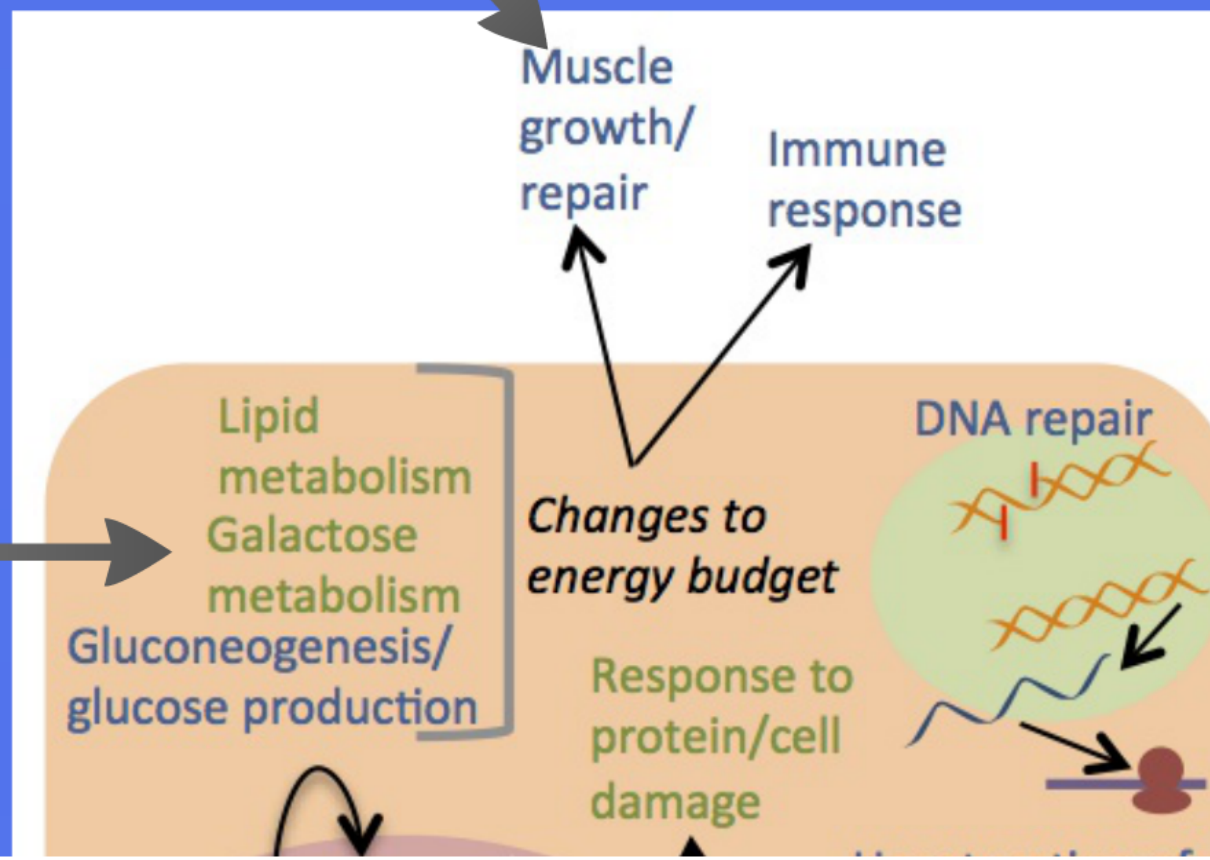

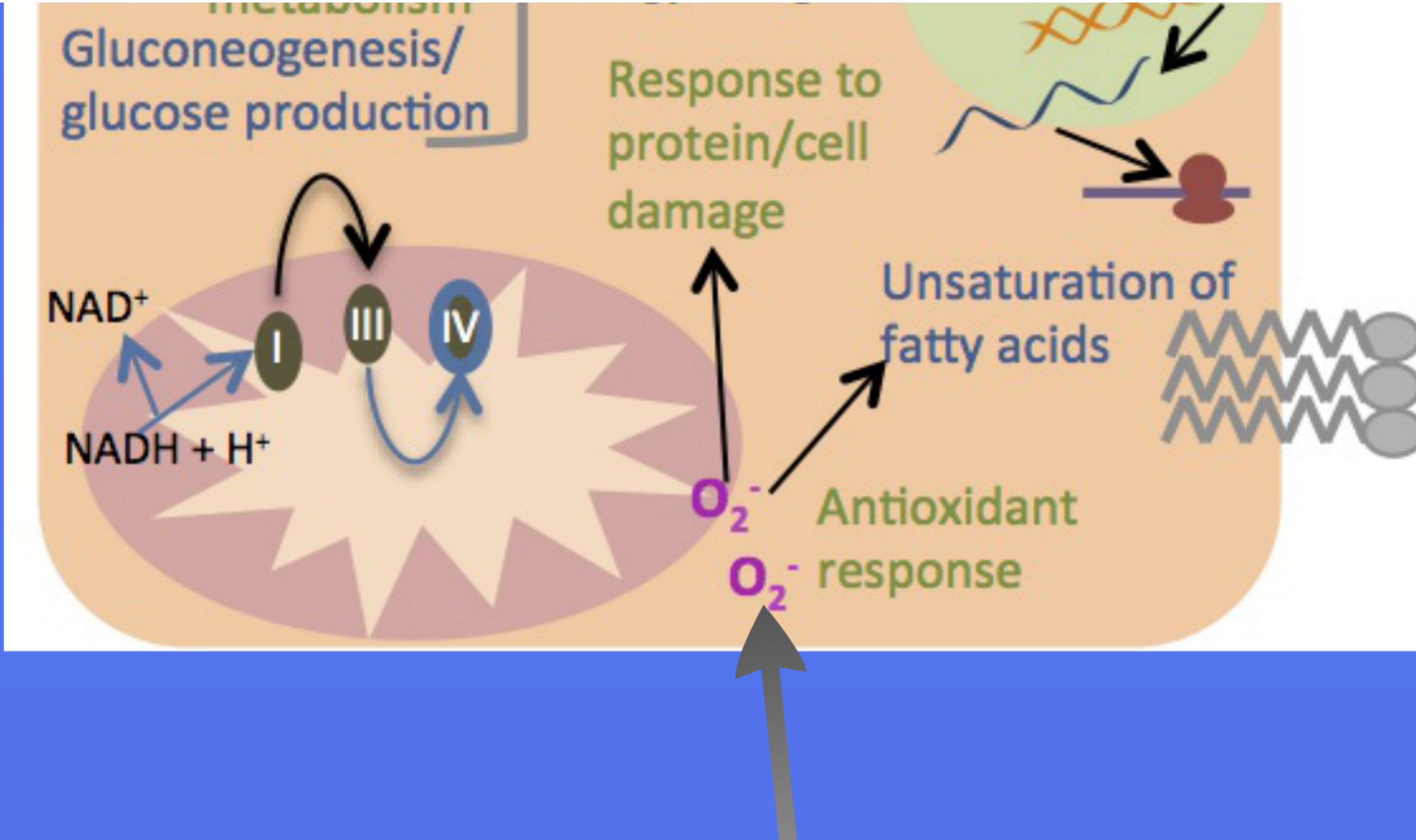

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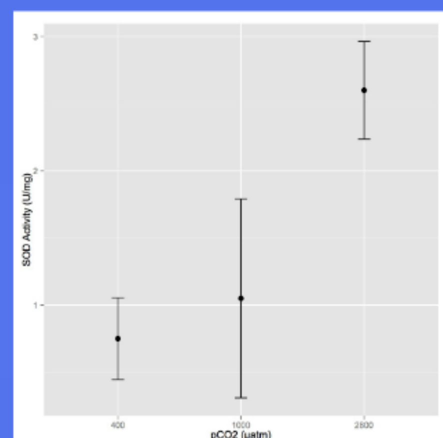

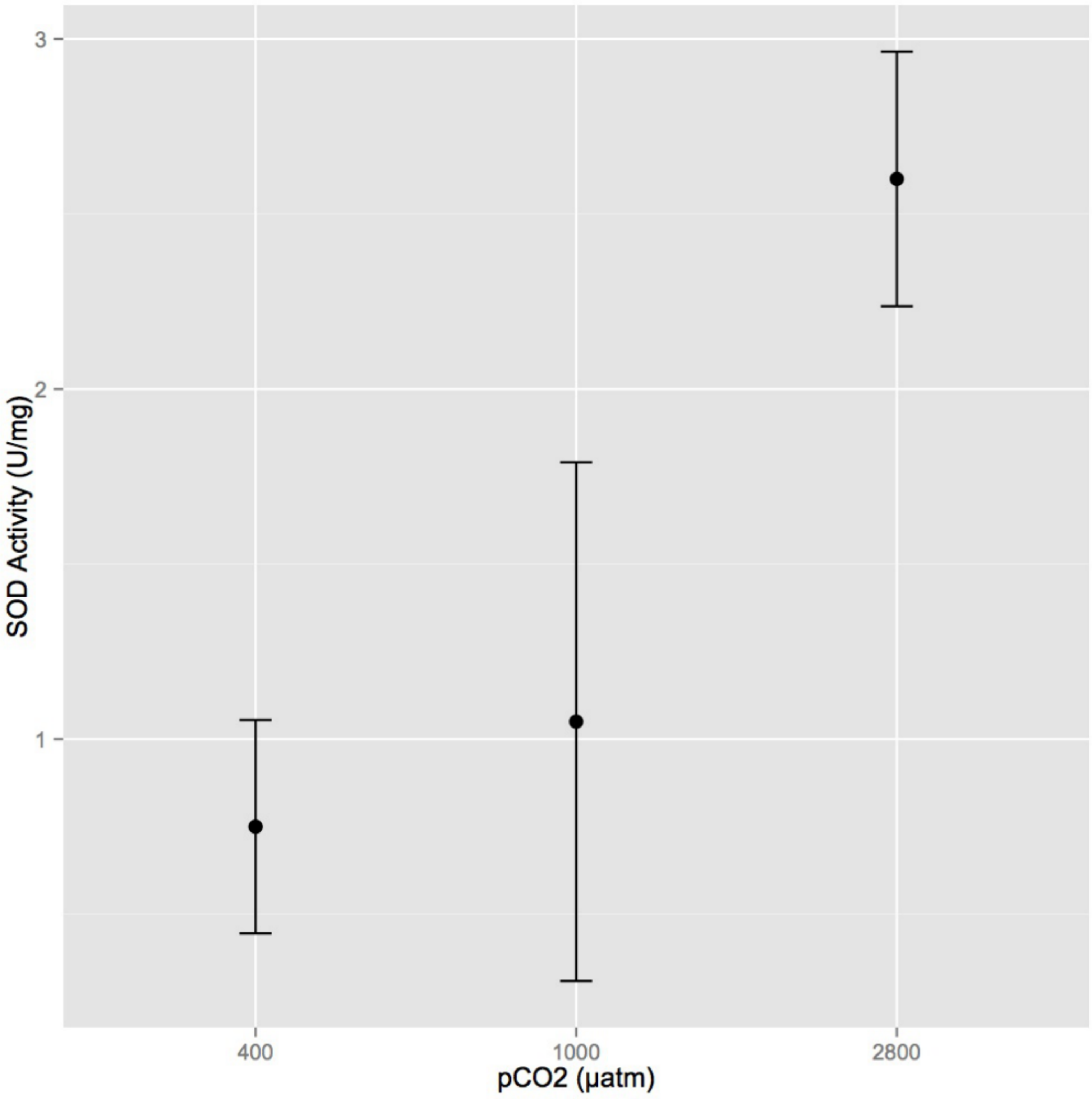

SOD Activity (U/mg)
3
2
1
400
1000
2800
pCO2 (µatm)

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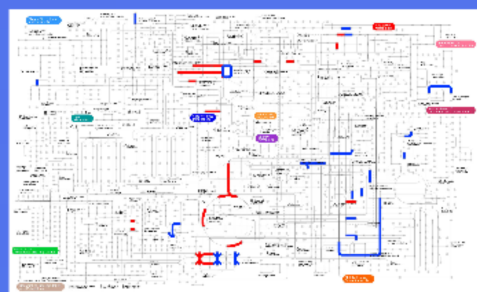
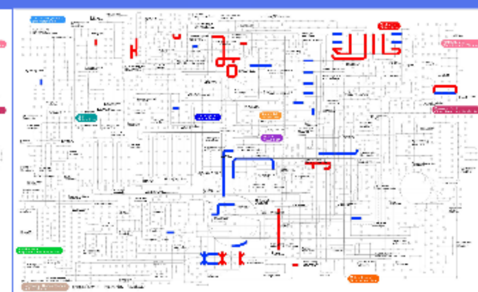
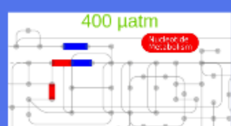
Mechanical stress stimulates a general stress response in oysters.
How is the general stress response affected by ocean acidification? 400, 2800 µatm



Response at 400 µatm



Response at 2800 µatm



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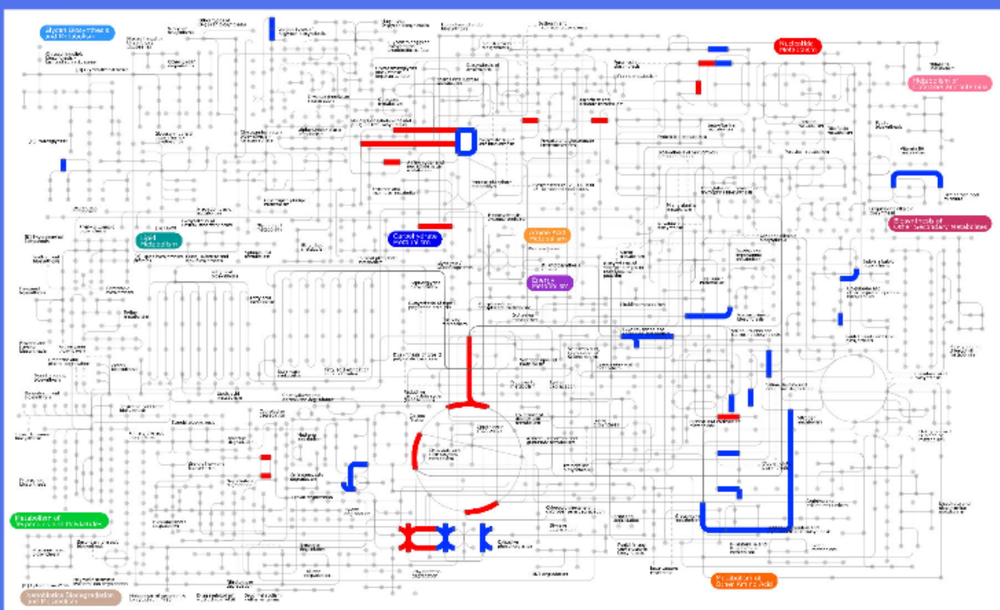

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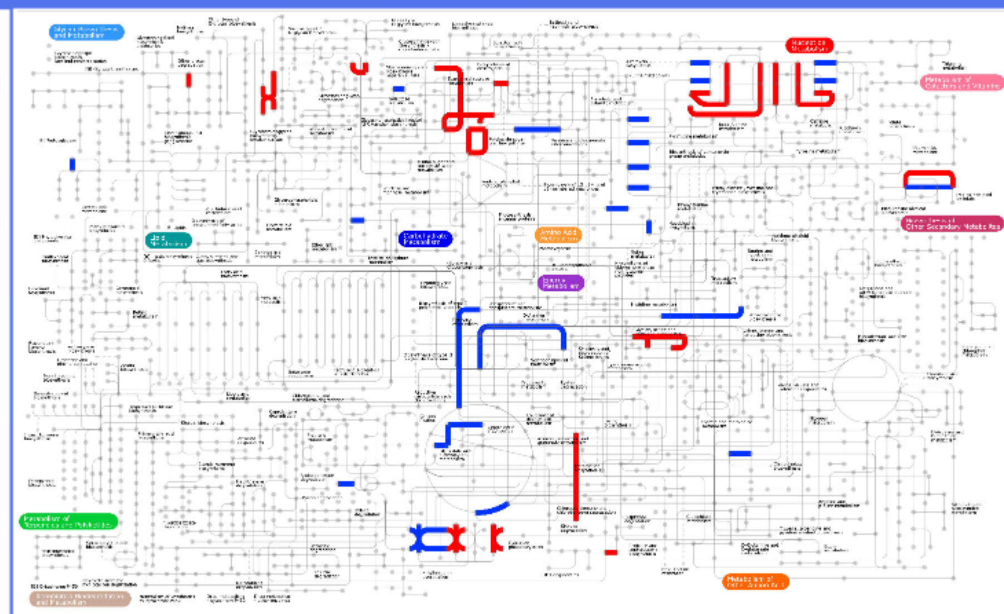

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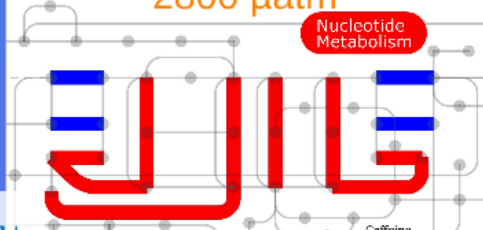
2800 µatm



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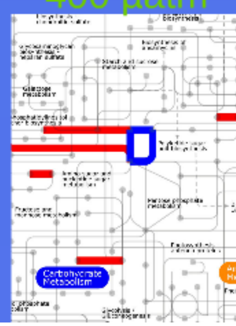
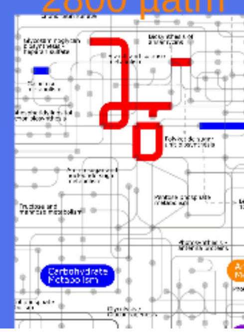


2800 µatm



Carbohydrat
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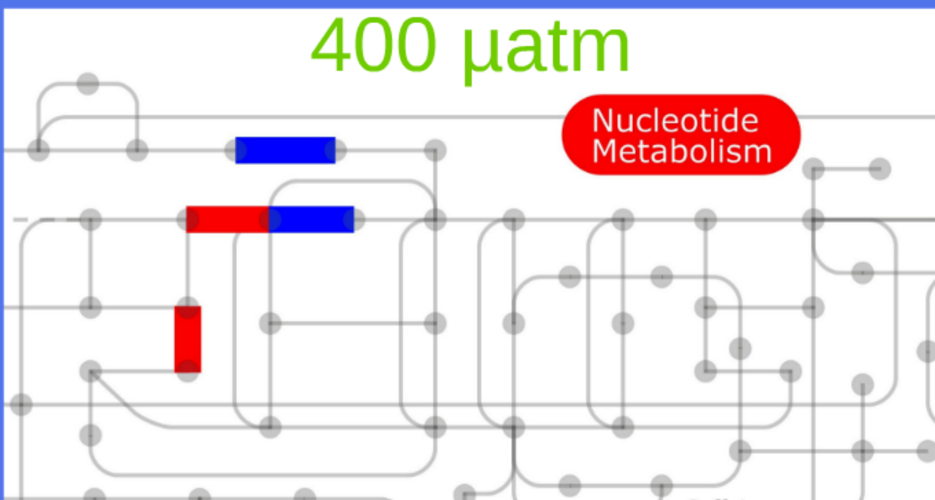
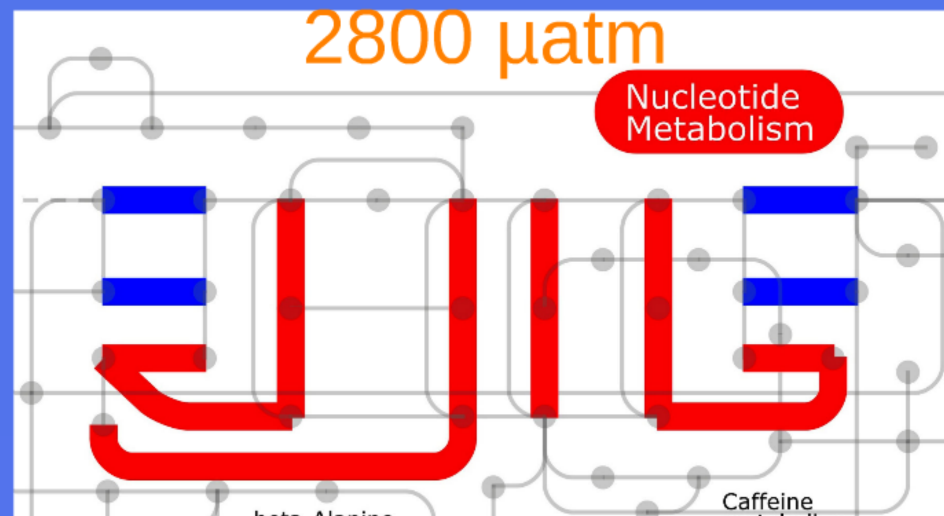
more affecte

Response at 40





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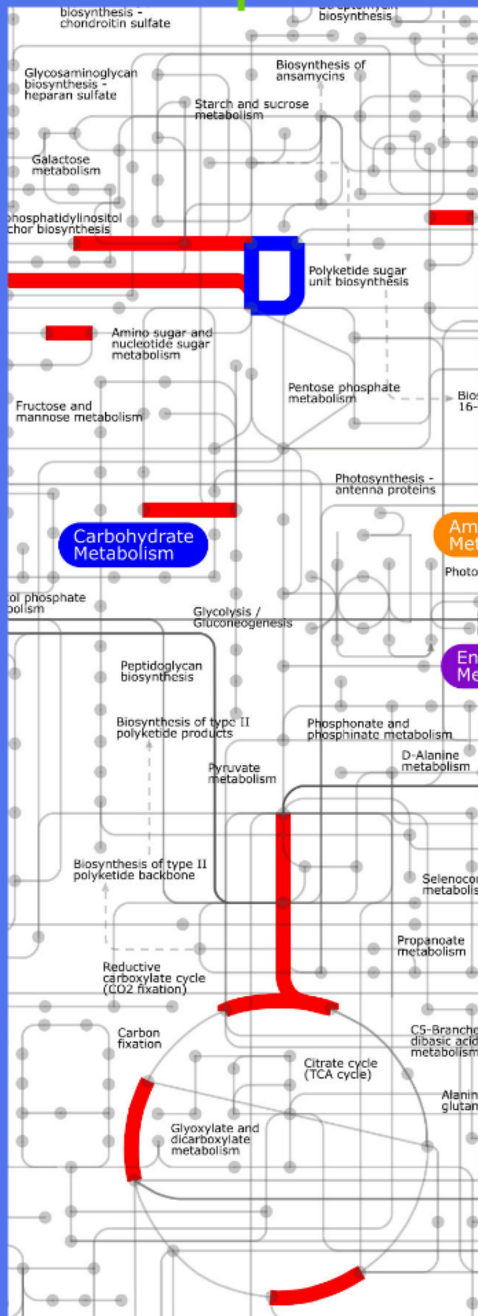
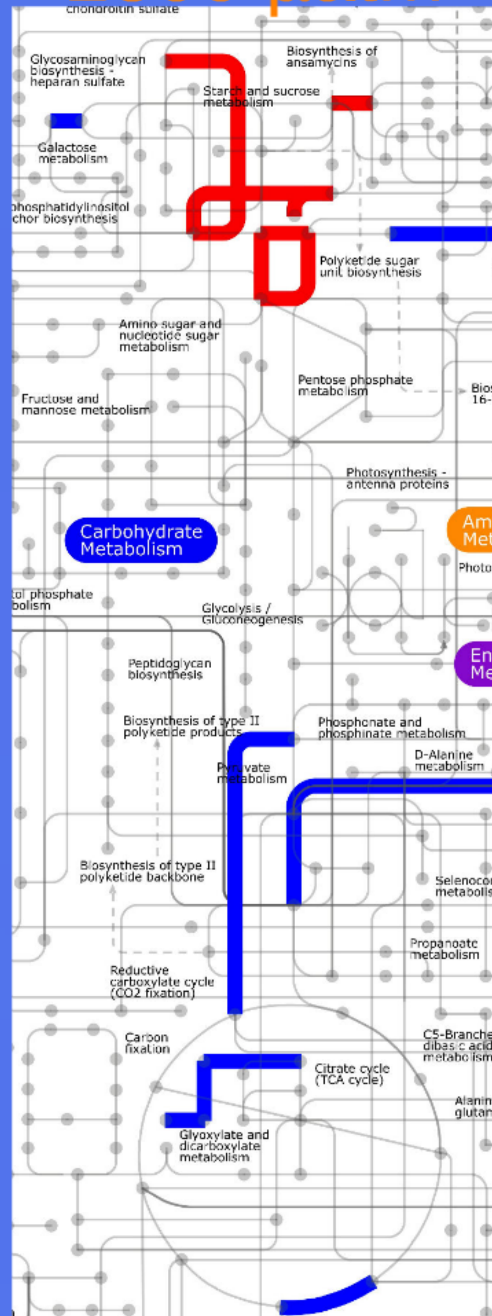


2800 µatm



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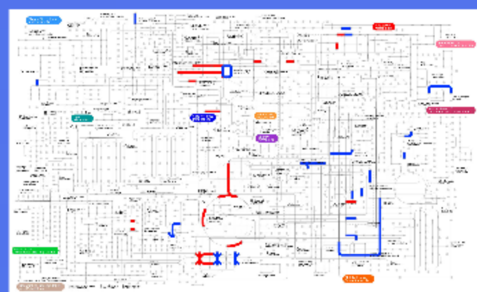

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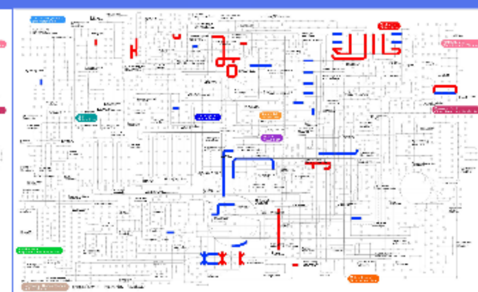
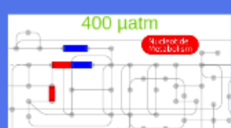


Response at 2800 µatm



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Conclusions

The impacts of ocean acidification are negative, but the key to acclimatization or adaptation lies in the **variability of responses**.

This variability has been observed between species, habitats, family groups, and offspring of adults exposed to difference pCO2.

Ecological history may play a large role in resilience to ocean acidification.

In adult bivalves, increased access to food mitigates the effects of ocean acidification.

This makes the adult bivalve response to ocean acidification a question of energetic resources.

Could resistance to ocean acidification lie in the phenotypes that more efficiently use energetic resources?

Prezi

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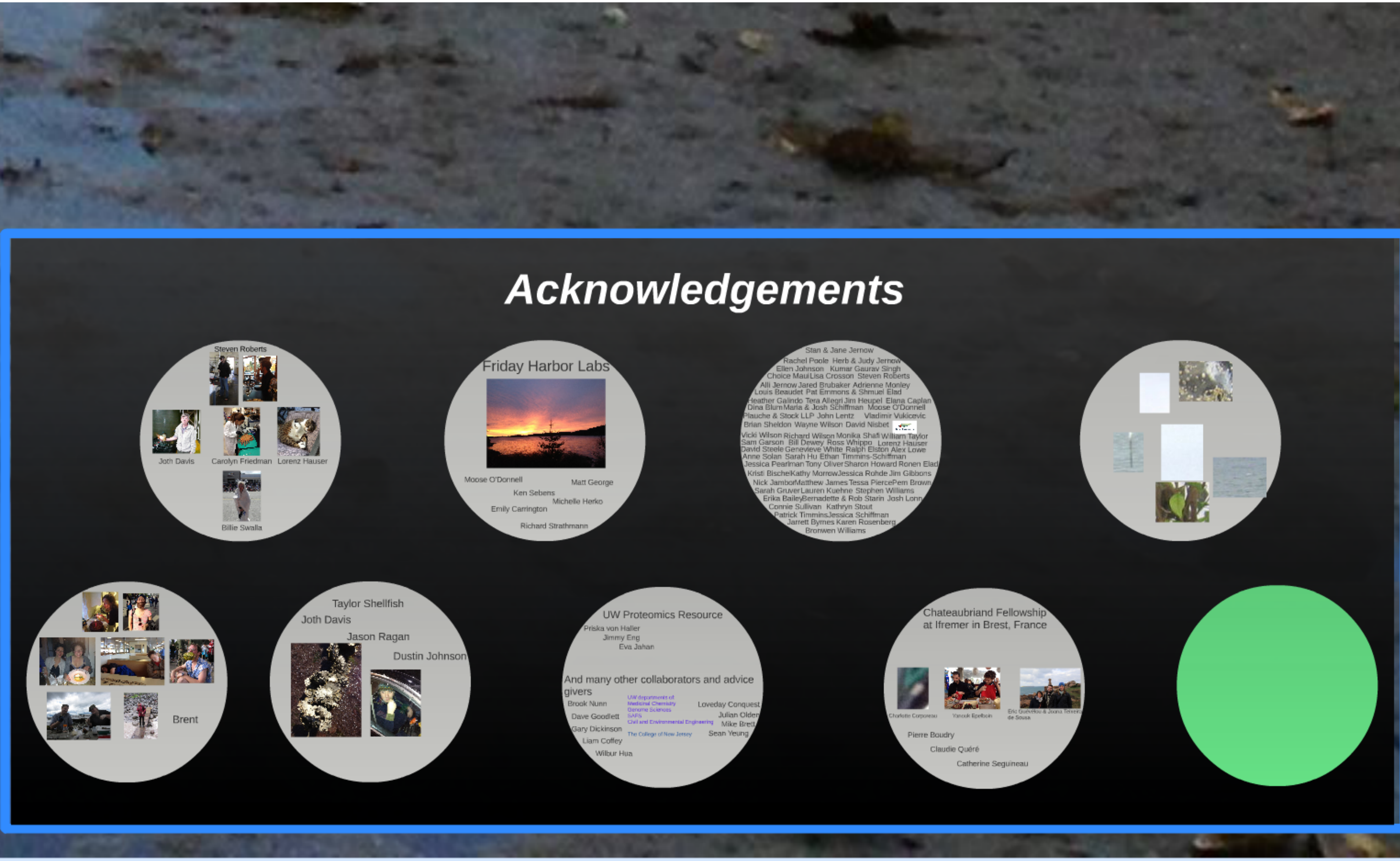

Acknowledgements
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Rachel Poole Herb & Judy Jernow
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Vicki Wilson Richard Wilson Monika Shafi William Taylor
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Jarrett Byrnes Karen Rosenberg
Bronwen Williams
Brent
Taylor Shellfish
Joth Davis
Jason Ragan
Dustin Johnson
UW Proteomics Resource
Priska von Haller
Jimmy Eng
Eva Jahan
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Brook Nunn
Dave Goodlett
Gary Dickinson
Liam Coffey
Wilbur Hua
UW departments of
Medicinal Chemistry
Genome Sciences
SAFS
Civil and Environmental Engineering
The College of New Jersey
Loveday Conquest
Julian Olden
Mike Brett
Sean Yeung
Chateaubriand Fellowship
at Ifremer in Brest, France
Pierre Boudry
Claudie Quéré
Catherine Seguineau