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Combined effects of seawater acidification and Arsenic in *Crassostrea gigas* and *C. angulata*: oxidative stress and biomineralization enzymes activity assessment

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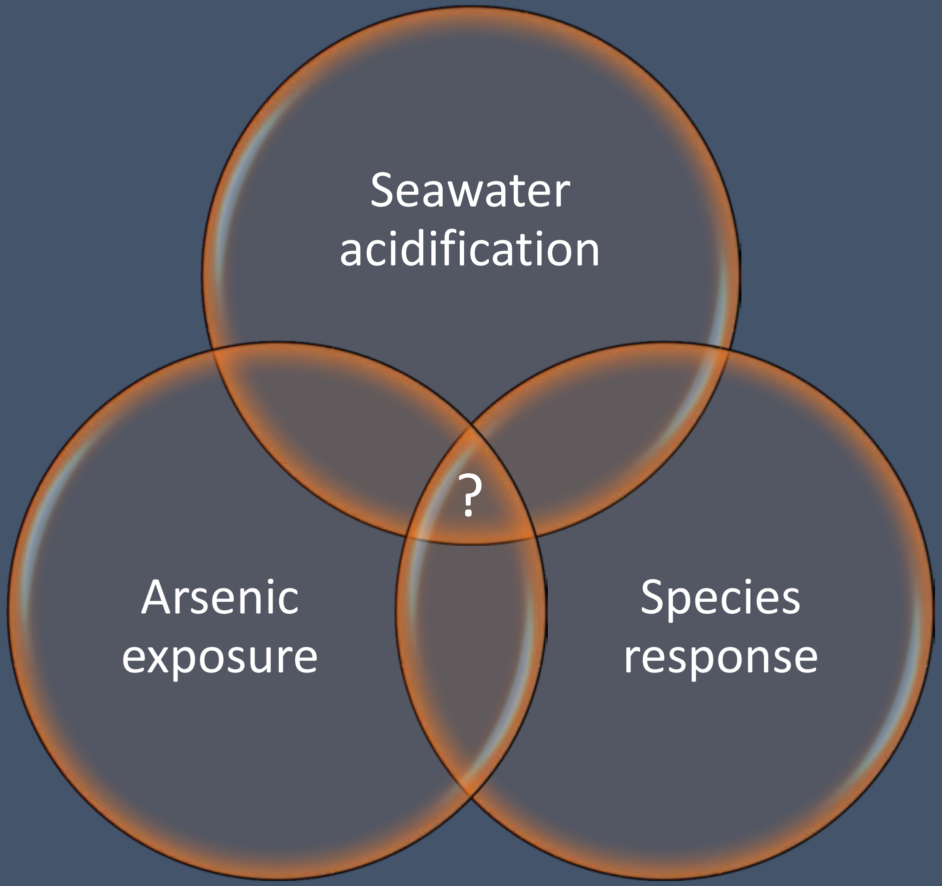
Scope

Bivalves under seawater acidification and arsenic exposure



Impacts on marine bivalves by arsenic and acidification individually, are relatively well documented

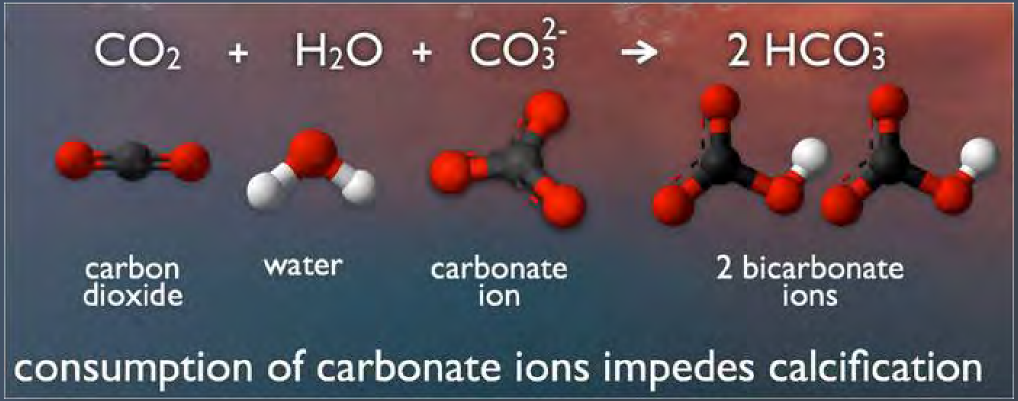
Interactions between acidification and arsenic exposure are still to be studied

The performance of different species under these stressors may influence population dynamics

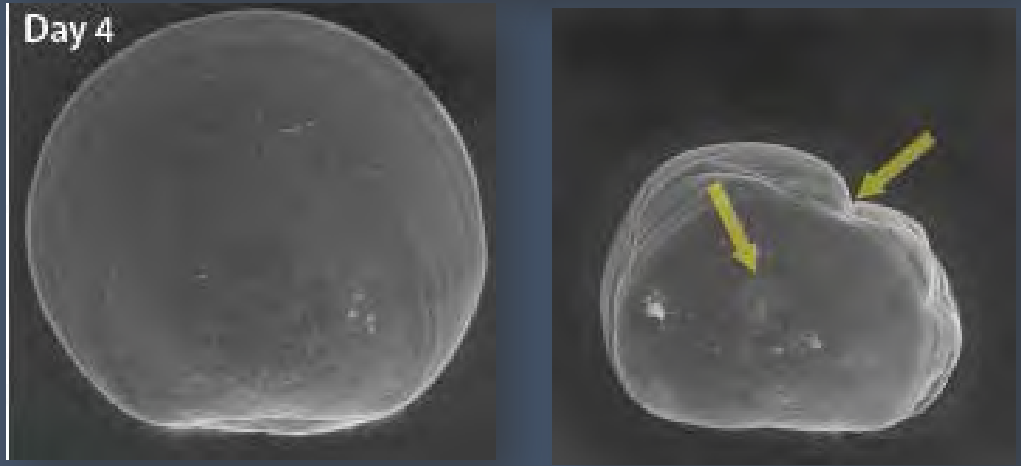
Introduction

Seawater acidification and biomineralization

- Ocean pH is predicted to decrease 0.4 units by 2100 (IPCC 2013)
- Biomineralization processes are affected by both acidification ($CaCO_3$ dissolution) and low carbonate ion availability
- Studies have shown impats on a variety of taxa (e.g bivalves, corals, decapods, equinoderms, diatoms)



www.pmel.noaa.gov



Oyster larvae (healthy, left) (under acidification right). Micrograph by OSU.

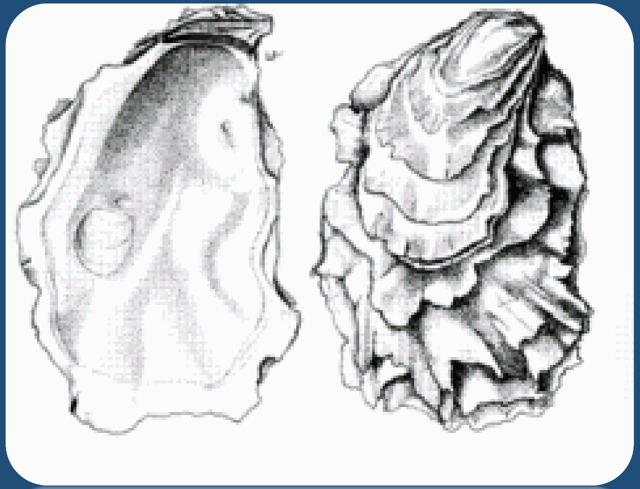
www.worldviewofglobalwarming.org/images13/03WAElectronMicroViewOysterLarvaeAcidifCO2DamageOSU.jp

Introduction

Arsenic

- Arsenic is one of the most important pollutants worldwide
- Arsenic has shown to be one of the most bioaccumulated elementes by bivalves, inducing toxicity to both organisms and humans
- The combined effect of arsenic with ocean acidification is not known

Objectives



http://www.thefishsite.com/

- To compare the performances of *Crassostea gigas* and *C. angulata* under a seawater acidification scenario combined with arsenic exposure, by assessing changes on biochemical parameters: namely biomineralization enzymes, and oxidative stress related markers

- Does the effect of low pH and As, acting alone or in combination, influence each species performance?

- Do species respond differently to tested conditions?

Materials and Methods

Experimental organisms

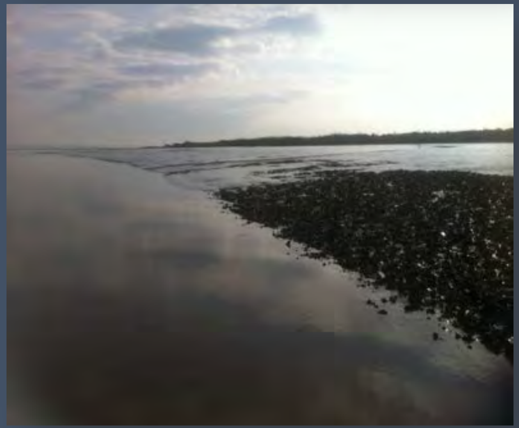
Crassostrea gigas

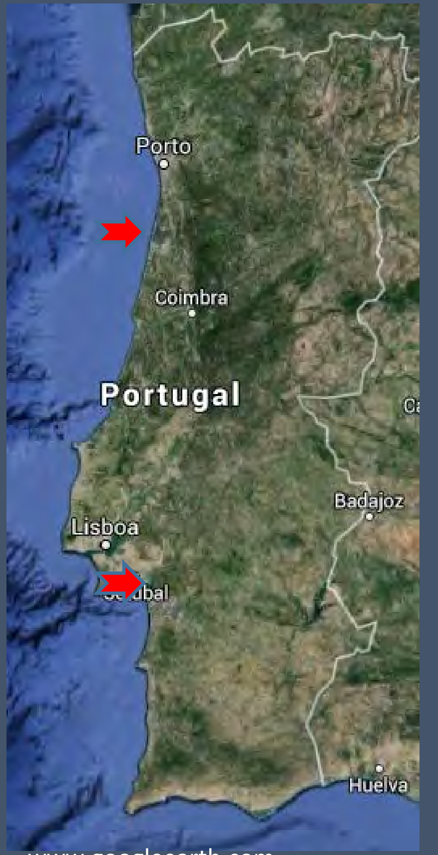
- Aquacultured specimens (Ria de Aveiro estuary, NW Portugal)
- Worldwide distributed species

www.ostraria.pt

Crassostrea angulata

- Wild specimens (Sado estuary, SW Portugal)
- Closely related native species, from SW Portugal





www.googleearth.com

Materials and Methods

EXPERIMENTAL DESIGN

- Acclimation 1 month
- Daily fed, and 10% water change
- 4 conditions tested:
  - Control pH=7.8; [As]=0 mg.L^{-1}
  - [As] = 2.6 mg.L^{-1}
  - pH = 7.3
  - $pH_{7.3}$ + [As] $_{2.6\ mg/L}$
- 20L individual tanks, 3 oysters per tank, 3 fold replicated conditions
- 28 day exposure chronic assay
- pH was continously monitored and controlled (pH STATsystem AQUAMEDIC)

Parameters

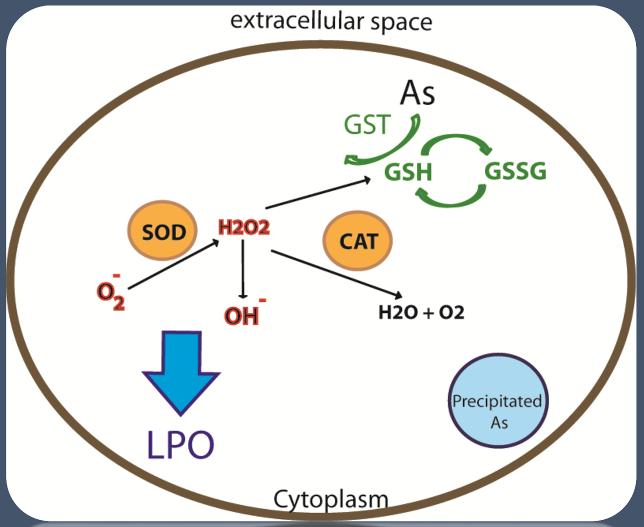
❖pH and Arsenic have shown to induce oxidative stress

❖**Oxidative stress** was measured through conventional biomarkers

❖**Biomineralization** related enzymes were used to assess possible effects on calcification

Oxidative stress:

- Lipid Peroxidation (LPO)
- Catalase (CAT)
- Superoxide dismutase (SOD)
- Glutathione-S-Tranferases (GSTs)



Biomineralization:

- Carbonic anhydrase (CA)
- Alkaline phosphatase (PhoAlk)
- Acid phosphatase (PhoAc)

Multivariate and statistical analysis: Primer 6.0

Results

- Arsenic quantification by IPCMS, certified laboratory
- Soluble fraction
- Insoluble fraction

Assay bioaccumulation

- Both species accumulated more As in the soluble fraction (2.6 µg As.g FW $^{-1}$) than in the insoluble fraction (0.9 µg.g FW $^{-1}$), with no significant differences among treatments neither between species
- Metals and metalloids in the soluble fraction have been shown to be more toxic to organisms and more bioavailable to higher trophic levels

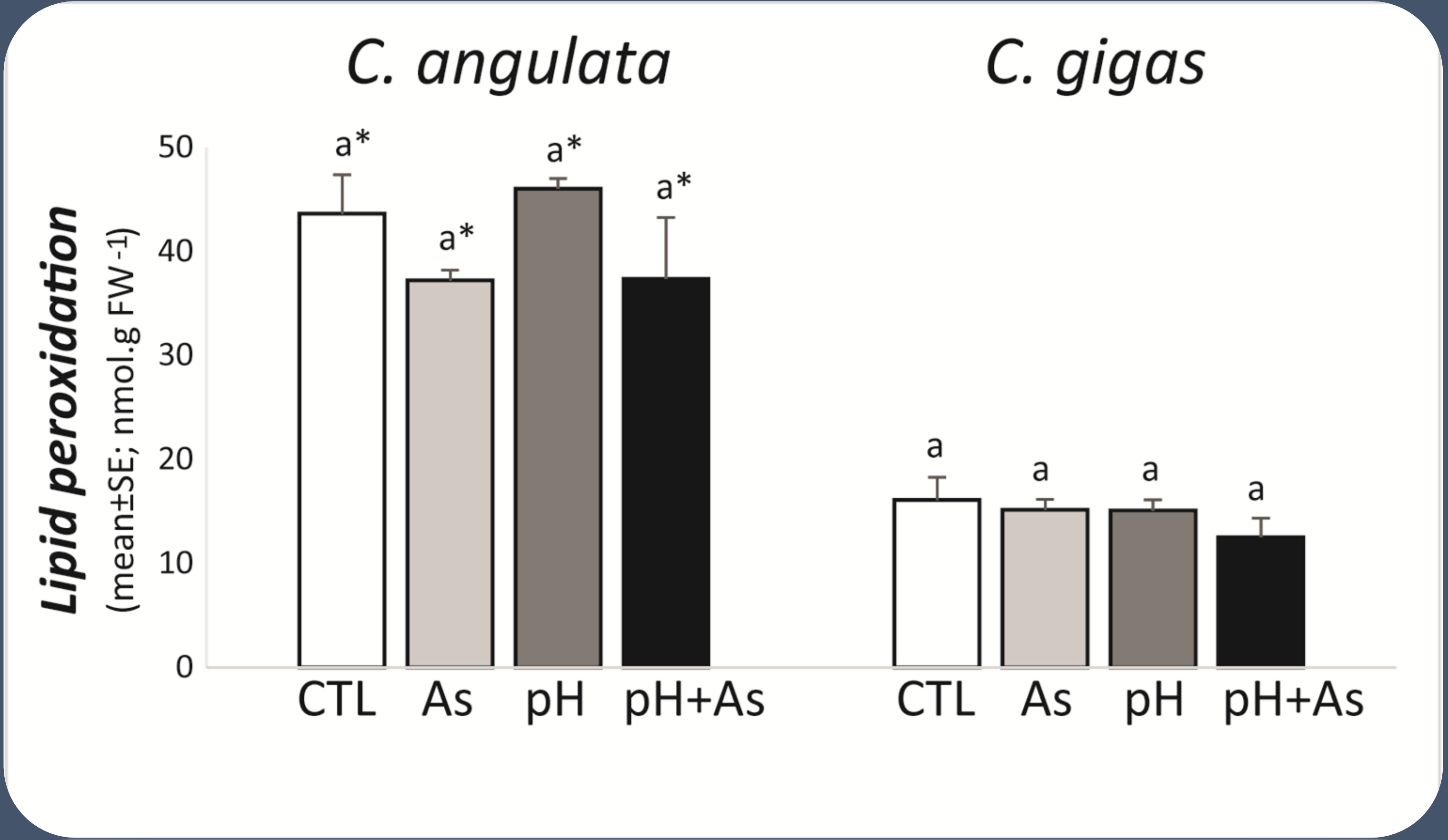
Results

❖**LPO** was **significantly higher** in *C. angulata* than in *C. gigas* for all treatments

❖**No significant** differences between treatments within species

Note: *C. angulata* presents higher LPO in the field (own data not shown)

Lipid peroxidation

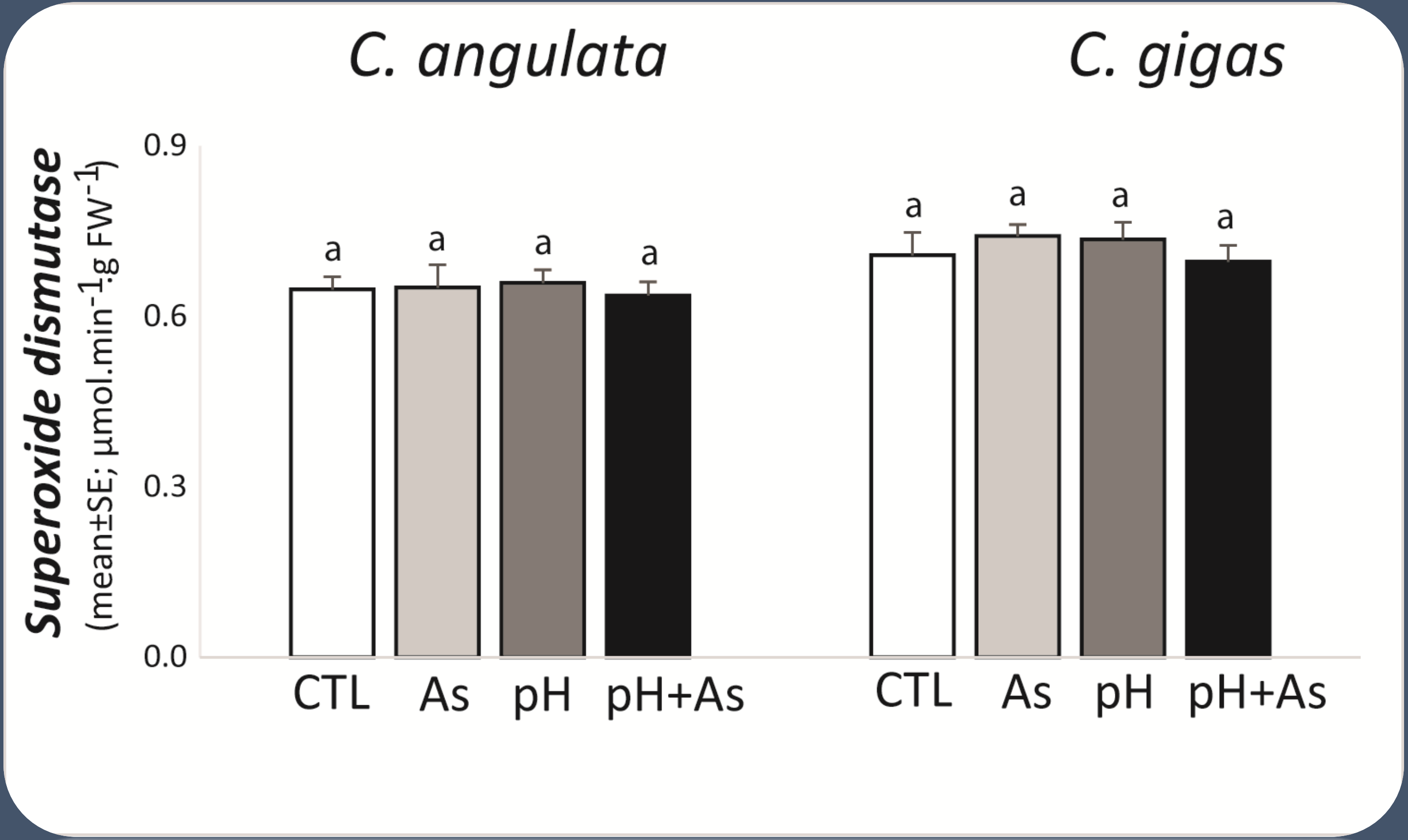


Diff. letters *p* ≤0.05 within species
* *p* ≤ 0.05 between species

Results

- No significant differences between species
- No changes among treatments

Superoxide dismutase



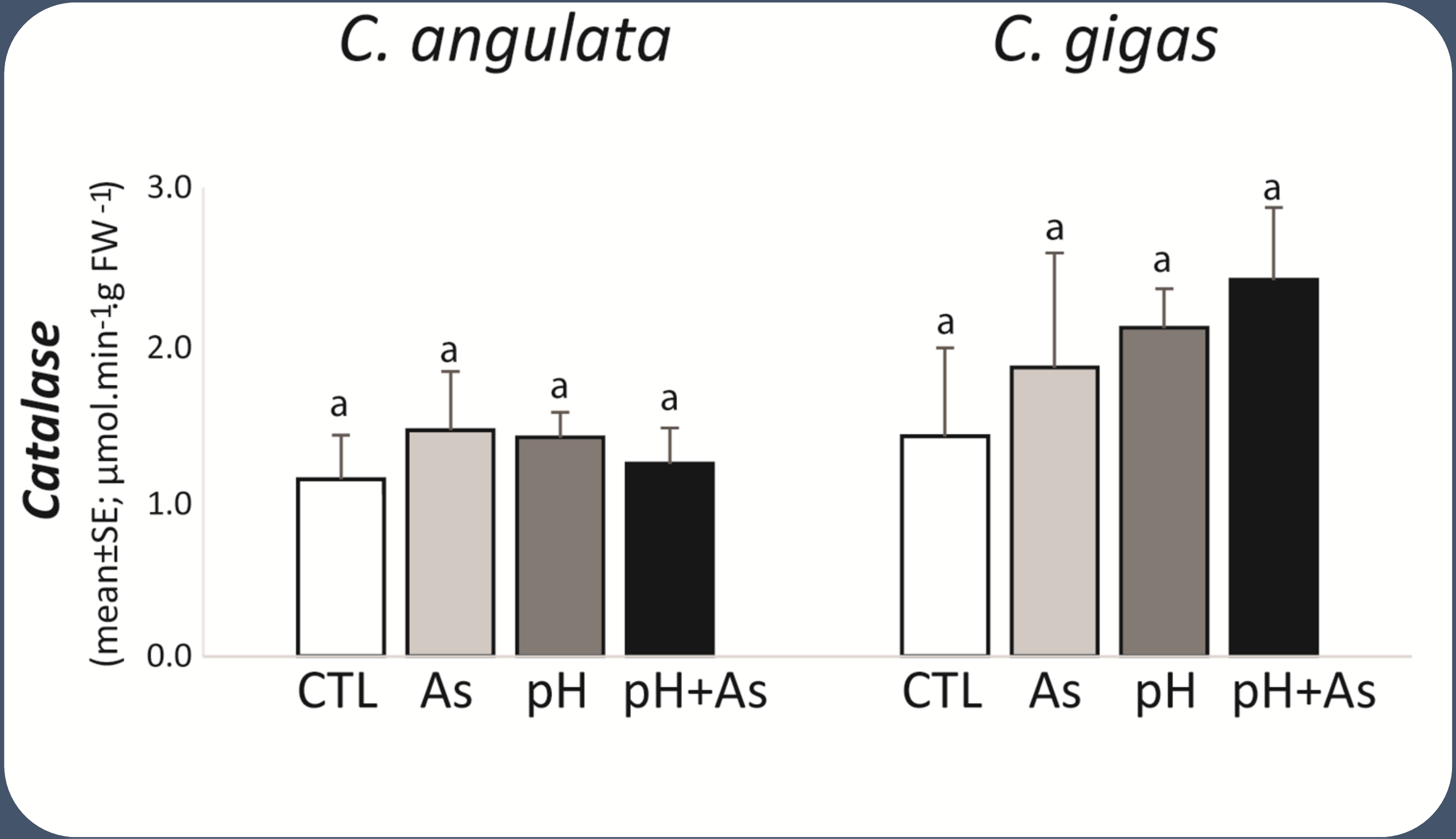
Diff. letters $p \leq 0.05$ within species
* $p \leq 0.05$ between species

Results

❖No significant differences between species

❖*C. gigas* shows an increasing CAT activity, especially when exposed to pH+As (70% increase comparing to control)

Catalase



Diff. letters $p \leq 0.05$ within species
* $p \leq 0.05$ between species

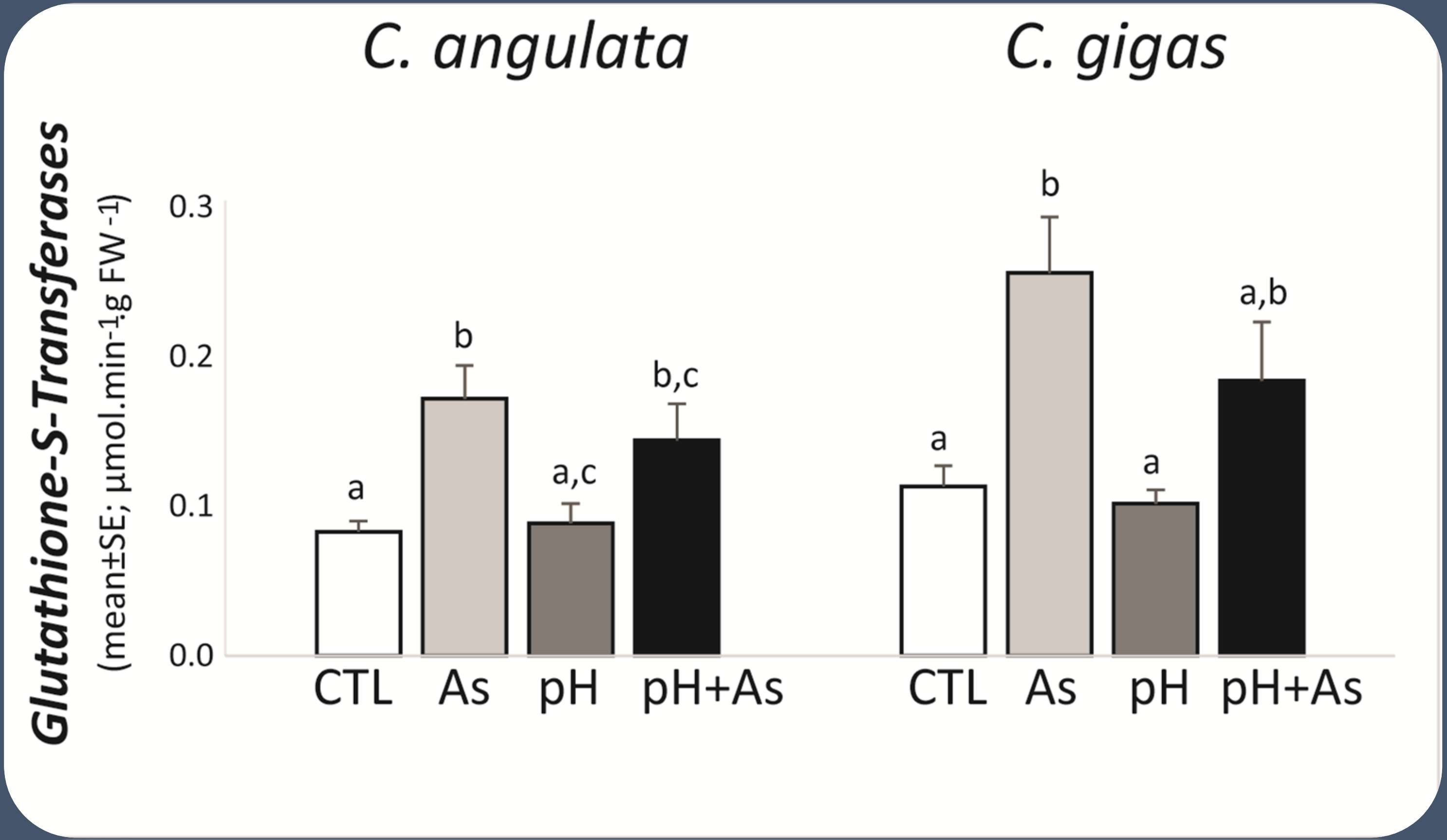
Results

❖Both species presented a similar pattern for **GSTs**

❖**GSTs** showed higher activity in **As** exposed oysters

❖pH did not show effects on **GSTs** activity

Glutathione-S-Transferases



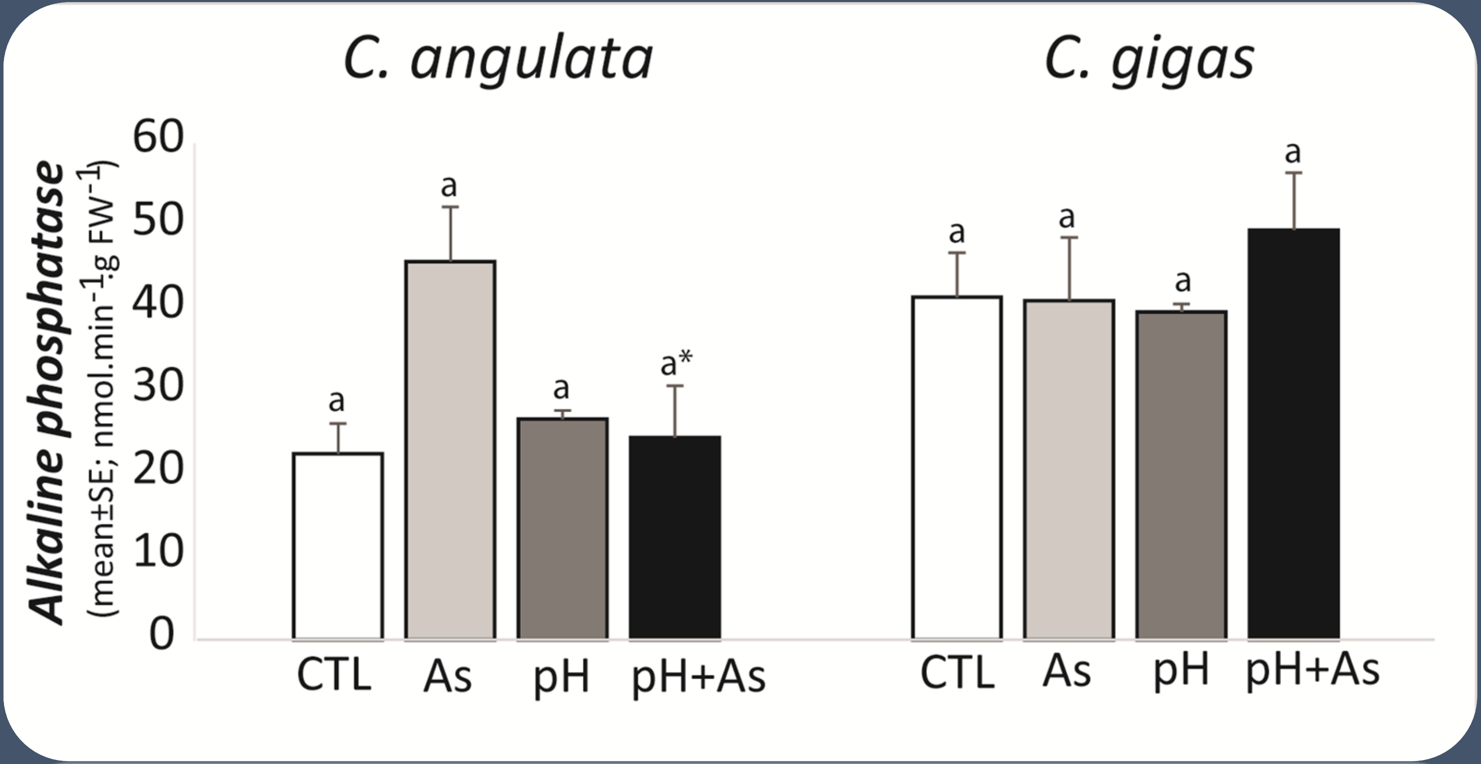
Diff. letters $p \leq 0.05$ within species

* $p \leq 0.05$ between species

Results

- *C. gigas* presented higher Alk Pho activity than *C. angulata* in all treatments except for As
- *C. angulata* presented higher Alk Pho activity when exposed to As
- ALK Phos was significantly different between species for pH+As

Alkaline phosphatase



Diff. letters $p \leq 0.05$ within species
* $p \leq 0.05$ between species

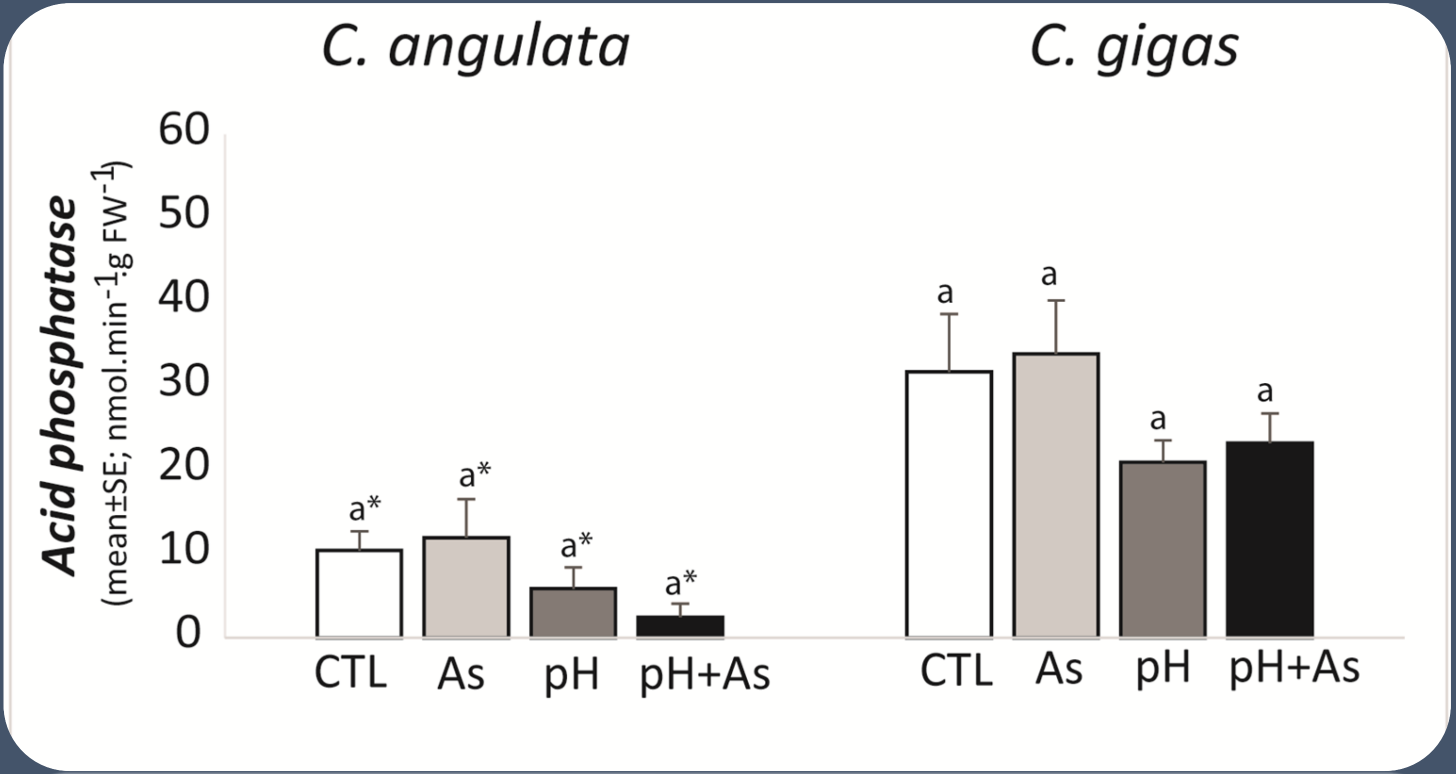
Results

❖Phos Ac was significantly lower in *C. angulata* for all treatments

❖Phos Ac activity in *C. angulata* reduced 44% relative to control in low pH, and 76% for pH+As

❖Phos Ac activity in *C. gigas* reduced 34% (pH), and 27% (pH+As)

Acid Phosphatase



Diff. letters $p \leq 0.05$ within species
***** $p \leq 0.05$ between species

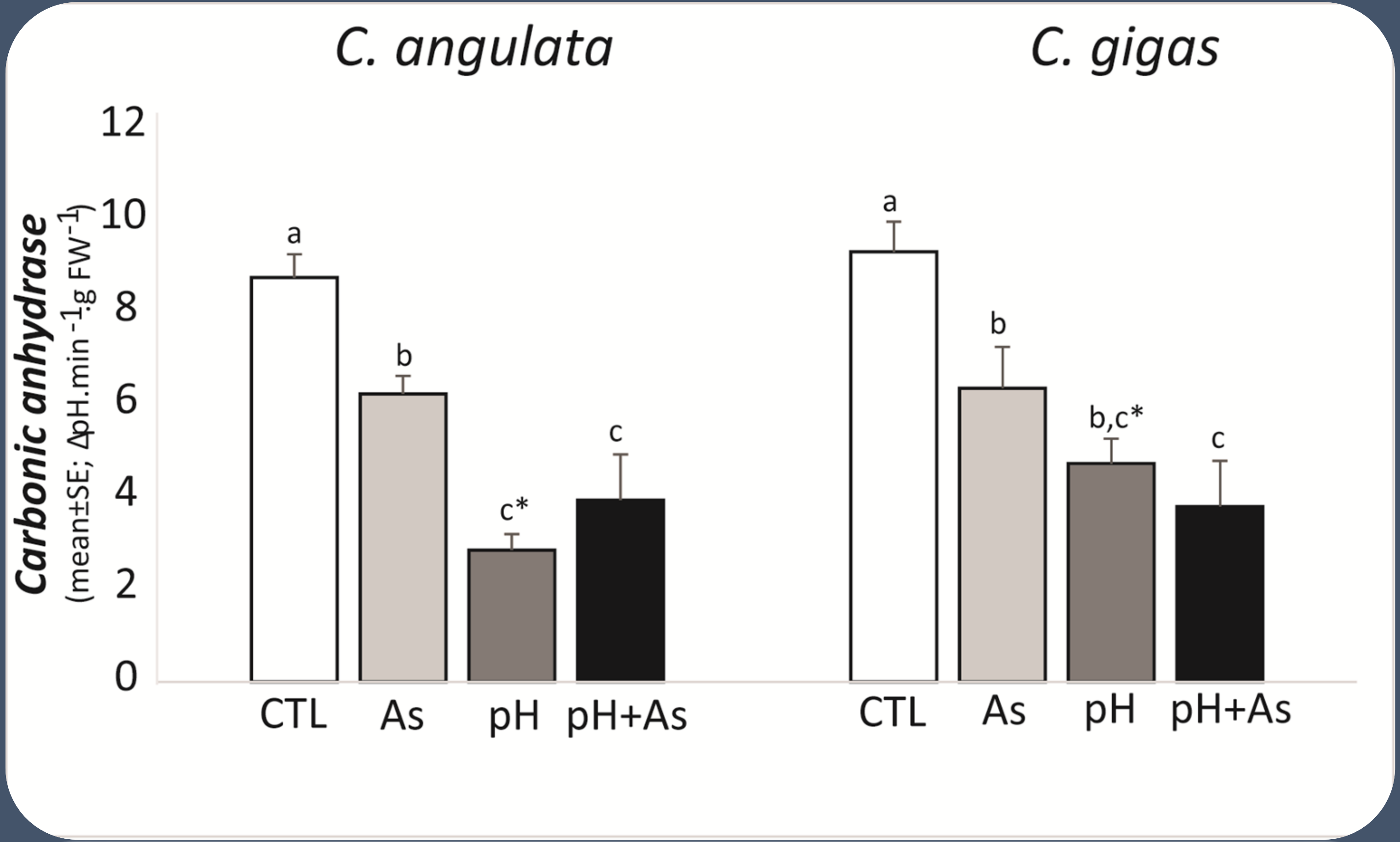
Results

❖Both species presented similar results for **CA**

❖**CA** activity was affected by both As exposure and pH

❖ *C. angulata* showed higher inhibition of **CA** activity for low pH (67 % lower than control) comparing to 49% in *C. gigas*

Carbonic anhydrase



Diff. letters $p \leq 0.05$ within species

* $p \leq 0.05$ between species

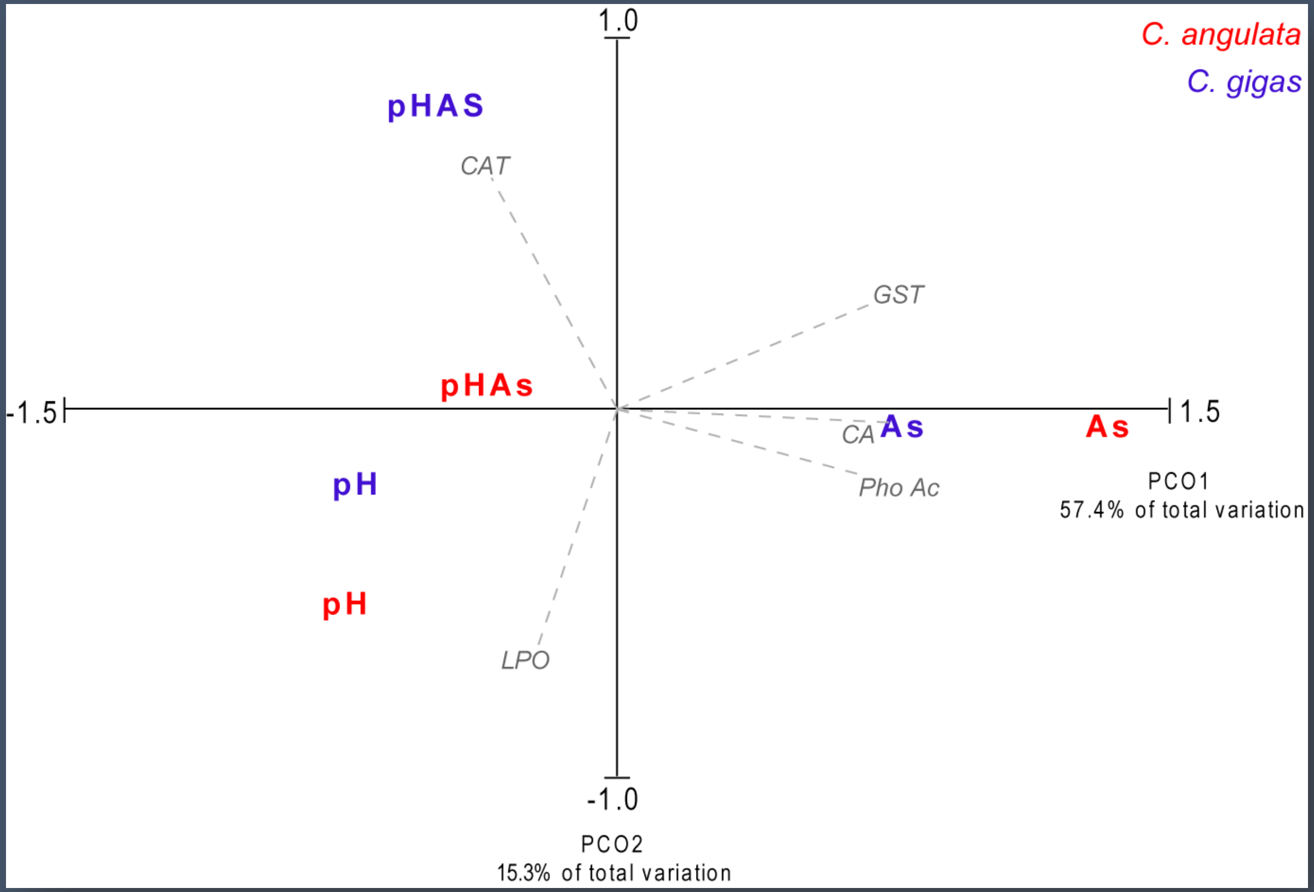
Results

- Clear separation between all treatments, where species respond biochemically within the same pattern

- Descriptors that characterize treatment separation between As and low pH treatments were (GST, Pho Ac, and CA)

- Descriptors that characterize treatment separation between low pH and pH+As, were LPO and CAT respectively

Principal components ordination (PCO)



r> 0.75

Discussion

http://northislandexplorer.com/molluscs/giantpacificoyster.htm

- Results show that adult oysters can be affected by acidification, bringing new insights these species ecophysiology, since most studies focus on larvae and seeds
- Low pH signifincantly affected carbonic anhydrase, indicating this enzyme as a good biomarker for seawater acidification related stress assessment
- The combined effect of low pH and As did not appear to be cumulative
- *C. angulata* appears to be more sensitive to low pH conditions than *C. gigas*, generally showing a steeper response to stressors (e.g Phosp Ac and CA)

Does the effect of low pH and As, acting alone or in combination, influence each species performance?

- Both species showed biochemical alterations for tested conditions
- PCO showed a clear separation between treatments in relation to control
- CA, GSTs showed significant changes between tested conditions and control oysters
- The **combined** effect of low pH and As did not appear to be cumulative for the majority of parameters assessed

Concluding remarks

Do species respond differently to tested conditions?

- Although the performance of *Crassostea gigas* and *C. angulata* followed a similar pattern in response to tested conditions
- Species response varied in intensity
- *C. angulata* showed higher sensitivity to low pH (CA, Phos Ac)
- *C. gigas* showed higher Phos Alk activity than *C. angulata* for low pH + As
- *C. gigas* presented higher GSTs activity than *C. angulata,* indicating a better response towards As

Concluding remarks

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Acknowledgments