# OCEAN ACIDIFICATION IMPACTS SURVIVAL OF JUVENILES AND REDUCES SHELL RESISTANCE OF ADULT ABALONE *H. TUBERCULATA*

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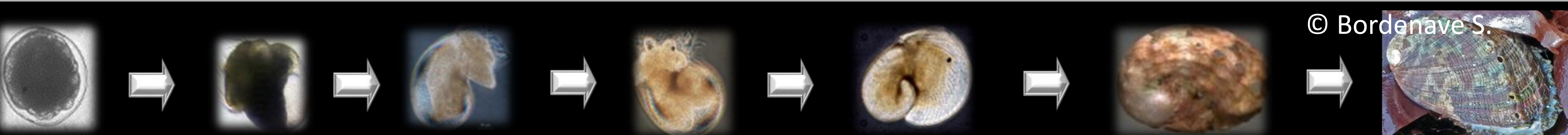
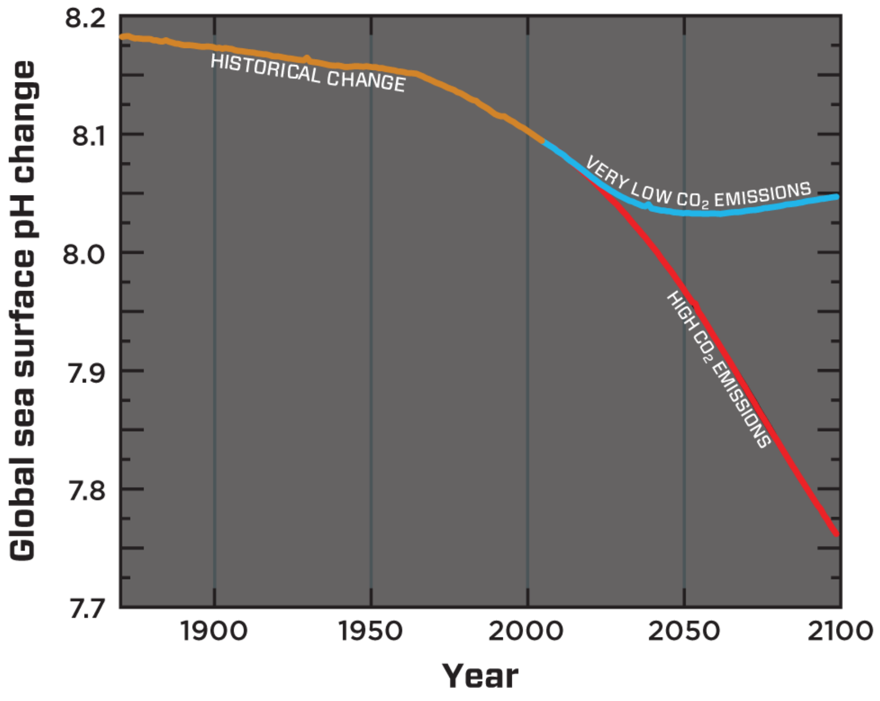


# Ocean acidification : a major global stressor

1- Reduced pH
2- Modified carbonate system equilibrium
- ↘ calcium carbonate saturation state (Ω)
- ↗ the concentration of dissolved inorganic carbon (DIC)

According to the most pessimistic predictions (RCP 8.5 scenario, IPCC), surface ocean pH should decrease up to 0.3 unit by 2100.

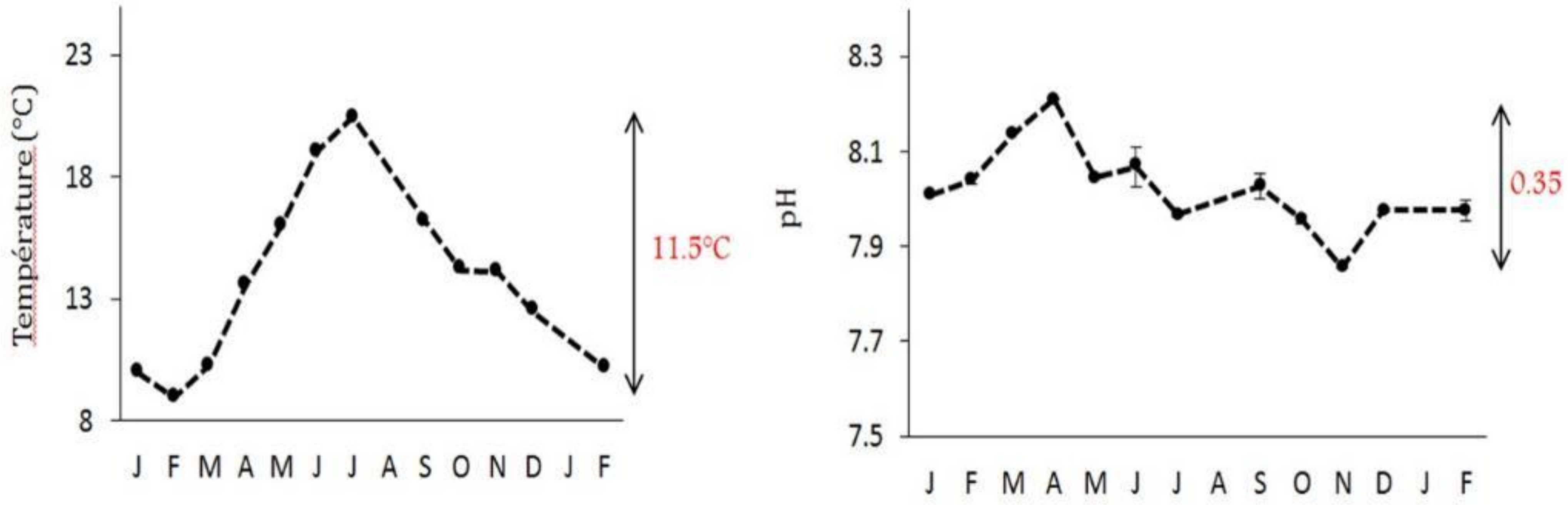
OA and GW already affecting
⇨ shellfish industry and local economies (Petit, 2018)
⇨ barrier reef




*Surface pH change based on the RCP 8.5 (red) prediction, RCP 2.6 (blue) .Adapted from Bopp et al., 2013*

# pH in coastal marine system : seasonal variation

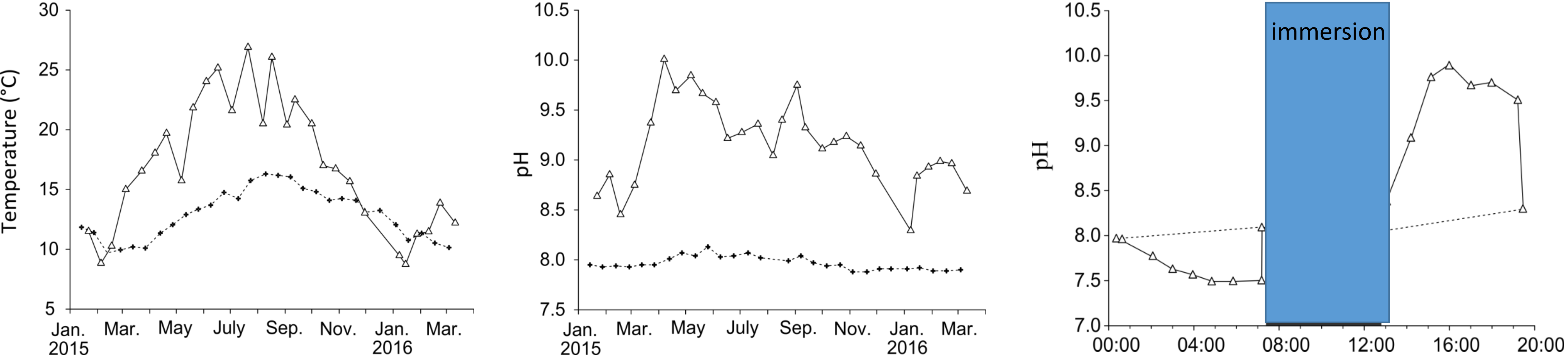
Seasonal variability in Maerl bed, Bay of Brest (Brittany, France)




*Qui Minet et al. (2018) Estuarine, Coastal and Shelf Science*

# pH in coastal marine system : seasonal AND diurnal variation

Seasonal and diurnal variability of temperature and pH in intertidal rockpool (Roscoff, Brittany, France)




Legrand *et al.* (2018) *Regional Studies in Marine Science*

# Acidification on farm : frequently observed but not often controlled

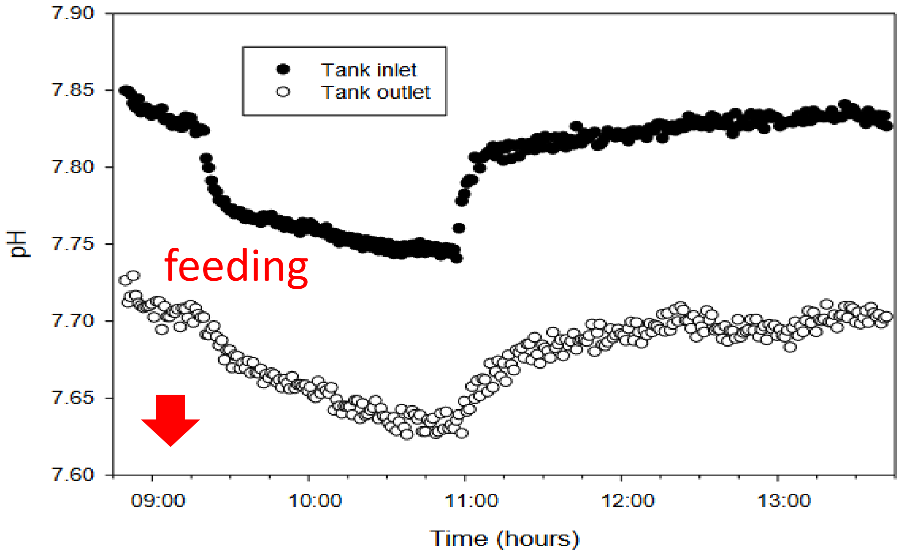
## FLOW-THROUGH SYSTEM

=> water pumped in shallow environment

## HIGH INTENSITY RECIRCULATED AQUACULTURE SYSTEM

- ⇒ Rapid accumulation of metabolic carbon dioxide
- ⇒ Respiration of abalone and bacteria
- ⇒ Influenced by the activity in the tanks (feeding)

**pH measured in a high intensity recirculated pilot aquaculture system**




(From Wright, 2011)

# OCEAN ACIDIFICATION

affects organisms **producing calcium carbonate shells**, tests or skeletons, such as molluscs, corals and echinoderms, to different extents

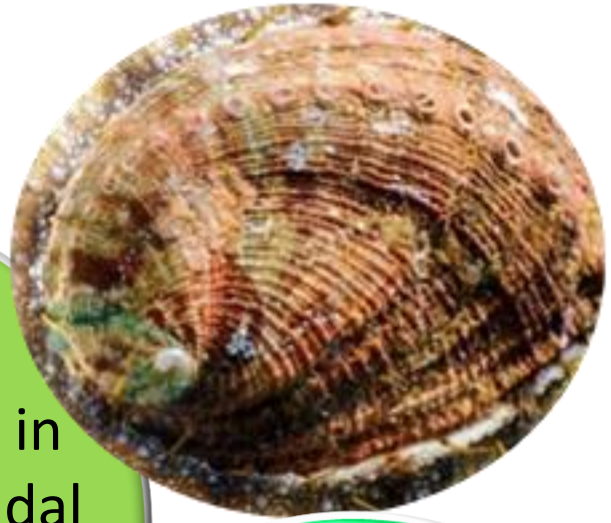
(Hendriks *et al.,* 2010; Hofmann *et al.*, 2010; Wittmann and Pörtner, 2013; Cyronak *et al.,* 2016).

# MOLLUSCS VULNERABLE

Because they regulate only partly their acid-base balance

(Fabry, 2008; Gazeau *et al.*, 2013; Kroeker *et al.*, 2013; Parker *et al.*, 2013)

ABALONE
A mollusc living in intertidal – subtidal environment, with aragonite shell



Abalone : fished and farmed species

# OBJECTIVE

Study the effect of a **low ocean pH (7.7)** on **adult abalone during reproduction conditioning** and on their **offspring** using a **multivariate approach**

# ANIMALS

Abalone

- 3.5 years of age
- 5 cm
- 3-week acclimation

# TREATMENTS

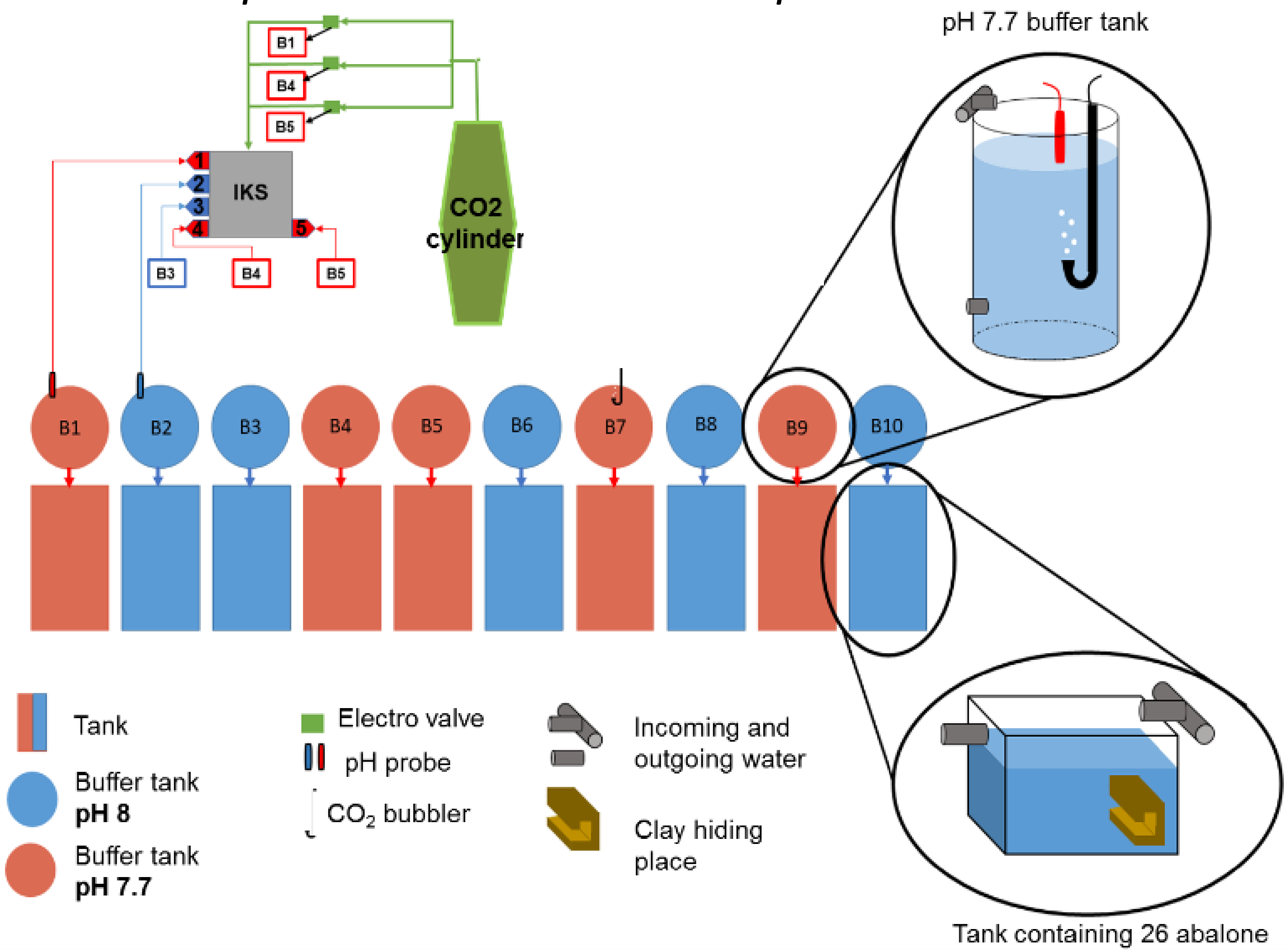
**Actual pH (8.0 – 8.1)**

**Low pH (7.7)**

# EXPERIMENTAL SET-UP

- $CO_2$ bubbling in buffer tank
- 5 replicate (tank) per treatment

*pH control and tank setup*



# Multivariate approach

*Experimental setup*


**BIOLOGY**
- Growth
- Survival
- Morphology

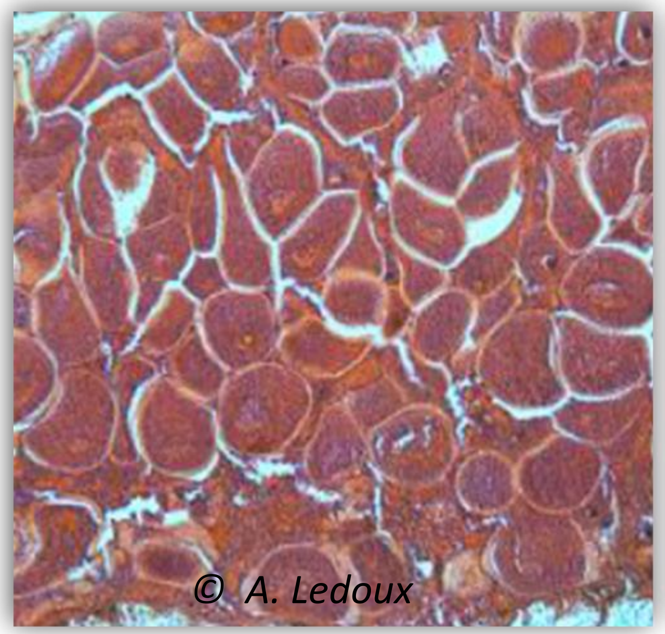
**BEHAVIOUR**
- Diurnal rhythm
- Feeding behaviour
- Responses to stressors

**PHYSIOLOGY**
- Metabolism
- Immunity
- Calcification
- Reproduction

# GROWTH and REPRODUCTION

- Growth and mortality of adults
- Gonad maturation and ratio
- Number of ovocytes and spermatozoids
- Fertilization

FEMALE




MALE

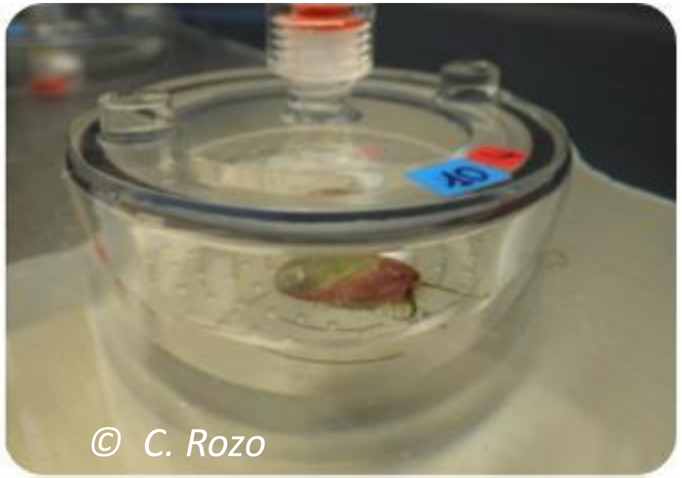

# METABOLISM and IMMUNITY

***Immune parameter***

- Phagocytosis efficiency

***Metabolism parameters***

- Respiration
- Excretion
- Haemolymph pH




*Respiratory chamber*

# CALCIFICATION

## Shell fracture force


*force bench (Instron 5543) used for the shell fracture measures*

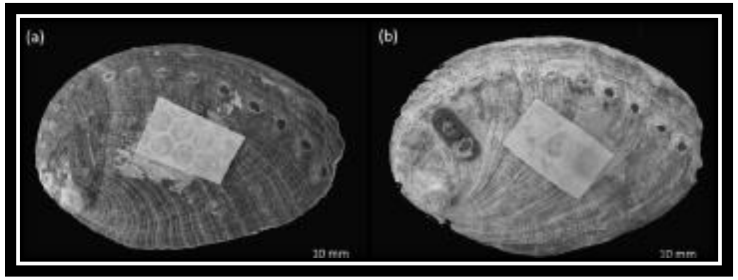
## Shell thickness and microstructure

with Scanning Electron Microscopy

## Net shell calcification

## Shell color pattern

in grey value



# BEHAVIOUR

## *DIURNAL AND FEEDING BEHAVIOUR*

## *SPAWNING BEHAVIOUR*

# BEHAVIOUR

## *HIDING TEST*


## *PREDATOR TEST*

*Starfish contact*

## *RIGHTING TEST*

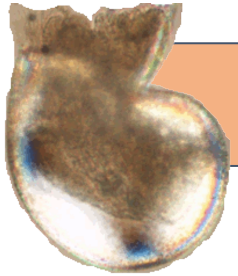
***Statistical model***

*⇨ mixed model with treatments as fixed effect, and the tank as random factor*

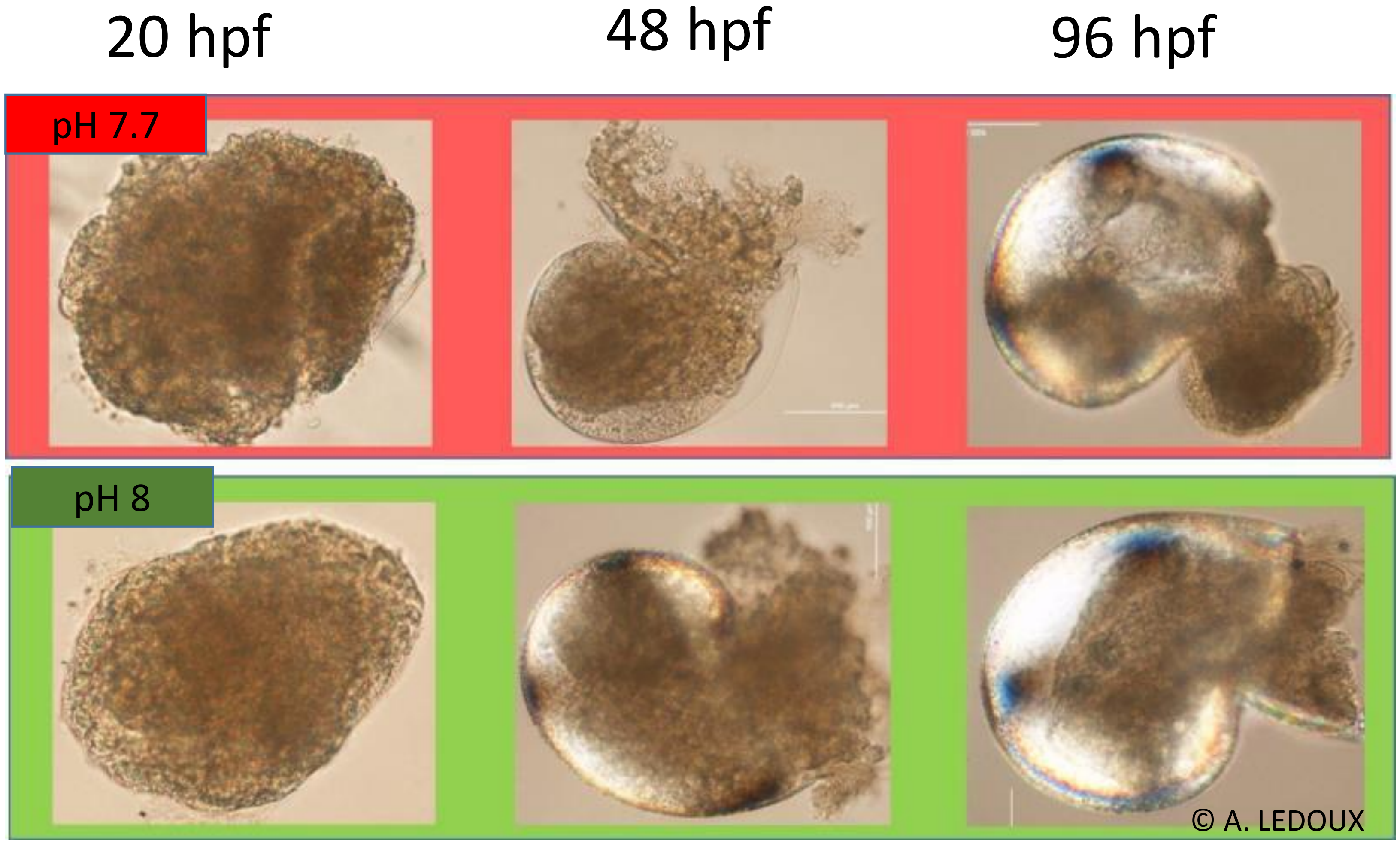
*3-week-acclimation* | *4-month-exposition*

| | | 1rst month | 2nd month | 3rd month | 4th month |
|---|---|---|---|---|---|
| **Actual pH (8.0 – 8.1)** | | | | | |
| **Low pH (7.7)** | | | | | |

| MEASURES | 1rst week | 2nd month | 3rd month | 4th/ 5th month |
|---|---|---|---|---|
| Diurnal, feeding and spawning behaviour |  n = 26/tank | |  n = 20/tank |  n = 34/treat |
| Hiding, predator and righting test |  n = 20/treat | |  n = 20/treat | |
| Phagocytosis activity, haemolymph pH |  n = 10/treat |  n = 10/treat | |  n=10 /treat |
| Respiration, excretion |  n = 10/treat |  n = 10/treat |  n = 15/treat | |
| Gonad index |  n = 10/treat |  n = 20/treat |  n = 15/treat |  n = 25/treat |
| Gonad maturation | | | |  n = 10/treat |
| Calcification rate |  n = 10 /treat |  n=10/treat |  n=15 /treat | |
| Shell weight, shell thickness, shell fracture force | | | |  |



# LARVAL VIABILITY AND GROWTH




- **Shell calcification** and **morphology of larvae**

=> Body abnormalities of larvae

- **Viability** and **growth** of larvae and juveniles at 20 hpf, 48 hpf, 96 hpf, 9 days, 2.5 months and 5 months

# BEHAVIOUR

NO EFFECT on **acute behavioural stress responses**

⇨ Hiding, predator and righting test

NO EFFECT on **basal behavioural responses**

⇨ Diurnal behaviour during a 48h observation

⇨ Feeding behaviour during a 4-month duration

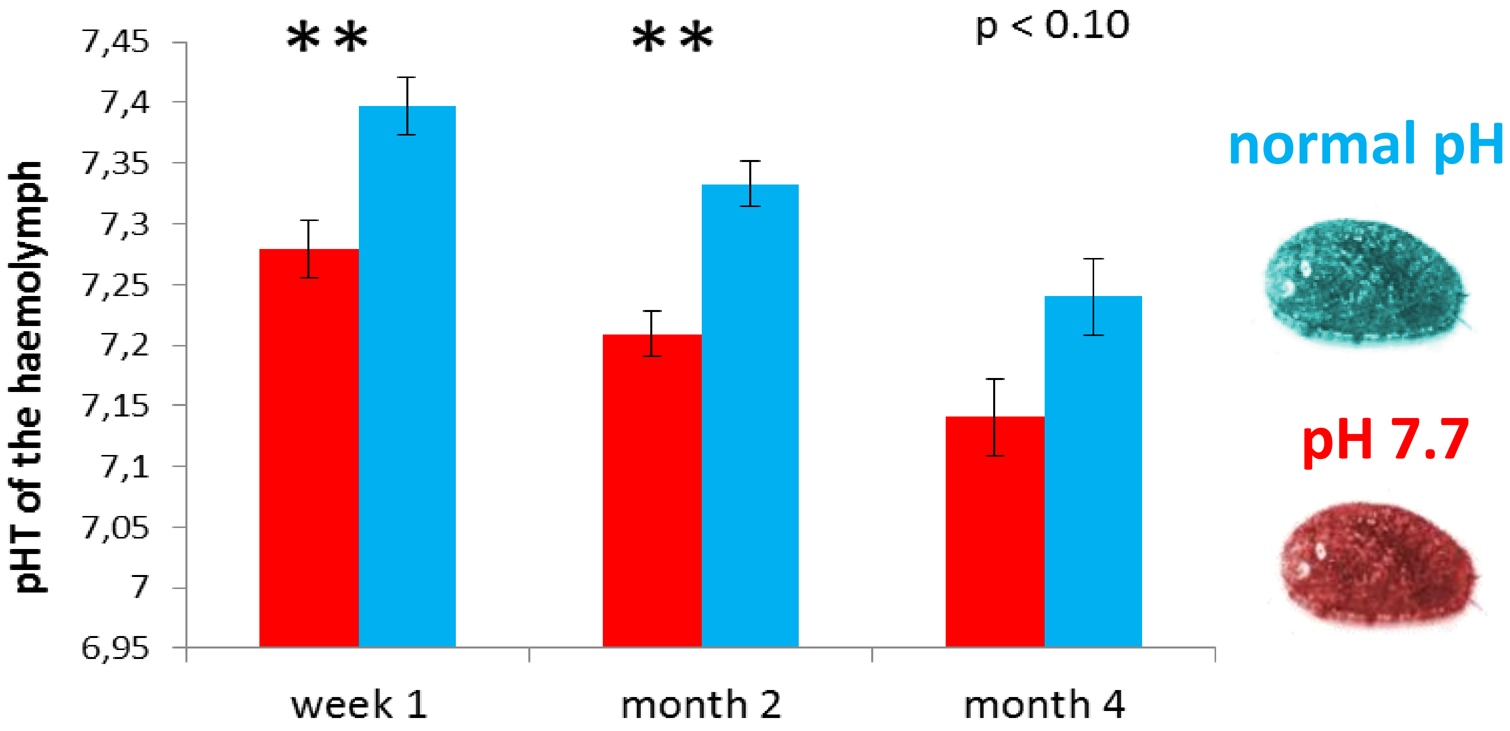
⇨ Spawning behaviour

In contradiction with reduced reactivity observed in fish *(Cripps et al., 2011, Dixon et al. 2010, Porteus et al., 2018)*.
However, in some species such as echinoderms, reported effects of OA are not so pronounced *(Cohen-Rengifo, 2018)*

# IMMUNITY and METABOLISM

EFFECT on **pHT of the haemolymph**

⇨ *abalone exposed to low pH had a reduced haemolymph pHT*




NO EFFECT on **phagocytosis efficiency**

NO EFFECT on **respiration** or **excretion**

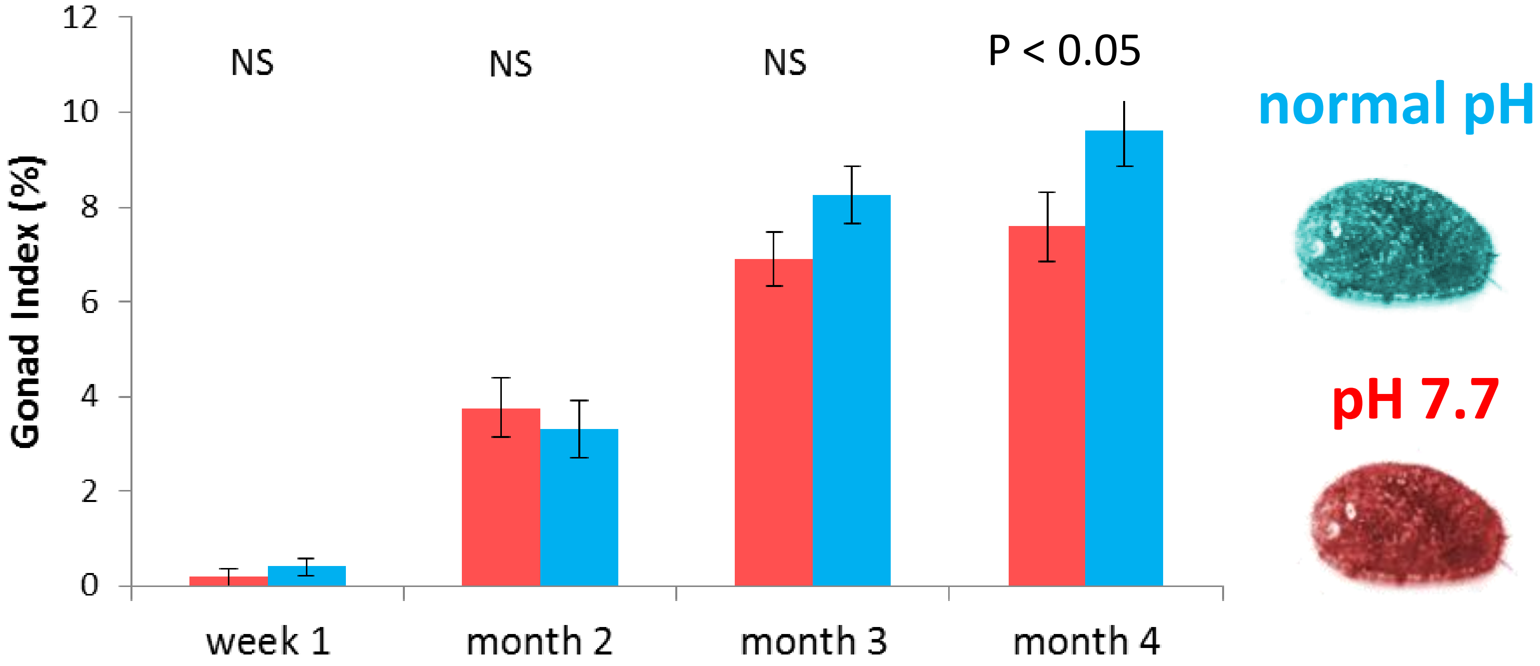
No effect on respiration and excretion traits reported in others species *(Mussels : Benitez et al. 2018; Sydney rock oyster : Scanes et al. 2018)*

# GROWTH & REPRODUCTION

A WEAK EFFECT on reproduction : a reduction of the **gonad ratio** but NO EFFECT on maturity

NO EFFECT on **mortality**

A **REDUCED SHELL LENGHT** after 5 months of exposition

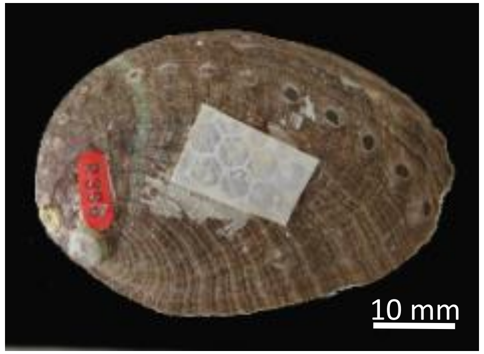



Effect on the reproductive status reported in the sea urchin *(Kurihara et al., 2013)* and Sydney rock oyster *(Scanes et al., 2018)* but not always *(Moulin et al., 2015)*

# CALCIFICATION

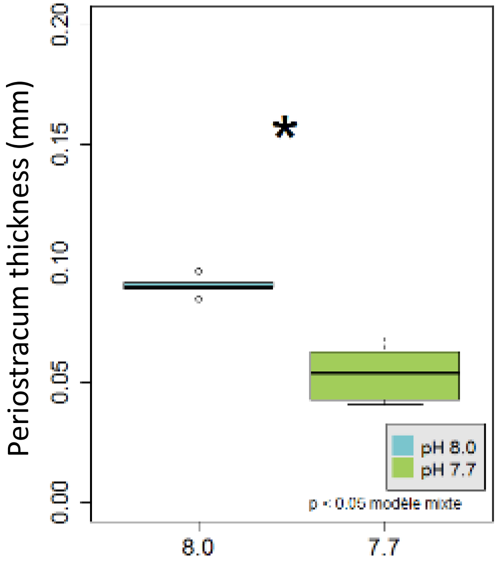
A STRONG EFFECT with a REDUCTION of the net calcification rate, a grey coloration of the shell , and reduction of periostracum thickness and structure ($p < 0.01$)

**normal pH**




**pH 7.7**

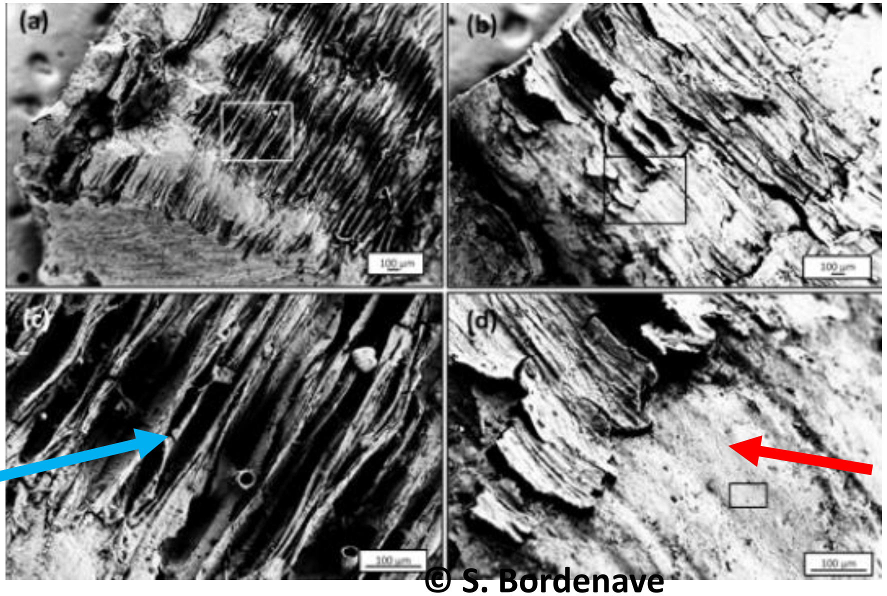

*Periostracum thickness*




homogenous surface with the typical ridge and groove pattern.

**Scanning electron microscopy of the outer shell surface (periostracum)**

**normal pH** **pH 7.7**



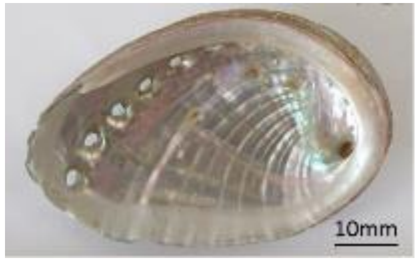

delamination of organic layer and revealing the underlying spherulitic layer.

© S. Bordenave

# SHELL CALCIFICATION

- Partial dissolution of the nacre surface, irregular or damaged and a reduced shell fracture force lower in low pH treatment

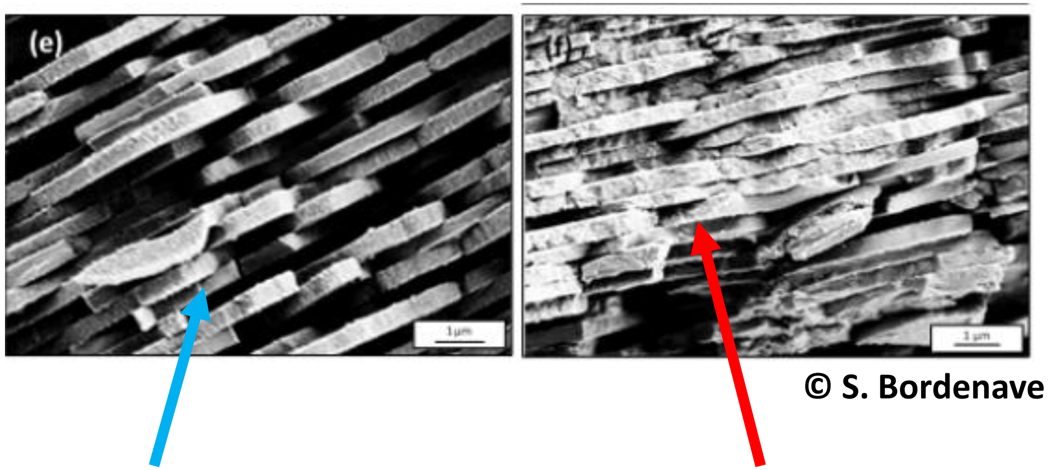
**normal pH**




**pH 7.7**


**Scanning electron microscopy of the nacre**

**normal pH** | **pH 7.7**

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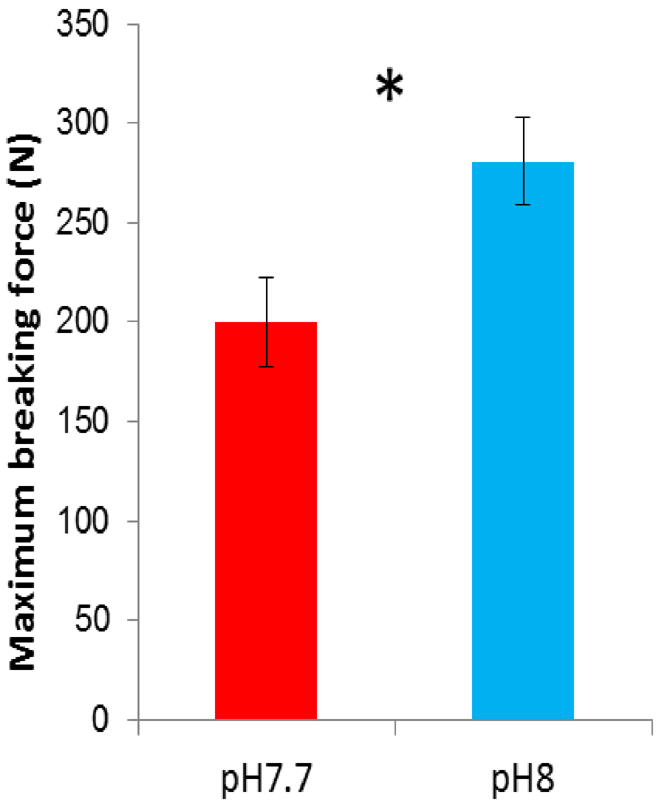
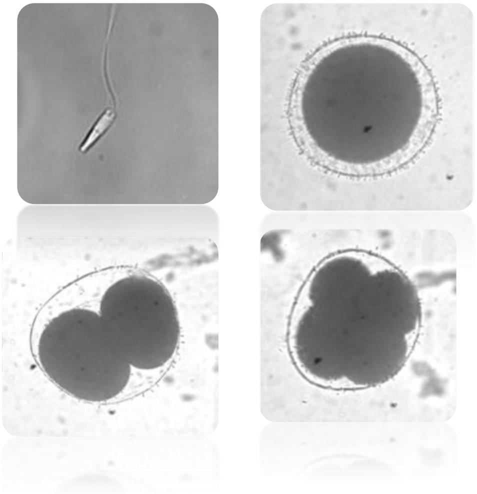
regular stacks of aragonite platelets

Disorganized and pitting corrosion within the aragonite platelets

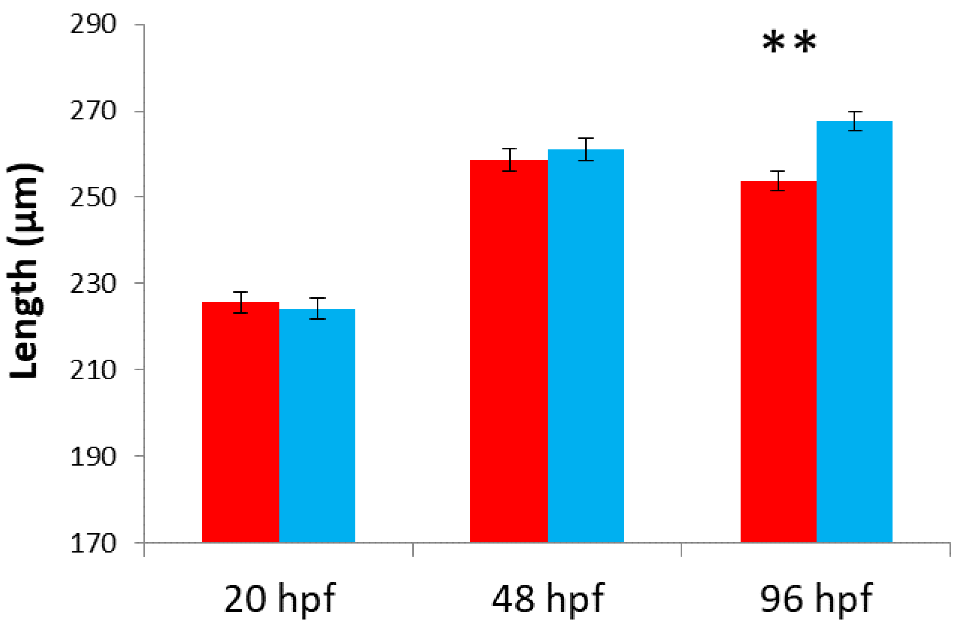


# LARVAL VIABILITY AND GROWTH

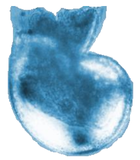
No EFFECT on **gamete production** and **fertilization**



An **increase** of the **% of shell** and **body abnormalities** at 48hpf and 96 hpf, a **reduction** in **shell calcification** and **length** at 96 hpf of larvae exposed to low pH




**normal pH**



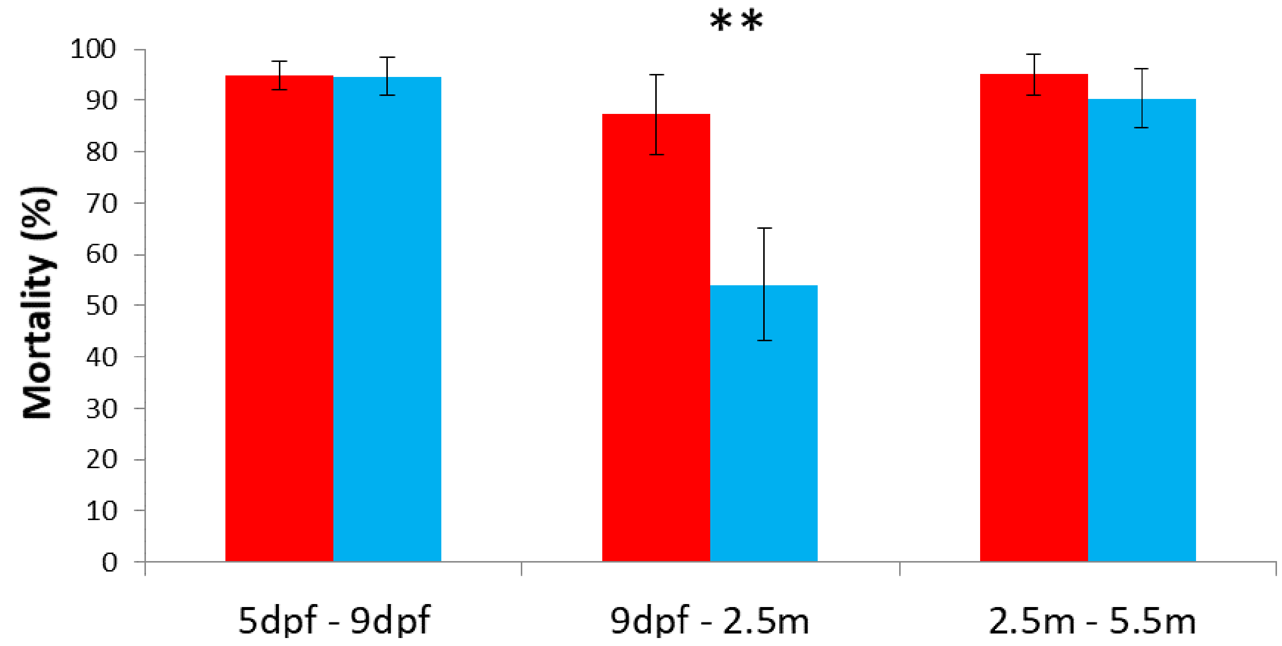
**pH 7.7**

Results in accordance with *Wessel et al. 2018* and *Byrne 2011*

# POST-LARVAE and JUVENILES

No effet on larval fixation

An **increase** of **mortality** between post-fixation and 2.5 months of age of juveniles exposed to low pH




normal pH

pH 7.7

# SUMMARY
# EFFECT OF OCEAN ACIDIFICATION ON ADULT ABALONE

= behaviour
= respiration / excretion
= immune status
= mortality

⇨ No effect

(↘) gonad investment

⇨ Reduced energy level
Reproduction: one of the most sensitive function

↘ shell growth
↘ shell fracture force
↘ haemolymph pHT

⇨ Potential impacts on acid-base regulation and shell calcification

OA and **adult** abalone
=> no effect on vital function
=> probably less sensitive to pH decrease compared to juvenile and larval stages because the main physiological functions were not impacted

# SUMMARY
## EFFECT OF OCEAN ACIDIFICATION ON LARVAE AND JUVENILE PROGENIES

⇨ No effect observed for gametes, eggs and larvae before shell formation (before 20 hpf)

⇨ No effect observed after 3 months of age

**48hpf and 96hpf larvae**

↗ shell and body abnormalities

↘ shell calcification

↘ length

**9 dpf – 2.5 month old juvenile**

↗ mortality

**Larvae and young juveniles** before 3 months of age : **the most sensitive stage** resulting in high malformation and important mortality

⇨ Even if **broodstock** was **acclimated** during 5 months to low pH, progenies were sensitive to the effects of ocean acidification in the first 3 months of age

# JUST THE FIRST STEP …

*Further studies need to be performed*

1- Interaction between ocean acidification and **global warming** ?
2- Interaction between ocean acidification and **food resources** ?
And the **trophic chain** (complex ecosystem)?

*Various mitigation strategies practicable in the future to maintain sustainable abalone population in the wild as well as in aquaculture system*

1- Possibility to select families more resistant to acidification ?
2- Epigenetic mechanism = **conditioning** the broodstock or larvae to OA ?

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