Blue diatoms as a potential for sustainable shellfish aquaculture

By

Fiddy S. Prasetiya*, Priscilla Decottignies, Michèle Morançais, Luc A. Comeau, François Turcotte, Romain Gastineau, Iskandar, Toto Subroto, Yudi N. Ihsan, Réjean Tremblay, Bruno Cognie, Christophe Stavrakakis and Jean-Luc Mouget

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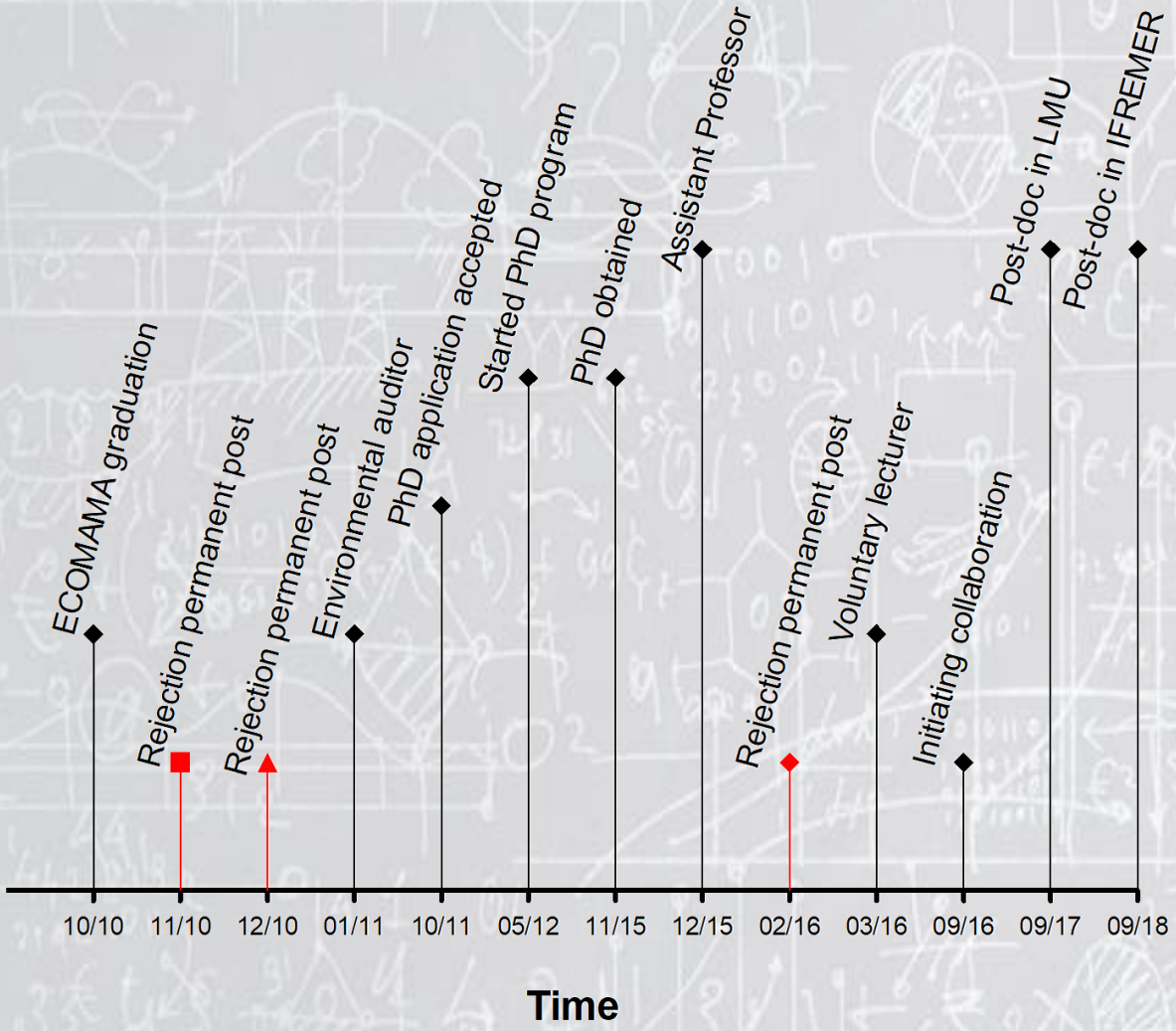


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Challenges for Indonesian young researchers

- Limited research funding sources
- Complexity for obtaining national grants applications
- Less funding opportunity for basic research
- Lack of transparency, unclear & long delay of funding

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NEWS · 20 FEBRUARY 2018

Indonesian scientists hamstrung by year-long funding delay

The country's dedicated science fund has failed to raise enough money to finance projects.

Research collaboration to minimize the gap..

Marie Skłodowska - Curie Actions

H 2020

GHaNA 2017-2021

The Genus Haslea, New marine resources for blue biotechnology and Aquaculture

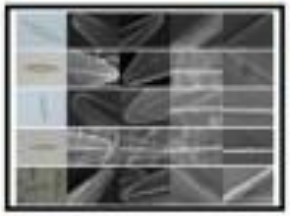
Public-private partnership: 13 countries, 22 institutions (budget: 1.6M€)

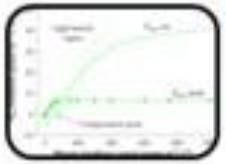
Lead institution: (France) Le Mans Université

EA 2160 Mer-Molécules-Santé
FR CNRS 3473 IUML
Lead: Jean-Luc.Mouget@univ-lemans.fr
Co-lead: PerkinsR@cardiff.ac.uk

Diatoms from the genus *Haslea*

Antimicrobials
Aquaculture
Biodiversity
Blue biotechnology
Cosmetics
Ecophysiology
Genomics
High-value compounds
Lipids
Marennine
Oysters
Photobioreactors
Phylogeny
Pigments
Process engineering
Reproduction
Strain maintenance





Participant Countries:
France
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Denmark
Greece
Switzerland
Poland
Spain
USA
Canada
Australia
Algeria
Indonesia
Vietnam

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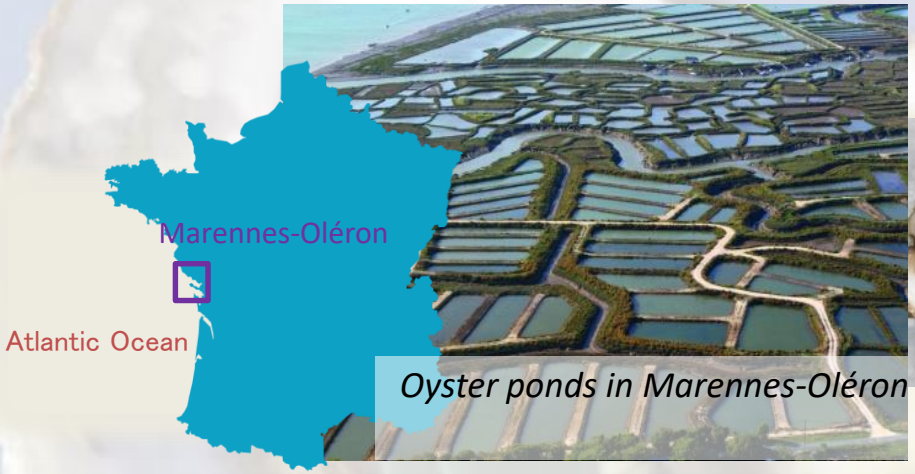
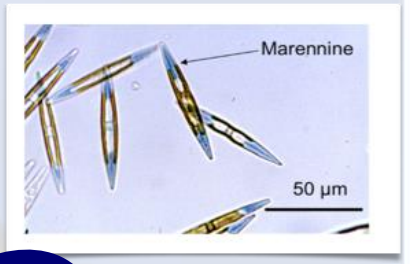
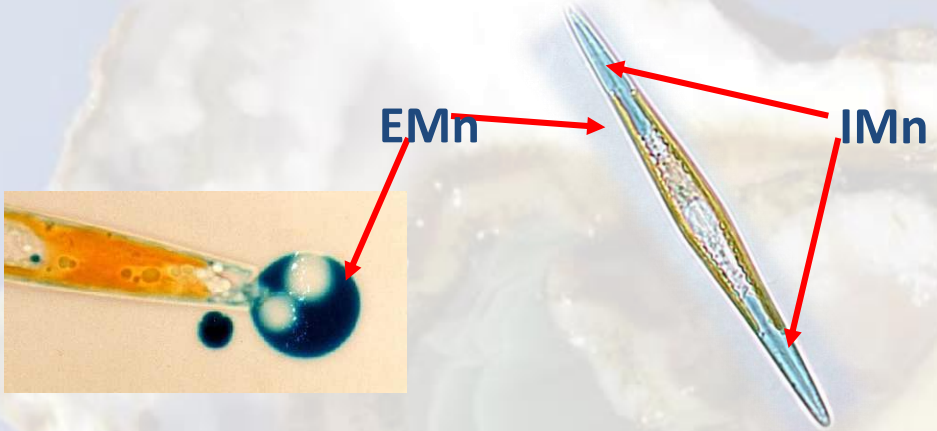
Haslea ostrearia and oyster greening





- Oyster greening:
  - Erratic proliferation of *H. ostrearia* & pigment released in oyster ponds
  - Marennine fixation on oyster gills
  - Added economic value

Haslea ostrearia and Marennine

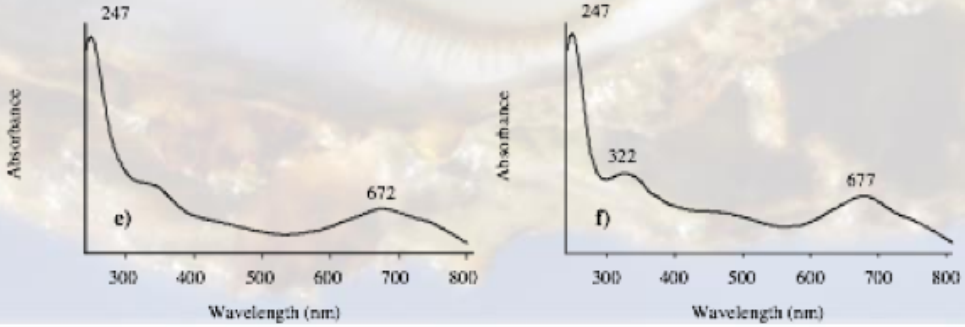


Marennine molecule structure?

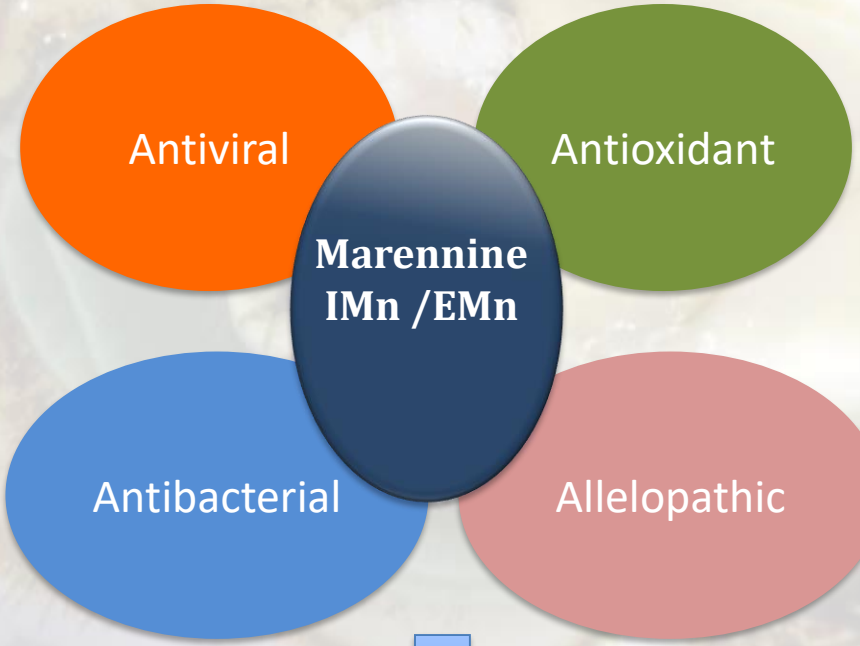
Unknown:

- Pouvreau *et al*. (2006): Polyphenolic
- Gastineau *et al*. (2014): Glycosidic

Characteristics	Type of pigment	
	IMn	**EMn**
Localization	Apical axis of the cell	Released into the medium
Molecular weight	10.7 kDa	9.8 kDa



Biological activities of marennine *in vitro*



Purified EMn and IMn

HSV-1

Gastineau *et al*. (2012)

Purified EMn and IMn

Pouvreau *et al*. (2008)

Purified EMn and IMn

Vibrio tubiashii
V. aestuarianus
V. coralliilyticus
V. Tasmaniensis

Gastineau *et al*. (2014)

***H. ostrearia* in co-culture**

Skeletonema costatum
Chaetoceros calcitrans
Tetraselmis suecica

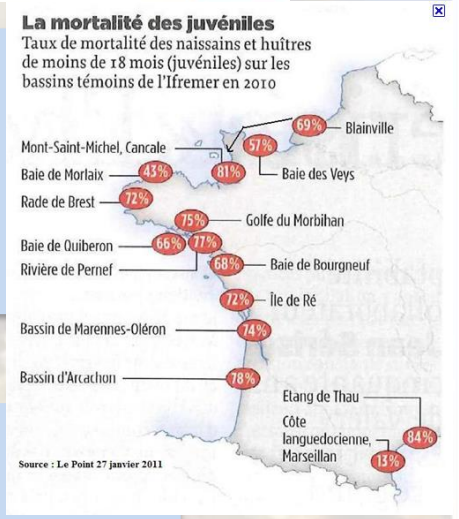
Prasetiya *et al*. (2016)

Oyster aquaculture

- France is the main oyster producer in Europe (87.4%)

However…

- Pathogen infections
  - Disease outbreaks (OsHV-1, *Vibrio aestuarianus*)
  - Mass mortality: juveniles (2008), adults (2013)

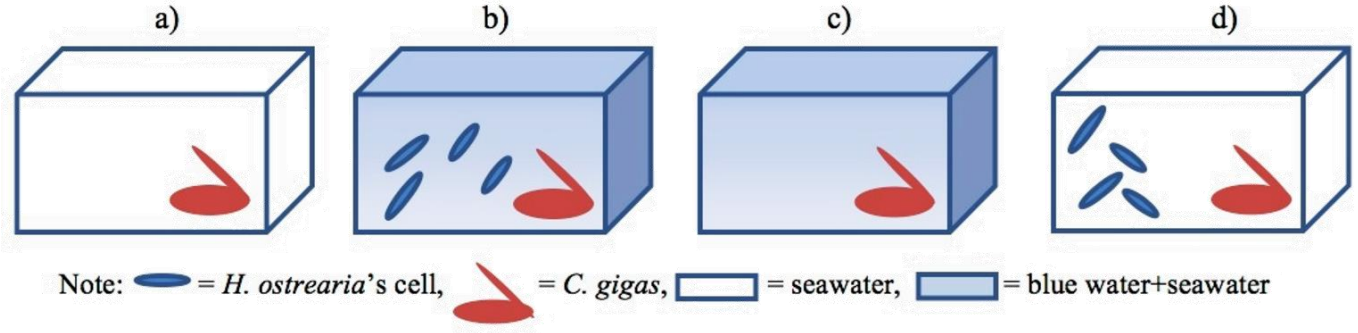


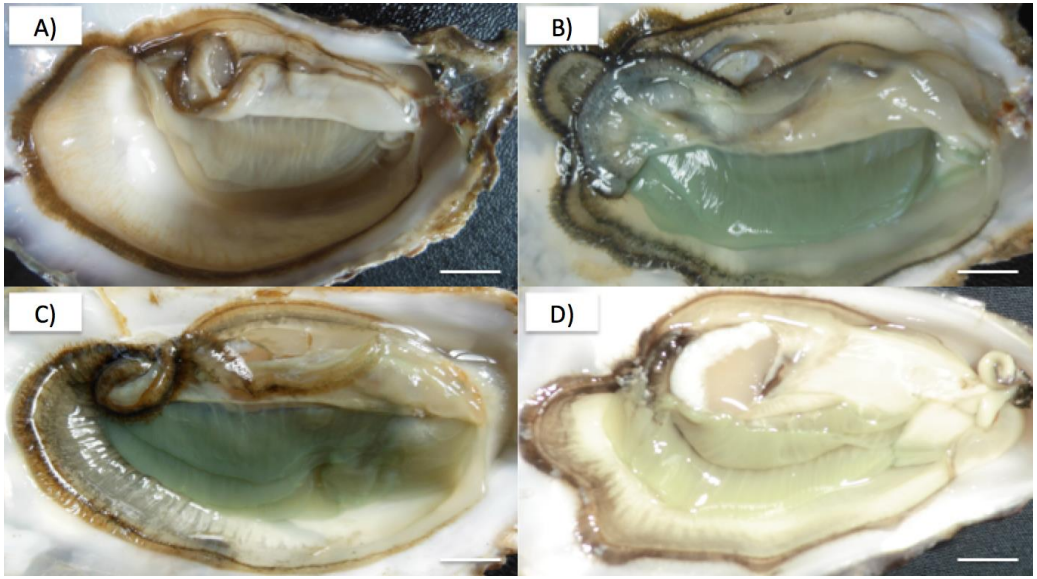
- Stock depletion & Economic loss

Assessment for utilization of marennine in shellfish aquaculture

- How the greening is occurred?
- How Haslea affect oyster's feeding behavior?
- Consequence of greening on functional responses of bivalve?
- Prospective application of marennine in shellfish aquaculture?

Is the greening only due to EMn in solution, or to the diatoms (IMn) consumed by oysters?





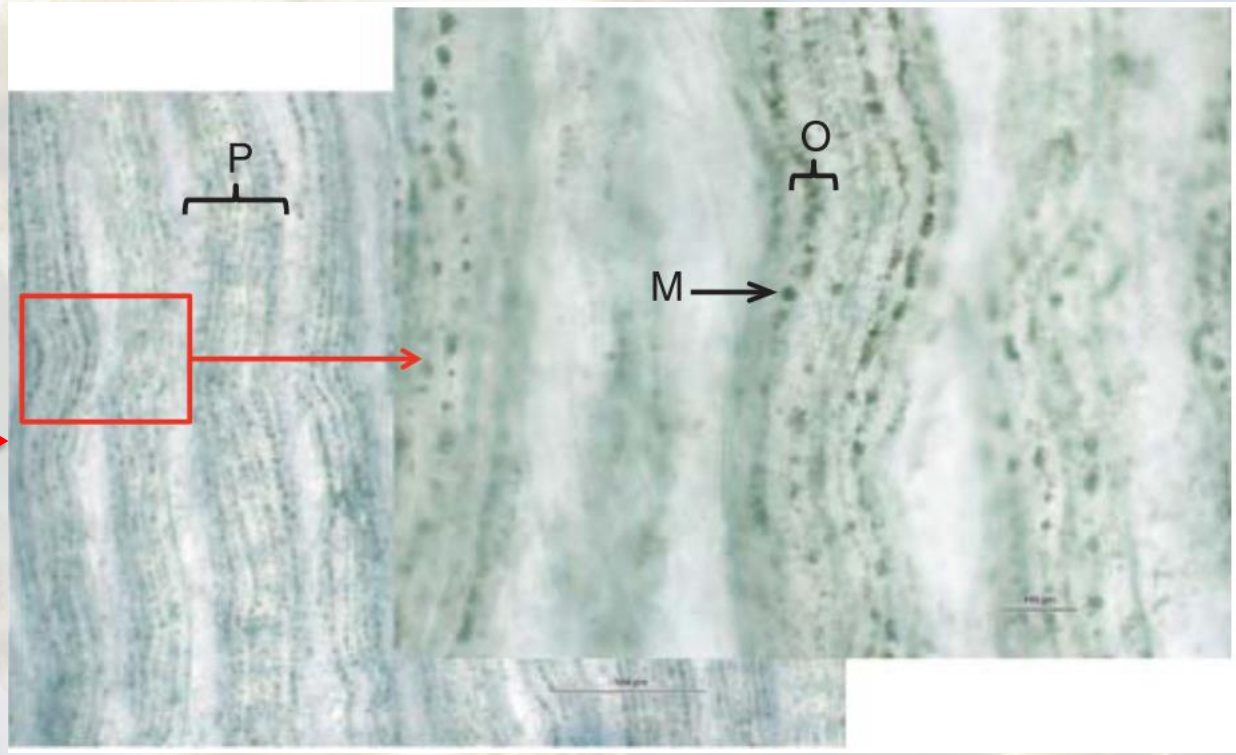
a) Control (without marennine and *H. ostrearia*)
b) H. ostrearia + blue water (IMn + EMn, 72h)
c) Blue water (supernatant) (EMn, 72h)
d) H. ostrearia cells (IMn, 12 weeks)

Persistency of greening

No de-greening after 12 weeks

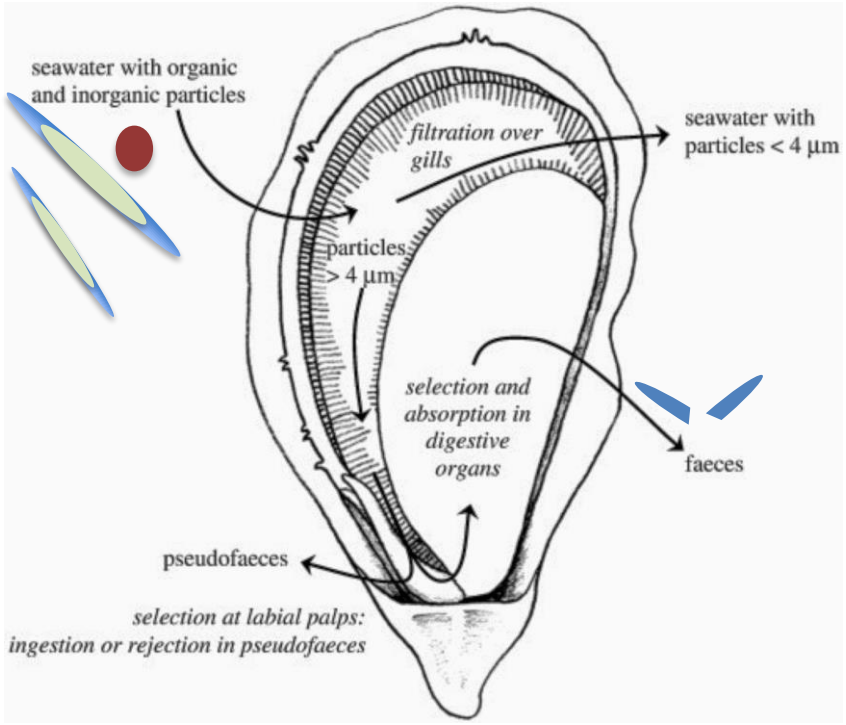
Marennine fixation is persistent

Marennine fixation on the gills



Marennine fixation in mucocytes

Haslea on feeding behaviour



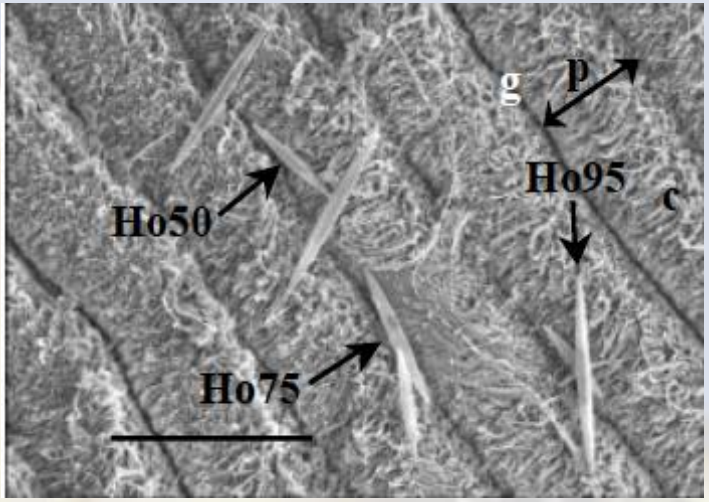
Cognie (2001), Barille *et al*. (2001)

Does the size really matter?

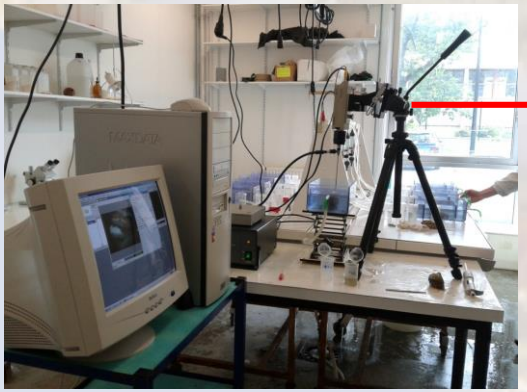
Photo: JD Billaud

Part 2: Role of size in preingestive selection of *H. ostrearia* in *C. gigas*

- Experiment A: Scanning electron microscopy (SEM)



- Experiment B: Video endoscopy directed-sampling



DT

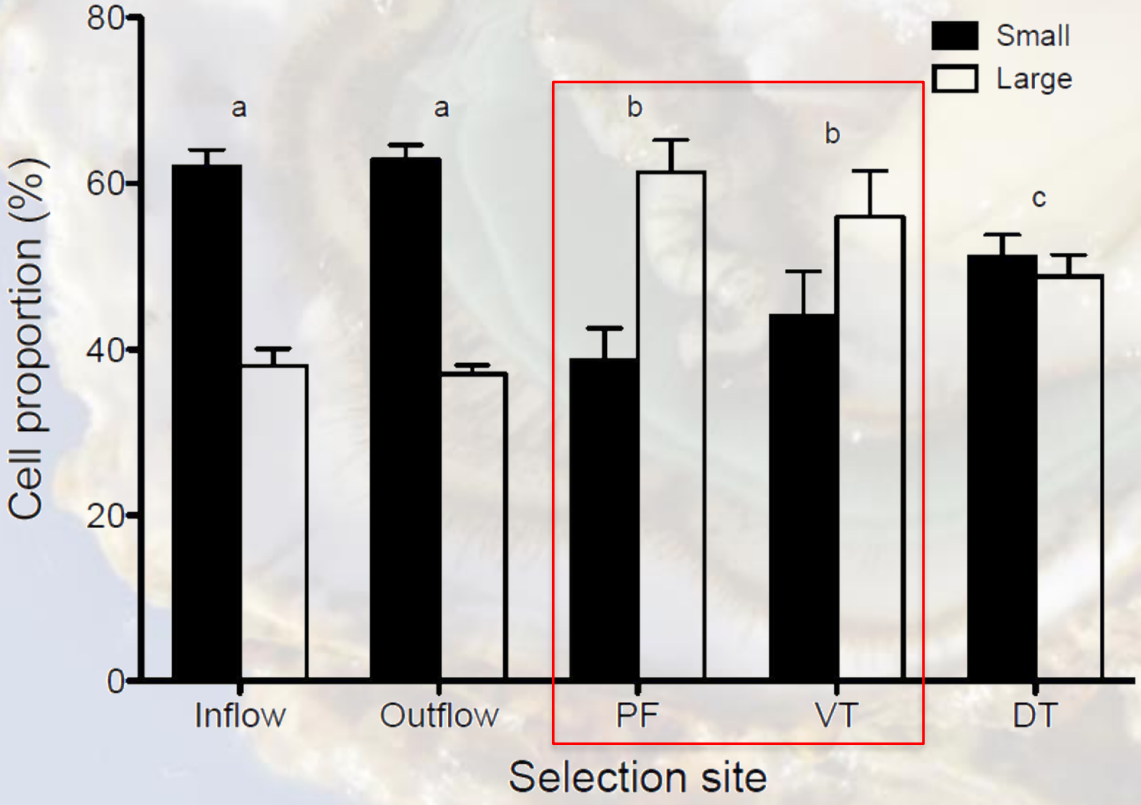
VT

Data analysis:

- Homogenity & Normality test
- T-test (XLSTAT)

Experiment B:

Video endoscopy observation



Rejection of larger cells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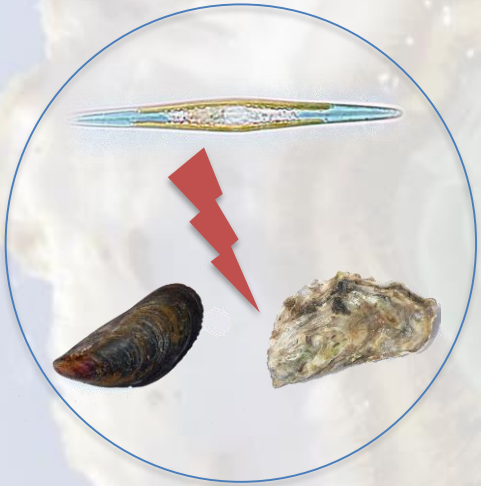
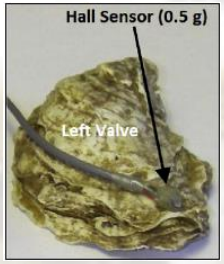
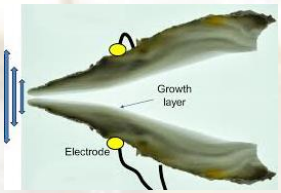
Valve activity experiment (short-term effect)

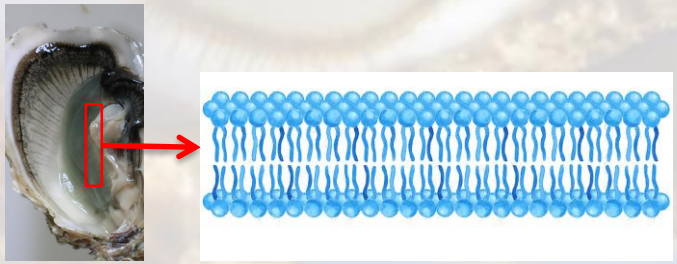






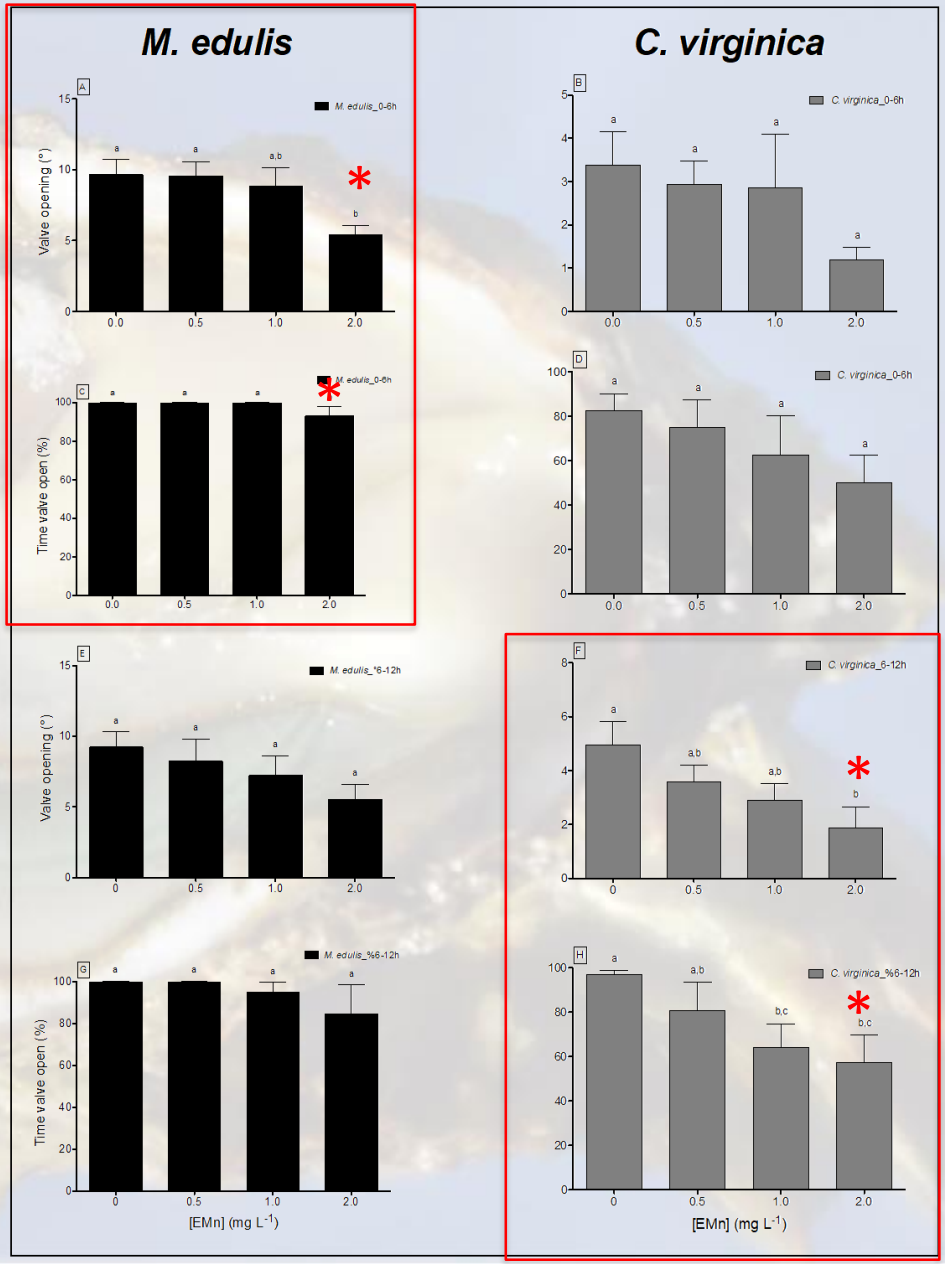
Analysis of Scope for Growth

Lipids and FAs analysis (long-term effect)



Behavioral traits

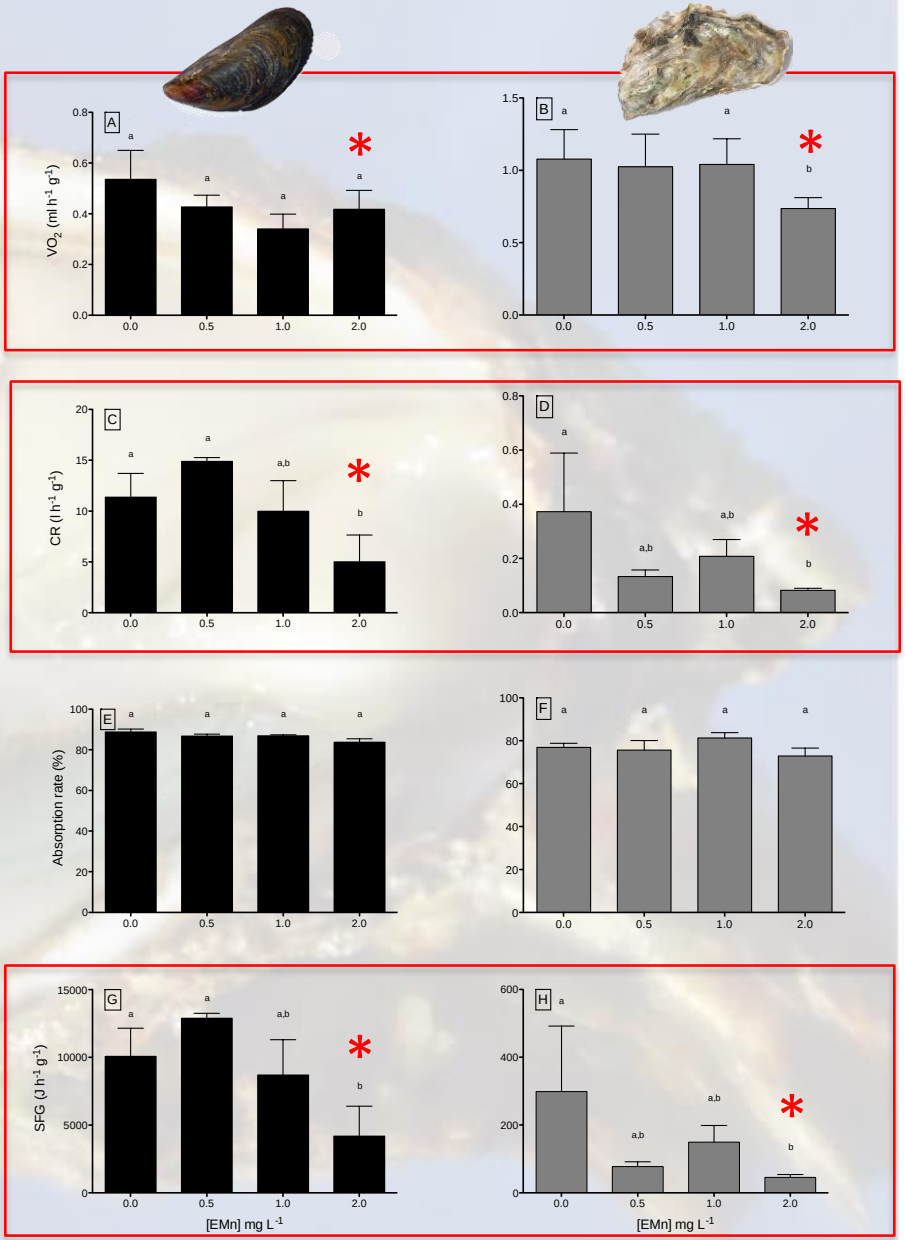
- Significant effect on mussel's acclimation phase
- Post-acclimation effect on oyster



Physiological traits

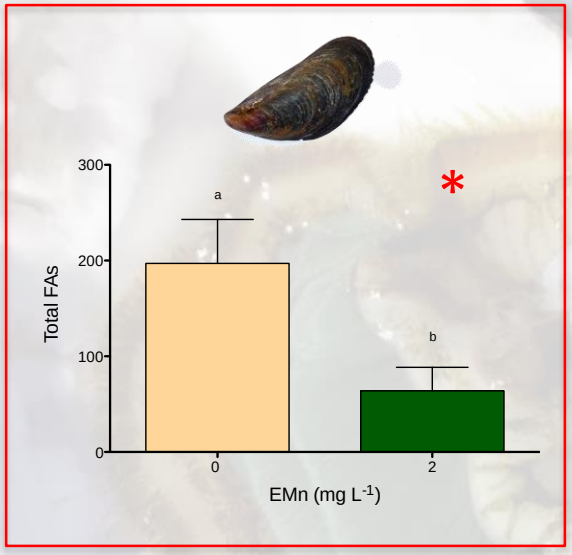
Parameter	Significancy	Animal
VO_2	S	Oyster
CR	S	Mussel, oyster
AR	NS	Mussel, oyster
SFG	S	Mussel, oyster

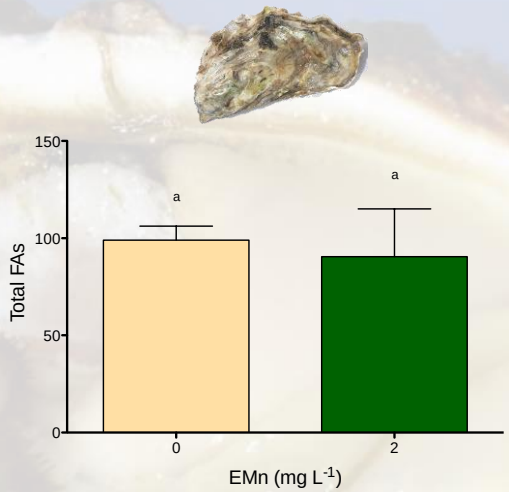
S = Significant
NS = Non significant



Part 3: Consequences of greening by marennine on the integrative response of bivalve

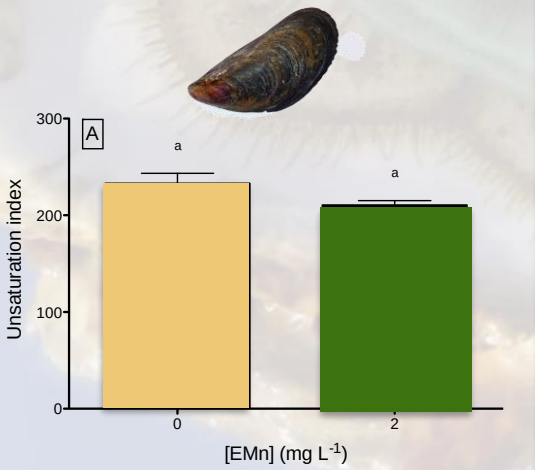
- NL, energetic reserves

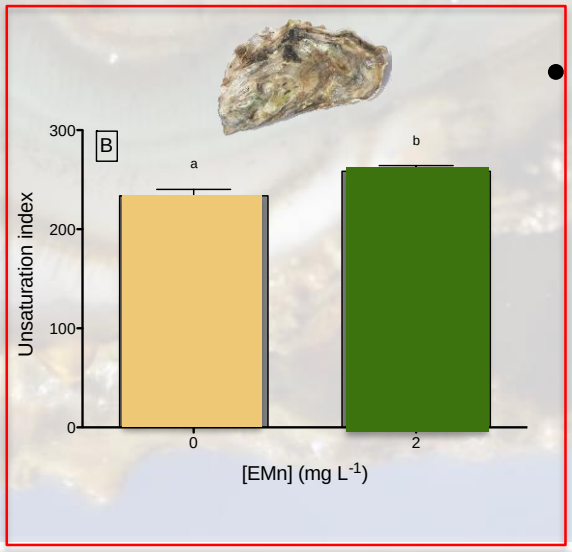




- *S in FAs composition (PERMANOVA)
  - 18:3 n-6; 20:5 n-3 (SIMPER)

- PL, membrane saturation





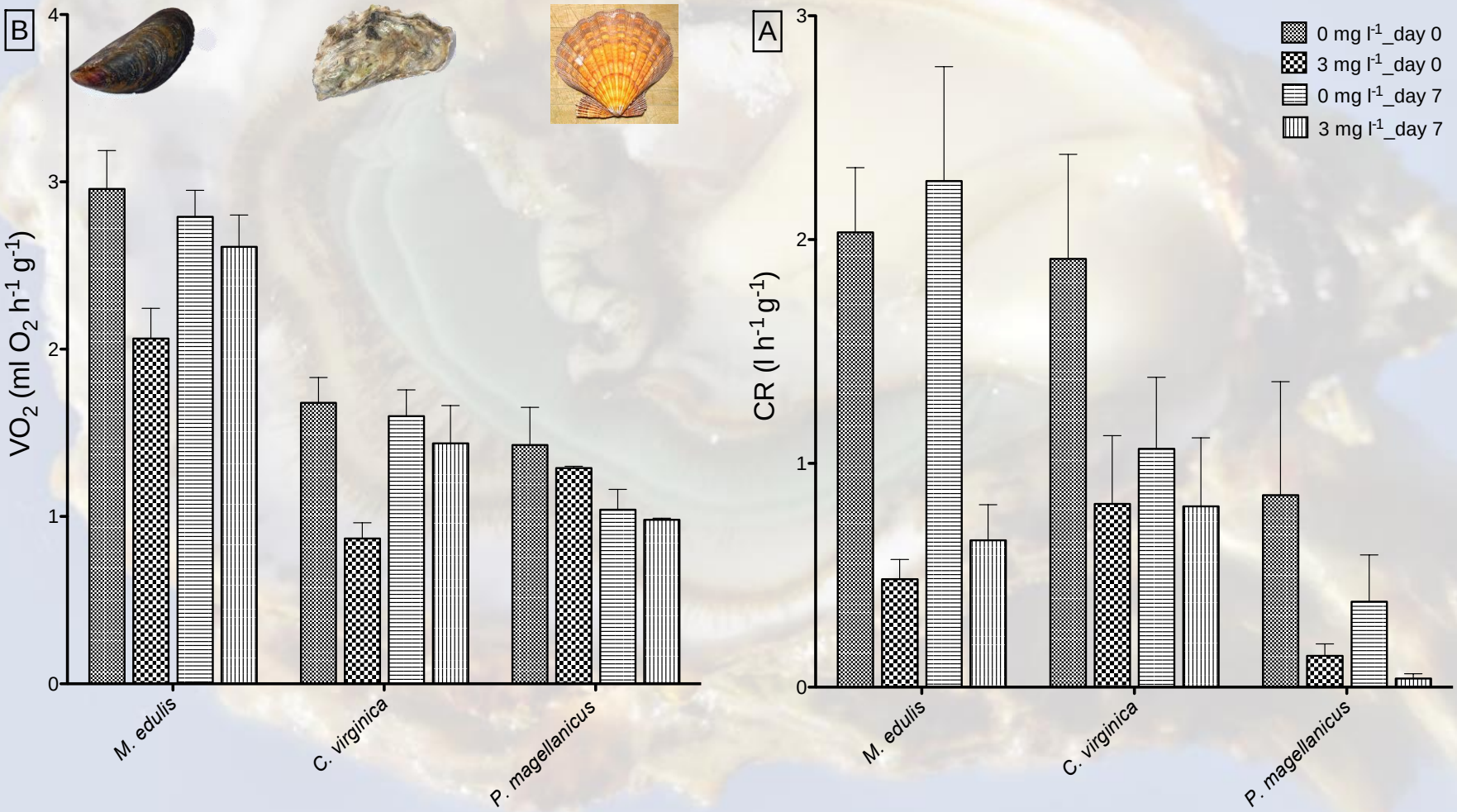
- *S in UI

  + 18:2 n-6, 20:3 n-3, 22:6 n-cis

  - 18:3 n-6

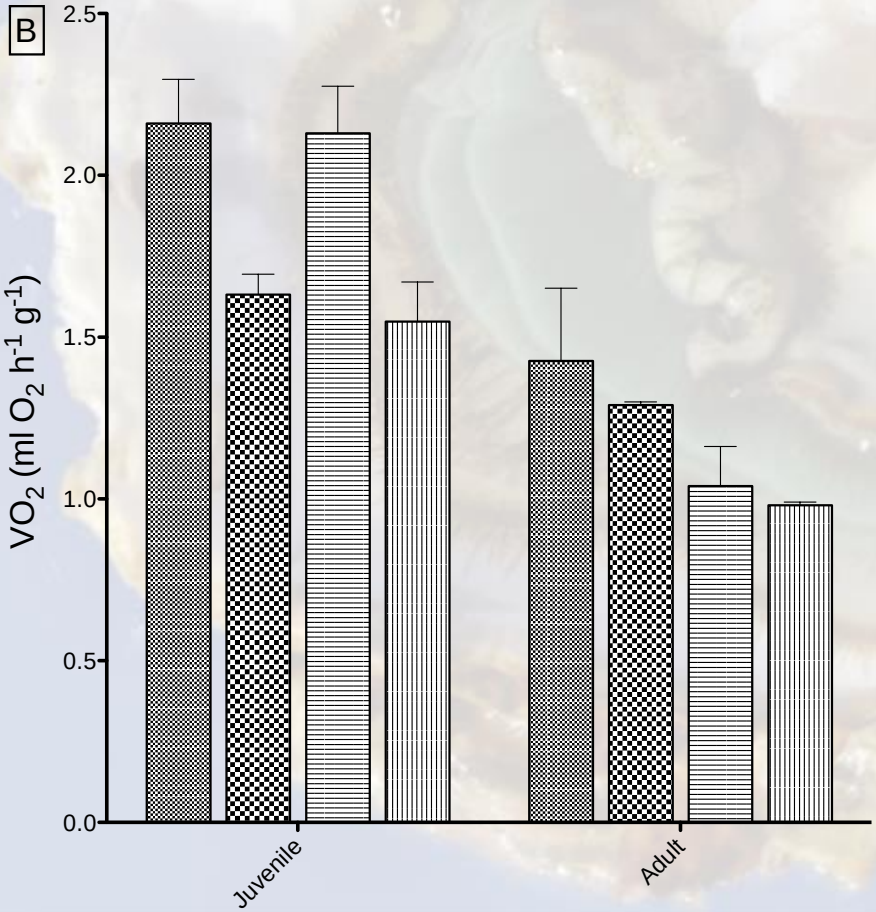
Physiological traits

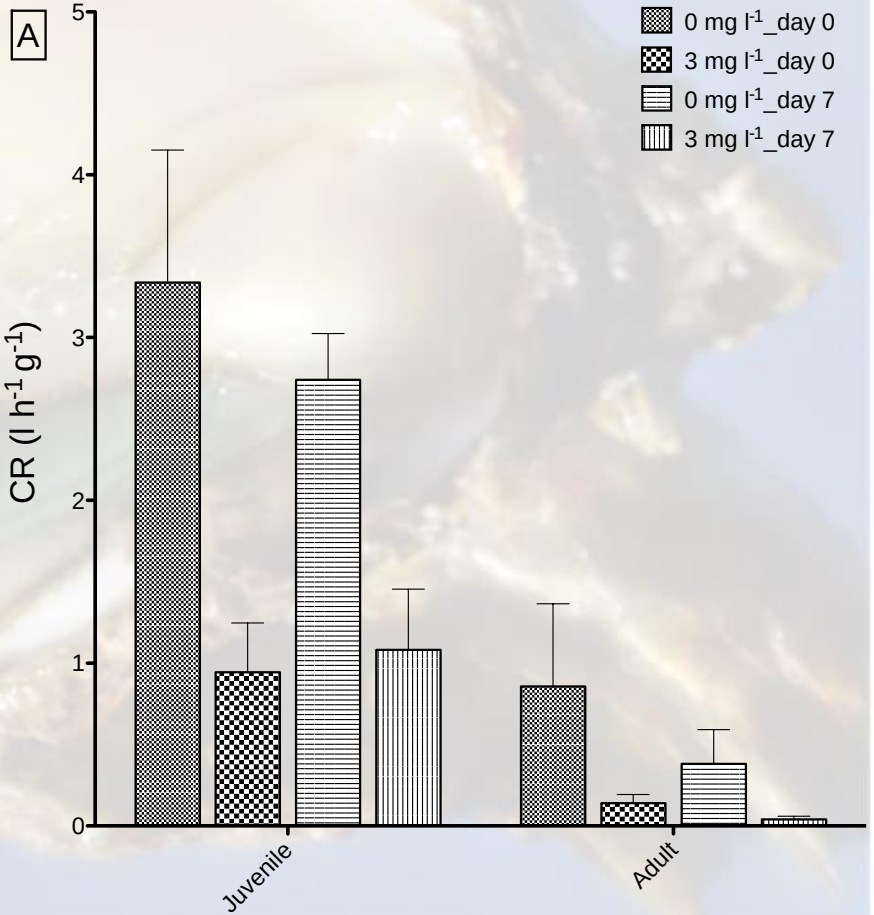
- Species specific



Physiological traits

- Age-specific

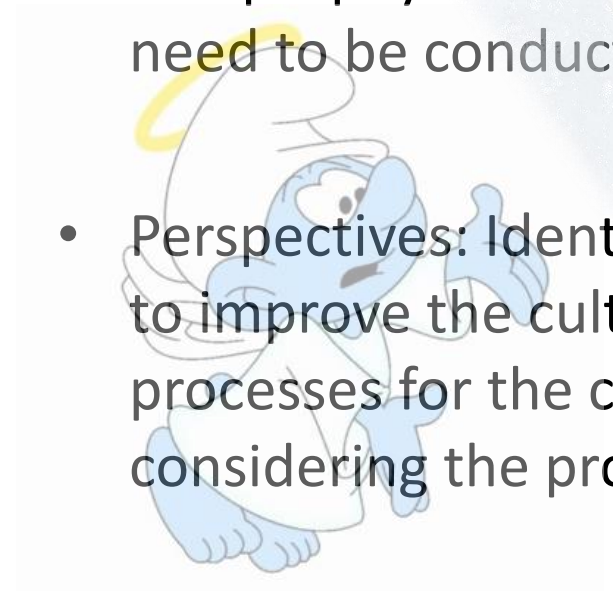


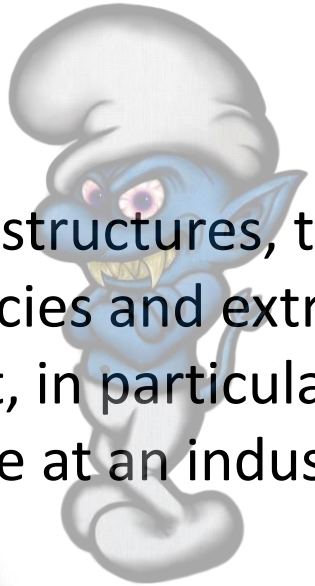


Conclusion and perspectives

- *Haslea* and its marennine (-like) pigments are promising sources of natural compounds with potential applications with probiotic and prophylactic benefits in aquaculture. Yet further studies need to be conducted..

- Perspectives: Identification on chemical structures, toxicity and to improve the cultivation of *Haslea* species and extraction processes for the compounds of interest, in particular when considering the production of marennine at an industrial scale.





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Dank u well!
Merci!
Terima Kasih!

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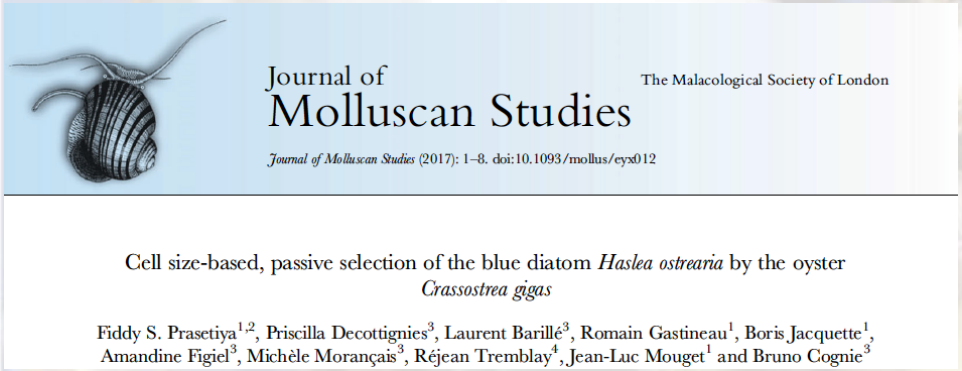

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The Malacological Society of London

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Cell size-based, passive selection of the blue diatom *Haslea ostrearia* by the oyster *Crassostrea gigas*

Fiddy S. Prasetiya[1,2], Priscilla Decottignies[3], Laurent Barillé[3], Romain Gastineau[1], Boris Jacquette[1], Amandine Figiel[3], Michèle Morançais[3], Réjean Tremblay[4], Jean-Luc Mouget[1] and Bruno Cognie[3]

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Effect of marennine produced by the blue diatom *Haslea ostrearia* on behavioral, physiological and biochemical traits of juvenile *Mytilus edulis* and *Crassostrea virginica*

Fiddy S. Prasetiya [a,e], Luc A. Comeau [b], Romain Gastineau [a], Priscilla Decottignies [c], Bruno Cognie [c], Michéle Morançais [c], François Turcotte [d], Jean-Luc Mouget [a], Réjean Tremblay [d,*]

J Appl Phycol (2016) 28:2241–2254

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Does allelopathy affect co-culturing *Haslea ostrearia* with other microalgae relevant to aquaculture?

Fiddy S. Prasetiya [1] · Ikha Safitri [1] · Ita Widowati [2] · Bruno Cognie [3] · Priscilla Decottignies [3] · Romain Gastineau [1] · Michèle Morançais [3] · Eko Windarto [1] · Réjean Tremblay [4] · Jean-Luc Mouget [1]

Mar. Drugs **2014**, *12*, 3161-3189; doi:10.3390/md12063161

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Review

Marennine, Promising Blue Pigments from a Widespread Haslea Diatom Species Complex

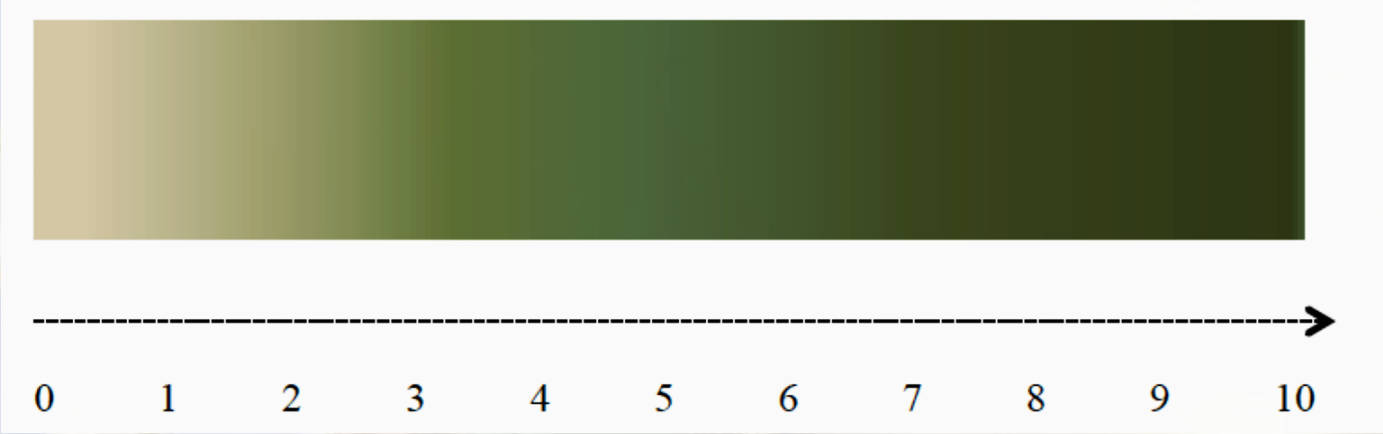
Romain Gastineau [1], François Turcotte [2], Jean-Bernard Pouvreau [3], Michèle Morançais [4], Joël Fleurence [4], Eko Windarto [1], Fiddy Semba Prasetiya [1], Sulastri Arsad [1], Pascal Jaouen [5],

Contact information:

fsembapr@gmail.com

https://www.researchgate.net/profile/Fiddy_Prasetiya

Greening estimation: Semi-qualitative method



Quantitative method

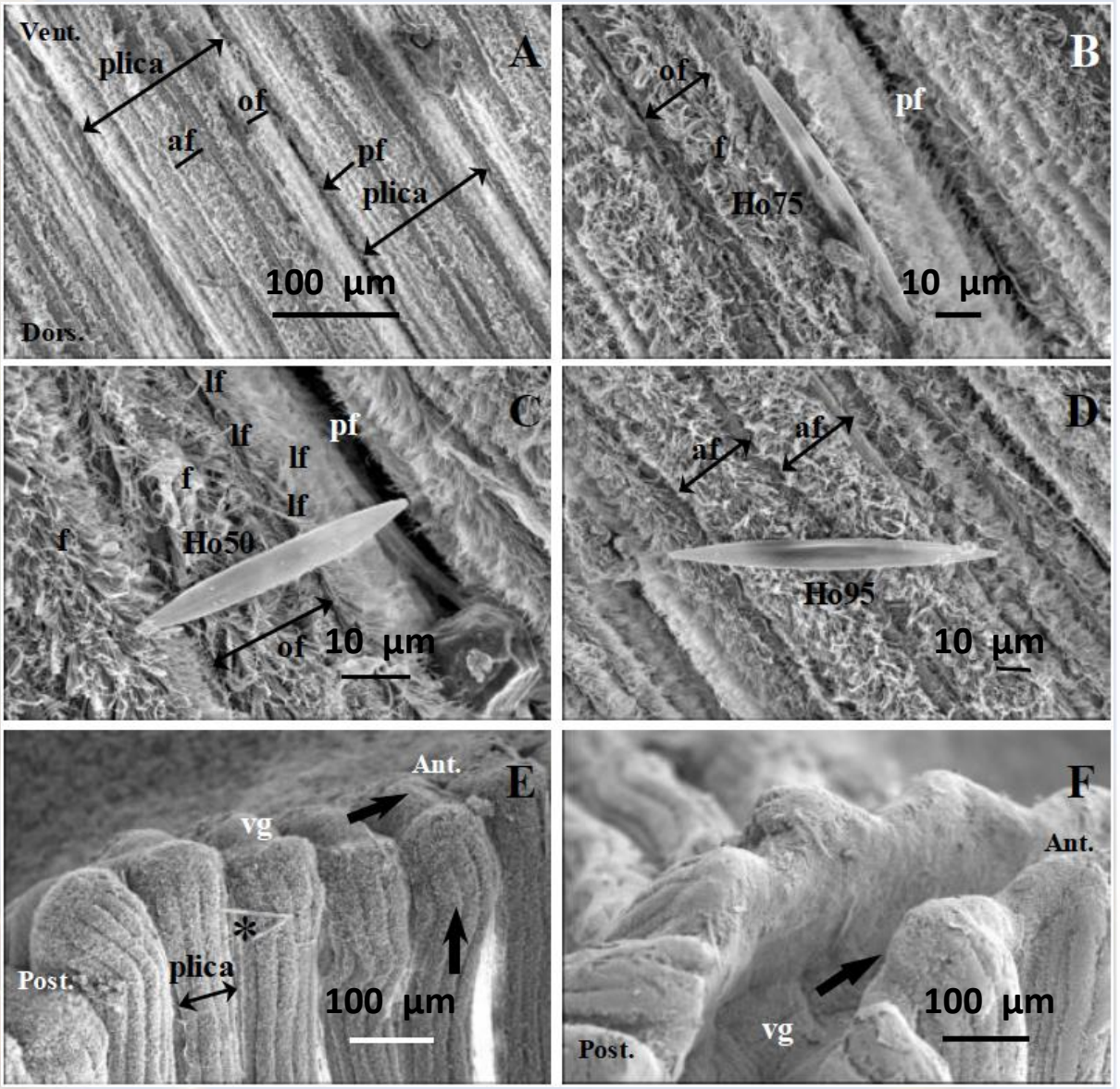
Grounded freezed gills + Urea 8M

Heated 50°C, 24h,centrifuged 8500 rpm

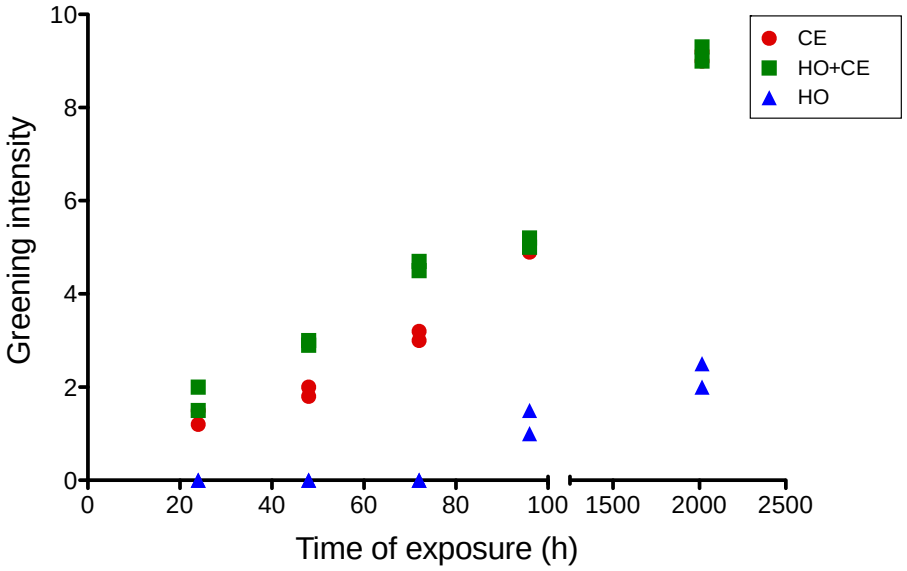
Spectro-UV vis on supernatant

Experiment A: SEM observation

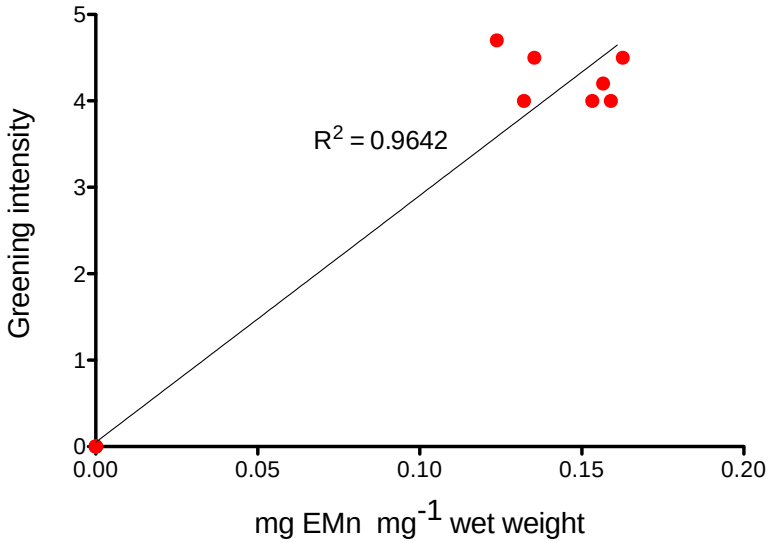
3 populations differing in cell size



Greening estimation: Semi-qualitative



Semi-qualitative vs quantitative



Marennine & gill interaction

Fresh gills, dissected & washed

Immersion, 3-30 kDa ultra-filtered EMn

Fixation, 10% formaldehyde, 10d

Cryostat sectionning, 40 micron

Observation under microscope

Greening experiment

BW: Blue water

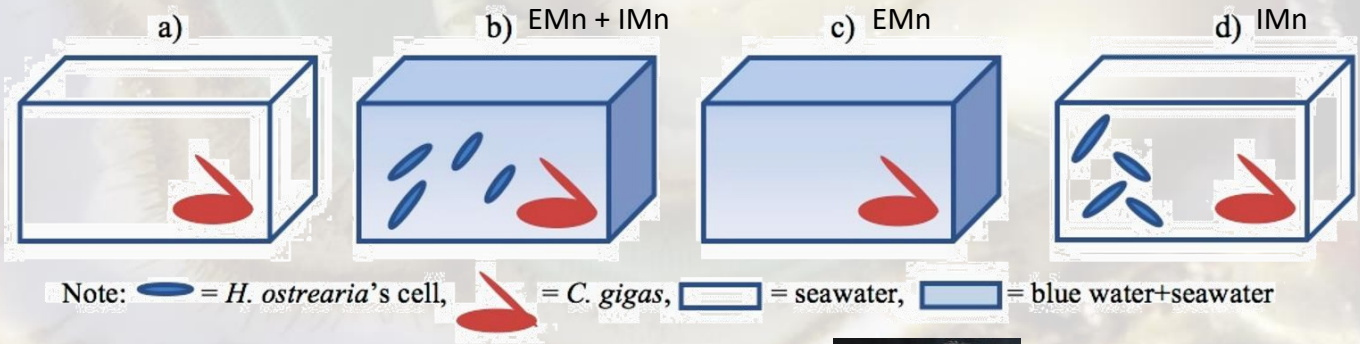
S. costatum 30-120 10^3 cell mL^{-1} (Control)

H. ostrearia + BW, 5 mg L^{-1}

BW, 5 mg L^{-1}

H. ostrearia, 30-120 10^3 cell mL^{-1}

BW removal, centrifugation



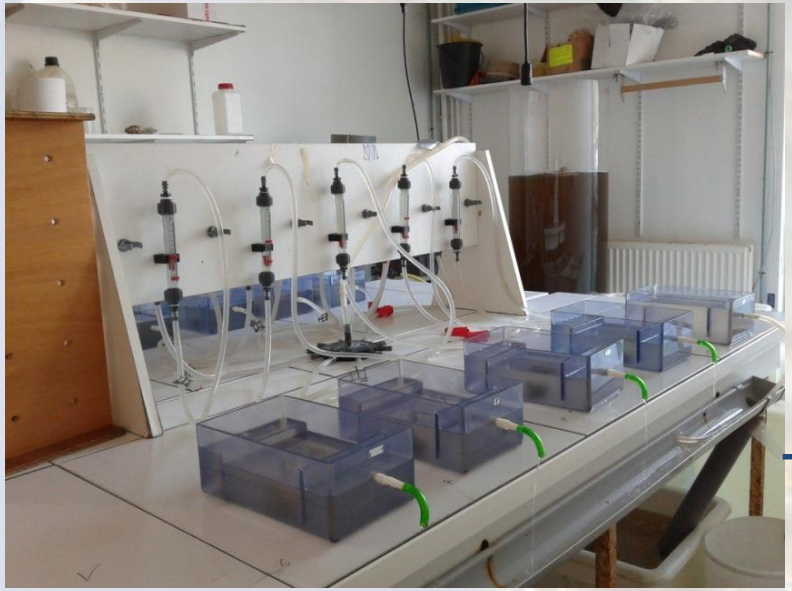
Placed in normal medium, 12 weeks

Greening quantification

Marennine on CR

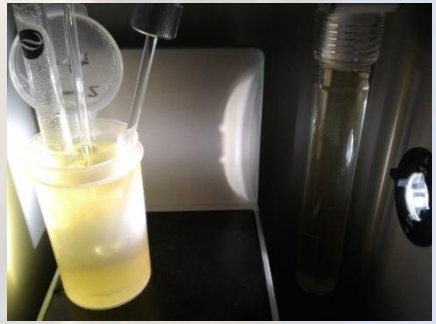
C. gigas juveniles + EMn (5 mg/L, n=8)

C. gigas juveniles (control, n=8)



Outflow

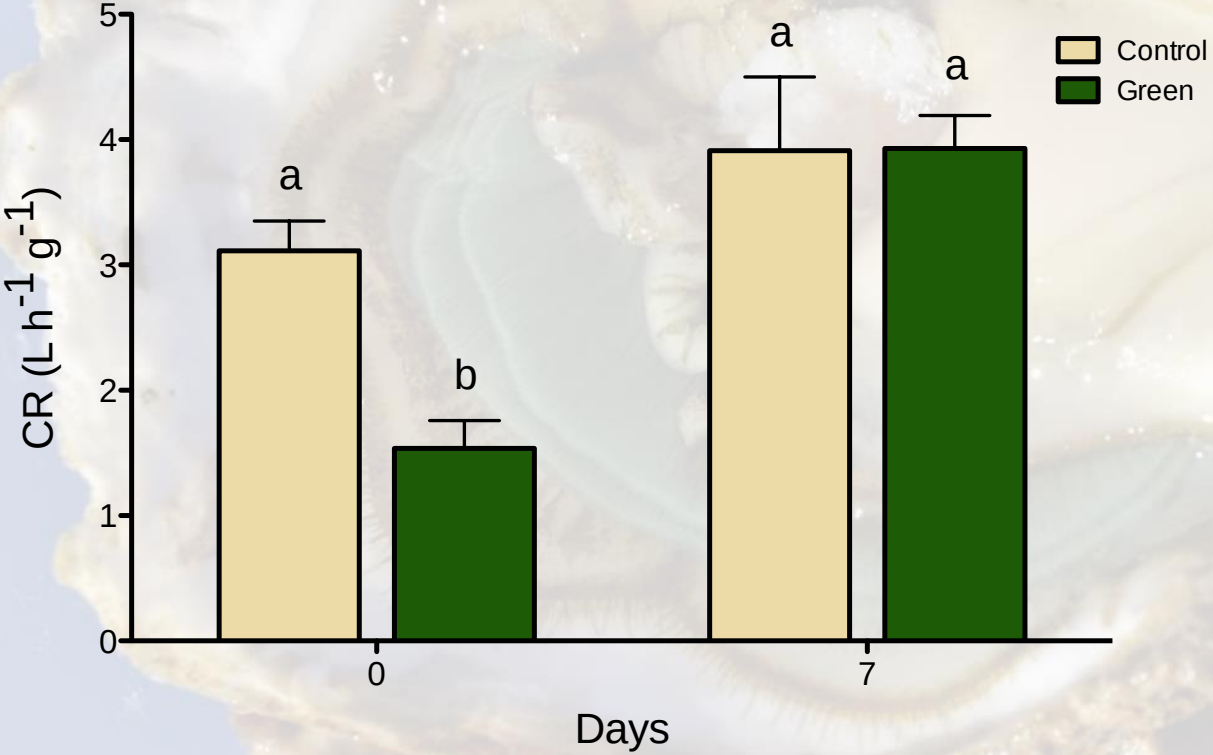
Sampling at outflow with time T_0 T_{20} T_{40} & T_{60} (min) for each group



Samples were analyzed by particle counter multisizer

Analysis of CR

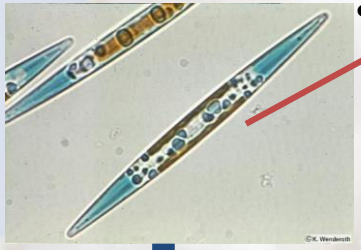
Effect of marennine on Clearance Rate (CR)



Initial response

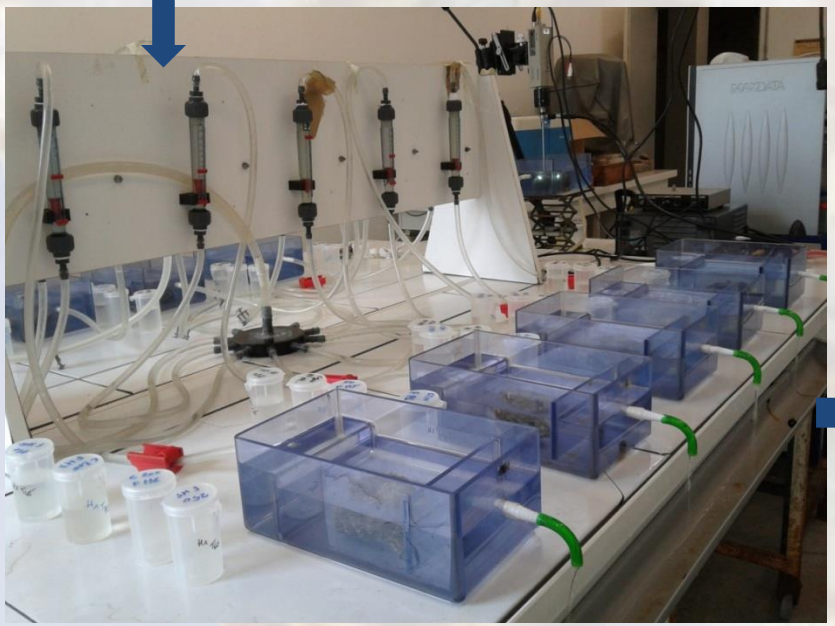
Recovery ability

Part 2: Role of size in preingestive selection of *H. ostrearia* in *C. gigas*

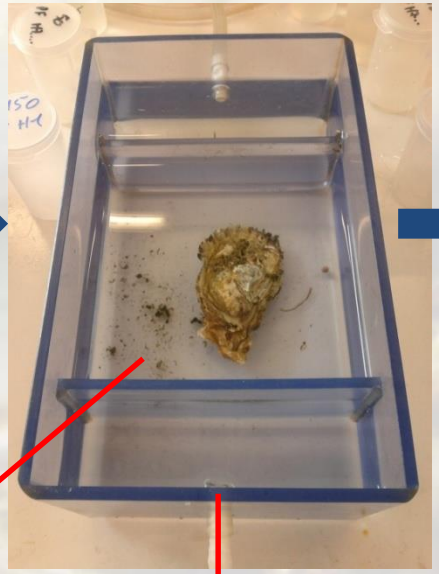


- Different sizes of *H. ostrearia* populations (Experiment A & B), 60 L, (3.10^6cells.l^{-1})

- 10 adult oysters were acclimated, flow rate 10$l.h^{-1}$



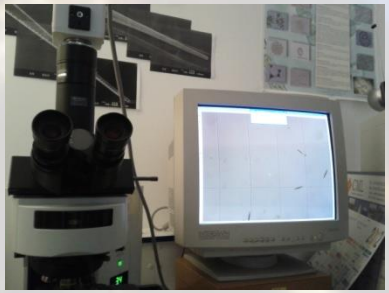
Flow-through chamber system (FTC)



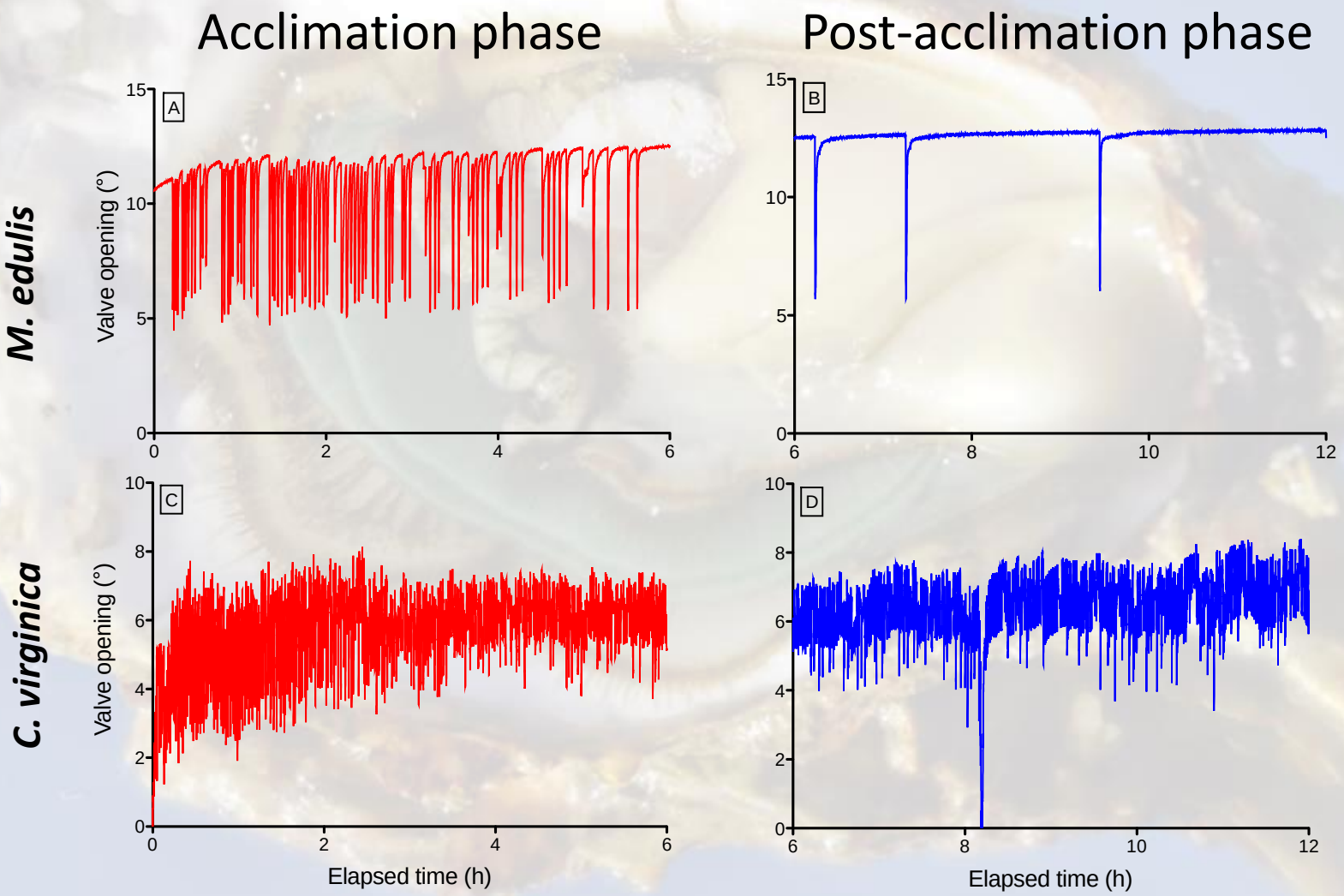
PF

Outflow

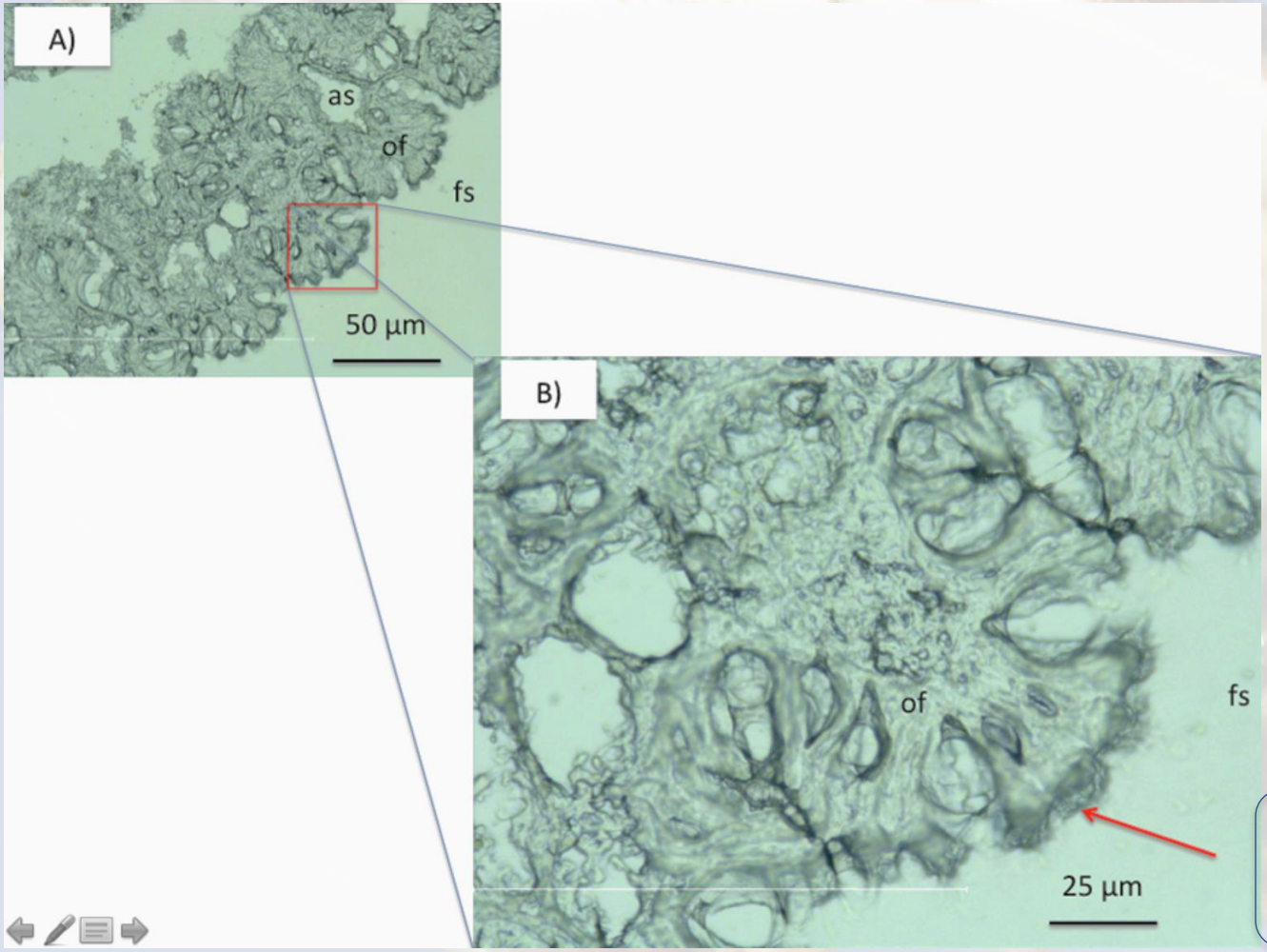
- Sampling each 15', during 1h, at outflow
- Sampling of PF at the end of observation
- Fixed with Lugol & counted with biometric microscope



Behavioral traits (short-term)



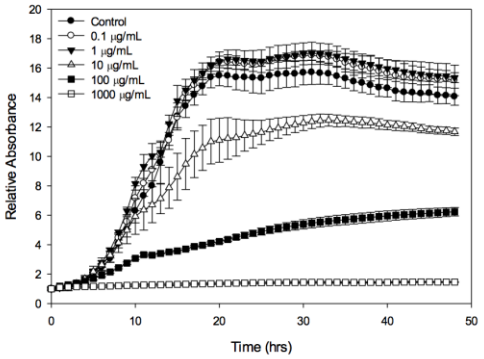
Marennine fixation on the gills



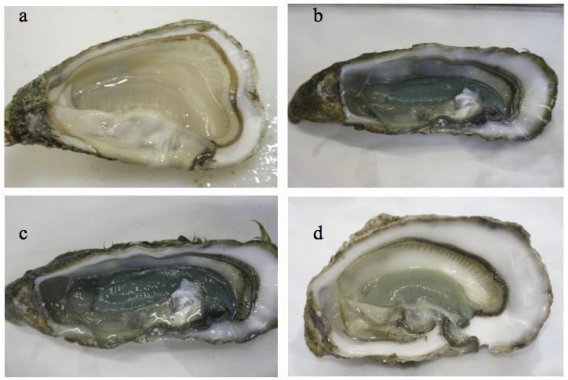
Marennine fixation in mucocytes

Aktivitas Biologis Pigmen Marenin untuk Budidaya Perairan

Bakteri patogen kerang (*Vibrio splendidus, V. aestuarianus, ...*)

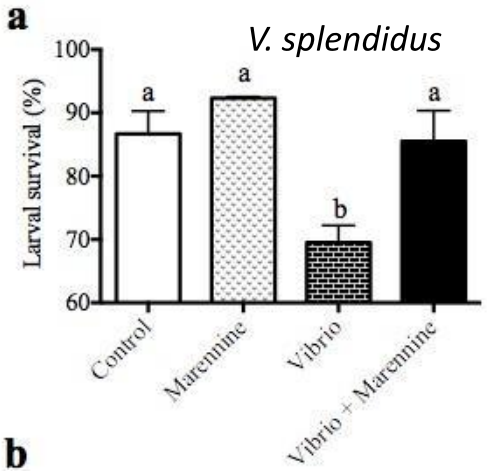


EC_{50}: 2.89 mg L^{-1}
V. splendidus



Crassostrea gigas Prasetiya et al., 2018

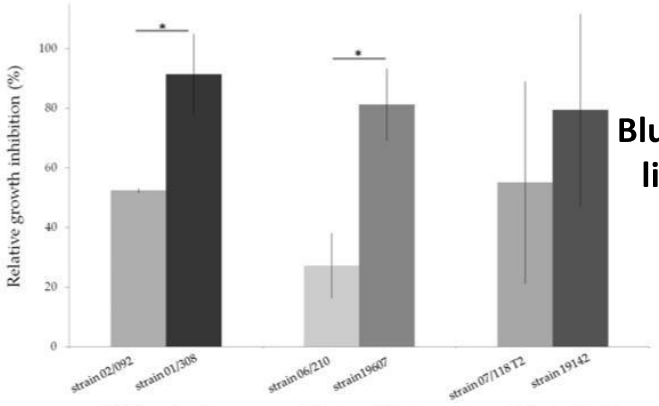
In vivo antibacterial effect



Antibacterial activity
in vitro

The inhibitory effect is concentration-, strain-, species-dependent

Mytilus edulis



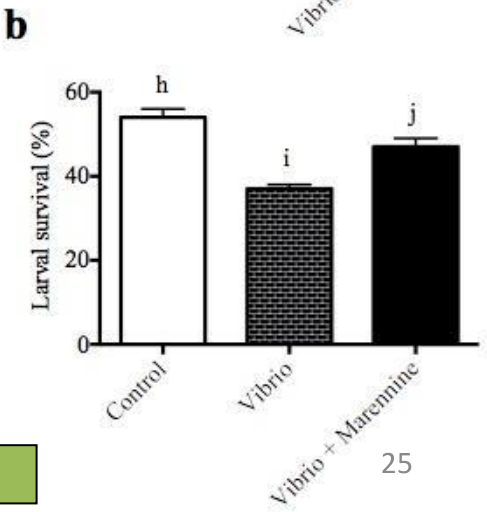
V. aestuarianus *V. coralliilycus* *V. tubiashii*

Falaise et al (2016)

Blue *Haslea* / marennine-like pigments could be used as prophylactic agents

Plactopecten magellanicus

Turcotte et al (2016)



Potensi Aplikasi *Haslea* dan Marennine di Bidang Budidaya Perikanan


Larva udang (Bali)

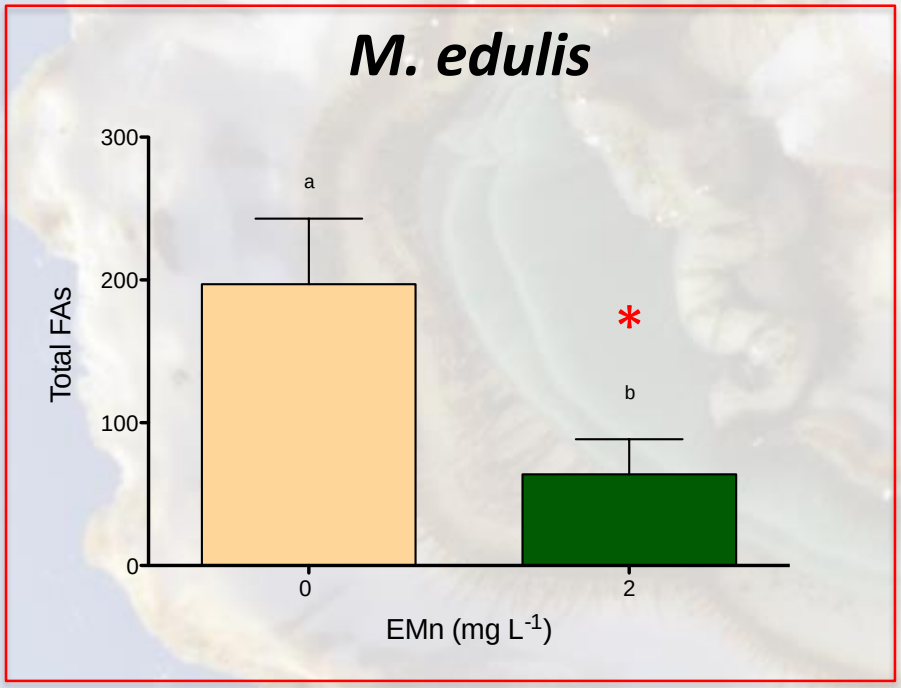

Kerang abalone (Pangandaran)

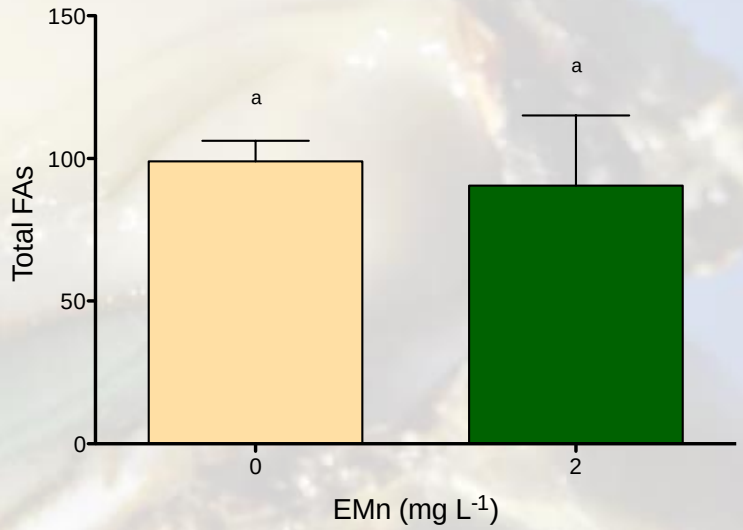

Udang vanname (Pangandaran)


Kerang mutiara (Bali)

Biochemical traits

- NL, energetic reserves

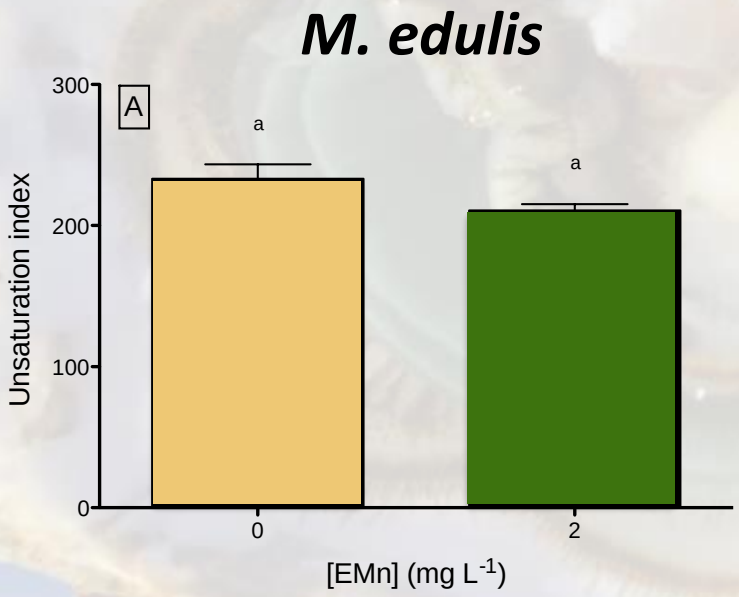


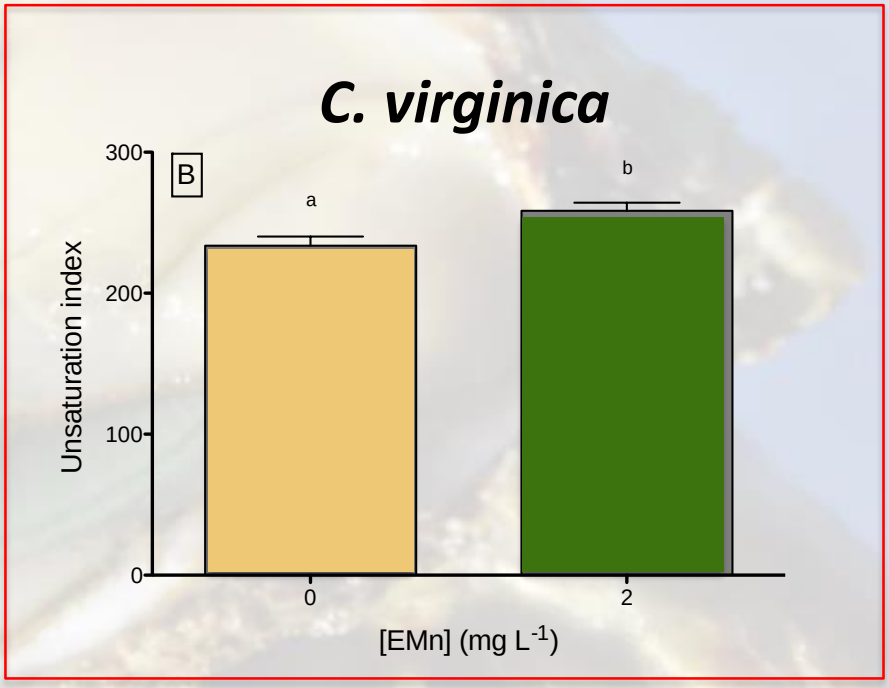


- Significantly different in FAs composition (PERMANOVA)
  - 18:3 n-6; 20:5 n-3 (SIMPER)

Biochemical traits

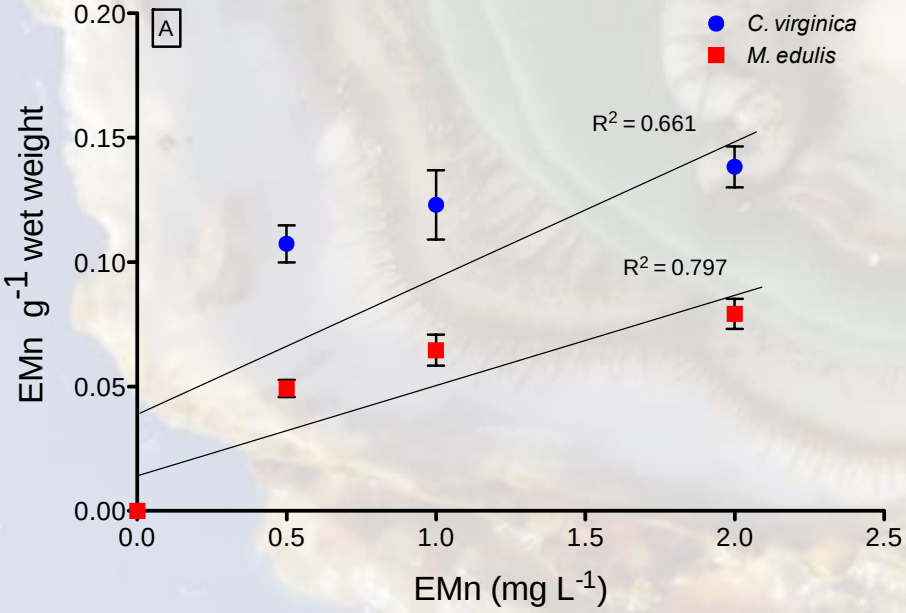
- PL, membrane saturation

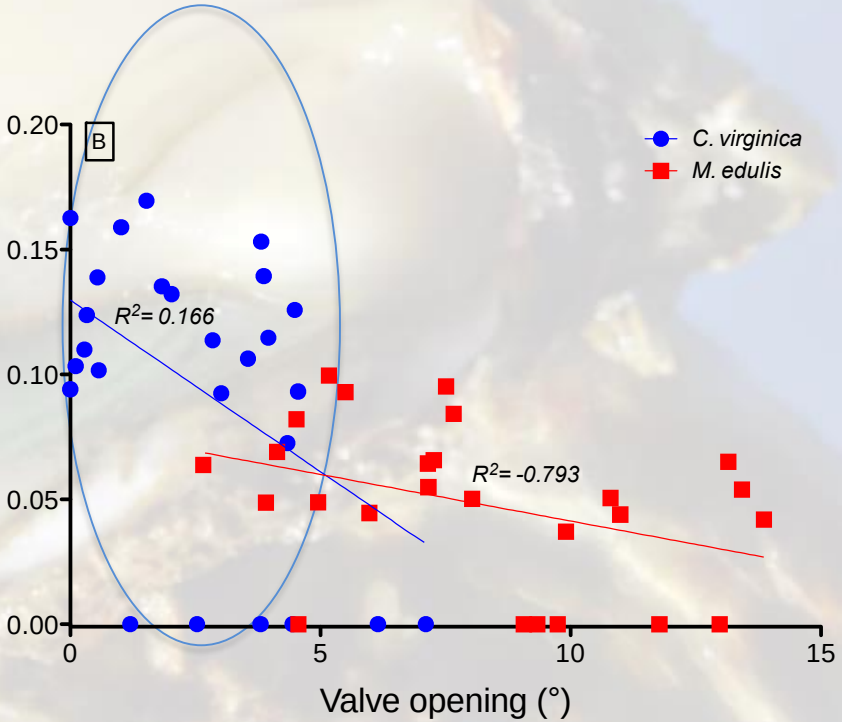




Behavioral traits

- Relationship between EMn on the gill and valve opening



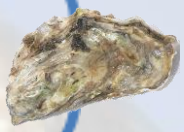


Valve activity experiment (short-term effect)

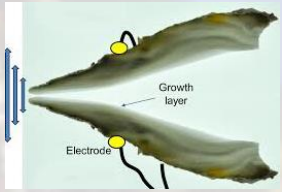
Sample preparation

(n = 32)

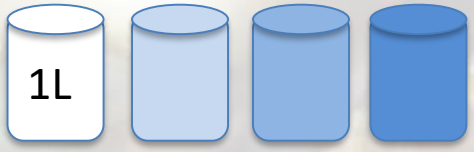
(n = 32)



- Installation of Hall's element sensor



- Exposed to 0, 0.5, 1 and 2 mg L^{-1}



- Recording of valve activity (in degree)
  - Acclimation phase
  - Post-accclimation phase
- EMn quantification on the gill

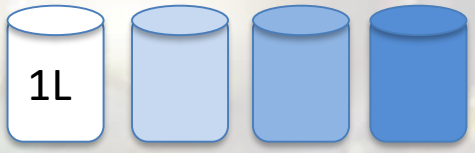
SFG experiment

Sample preparation

(n = 32)

(n = 32)

- Exposed to 0, 0.5, 1 and 2 mg L^{-1}

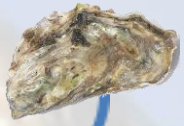


- Measurement of:
  - O_2 consumption
  - Clearance rate (CR)
  - Absorption rate (AR)
  - SFG

Lipids and FAs analysis (long-term effect)

- Min. 100 mg of tissue wet weight (gills - digestive glands)


(n = 16)

(n = 16)


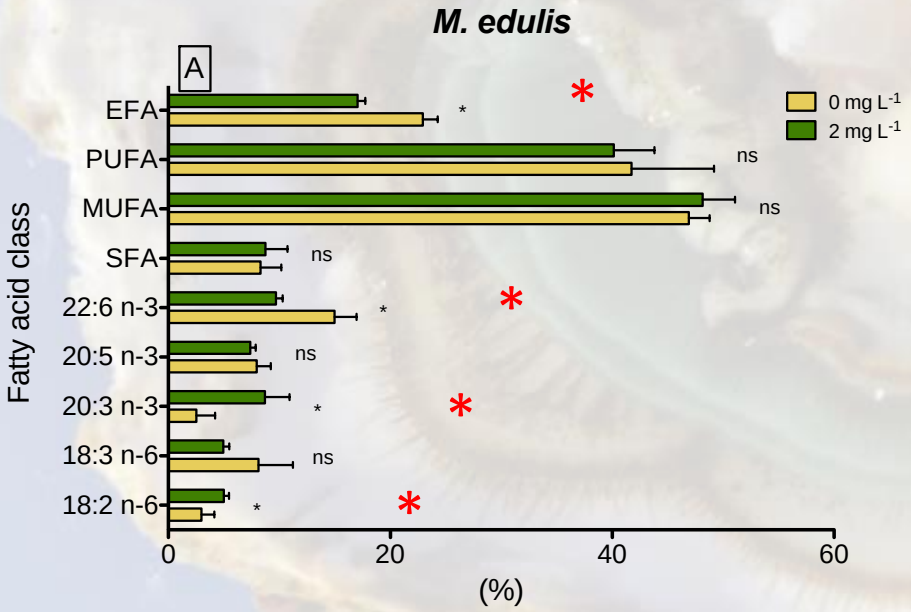
- Lipid extraction
- Separation of neutral (NL) and polar lipids (PL)
- Methylation
- Purification
- Quantification in GC MS

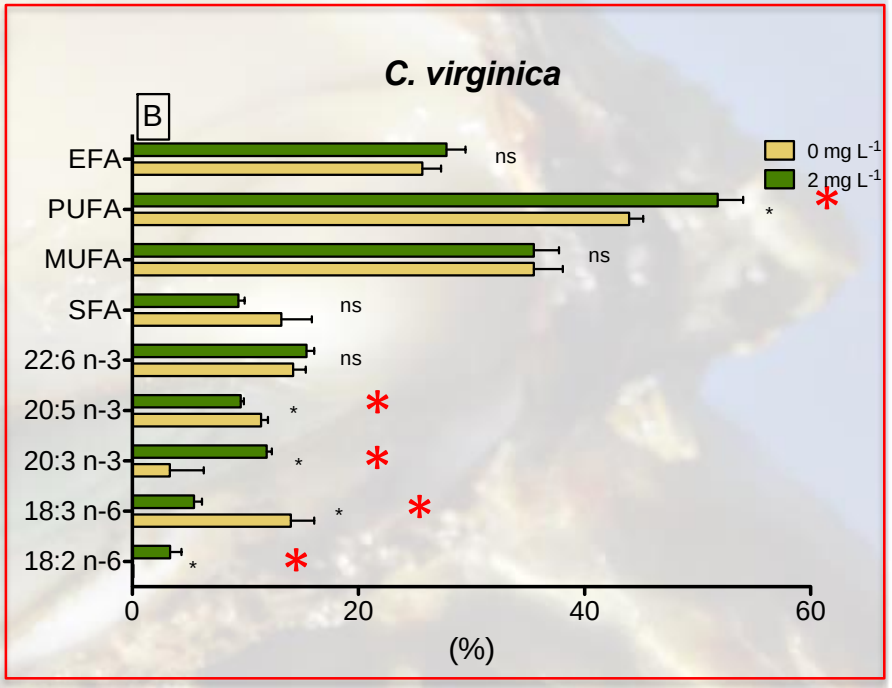
Statistical analysis

- One-way ANOVA,
- Multivariate analysis of variance PERMANOVA+

Biochemical traits

- PL, membrane saturation





- Significant correlation, UI (unsaturation index) vs PUFA

  \+ 18:2 n-6, 20:3 n-3, 22:6 n-cis

  \- 18:3 n-6