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 Cogne, Christophe Stavrakakis and Jean-Luc Mouget
Up and down postgraduation
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
Research collaboration to minimize the gap..
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
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

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Type of pigment</th>
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<tbody>
<tr>
<td></td>
<td>IMn</td>
</tr>
<tr>
<td>Localization</td>
<td>Apical axis of the cell</td>
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<tr>
<td>Molecular weight</td>
<td>10.7 kDa</td>
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</table>
Biological activities of marennine *in vitro*

Purified EMn and IMn

*H. ostrearia* in co-culture

*Skeletonema costatum*

*Chaetoceros calcitrans*

*Tetraselmis suecica*

Pouvreau et al. (2008)

Purified EMn and IMn

Antiviral

HSV-1

Gastineau *et al.* (2012)

Antioxidant

*Marennine IMn /EMn*

Antibacterial

V. tubiashii

V. aestuarianus

V. coralliilyticus

V. Tasmaniensis

Gastineau *et al.* (2014)

Allelopathic

H. ostrearia in co-culture

Prasetya *et al.* (2016)

Natural antibiotic for aquaculture
Oyster aquaculture

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

- Pathogen infections
  - Disease outbreaks (OsHV-1, *Vibrio aestuarianus*)

- Stock depletion & Economic loss

However...
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?
Part 1: Greening of oyster by *H. ostrearia*

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)
Part 1: Greening of oyster by *H. ostrearia*

Persistency of greening

No de-greening after 12 weeks

Marennine fixation is persistent
Part 1: Greening of oyster by *H. ostrearia*

Marennine fixation on the gills

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

**Haslea on feeding behaviour**

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

Does the size really matter?
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

**Data analysis:**
- Homogenity & Normality test
- T-test (XLSTAT)
Experiment B:
Video endoscopy observation

Part 2: Role of size in preingestive selection of *H. ostrearia* in *C. gigas*
Part 3: Consequences of greening by marennine on the integrative response of bivalve

Valve activity experiment (short-term effect)

Analysis of Scope for Growth

Lipids and FAs analysis (long-term effect)
Part 3: Consequences of greening by marennine on the integrative response of bivalve

Behavioral traits

- Significant effect on mussel’s acclimation phase
- Post-acclimation effect on oyster
### Physiological traits

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Significance</th>
<th>Animal</th>
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<tbody>
<tr>
<td>VO₂</td>
<td>S</td>
<td>Oyster</td>
</tr>
<tr>
<td>CR</td>
<td>S</td>
<td>Mussel, oyster</td>
</tr>
<tr>
<td>AR</td>
<td>NS</td>
<td>Mussel, oyster</td>
</tr>
<tr>
<td>SFG</td>
<td>S</td>
<td>Mussel, oyster</td>
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</table>

S = Significant
NS = Non significant
Part 3: Consequences of greening by marennine on the integrative response of bivalve

- **NL, energetic reserves**

- **PL, membrane saturation**

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

- **S in UI**
  - + 18:2 n-6, 20:3 n-3, 22:6 n-cis
  - - 18:3 n-6
Part 3: Consequences of greening by marennine on the integrative response of bivalve

Physiological traits
• Species specific
Part 3: Consequences of greening by marennine on the integrative response of bivalve

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.
Thank you!
Dank u well!
Merci!
Terima Kasih!
Related publications

Book chapter: Marennine-Like Pigments: Blue Diatom or Green Oyster Cult?

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

Contact information:
fsembapr@gmail.com
https://www.researchgate.net/profile/Fiddy_Prasetiya
Part 1: Greening of oyster by *H. ostrearia*

**Greening estimation: Semi-qualitative method**

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<th>8</th>
<th>9</th>
<th>10</th>
</tr>
</thead>
</table>

**Quantitative method**

- **Grounded freezeed 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

Part 2: Role of size in preingestive selection of *H. ostrearia* in *C. gigas*
Greening estimation: Semi-qualitative

Semi-qualitative vs quantitative

$R^2 = 0.9642$

Time of exposure (h)

Greening intensity

mg EMn mg$^{-1}$ wet weight

Greening intensity
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

Part 1: Greening of oyster by *H. ostrearia*
Part 1: Greening of oyster by *H. ostrearia*

**Greening experiment**

- **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**
Part 1: Greening of oyster by *H. ostrearia*

**Marennine on CR**

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

*C. gigas* juveniles (control, n=8)

Sampling at outflow with times $T_0$, $T_{20}$, $T_{40}$, and $T_{60}$ (min) for each group

Samples were analyzed by particle counter multisizer

Analysis of CR
Part 1: Greening of oyster by *H. ostrearia*

Effect of marennine on Clearance Rate (CR)

- **Initial response**
- **Recovery ability**

- **Days**
  - CR (L h$^{-1}$ g$^{-1}$)
  - Control
  - Green

- **Legend**
  - Control
  - Green
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, \(3.10^6\) cells l\(^{-1}\)

- 10 adult oysters were acclimated, flow rate 10 l h\(^{-1}\)

- Sampling each 15’, during 1 h, at outflow

- Sampling of PF at the end of observation

- Fixed with Lugol & counted with biometric microscope
Behavioral traits (short-term)

Acclimation phase

M. edulis

Post-acclimation phase

C. virginica

Elapsed time (h)

Valve opening (°)

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

Marennine fixation in mucocytes
**Aktivitas Biologis Pigmen Marenin untuk Budidaya Perairan**

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

**Antibacterial activity in vitro**

\[ EC_{50} : 2.89 \text{ mg L}^{-1} \]

*V. splendidus*

In vivo antibacterial effect

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

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

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

Falaise et al (2016)

Placochacten magellanicus

Turcotte et al (2016)
Potensi Aplikasi *Haslea* dan Marennine di Bidang Budidaya Perikanan

- Kerang abalone (Pangandaran)
- Kerang mutiara (Bali)
- Udang vanname (Pangandaran)
- Larva udang (Bali)
Biochemical traits

- NL, energetic reserves

**M. edulis**

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

**C. virginica**
Biochemical traits

- PL, membrane saturation

**M. edulis**

**C. virginica**

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

• Relationship between EMn on the gill and valve opening

Part 3: Consequences of greening by marennine on the integrative response of bivalve
**Valve activity experiment (short-term effect)**

**Sample preparation**

- (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)
- EMn quantification on the gill

**Acclimation phase**

**Post-acclimation phase**

(n = 32)
Part 3: Consequences of greening by marennine on the integrative response of bivalve

SFG experiment

Sample preparation

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

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

(n = 32)
Lipids and FAs analysis (long-term effect)

- Min. 100 mg of tissue wet weight (gills - digestive glands)
- Lipid extraction
- Separation of neutral (NL) and polar lipids (PL)
- Methylation
- Purification
- Quantification in GC MS

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

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