

EFFECT OF ENVIRONMENTAL AND NUTRITIVE CONDITIONINGS ON DEFENSE MECHANISMS OF OYSTER *Crassostrea gigas* DURING AN ANNUAL CYCLE

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How can we respond to this problem ?

➤ Two types of experiments :

- *In situ* : two different environmental conditions
- *Ex situ* : in controlled conditions at the experimental shellfish hatchery of Argenton (Ifremer, Finistere)

➤ Experimental animals :

- Oysters *Crassostrea gigas*
- pool of 15 families provided by MOREST program
- a large genetic variability
- F1, one year-old oysters

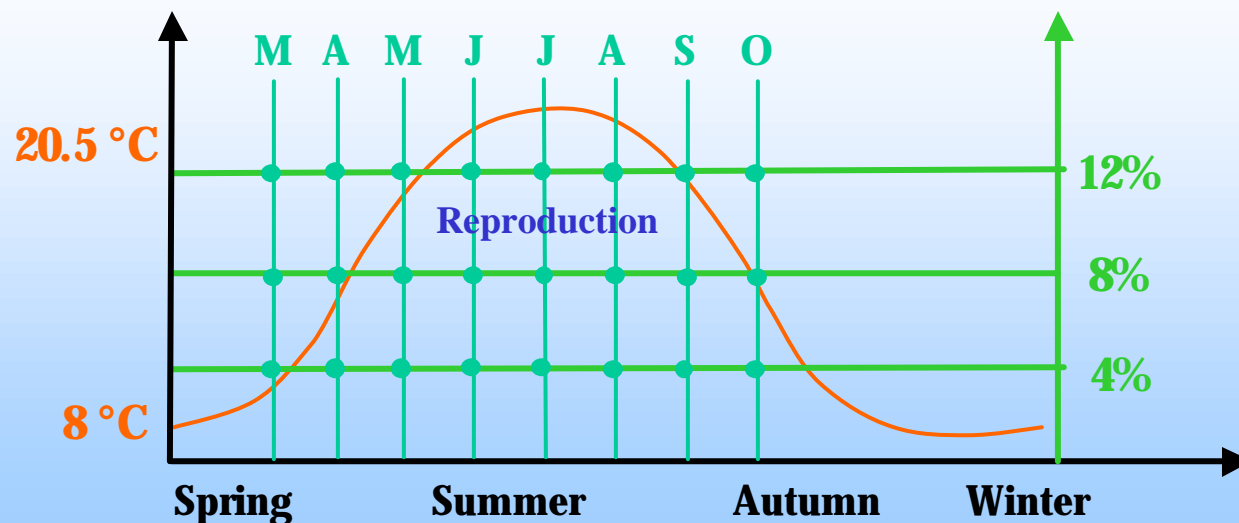
In situ : two different sites

- **Baie Des Veys (BDV, Normandy) :**
 - High food availability
 - No or partial spawning
- **Marennnes (Charente) :**
 - Low food availability
 - Spawning



Ex situ : Dietary Conditioning Experiment

- Oysters were conditioned 1 year with :
 - Three different algae levels : 4%, 8% and 12% of algal dry weight / oyster dry weight (4% = Marennes ; 12% = BDV)
 - Mixture of 4 algae : *C. calcitrans*, *S. costatum*, *T-Isochrysis*, *T. chui*
- Temperature cycle and sampling dates :



Sampling protocol



In situ



Once a month

Hatchery



Laboratory :

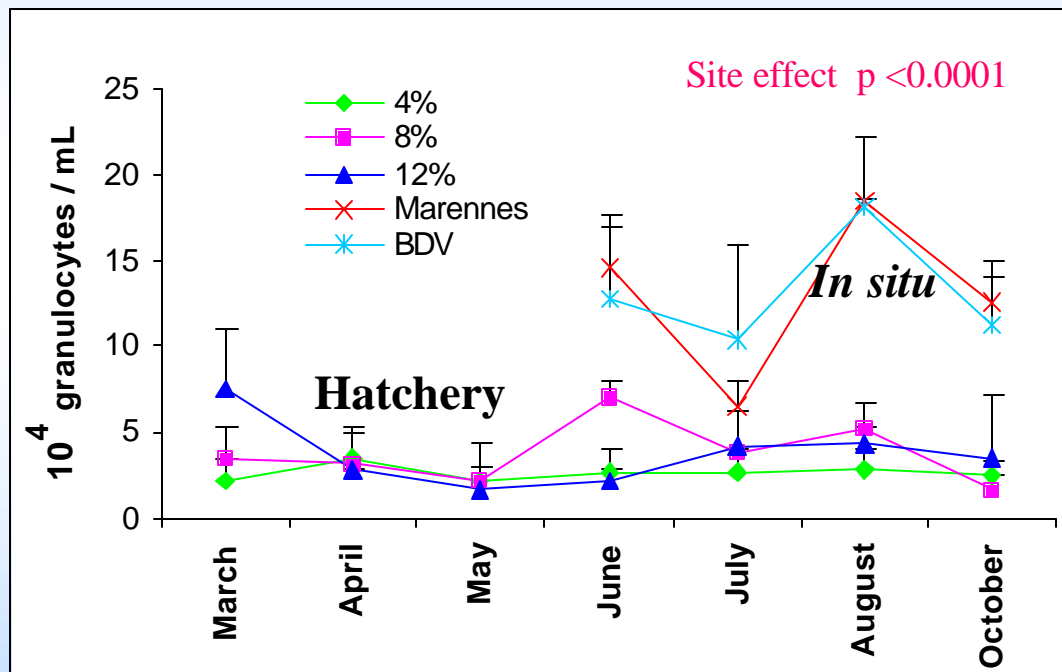
Day 0 : Oysters are notched and stored in sea water for 24h

Day 1 : Oyster bleedings and flow cytometry analysis

Immune parameters analyzed by flow cytometry

- Hemocyte concentration
- Percentage of dead cells (propidium iodide)
- Phagocytic activity (fluorescent beads)
- “Oxidative burst” activity : synthesis of reactive oxygen intermediates (2’7’ dichlorofluorescein diacetate)
- Adhesive capacity (SYBR Green)

Hemocyte concentration



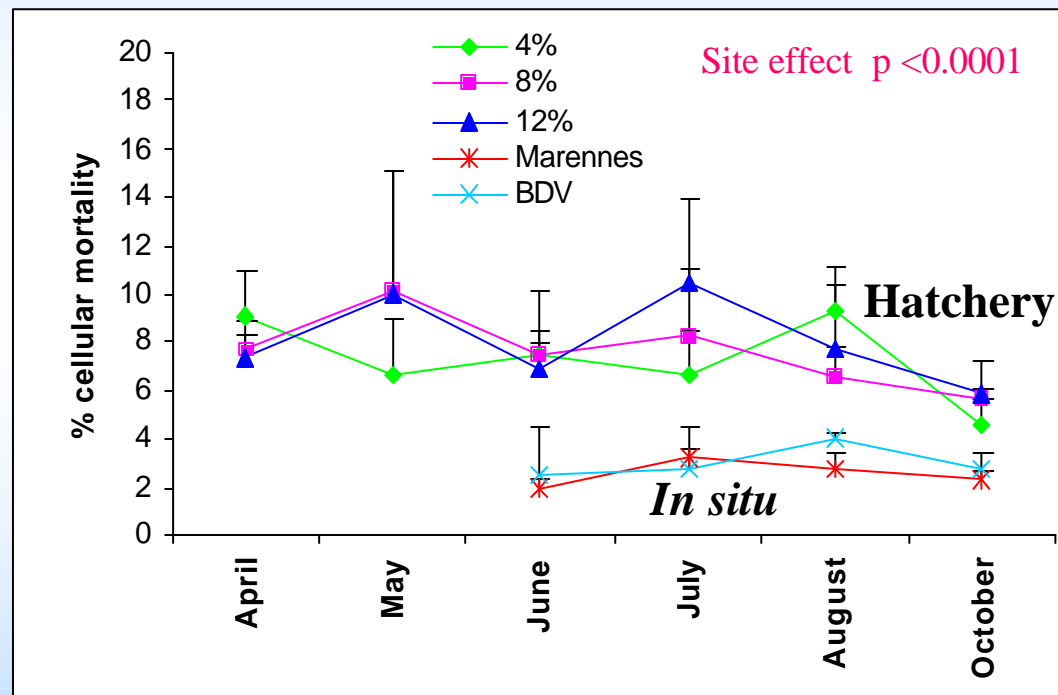
➤ **Hatchery : no difference in granulocyte counts between dietary treatments**

➤ **Rearing conditions effect :**

■ **Field > Hatchery**

➤ **Higher total hemocyte concentration in field oysters : more granulocytes and hyalinocytes than for oysters reared in hatchery**

Percentage of dead hemocytes

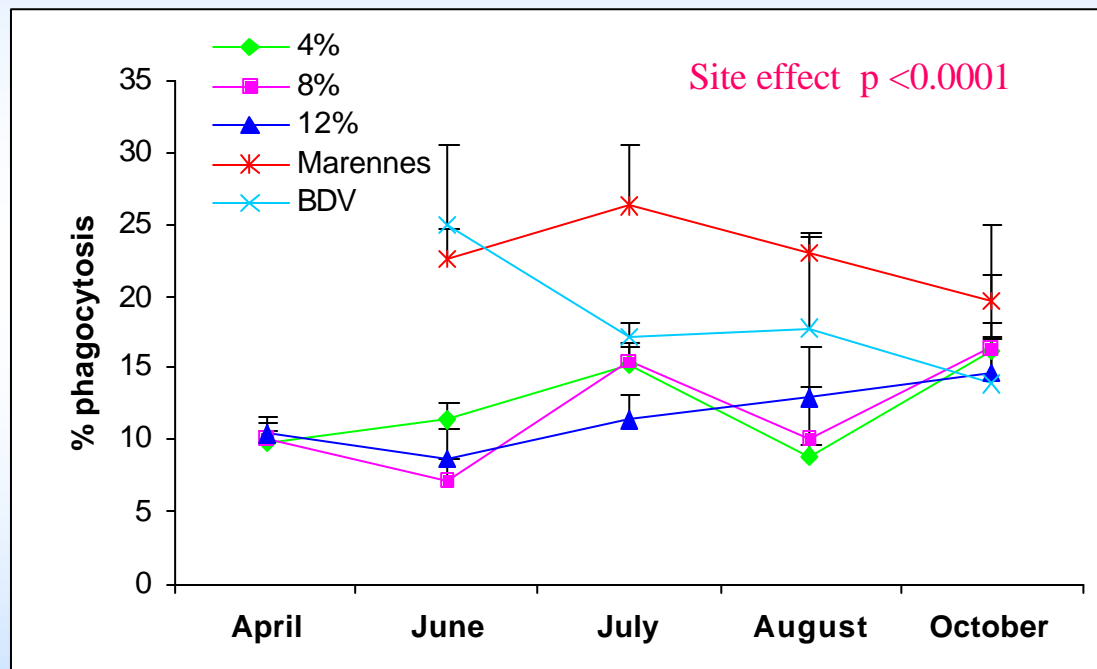


- Hatchery : no difference in percentage of dead hemocytes between dietary treatments.
- Rearing condition effect :
 - Field < Hatchery

➔ Differences between field and hatchery suggest rearing conditions are better in the field.

Phagocytic activity :

% of cells that engulfed beads

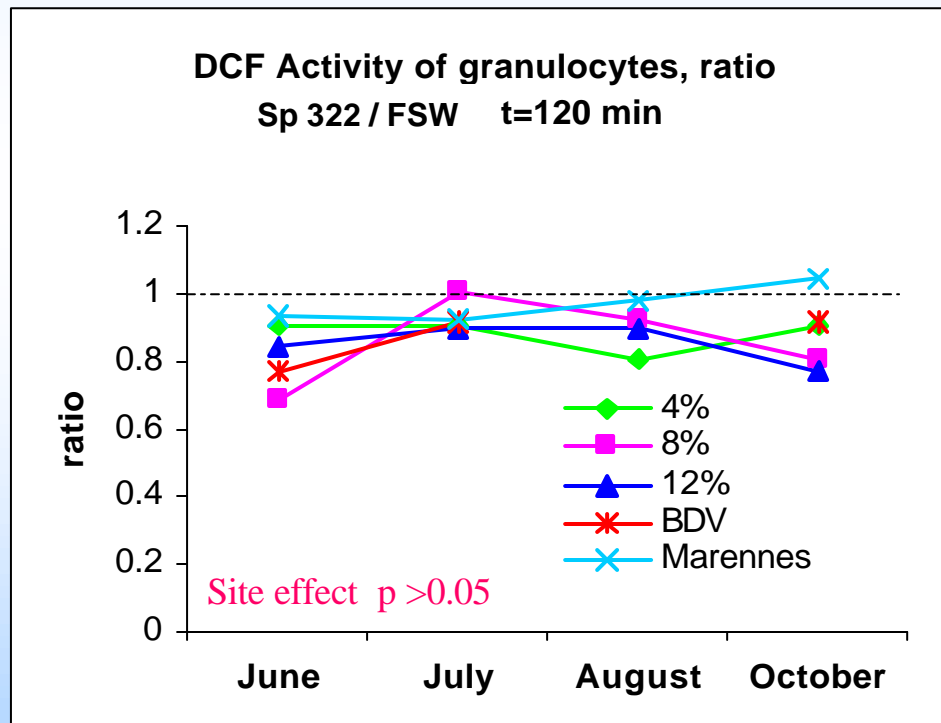


- Hatchery : no difference in phagocytic activity between treatments
- Rearing conditions effect :
 - Field > Hatchery
 - Marennnes > BDV

➔ In field, higher phagocytic activity associated with higher number of granulocytes

Oxidative burst activity :

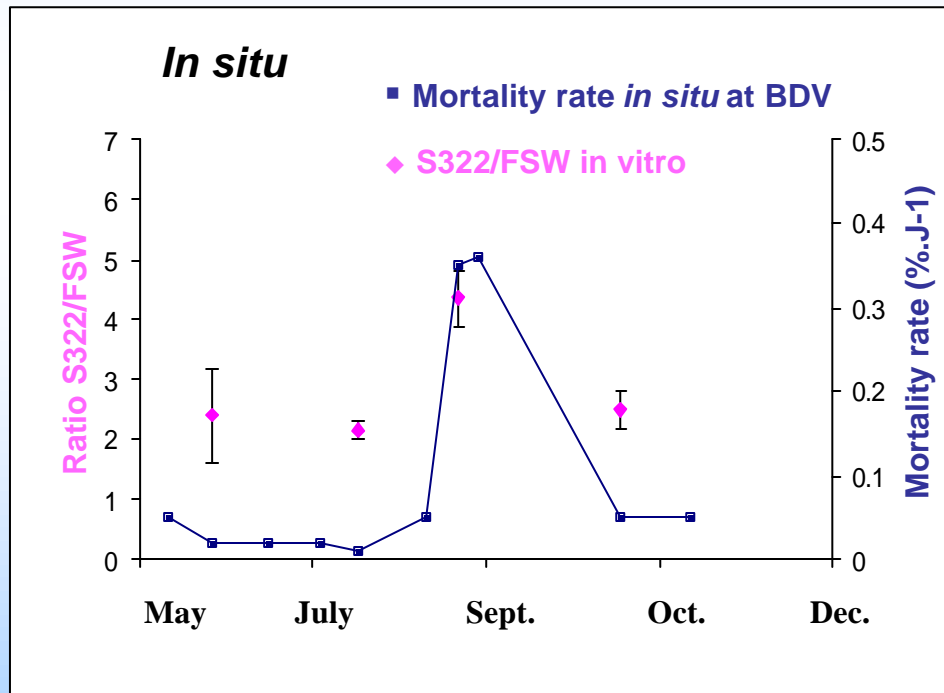
Activity of hemocytes in presence of the pathogenic *Vibrio* sp. 322 /
activity of hemocytes in presence of FSW



- Ratio < 1 = inhibition of hemocyte oxidative burst activity by *Vibrio* sp. 322
- No nutritive conditioning neither rearing conditioning effect
- Hemocytes more sensitive to the pathogenic *Vibrio* sp. 322 in June

Adhesive capacity :

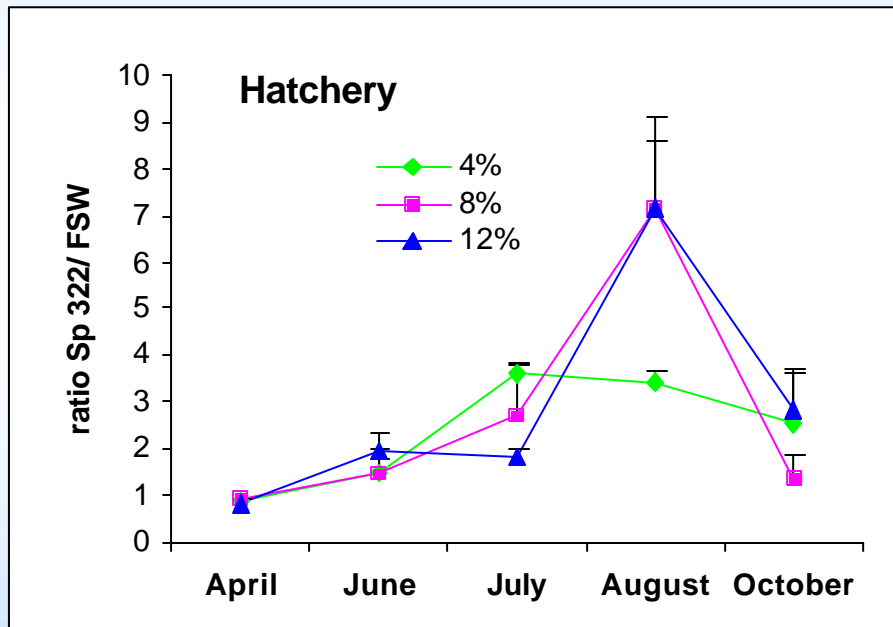
% of cells in presence of the pathogenic *Vibrio* sp. 322 / % of cells in presence of FSW



- Ratio > 1 = inhibition of adhesive capacity by *Vibrio* sp. 322
- In September, highest sensitivity of hemocytes to the pathogenic *Vibrio* sp. 322
- Correlated with mortality events observed in the field.
- Same results for oysters reared in Marennes

Adhesive capacity :

% of cells in presence of the pathogenic *Vibrio* sp. 322 / % of cells in presence of FSW



- Ratio >1 = inhibition of adhesive capacity by *Vibrio* sp. 322
- In August, highest sensitivity of hemocytes to the pathogenic *Vibrio* sp. 322

➔ But, no relationship with mortality events.

Only oysters fed 8% algae level were experienced high mortalities from May until September.

Summary of immune responses

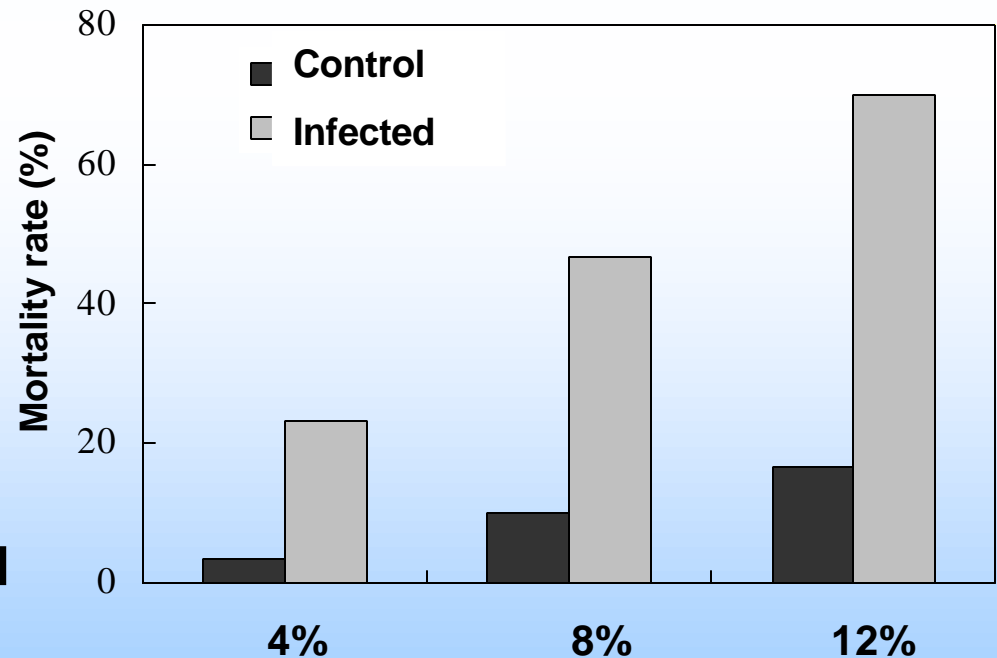
	Dietary effect	Site effect
Hemocyte counts	NS	Marennnes = BDV > Hatchery
Cell mortality	NS	Marennnes = BDV > Hatchery
Phagocytosis	NS	Marennnes > BDV > Hatchery
Adhesive capacity : Susceptibility to <i>vibrio sp 322</i>	No correlation with mortality events	Correlated with mortalities

Do nutritive and rearing conditionings affect defense mechanisms of *Crassostrea gigas* during an annual cycle ?

➤ Nutritive conditionings **in hatchery** do not affect immune responses of oysters

➤ But, oysters fed 12% algae were more sensitive to an experimental infection with *Vibrio lentus* (Melanie Gay)

➔ Immune system depressed

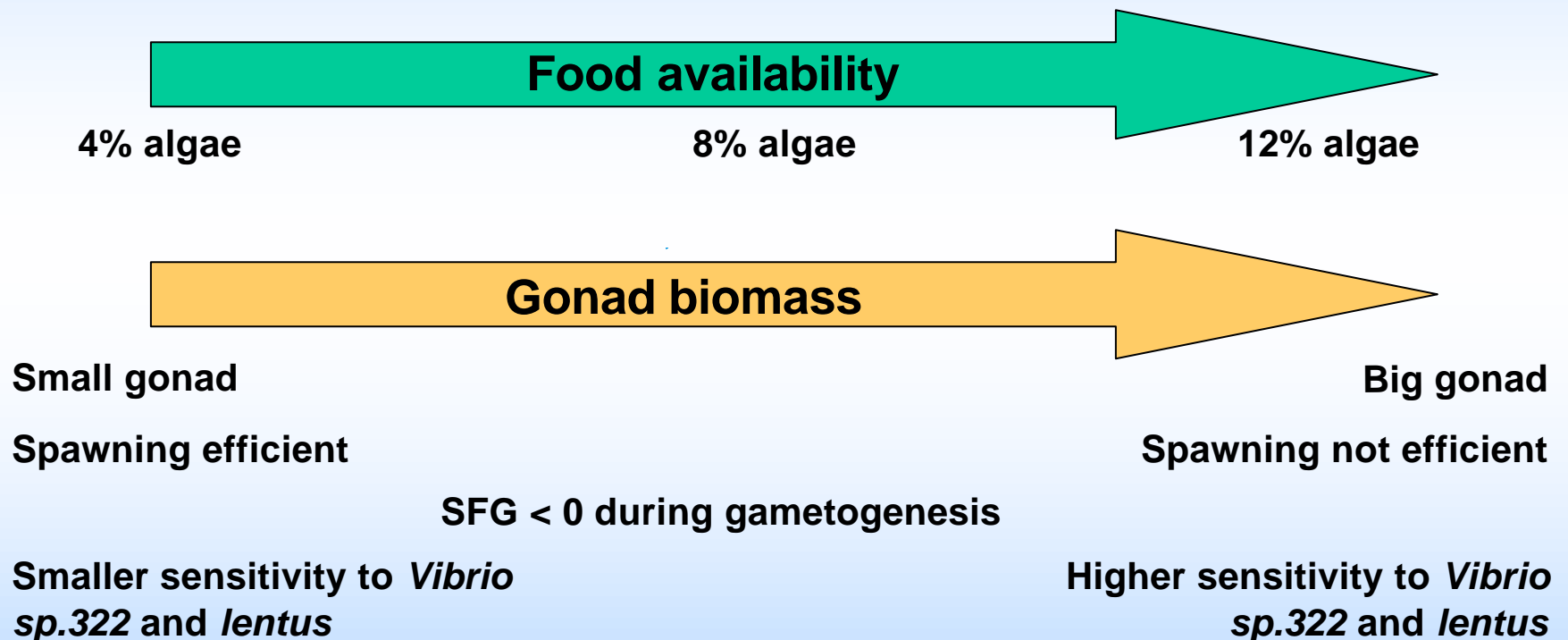


Do nutritive and rearing conditionings affect defense mechanisms of *Crassostrea gigas* during an annual cycle ?

- Nutritive conditionings **in hatchery** do not affect immune responses of oysters
- In contrast, **rearing conditions** (site effect: hatchery vs field) affect immune responses

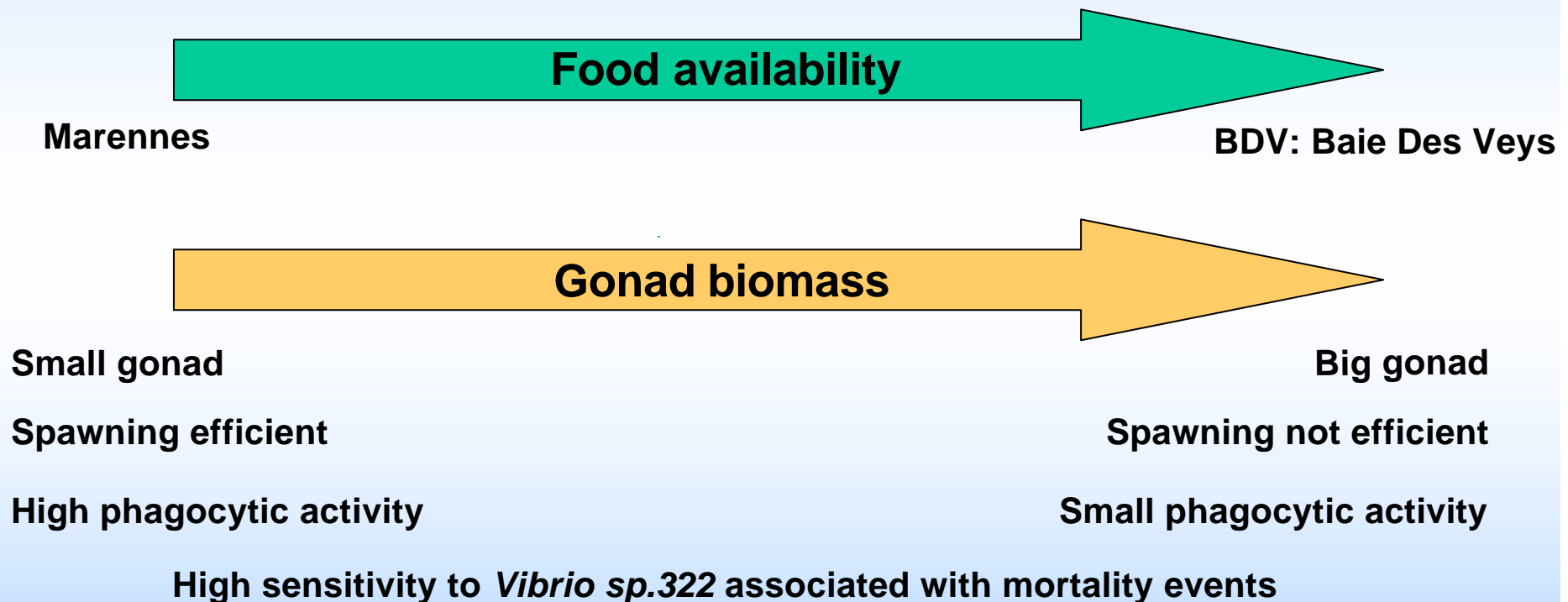
Oysters reared in field showed a “better” immune system than those reared in hatchery : more hemocytes, more granulocytes, higher phagocytosis activity, best cell viability. This correspond to the characteristics of “TOP form” oysters (Lambert *et al.*, NSA 2003).

Do relationship between physiological and immune parameters exist **in hatchery** ?



→ The more oysters spend energy in reproductive process, the more their immune system is depressed and the more oysters are sensitive to infection

Do relationship between physiological and immune parameters exist **in Field** ?



→ The more oysters spend energy in reproductive process, the more their immune system is sensitive

Conclusion

- **In hatchery, nutritive conditionings induce different reproductive status but do not affect the immune system.**
- **Our experiment suggests rearing conditions are better in field than in hatchery. Oysters reared in field have a “better” immune system.**



Future approaches

- **Precise the relationship or competition between immune system and reproductive cost in hatchery as well as in field ? Would it be better for oysters to spend energy in reproductive process or in immune system ?**
 - ➔ **Use triploids as control ?**
- **Improve the rearing conditioning in hatchery in order to be closer to field conditioning**

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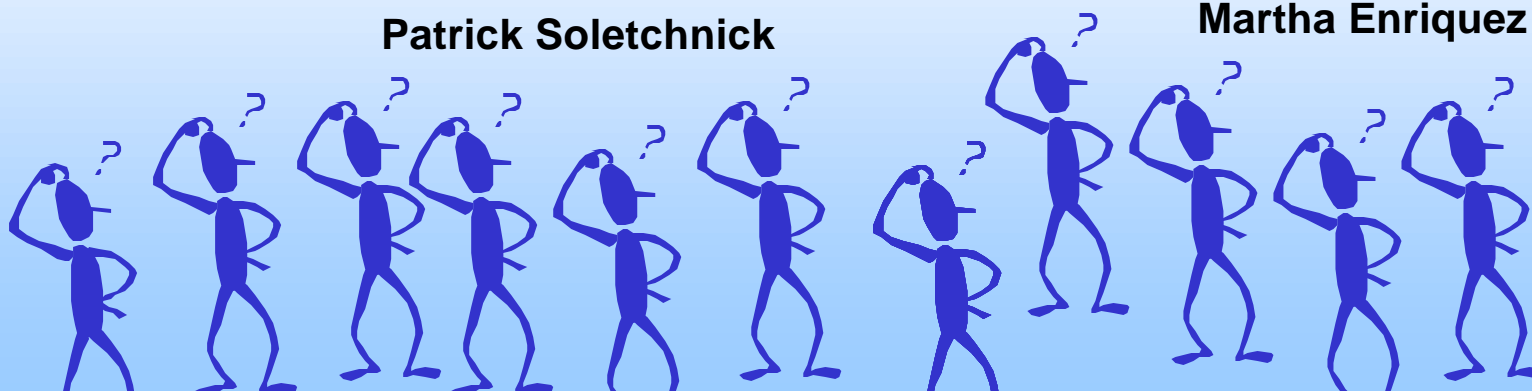
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