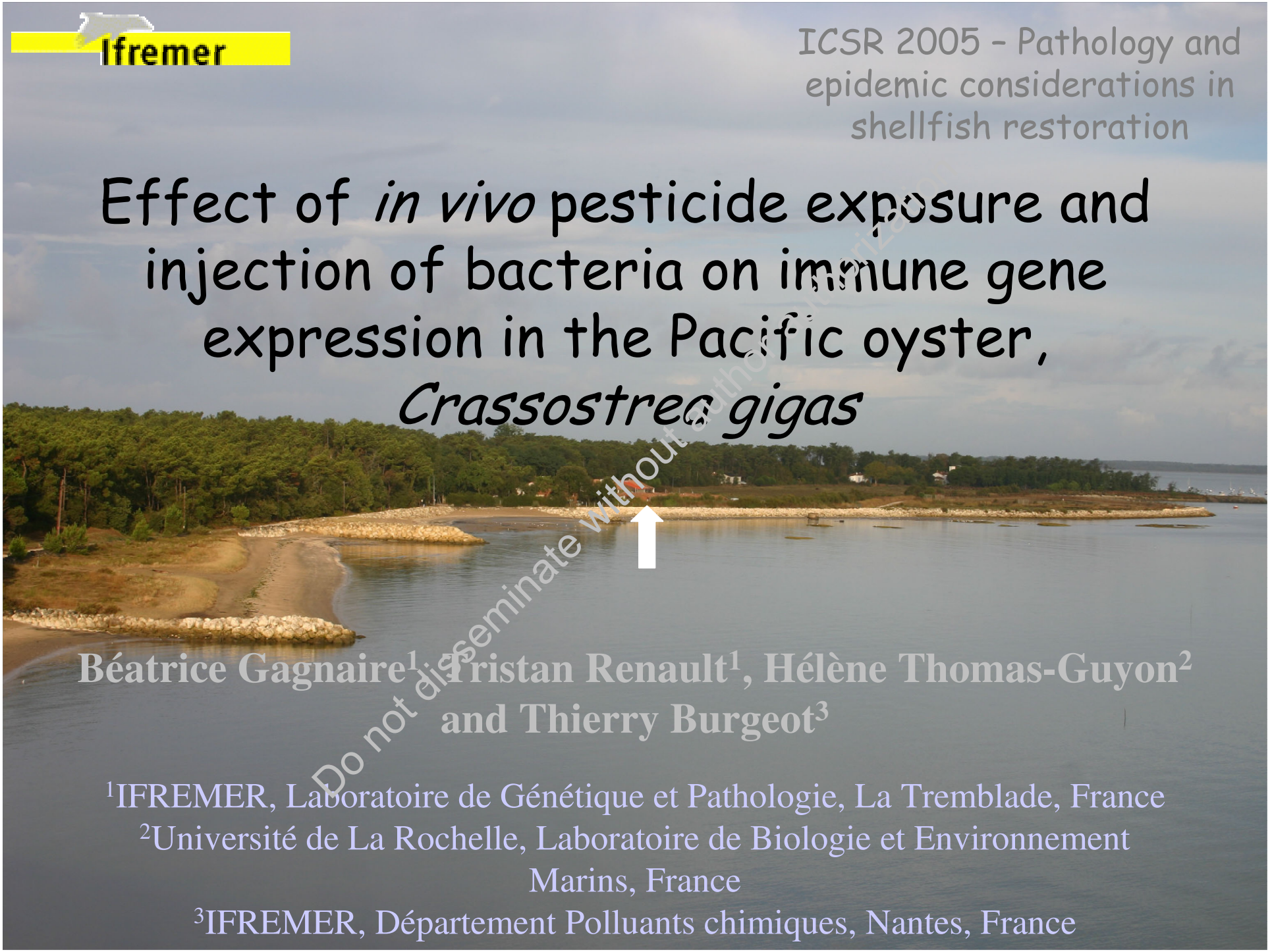


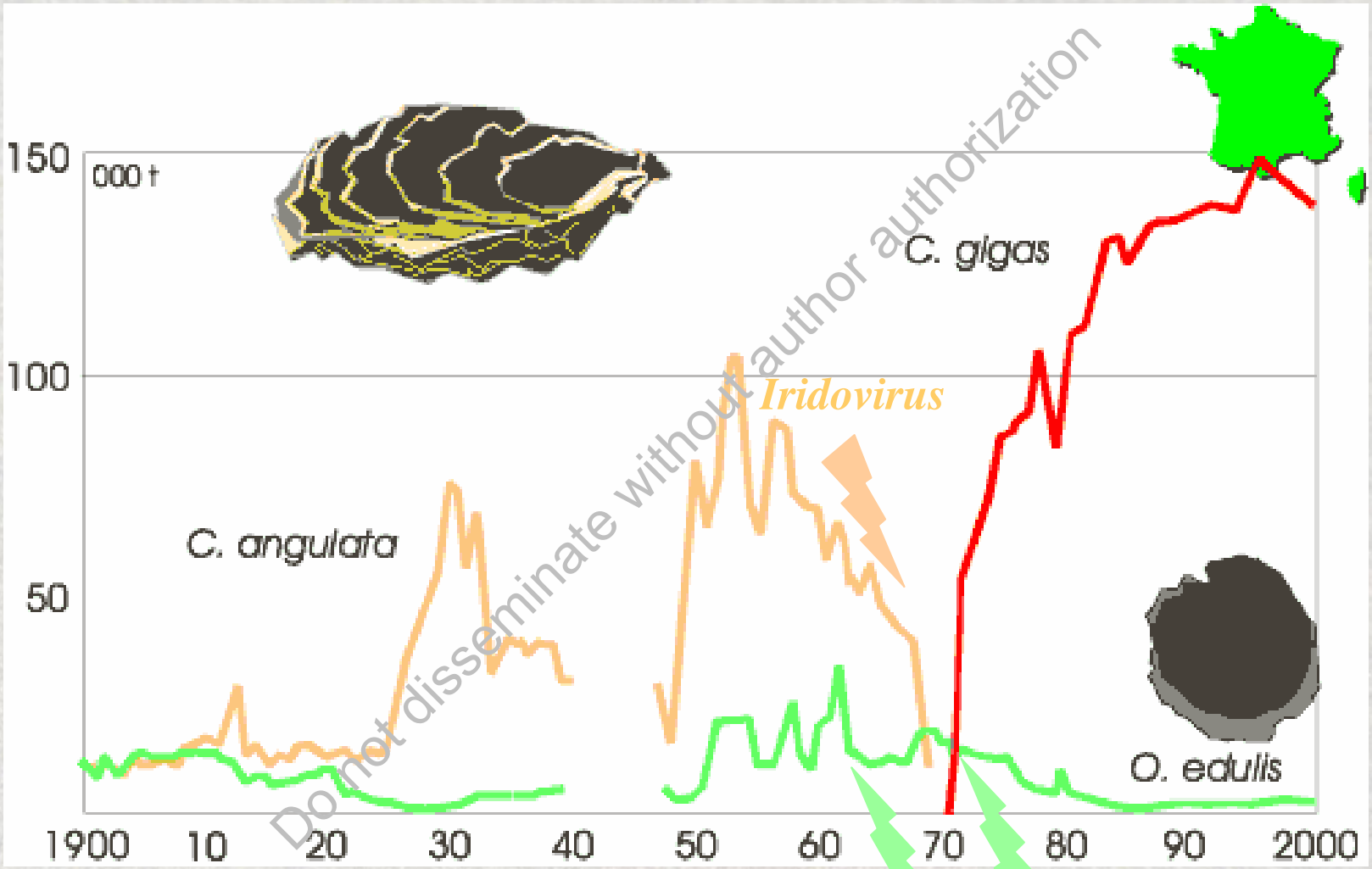
ICSR 2005 – Pathology and epidemic considerations in shellfish restoration

Effect of *in vivo* pesticide exposure and injection of bacteria on immune gene expression in the Pacific oyster, *Crassostrea gigas*

Béatrice Gagnaire[1], Tristan Renault[1], Hélène Thomas-Guyon[2] and Thierry Burgeot[3]

[1]IFREMER, Laboratoire de Génétique et Pathologie, La Tremblade, France
[2]Université de La Rochelle, Laboratoire de Biologie et Environnement Marins, France
[3]IFREMER, Département Polluants chimiques, Nantes, France

French oyster production



Pollutants, diseases and oyster farming

- Rearing areas = estuary areas receiving pollutants
  - Industry : heavy metals, PCBs
  - Boats : PAHs
  - **Agriculture : pesticides**

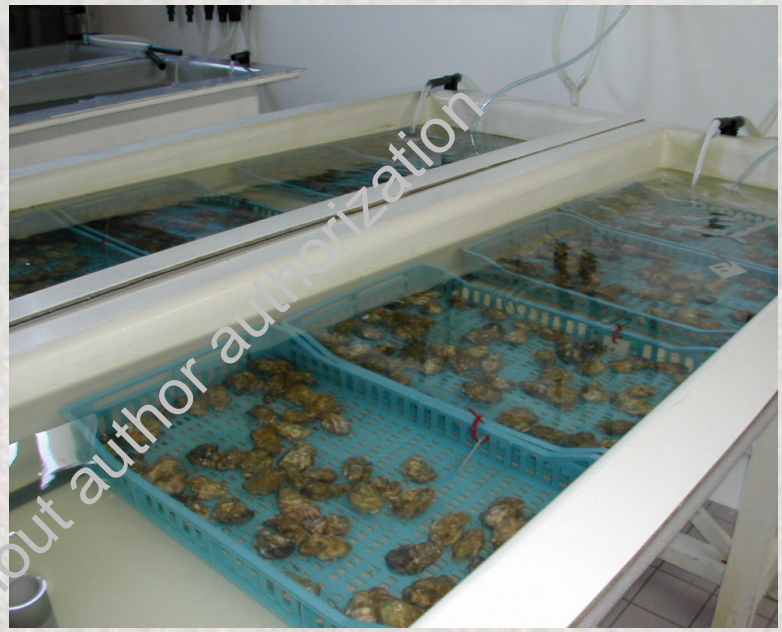
- *Crassostrea gigas*
  - Sedentary
  - filter-feeding

➡ Effects of pollutants on oyster defence capacities?

- Presence of infectious agents in *C. gigas*
  - Rearing conditions
  - No vaccination

➡ Relations between pollutants and susceptibility to diseases?

In vivo pesticide exposure



- 7 days : control (C) and pesticides (P) (3 experiments)
- Mixture of 8 pesticides:
  - Alachlor, metolachlor, atrazine, diuron, glyphosate, terbutylazine, fosteyl aluminium, carbaryl
  - Environmental concentrations (0.05 to 0.8 µg.L^{-1})
- Flow cytometry: measurements of hemocyte parameters (mortality, esterase, ROS, phagocytosis)

Results

- Cell mortality: few variations
- ROS and esterase positive cells : P<C after 3 days only in the first experiment
- **Phagocytosis :**
  - P<C after 7 days of pesticide exposure
  - Reproducible results for the 3 experiments

→ Decrease of phagocytosis activity due to pesticide mixture

Bacteria injection

- After 7 days of pesticide exposure, injection **on C and P** of ASW or bacteria
  - mixture of 2 *Vibrio* isolated from mortality events (2.10^7 or 4.10^7 bacteria/oyster)
- Oyster mortality measurements
- RNA extraction from hemocytes after 24 h post-injection

Expression of genes involved in defence mechanisms (phagocytosis)

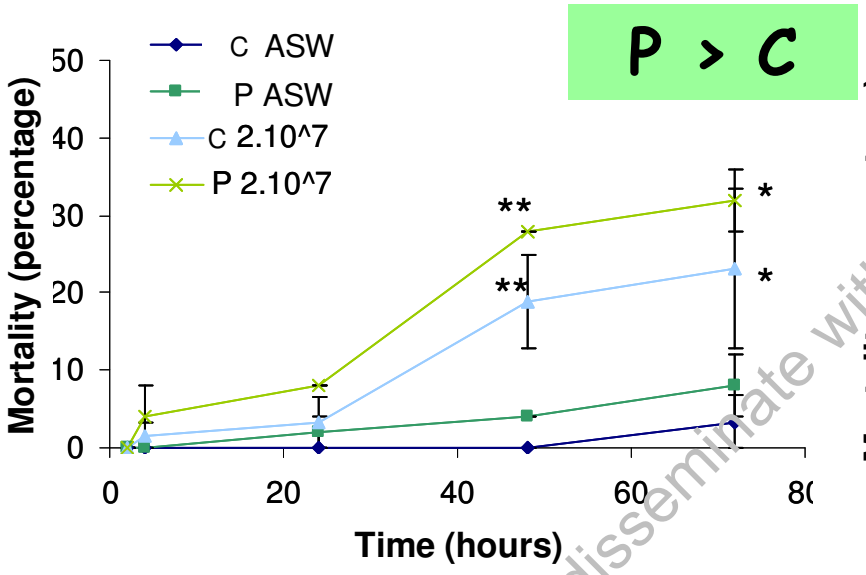
1. Receptor binding	2. Transduction	3. Cytoskeleton modification	4. Post-phagocytosis degradations and cellular protection mechanisms
Ficolin Galectin 4 LBP/BPI protein LPS/ß-1,3-glucan protein	Vav-3 protein Importin-a ECSIT DOCK 180 protein c-Src kinase	Ankyrin Cofilin Filamin Rho protein	Isocitrate deshydrogenase pro-Cathepsin L TIMP SOD Lysozyme Defensin

- RT
- Real-time PCR (Icycler, BioRad)
- Comparison to a reference gene (elongation factor)

Comparison between C+ *Vibrio* and P+ *Vibrio*

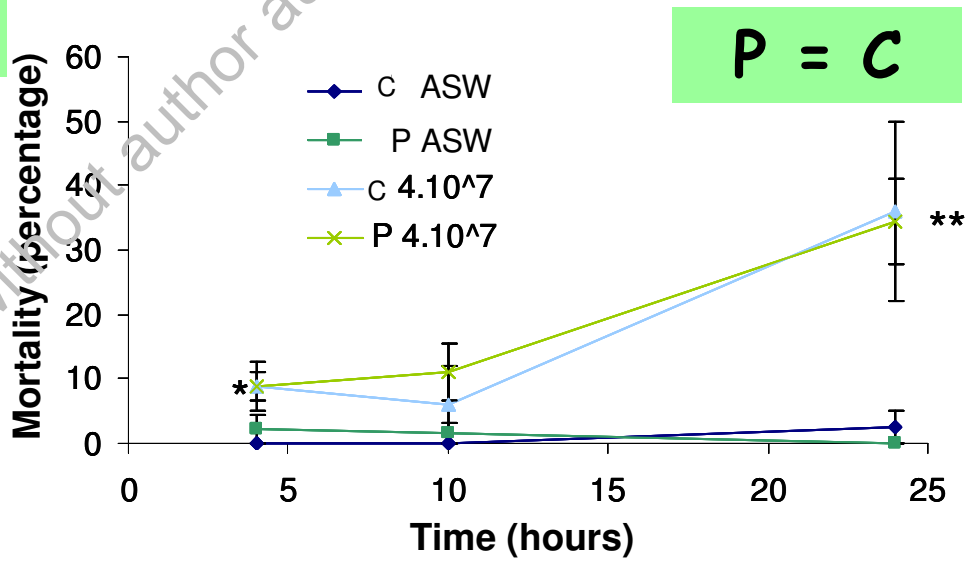
- Oyster mortality after injection

2.10^7 CFU/animal



P > C

4.10^7 CFU/animal



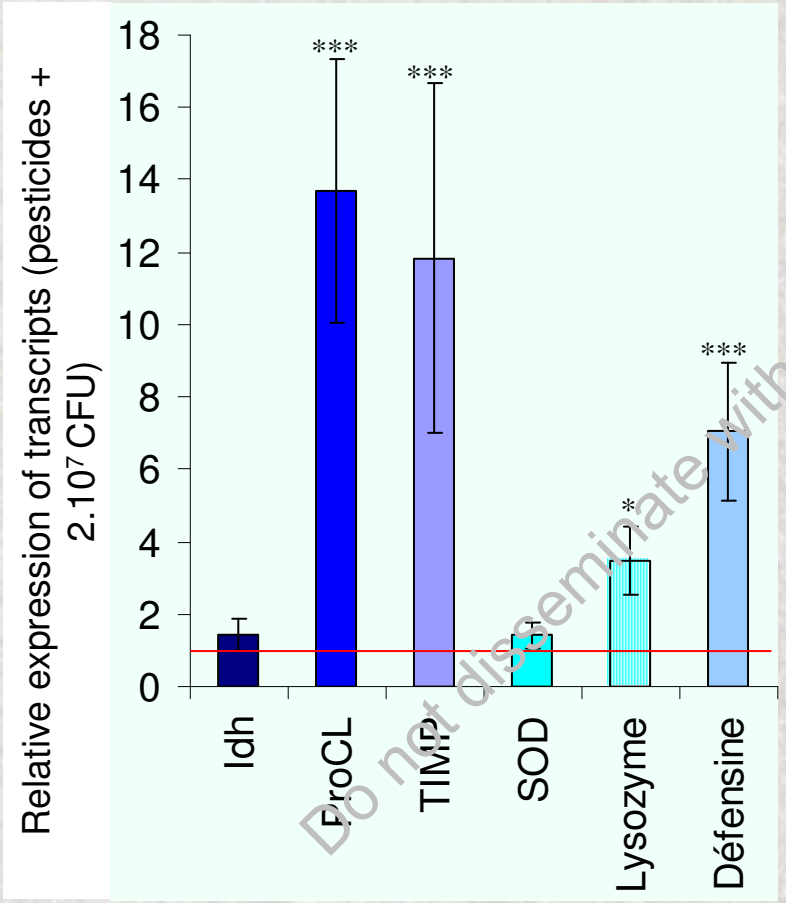
P = C

Higher mortality for contaminated oysters after injection of 2.10^7 CFU only

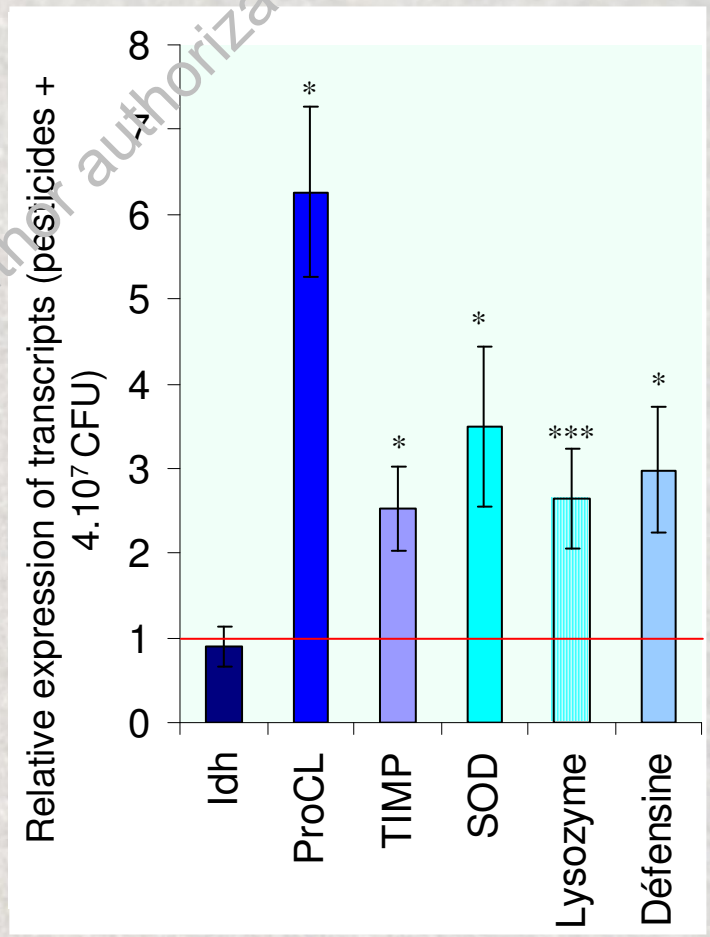
Comparison between C+ *Vibrio* and P+ *Vibrio*

- Phagocytosis gene expression at 24 h post-injection (P vs C):

2.10^7 CFU/animal



4.10^7 CFU/animal



Up-regulation of genes with pesticides, whatever the dose of bacteria may be

Discussion & Conclusion

- Decrease of phagocytosis after a 7 day-contamination period:
  - **susceptibility** of oysters to environmental contaminants
- Oyster mortality higher for P only with 2.10^7
  - 4.10^7: **too elevated** concentration to see differences
- Up-regulation of genes at 24 h post-injection of *Vibrio* in P compared to C:
  - **dysfunction** of host immune response: **harmful inflammation**
- Same gene response for both *Vibrio* concentrations:
  - No difference for mortality, but **response to disease**
- BUT **only one experiment**: need to confirm these results

Pesticides act on phagocytosis at cellular level and may disrupt the immune response to an infection