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

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French oyster production

**Graph**

- **Y-axis:** Tons
- **X-axis:** Years (1900-2000)
- **Graphs:**
  - *C. angulata*
  - *C. gigas*
  - *O. edulis*

**Key Events**

- **1970s:** Introduction of *C. gigas*
- **1990s:** *Iridovirus* outbreak
- **2000s:** Rapid increase in production

**Species**

- *Marteilia refringens*
- *Bonamia ostreae*
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
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)

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- 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

4.10^7 CFU/animal

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 $2 \times 10^7$
  - $4 \times 10^7$: 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
Thank you for your attention!!