In 2008, 2009 and 2010, widespread mortalities were reported among *C. gigas* farmed stocks causing severe losses, affecting all production sites of France and different EU member States. Reported cases of abnormal mortality were investigated and it appeared that mortality events in France, Ireland, and UK were associated to OsHV-1, predominantly the newly described μvar genotype (GenBank HQ842610). Until this work, there was no knowledge on the OsHV-1 μvar contamination of wild stocks of Pacific oysters in France. The two main objectives of this work are: to describe wild populations of Pacific oysters in Marennes Oleron bay; to characterize these populations regarding OsHV-1 infection and its prevalence for spat and adults in connection with the isolation level from reared stocks and the sampling periods. This study investigates detection of the virus in the wild population. The description of oyster populations was based on a specific spatiotemporal hydrodynamic model, GIS analysis and field work evaluation to specify the isolation level between reared and wild stocks. A statistical approach based on stratification of the data and random draw allowed to select 4 representative sites. Three sampling periods were targeted taking into account seawater temperature and presumed mortality risk, April, June, and October. According isolation level of sites tested, a significative difference appeared regarding OsHV-1 detection whatever the sampling dates. Detection of OsHV-1 in adults infected asymptomatically suggests the existence of persistent infections and provides elements to clarify the role of *C. gigas* adults as reservoir of the virus. Oyster wild stocks implanted near reared stocks may exchange infectious material according an 'Hydrodynamic' hypothesis and results can be relevant with 'transport' of the virus by currents (spreading) associated with the ability of OsHV-1 particles to be infectious for several days. The water may play a role in the transmission, but not in the conservation of infectious virus in the long term. This point should be further confirmed. This study highlights that wild stocks implanted with reared stock may be 'influenced' by them and *vice versa* but also the interest of the 'hydrodynamic' approach to select contrasted sites for survey or stocks management.
Situation of wild beds of oyster *C. gigas* regarding the virus OsHV-1 \( \mu \text{var} \):

Spatiotemporal description of OsHV-1 contamination in Pacific oysters wild beds in Marennnes Oleron bay (2010)

Pépin² Jean-François, Soletchnik¹ Patrick, Le Moine¹ Olivier, Robert¹ Stéphane, Lupo² Coralie, Geairon¹ Philippe, Seugnet¹ Jean Luc, Bernard³ Ismael

¹ Laboratoire Environnement Ressources Pertuis Charentais ; ² Laboratoire de Génétique et Pathologie – La Tremblade, ³ Laboratoire de Physiologie des Invertébrés – Brest, France
**Context of study**

- Epidemic situation has occurred in oysters farms since 2008
- Most of reared shellfish sites have been affected by mortalities, mainly associated with OsHV-1 detection

**Questions**

- What is the status of wild oyster stocks regarding OsHV-1 infection in Marennes Oleron bay?
- Is there difference for OsHV-1 prevalence between wild stocks from ‘isolated’ site vs wild stocks near reared livestock?
- Do adults from wild bed play the role of virus reservoir?

**Objectives**

1° - Describe wild populations of pacific oysters in Marennes Oleron bay
2° - Characterize wild oyster populations regarding OsHV-1 infection for spat and adults in connection with the isolation level from reared stocks
Situation of wild beds of oyster *C. gigas* regarding the virus OsHV-1 μvar

**Issues**

- Do ‘sanctuary places’ exist?

- Do virus ‘reservoir sites’ exist?

- What could the ability of ‘natural spreading’ be for OsHV1 between reared and wild stocks?

*Re-stocking projects...*
Sampling strategy: wild beds characterisation

Mapping limits of oyster wild beds + GIS analysis

Estimate level of occupancy on rocks +

Estimate density using pictures + Direct numeration

20 sites evaluated
**Sampling strategy:**

wild beds characterisation

Selection of sites to be tested:

1- Use of specific hydrodynamic model devoted to simulate oyster larvae spreading (tracer) to define isolation level from reared stocks (H₀: virus = tracer).

2- Stratification of data

3- Random draw: 4 Sites selected / 20 sites

**Strong isolation: 2 sites**

La Tranche; St Pierre

**Weak isolation: 2 sites**

Fouras; La Seudre
Sampling strategy: OsHV-1 characterisation

Sampling period

- April campaign
- June campaign
- October campaign

- «Threshold» temperature

- avril mai juin juillet aout septembre octobre
Sampling strategy: OsHV-1 characterisation

Diagnostic methods:

- DNA extraction (QIAgen kit)

- Real time PCR for detection and quantification on individual (DNA polymerase primer set)

- Conventional PCR (EU 175/2010) for OsHV-1 μvar genotype control on qPCR positive samples
Results: April OsHV-1 tests

OsHV-1 frequency detection

✧ OsHV-1 detected:
  ✧ in all adult stocks whatever the stock isolation level
  ✧ in spat stocks with the weak stock isolation level

✧ OsHV-1 not detected in spat from sites with strong isolation level (n=150)
Results: June OsHV-1 tests

OsHV-1 frequency detection

- OsHV-1 detected:
  - only in sites with the weak stock isolation level, in adult or spat stocks

- OsHV-1 not detected in sites with strong isolation level
Results: October OsHV-1 tests

OsHV-1 frequency detection

- OsHV-1 detected:
  - in sites with the weak stock isolation level, in adult or spat stocks
  - in adult stock with the strong stock isolation level from La Tranche

- OsHV-1 not detected in St Pierre Oléron site
More than 1300 live oyster samples tested, adults (n=460), spat (n=900), 66 samples detected OsHV-1 positive. All positive samples presented OsHV-1 μvar genotype (PCR test).

Wild adults oyster stocks tested were infected in all sites whatever isolation level, H₀: they could potentially act as a ‘reservoir’... but reared stocks also!

Interest of the ‘hydrodynamic’ approach to select contrasted sites:

- No detection of virus in spat from ‘isolated’ sites for the three times tested, April, June, October (n=538).
- Significative detection in spat from ‘weakly isolated’ sites (10.5% n=362).

During epidemic period (June) no detection of OsHV-1 in spats and adults from ‘isolated’ sites (n=159).

Oyster wild stocks implanted near reared stocks may exchange infectious material.

‘Hydrodynamic’ hypothesis: results can be relevant with ‘Transport’ of the virus by currents (spreading) in association with the ability of OsHV-1 particules to be infectious for several days. Hydrodynamic model could be helpful in risk management for re-stocking projects.