



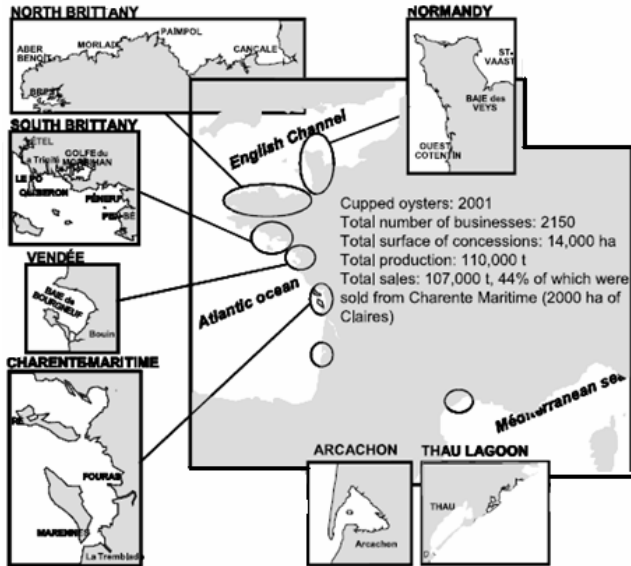
Experimental Ostreid herpes virus 1 (OsHV- 1) infection of the Pacific oyster *Crassostrea gigas*: kinetics of virus DNA detection by q-PCR in seawater and in oyster samples

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Introduction - States of Art



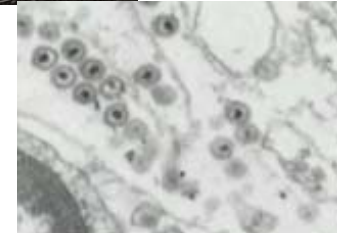
- ✓ France is the fourth producer of the world of cupped Pacific oysters *Crassostrea gigas* (with 120 000 tons per year)
- ✓ However, episodes of mortality can influence this production and cause important economical losses

✓ Oysters can be infected by different pathogens, including :

➤ Different bacteria (*Vibrio spp.*)



➤ The ostreid herpesvirus-1 (OsHV-1)



Introduction - States of Art

Host ranges for herpes-like virus ?

- ✓ Viral infections by herpes-like viruses in several species of molluscs associated with episodes of mortality



Crassostrea virginica

C. Gigas

C. angulata

Ostrea edulis

O. angasi

Tiostrea chilensis

Ruditapes decussatus

R. philippinarum

Pecten maximus

Haliotis diversicolor supertexta

Haliotis laevigata

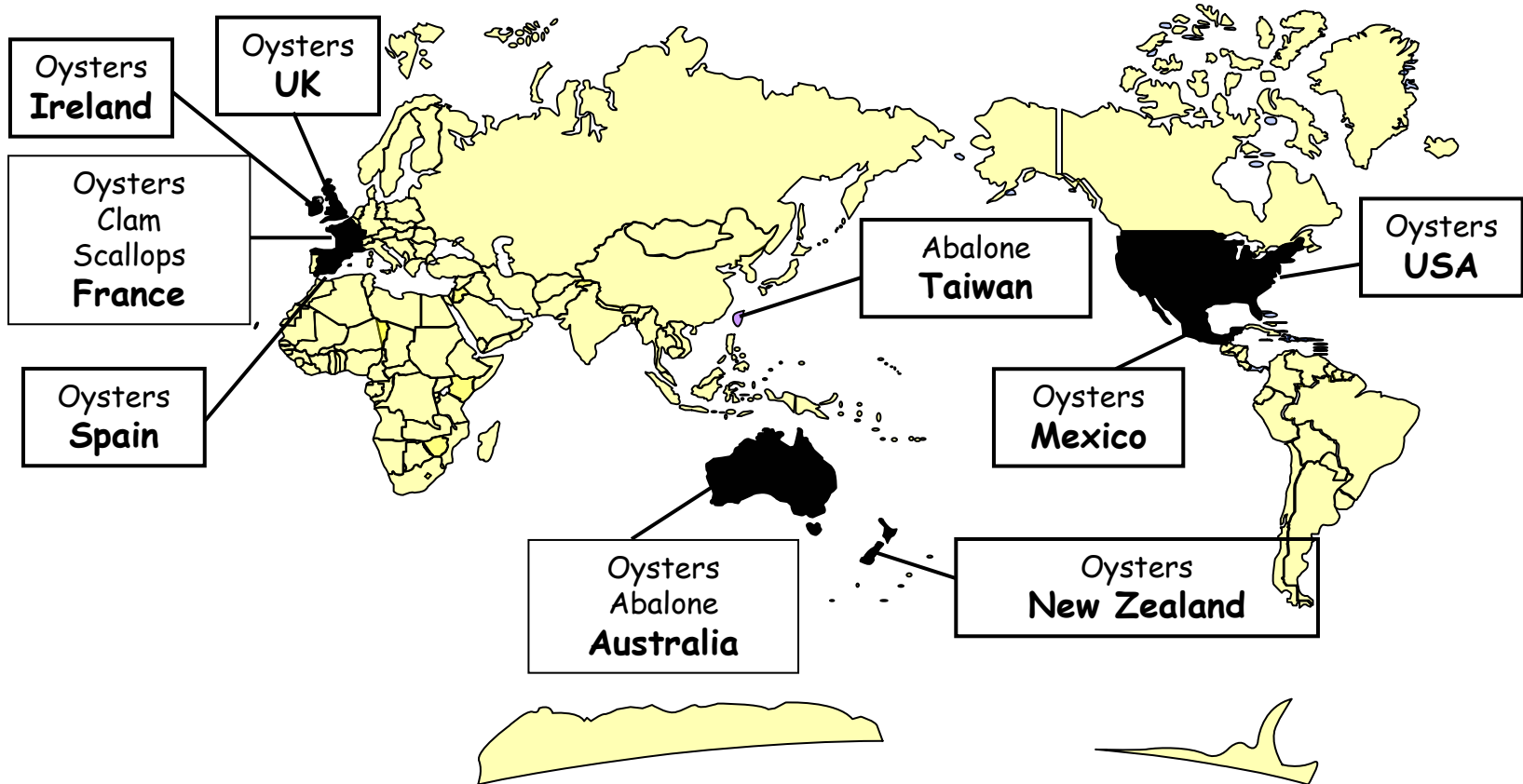
Haliotis rubra



Introduction - States of Art

Host ranges for herpes-like virus ?

✓ Viral infections by herpes-like viruses in different geographical areas



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Oysters mortalities & OsHV-1 in France ?

- ✓ A herpes virus infecting cupped Pacific oysters was observed for the first time in France in 1991 in larvae (90 to 100% mortality among larvae).
- ✓ Since, oyster summer mortalities were currently associated with the detection of the herpes virus OsHV-1



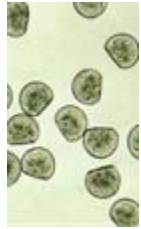
Velar lesions (arrow) of a *C. gigas* larva infected with OsHV-1

The last year ...

- In 2009, cupped Pacific oyster mortalities in France :
 - an average percentage of 80% of spat affected
 - with a large geographical distribution on French coasts
- Approximately 94% of batches analysed during the national survey were positives for the detection of OsHV-1 (quantitative-PCR method)

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To confirm the implication of OsHV-1 in oyster mortalities, different assays have been carried out in experimental conditions ...



On Larvae ...

- ✓ Demonstration of OsHV-1 experimental transmission from infected to axenic *C. gigas* larvae, associated with high mortality rates and detection of viral particles (Arzul *et al*, 2001)



On Spat ...

- ✓ Successful transmission of OsHV-1 from infected to healthy spat (less than 1 year old) with high mortality rates (associated with detection of viral DNA and viral particles).

Naturally
infected oyster spat
(sampled on the field)



Infectious
OsHV-1
suspensions



Intramuscular
injections
(in the adductor muscle)

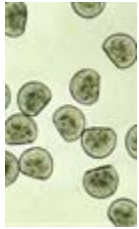


Mortality & qPCR



Introduction - States of Art

To confirm the implication of OsHV-1 in oyster mortalities, different assays have been carried out in experimental conditions ...



On Larvae ...

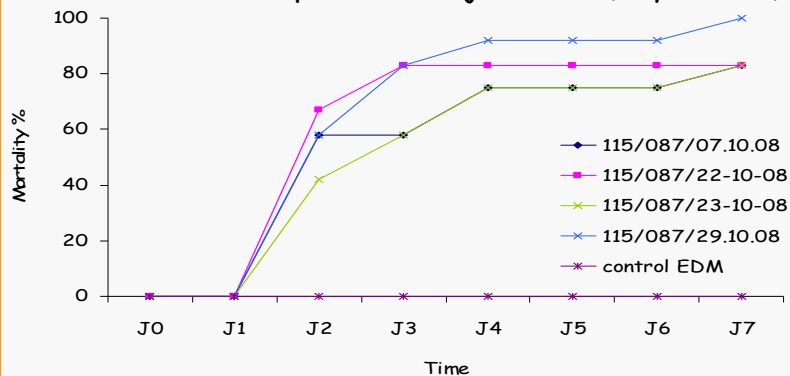
- ✓ Demonstration of OsHV-1 experimental transmission from infected to axenic *C. gigas* larvae, associated with high mortality rates and detection of viral particles (Arzul et al, 2001)



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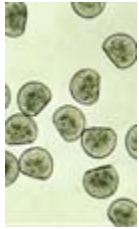
Cumulative mortality after IM injections of different infective suspensions in juveniles (<1 year old)



- High reproducibility of experimental assays (80% of mean mortality after 4 Days)
- Dead individuals showed high amounts of viral DNA (qPCR)
- It is possible to maintain sources of infective virus

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On Spat ...

- ✓ Successful transmission of OsHV-1 from infected to healthy spat (less than 1 year old) with high mortality rates (associated with detection of viral DNA and viral particles).

Nevertheless, this experimental infection protocol by intramuscular injection :

- Is not representative of "what happens in the field ?"
(*does not allow us to reproduce and to follow a natural way of OsHV-1 infection !*)

Objectives of this work

1°) To reproduce a more “natural” infection protocol by OsHV-1 in experimental conditions

Establishment of a protocol for **horizontal transmission of OsHV-1** based on the cohabitation between healthy and experimentally infected oysters by intramuscular injection.

2°) To Study the kinetics of viral DNA detection by qPCR in oysters samples and in seawater

Experimental methodology

1- Production of experimentally infected oysters

Intramuscular injection of the OsHV-1 suspension.



2- Cohabitation during 48 hours

Moribund oysters (2 days post-IM injection) were added in each aquaria containing healthy animals and then removed after 2 days.

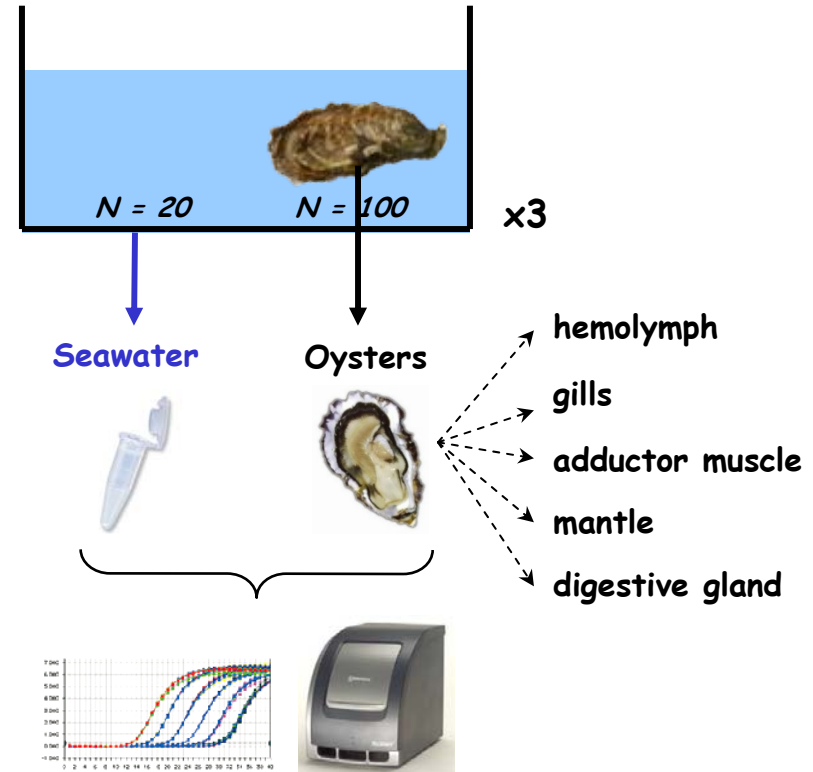
3- Collection of oysters and seawater samples

10 individuals were removed from each aquaria after different period of cohabitation (0, 6, 24, 36, 48, 72, 96, 120 and 148 h) and different oyster tissues were sampled.

4- DNA extractions and OsHV-1 viral DNA detection by qPCR analysis

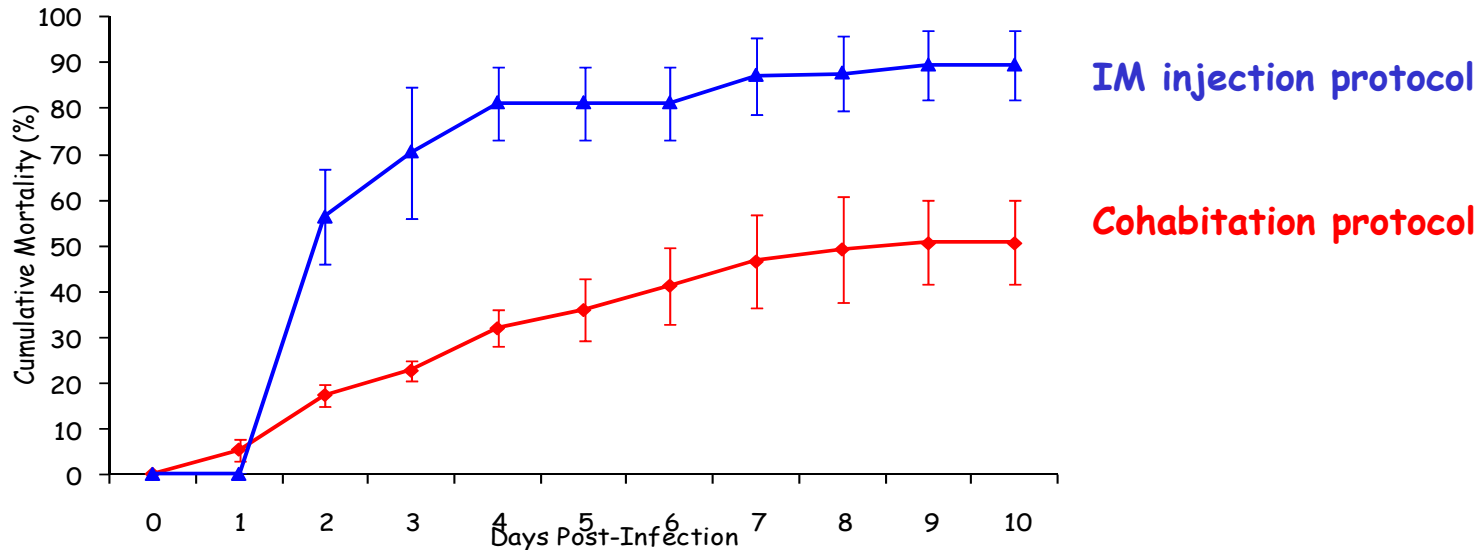
5- Monitoring of mortalities during cohabitation

in 3 other aquaria where no animals were collected



Results

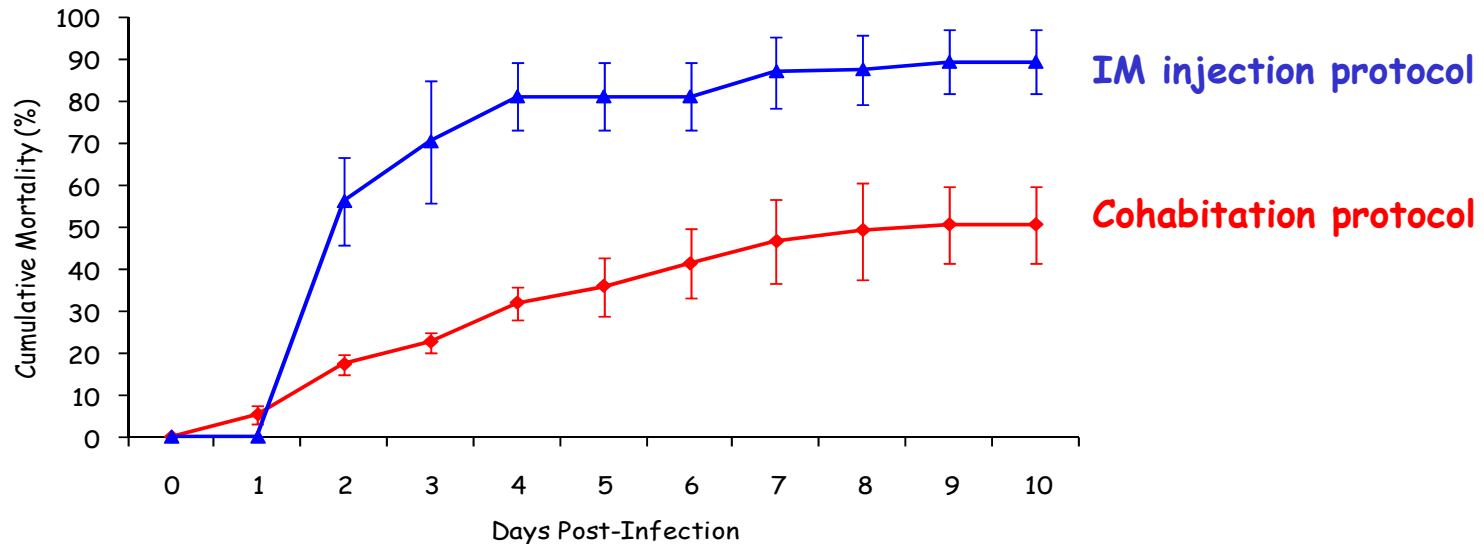
Oysters mortality



- Mortalities recorded in injection and cohabitation
- Differences in mortality rates between injected and cohabited animals (90% versus 50%) can be explained by the different routes of infection.
 - In the case of IM injection protocol, a mean mortality of 90% was observed 7 Days post-injection
 - In the case of cohabitation protocol, mortality increased more slowly to reach 50% at Day 8 after the beginning of cohabitation.

Results

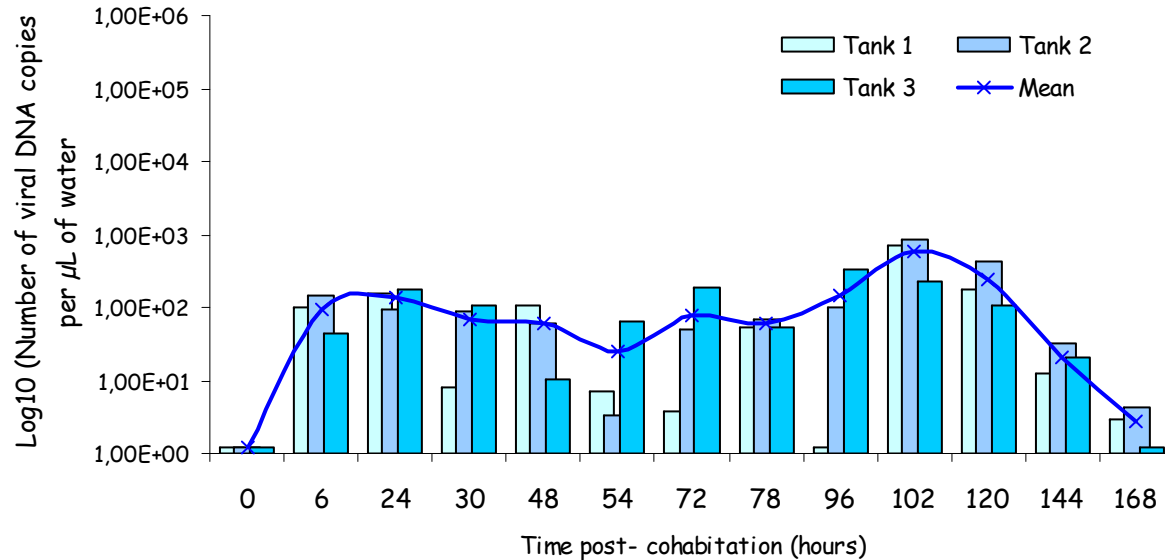
Oysters mortality



- Dead animals were all positives to the detection of OsHV-1 by qPCR
- Moreover, no pathogenic bacteria were evidenced in samples, attesting that mortalities occurring during the course of experiment can be attributed to OsHV-1 infection
- No mortalities were recorded in both negative controls

Results

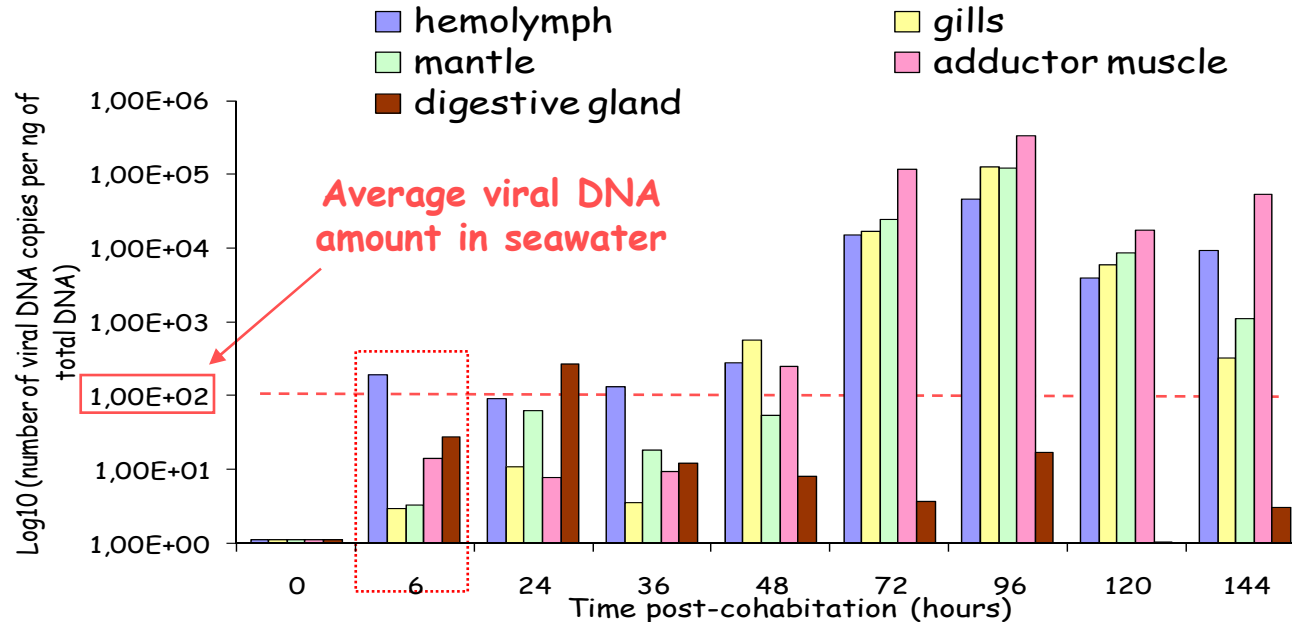
Viral DNA detection in seawater



- The amount of viral DNA in seawater increased to reach 1.10^2 DNA copies per μL of seawater and remained stable up to 48 h
- After 54 h, once that intramuscularly injected oysters were removed, the amount of viral DNA decreased slightly.
- Then, the number of viral DNA copies in seawater increased again at 72 h with a maximal amount of viral DNA of 1.10^3 DNA copies per μL of seawater at 102 h
- No virus DNA was detected in seawater sampled in both negative controls

Results

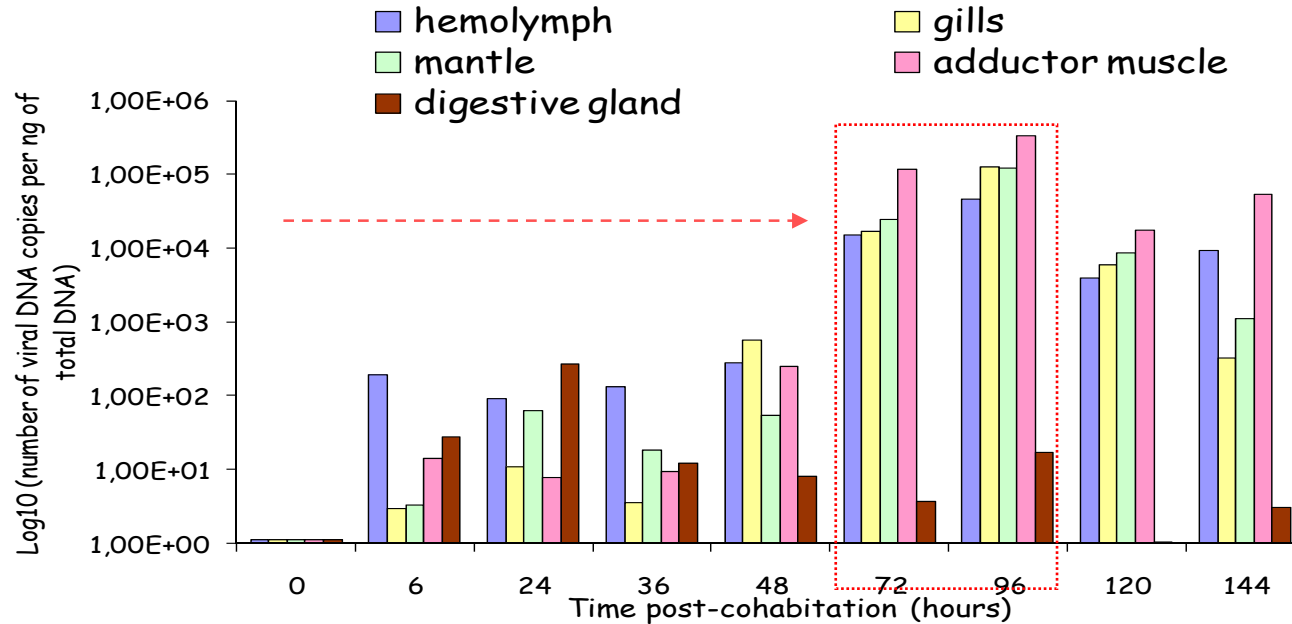
Viral DNA detection in oyster tissues



- After 6h of cohabitation, the highest average viral DNA amount was observed in hemolymph which was comparable to those quantified in seawater.
- This result can be interpreted as rapid penetration of the virus in the haemolymphatic system because of the open circulatory system present in oysters.

Results

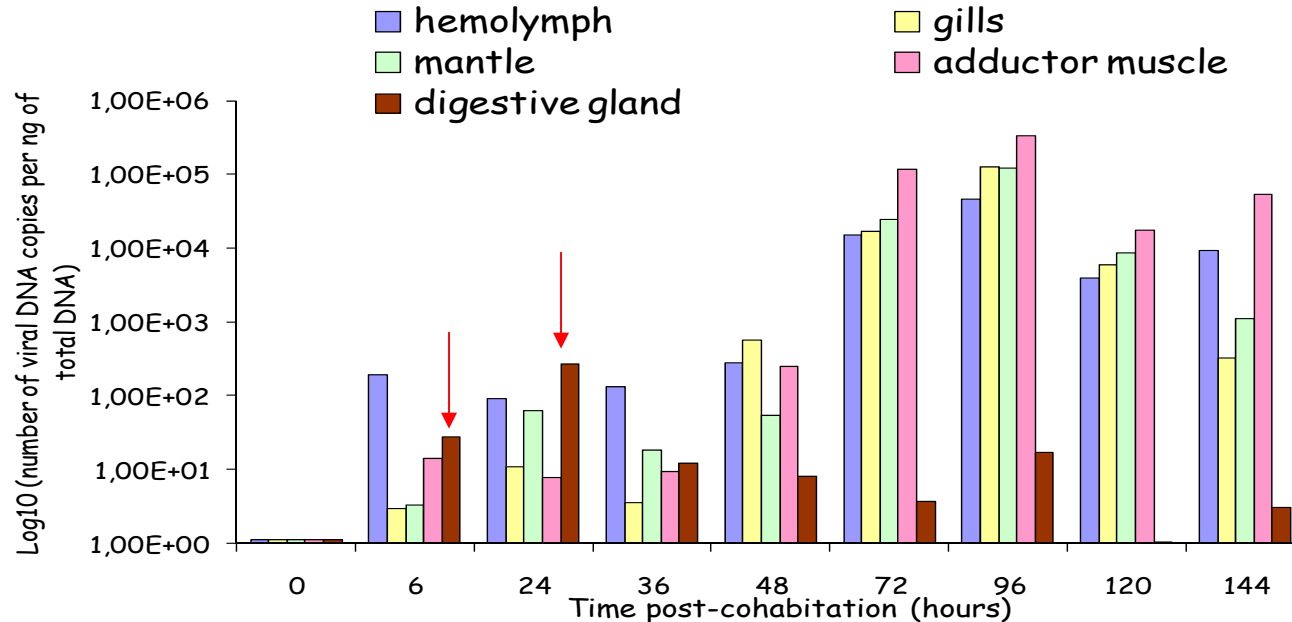
Viral DNA detection in oyster tissues



- A significant increase of the virus DNA amounts was observed from 72h to 96h post-cohabitation in all analysed tissues, except for the digestive gland.
- For longer period of cohabitation, results show a slight decline of viral DNA amounts in all tissues.

Results

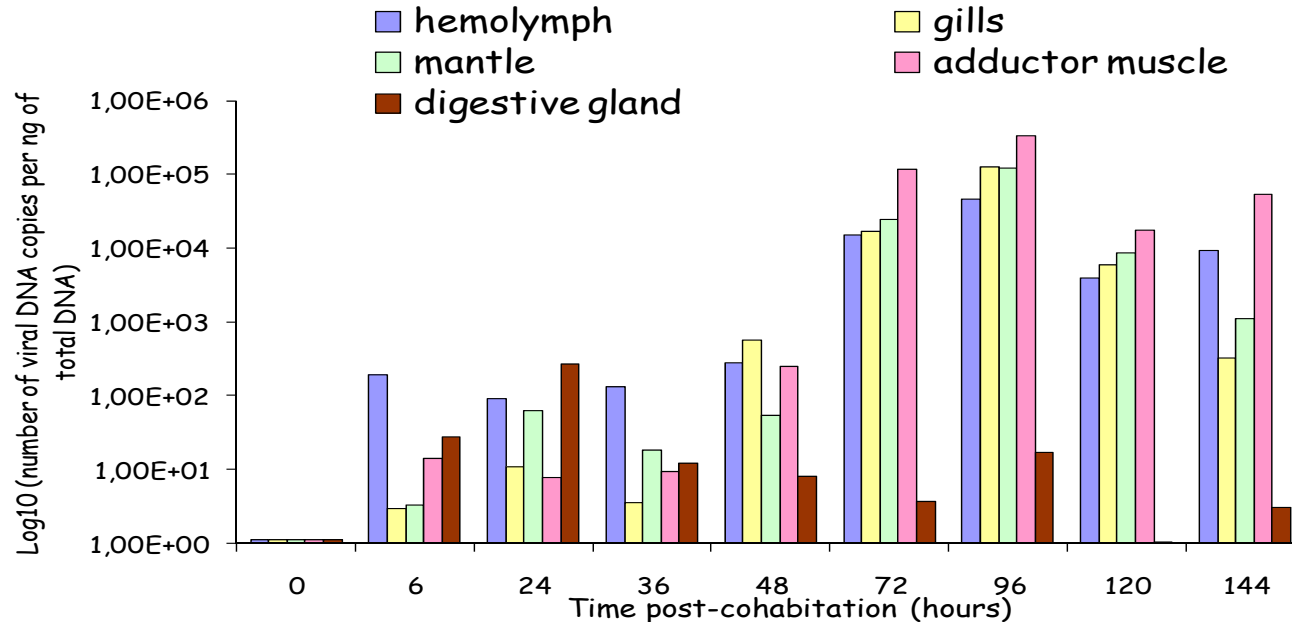
Viral DNA detection in oyster tissues



- Concerning the digestive gland, virus DNA amounts ranging from 1.10^2 to 1.10^3 DNA copies per ng^{-1} were detected early (6 h and 24 h), suggesting that this organ may play a role for the entry of the virus in oysters.
- However, high differences were observed for this tissue between experimental tanks, which can be explained by the presence of PCR inhibitors in this tissue.

Results

Viral DNA detection in oyster tissues



➤ Taken together, these results suggested that :

- OsHV-1 could first entry through the haemolymphatic system and the digestive gland (nutrition)
- and that viral particles could be transported by haemolymph to the different target organs before to engage an intense replication phase conducting to the development of the disease.

Conclusion



- We have established a new experimental protocol, based on cohabitation between infected and healthy animals, and allowing the horizontal transmission of OsHV-1.
- This cohabitation protocol :
 - appear more representative of "what happens in the field"
 - allow us to study the viral spread in experimental conditions.
- Taken together, our results allow us :
 - to have a better comprehension about the dissemination and the transmission of OsHV-1 in *C. gigas* during a mortality event
 - to precise the kinetics of OsHV-1 infection in *C. gigas*.
 - to precise viral DNA amounts associated to oyster mortalities in different organs tissues.

An aerial photograph of a wide, winding river, likely the Mississippi River, flowing through a vast, flat landscape. The river is a deep blue-green color. On the left bank, there are numerous rectangular plots, possibly aquaculture or agricultural fields. A long, multi-span bridge crosses the river in the lower middle section. The surrounding land is a mix of green vegetation and brownish fields. In the bottom right corner, there is a small cluster of buildings and a curved embankment. A speech bubble with a blue border and white background is positioned in the lower center of the image, containing the text "Thanks for your attention !!!".

Thanks for your attention !!!