

INFLUENCE OF TEMPERATURE ON DISEASE TRANSMISSION ASSOCIATED WITH OSTREID HERPES VIRUS OsHV-1 μ Var IN RELATION TO SURVIVAL OF JUVENILE *Crassostrea gigas*

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CONTEXT

Since 2008, heavy mortality of *Crassostrea gigas* juveniles in the field appears to be related to infection by the ostreid herpesvirus OsHV-1 μ Var (1). Mortality occurs at water temperature above 16°C while OsHV-1 μ Var is detected in dying oysters (2). Other pathogens, e.g. vibrios, may also play a role (3). Laboratory experiments in which diseased oysters are exposed to a specific pathogen by injection or cohabitation demonstrate a causal relationship (4). But they do not allow to reproduce the natural infection process occurring in the field.



OBJECTIVE

We examine the effect of seawater temperature on disease transmission and related mortality of oysters by applying an original **experimental infection protocol** called "MIR" (Mortality, Imprint & Revelation).

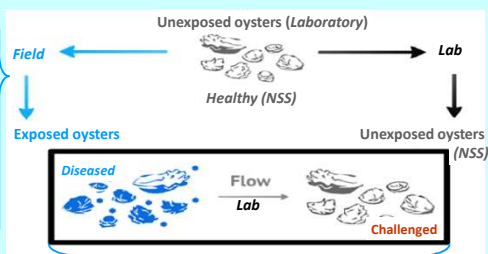
The final aim is to identify the thermal range for transmission of the disease responsible for heavy mortality of *C. gigas* juveniles and the relationship between temperature, OsHV-1 μ Var and vibrios.

MATERIAL & METHODS

Experimental infection protocol: MIR (Mortality, field Imprint & laboratory Revelation)

1. Imprint

Healthy naïve oyster spat (NSS, i.e. free of mortality, negative for OsHV-1 μ Var and *Vibrio* sp. (5)) is exposed for 10-15 days to field conditions in areas where mortality is occurring (Bay of Brest).



2. Revelation

Healthy naïve oyster spat (NSS) are cohabitating in the lab with previously field-exposed (diseased) oyster spat.

Laboratory experiment: testing the effect of water temperature

- 8 temperature values are tested (13.4°C, 14.4°C, 15.4°C, 16.2°C, 17.5°C, 21.9°C, 26.9°C, 29.0°C) on lab-challenged oyster spat.
- Lab-challenged spat held at 13.4°C for 40 days undergo a lab thermal challenge (LTC, (5)), i.e. 21°C over 30 days.

Microbiology:

- oyster samples are collected at different dates (0, 2, 4, 6, 13, 16 d) at each temperature level;
- qPCR assays to detect and quantify OsHV-1 μ Var are run by Idhesa (Quimper, FR) on duplicates of 3 pooled individuals (threshold value for detection: 10⁴ DNA copies of OsHV-1 μ Var per mg wet tissues, (6)).
- Vibrios are quantified on CHROMagar media.

Statistical analyses:

- **Regression models:** i) between temperature and final survival for field-exposed and lab-challenged oysters; ii) between temperature and the time needed to reach peak values of OsHV-1 μ Var DNA and vibrio in lab-challenged.

RESULTS

Survival (of field-exposed and lab-challenged spats)

- Seawater temperature (T°C) influences survival of both field-exposed and lab-challenged oysters (Fig. 1).
- The highest rate of decrease in survival and the lowest final survival were observed:

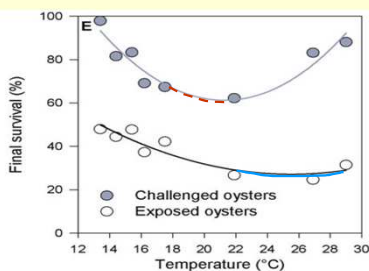


Figure 1. Survival at the end of 16 d at 8 different seawater temperatures of oysters previously exposed to field conditions and of naïve oysters challenged by cohabitation with exposed oysters.

for 16.2 <T°C <21.9
in lab-challenged oysters

for 21.9 <T°C <29.0
in field-exposed oysters

- At 13.4°C, final survival of lab-challenged oysters is 97.8% compared with only 48.0% in field-exposed animals.

Microbiology (of lab-challenged spat)

OsHV-1 μ Var

- OsHV-1 μ Var is detected in lab-challenged oysters less than 2 days (after cohabitation with field-exposed spat) whatever the seawater temperature.
- Maximum OsHV-1 μ Var DNA concentrations in lab-challenged oysters are similar within the range 16.2 <T°C <21.9.

Vibrios

- The onset of mortality (i.e. time elapsed before at least 5% mortality occurs) follows the outbreak of vibriosis by 2 days (intercept= 2, Fig. 2)
- A delay of 1 day (slope= 1, Fig. 2) in the vibriosis outbreak leads to a delay of 1 day in mortality.

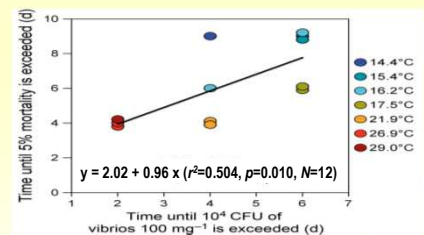


Figure 2. Relationship between the number of days required to observe at least 5% mortality and the number of days required to reach 10⁴ CFU of vibrios per 100 mg oyster flesh in naïve (lab-challenged) oysters held in cohabitation with field-exposed animals.

DISCUSSION & CONCLUSION

- The **MIR infection protocol** that includes NSS (i.e. "gold-standard" animals, (5)) is a realistic method to expose oysters to pathogens in the field → contracted disease is directly linked to observed mortality.
- **16.2 <T°C <21.9:** the optimal seawater temperature range for disease transmission, which agrees with field observations (2).
- **T°C=13.4°C:** 1/ when T°C=13.4°C: the survival of lab-challenged NSS is maximal;
2/ when held at 13.4°C for 40 days, lab-challenged oysters do not exhibit any mortality after 30 days at 21°C, i.e. recovery and no transmission of the virus to NSS → long-term holding at low T°C (<13°C) may allow to mitigate spat mortality.
- OsHV-1 μ Var is detected in lab-challenged spat within 2 days at all temperatures while vibrios are related to onset of mortality.

References.

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