Vibrio aestuarianus, a bivalve pathogen

Summary of work carried out over the past 15 years

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National Surveillance : Repamo network for the surveillance of mollusc health in France, and Reference laboratories: NRLs, EURL for mollusc diseases, Lydie Canier, Céline Garcia, Isabelle Arzul

Phd work: Yannick Labreuche (2004-2007), Patrick Azema (2013-2018), Leila Parizadeh (2014-2018), Aurélie Mesnil (2018 - 2022)

Research Projets: Morest, BIVALIFE, Vivaldi, ANR opopop, ANR GigasSat, ANR Envicopas, Actions DPMA Aestu, DGAl projet DS Inhybe

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Génomique des Vibrios – IFREMER CNRS, Roscoff
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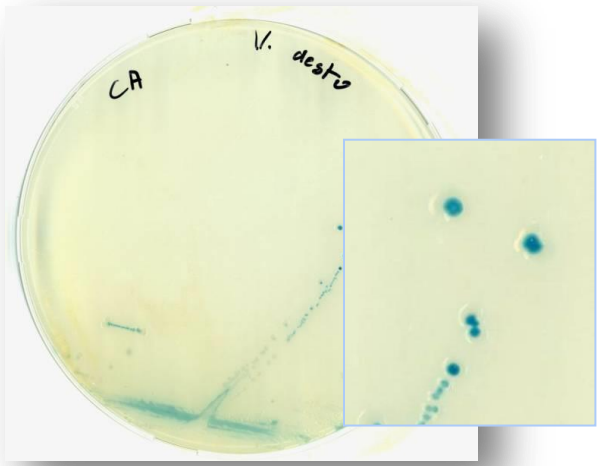
Oyster Industry Workshop, Ireland, 17th of April 2023 – Lydie Canier

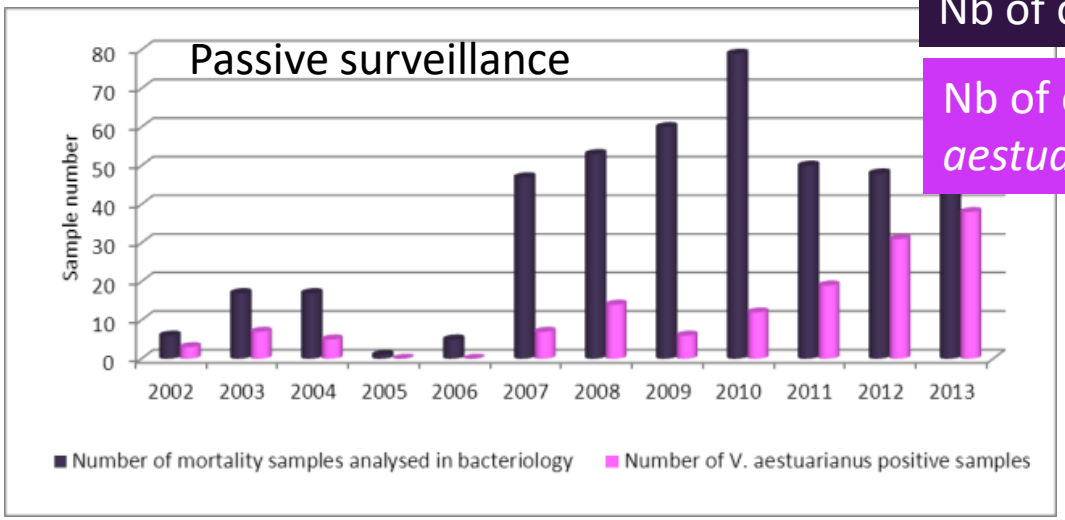
Plan

- General information
- Review of data from French National Surveillance
- EU distribution and genetic diversity
- Cycle of *V. aestuarianus*
- Factors influencing *V. aesturianus* dynamics
  - T°C / Salinity
  - Oyster age
  - Genetics
- Mitigation measures?

Vibrio aestuarianus

- Gram negative bacteria
- **Initially described in 1983 in the USA** from seawater, oysters, clams and crabs *(Tison & Seidler, 1983)* → ***type strain***
- **First detection in France in 2001** in the context of oyster mortality events (French research project MOREST – Garnier at . Al 2008)
- **Increased detection during oyster mortality investigations in 2011- 2012** (Repamo network = French surveillance network for mollusc diseases)



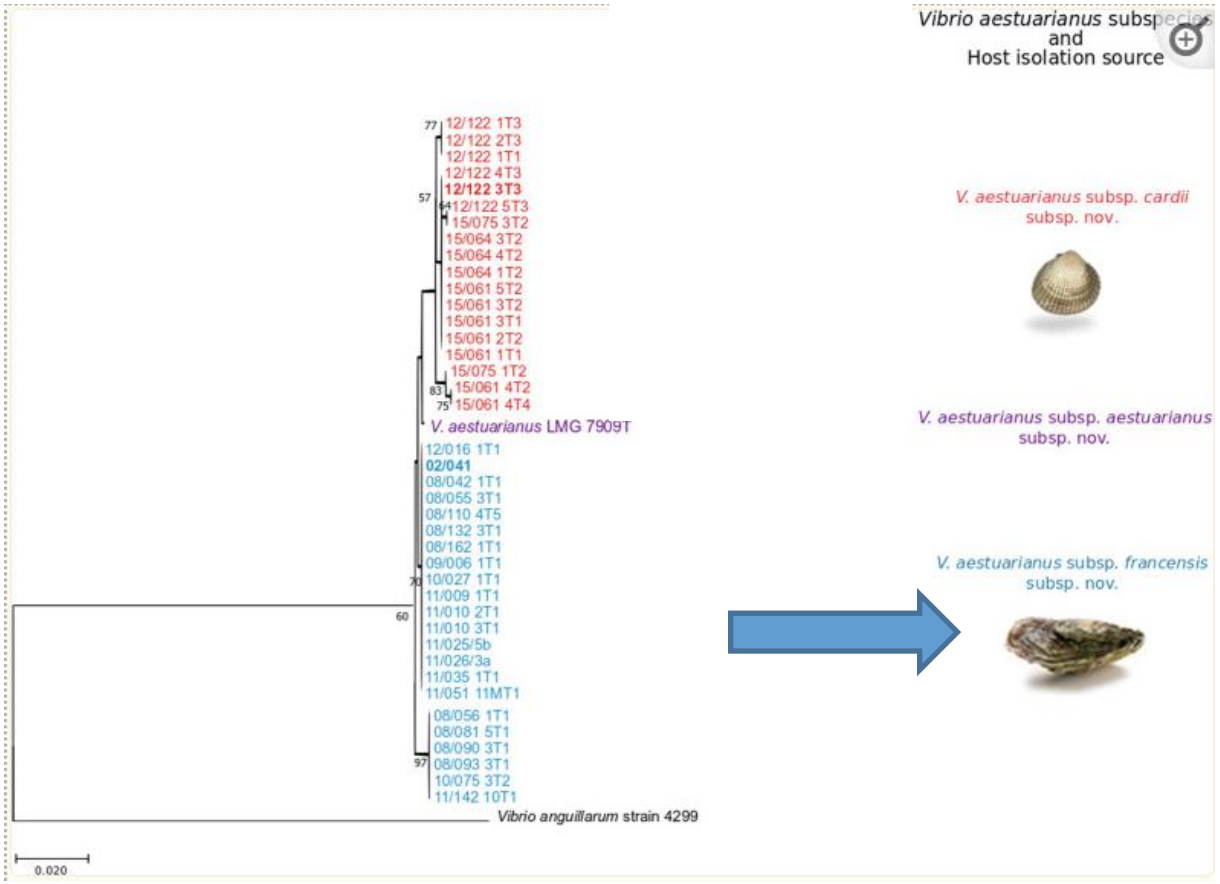


Nb of oyster samples analysed

Nb of oyster samples with *V. aestuarianus* detection

Vibrio aestuarianus

- **3 V. aestuarianus subspecies described**



Va Subsp. cardii
Pathogenic to cockles ,
not to pacific oysters

Va Subsp. aestuarianus
Not pathogenic
(First strain described)

Va Subsp. francensis
Pathogenic to Pacific oysters
Not to cockles

PCR used for diagnosis detects the 3 subsp.

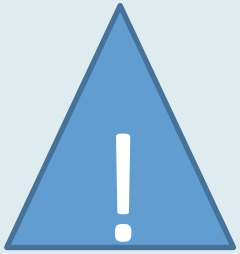
Garcia, C., Mesnil, A., Tourbiez, D., Moussa, M., Dubreuil, C., de Sa, A. G., ... & Travers, M. A. (2021). **Vibrio aestuarianus subsp. cardii subsp. nov., pathogenic to the edible cockles Cerastoderma edule in France, and establishment of Vibrio aestuarianus subsp. aestuarianus subsp. nov. and Vibrio aestuarianus subsp. francensis subsp. nov.** *International Journal of Systematic and Evolutionary Microbiology*, *71*(2).

Review of data from national surveillance in France

Passive surveillance (Repamo network) : investigation of oyster mortality events based on farmer notifications

1- Historical data

2 -Current situation in France



Sampling and diagnostic strategies changed over years

Mortality under reported

Affected Age classes

- Detection reported in all age classes
- More often detected in adults and Juveniles

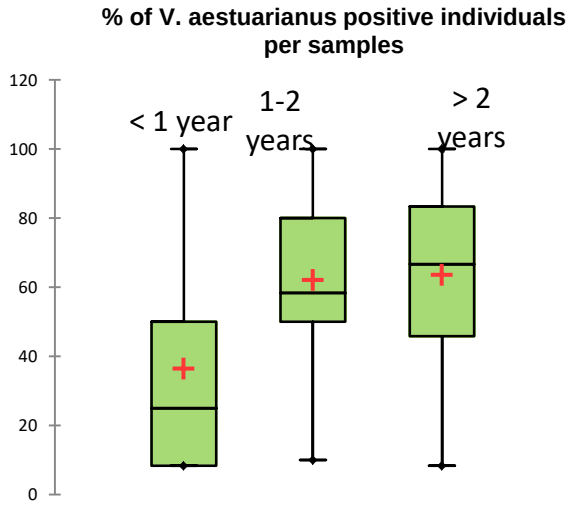
Detection of *V. aestuarianus* in oyster samples during mortality investigations – Repamo network

Numbre (%) of oyster samples reported positives for V. aestuarianus

	Spats	Juvéniles	Adults
2011	8/37 (22%)	1/2 (50%)	5/6 (83%)
2012	11/26 (42%)	3/5 (60%)	16/16 (100%)
2013	11/19 (58%)	5/7 (71%)	17/17 (100%)
2014	2/13 (15%)	7/8 (87%)	9/9 (100%)
Total 2011- 2014	**32/95 (34%)**	**16/22 (73%)**	**47/48 (98%)**

(2011-2014 = years with the highest numbers of oyster mortality investigations)

Higher number of oysters samples positives in adults and juvenile

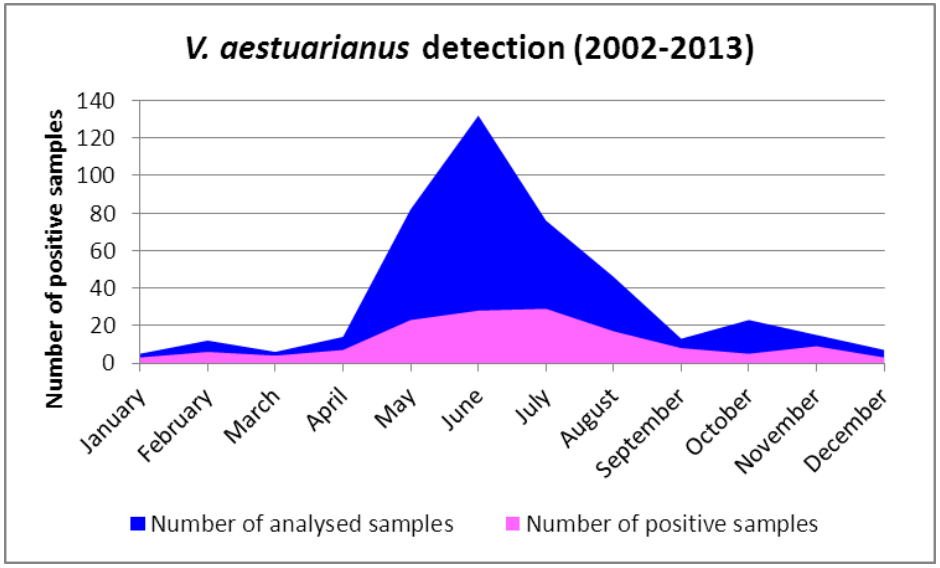


Distribution of detection frequency per samples for V. aesturianus in all samples found positive for V. aestuarianus between 2012 to 2019 (N=97 samples), categorised by age class

10 to 15 oysters analysed per sample
Among *V. aestuarianus* positives samples, more positive individuals in adults and juveniles than spats
Spats generally higly co-infected with OsHV-1

Detection period

- Detection possible all the year
- More often during the summer

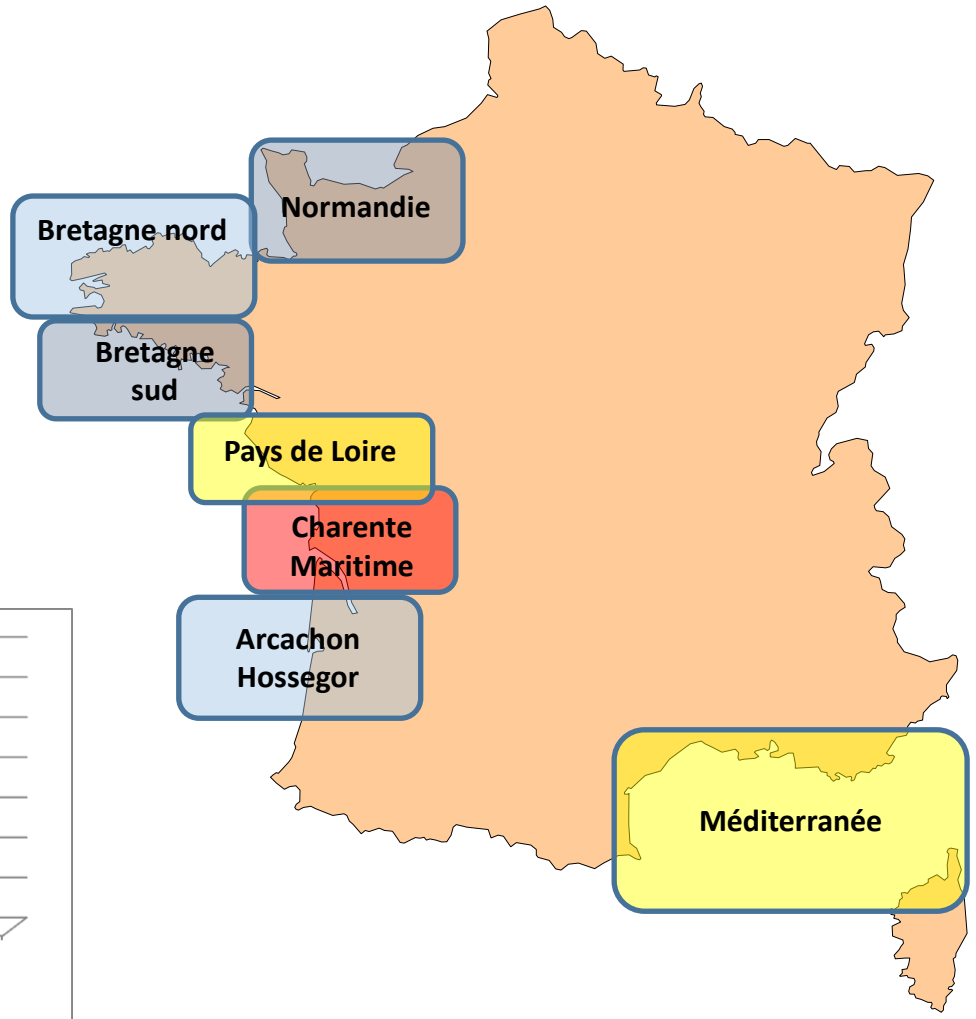


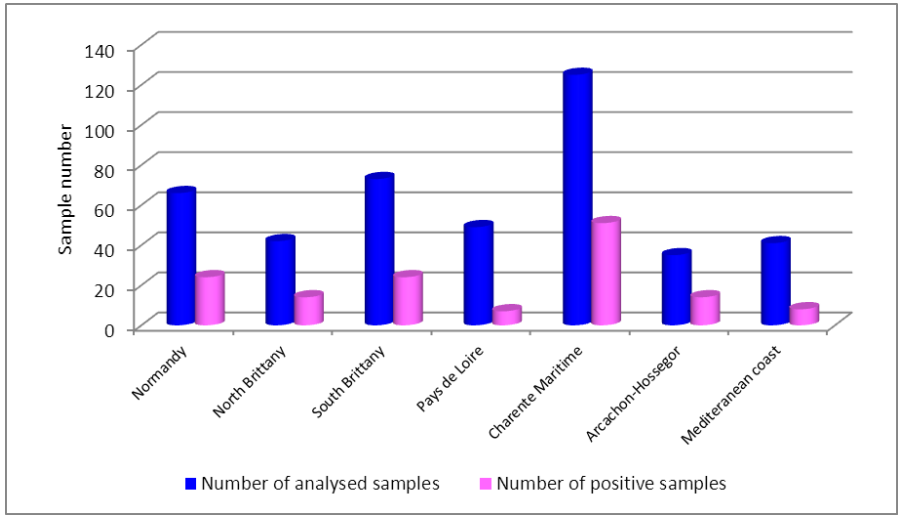
Mortality pattern

- Low mortality rate (~30%)
- Progressive, less sudden on the field in comparison with OsHV-1, seems to last on a long period

Distribution in France

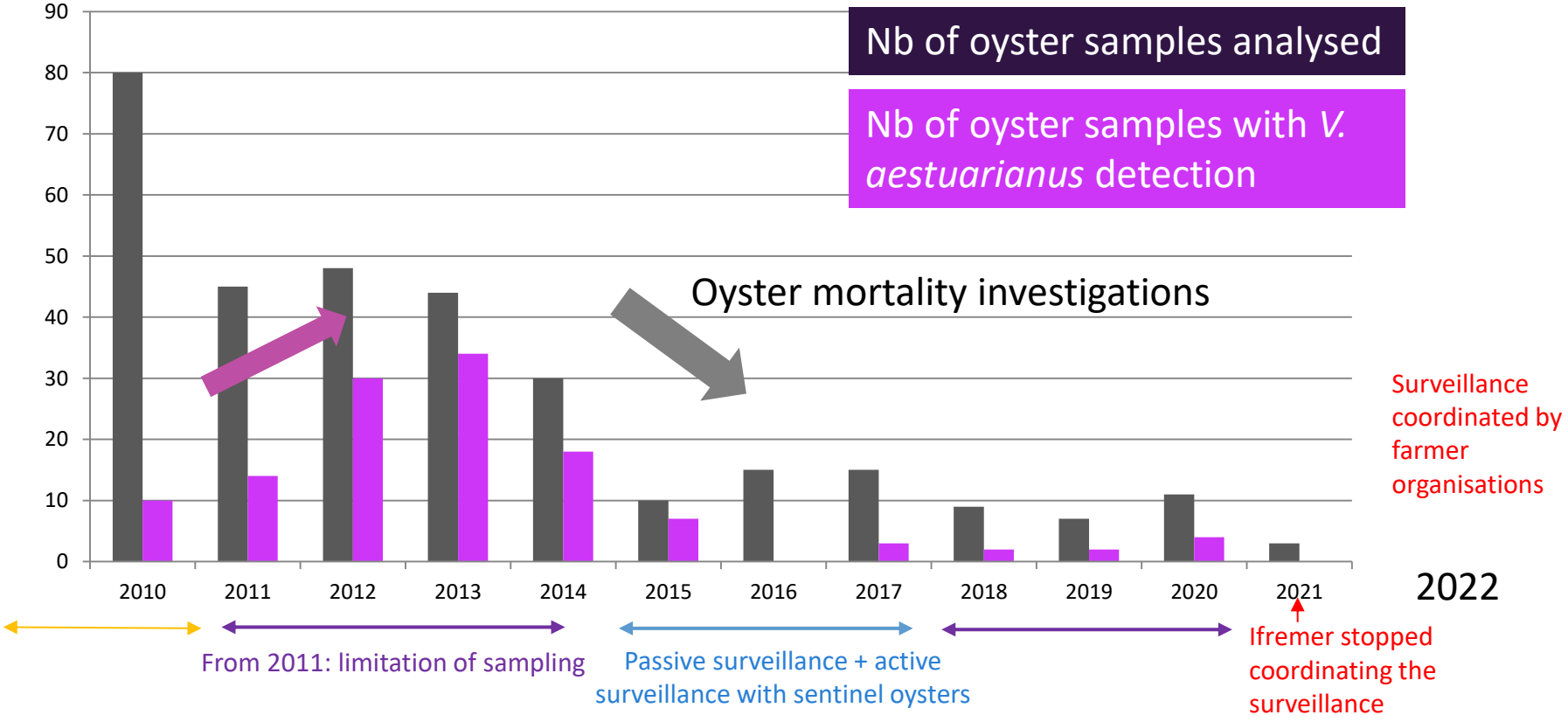
- Presence in all French oyster production areas
- Some sites seems more favourable (estuaries)





Vibrio aestuarianus

Oyster mortality investigations 2010- 2021



Only few reports of *V. aesturianus* over the last years
No detection in spats reported since 2015 in the context of national surveillance
But under-reporting of oyster mortality events (Change of surveillance system? Low mortality rate?)

V. aestuarianus Analyses not anymore supported by CA
Farmers looking for funding

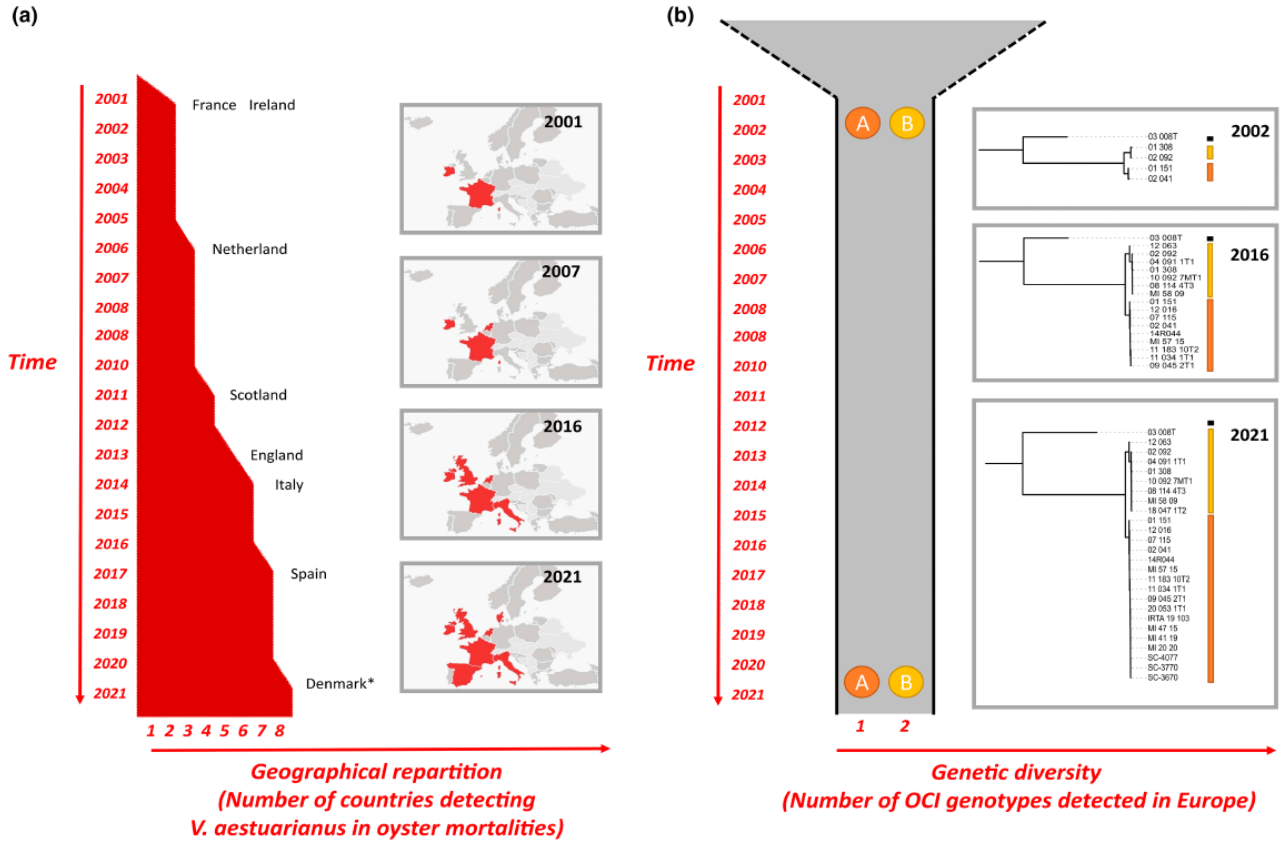
EU distribution and genetic diversity

- Survey performed in 2021 among NRLs for mollusc diseases to collect information on first detection of *V. aestuarianus* in EU countries

- Whole genome sequencing on strains from differents countries (France, Ireland, Spain, Italy, Scotland)

Vibrio aestuarianus

Distribution in Europe and genetic diversity



- **Presence in several EU countries**
- **Low diversity** among EU strains isolated from oysters
- **99% of isolated strains from oysters are pathogens**
- Pathogen adapted to oyster

Mesnil Aurelie, Jacquot Maude, Garcia Celine, Tourbiez Delphine, Canier Lydie, Bidois Audrey, Dégremont Lionel, Cheslett Deborah, Geary Michelle, Vetri Alessia, Roque Ana, Furones Dolors, Garden Alison, Orozova Petya, Arzul Isabelle, Sicard Mathieu, Charriere Guillaume, Destoumieux Garzon Delphine, Travers Agnes **Emergence and clonal expansion of Vibrio aestuarianus lineages pathogenic for oysters in Europe**. *Molecular Ecology* **IN PRESS**

Cycle of *V. aestuarianus*

OPOPOP project

Phd work Leila Parizadeh (2014- 2018)

V. aesturianus cycle

Pathogen free oysters (1500 oysters , >1 year old)
exposed in a pond containing V. aestuarianus-infected oysters
(ponds supposed to favor transmission)
Charente Maritime, France, from Oct 2014 to Feb.2015

Monitoring of mortality and environmental parameters
(temperature and salinity) each week during 5 months.

+ sampling in the oyster pond at 5 times (every 3 weeks) of:
- Oyster hemolymph and tissues (healthy)
- Sediment
- Water (filtration5 µm, 1 µm and 0.22)

PCR *V. aestuarianus*
Bacterial isolation

Observed mortalities **(pond)**

=> To check the presence of Va in environment and in healthy oysters before mortalities

Each week, batches of 20 healthy oysters were brought back to laboratory in one aerated-tanks with UV-treated seawater at **26°C**. Mortality observed in this tank was followed daily, and moribund animals were removed and analyzed.

=> To check presence of Va in healthy animals after thermic choc (induced mortalities)

Induced mortalities **in lab (26°C)**

1. *V. aestuarianus* natural mortality in claire ponds

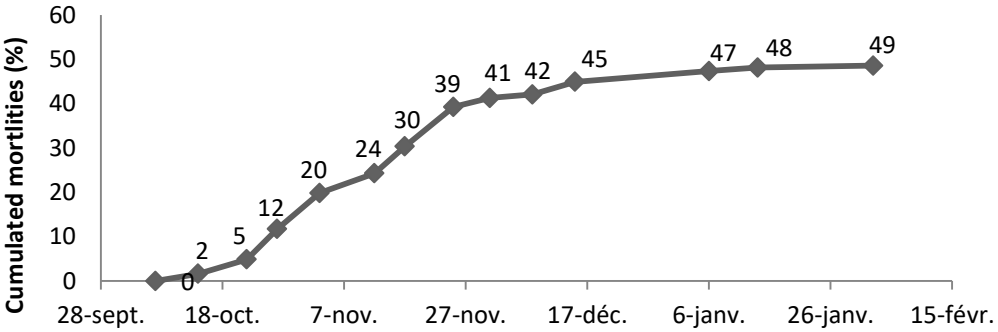
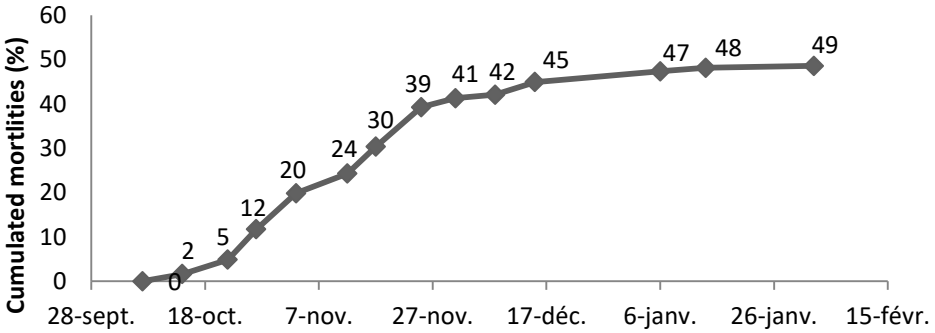


Figure 1: Cumulative mortalities recorded in a claire ponds.

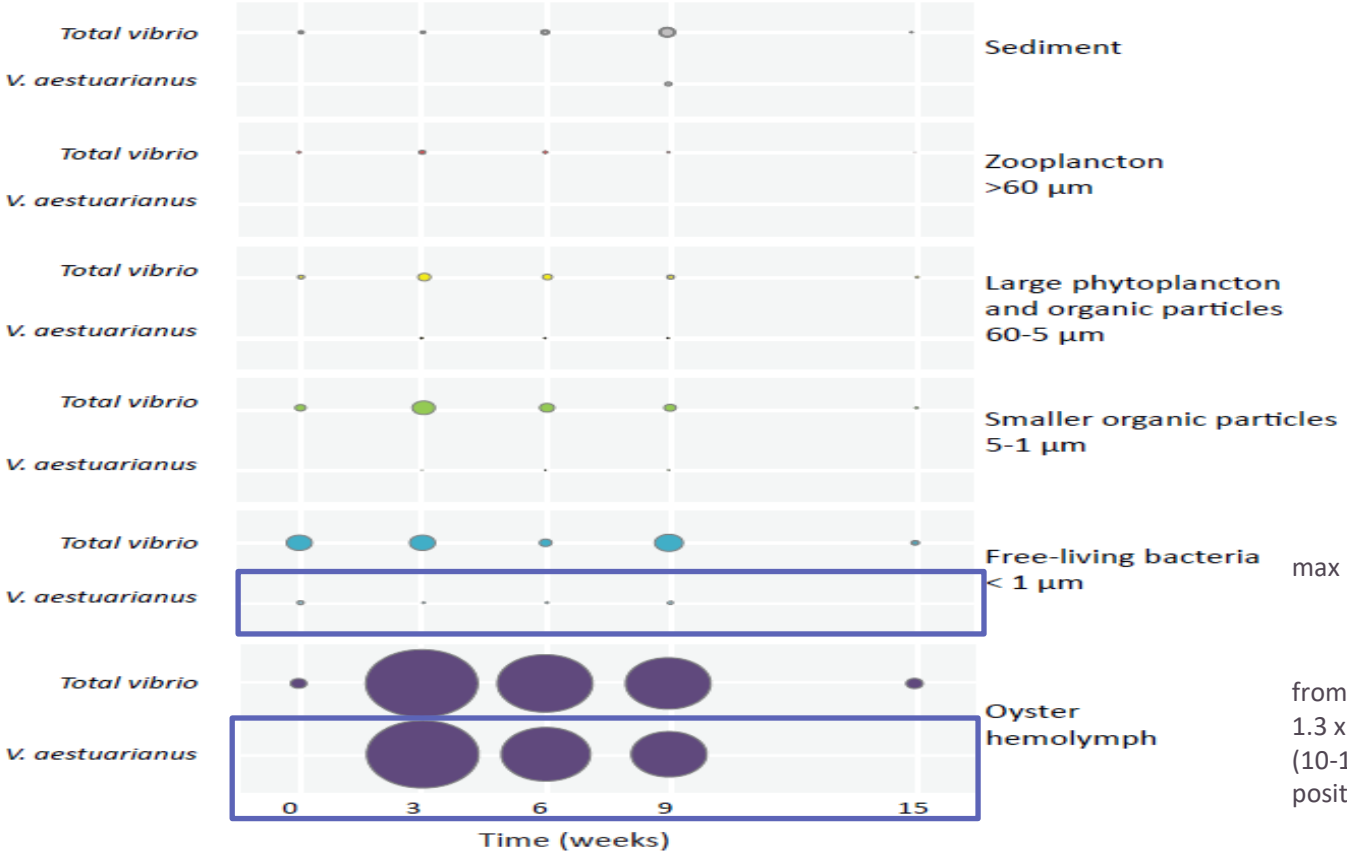
- **Mortality reached 45% (17/12/2014) after 3 months and stopped during winter.**
- In **moribund** animals: high amounts of *V. aestuarianus* DNA (>10^6 B/ml in hemolymph), absence of detection of herpes virus DNA, and isolation of 11 *V. aestuarianus* isolates
- Temperature around 21°C in October, decreased to 10°C in the beginning of December, when instant mortality decreased (from 13% per week between 17-25/11 to 1% per week between 01-08/12).
- Mean salinity = 31‰ (min 25.5‰, max 36 ‰)

2. *V. aestuarianus* positively associated with oysters

Small amount and few colonies from environmental samples



Few quantities of DNA before and during mortalities (max 1.95 x 10^3 B/L detected into 0.22 µm fraction
Presence in water only during mortalities

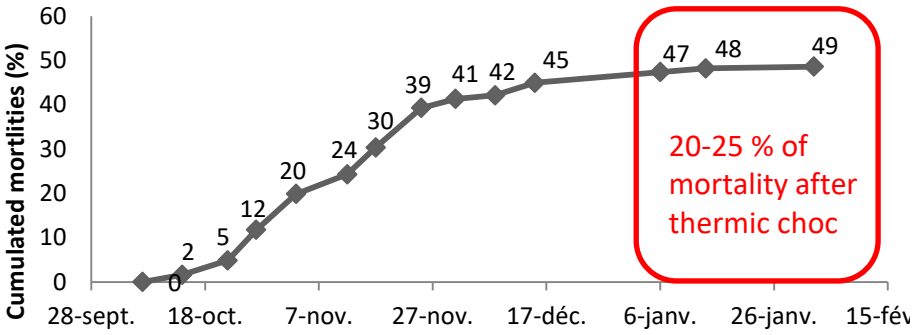


max 1.95 x 10^3 B/L

from 6.4 x 10^5 B/ml in October to 1.3 x 10^5 B/ml in December (10-15 % of healthy oysters positives)

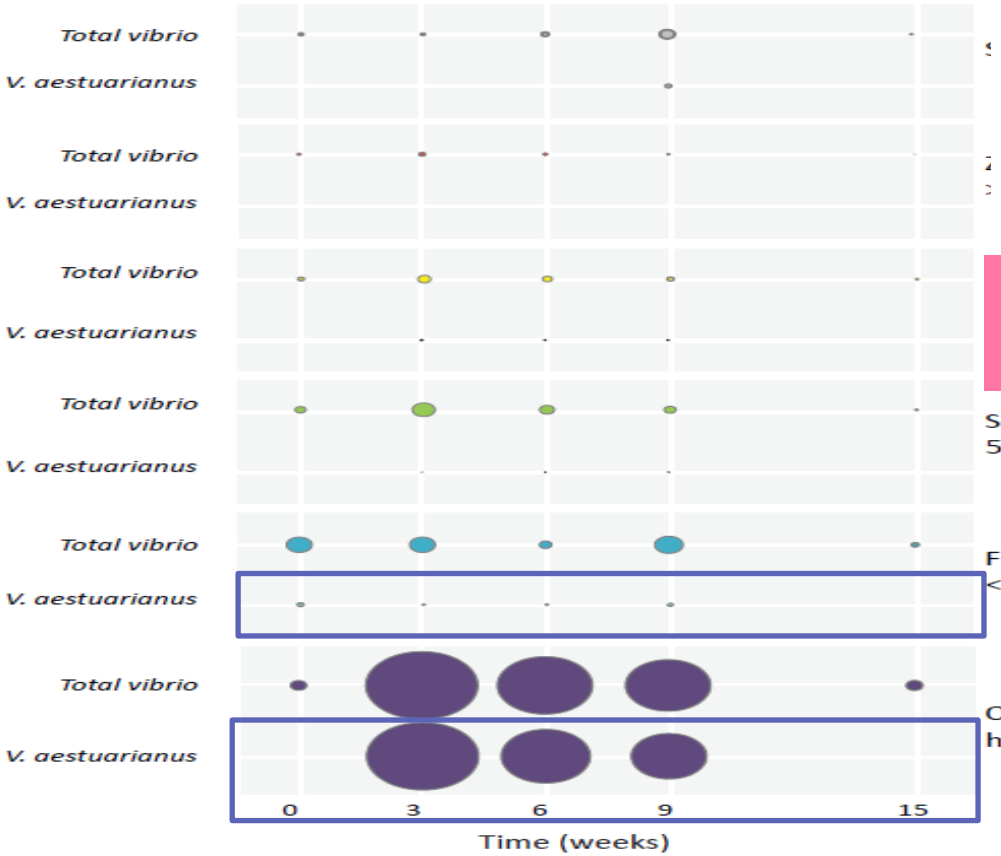
3. Persistence in oyster during winter?

Presence of bacteria into animals during winter, revealed by thermic choc



- In February, field mortality were stopped, DNA of *V. aestuarianus* was not detected, 20% of animals can be affected by mortality after thermic choc.

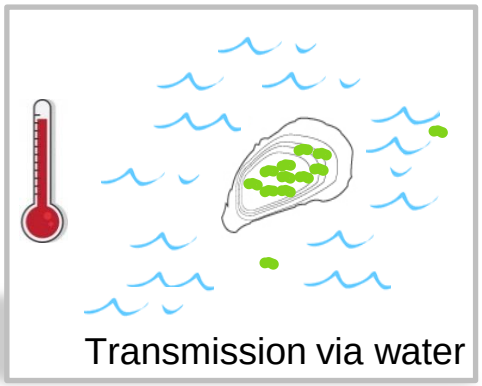
- Strains of *V. aestuarianus* were isolated from those moribund animals

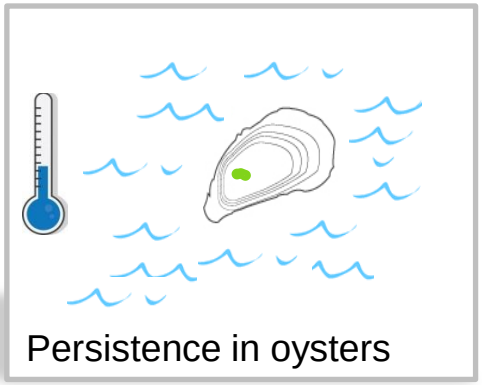


→ some bacteria can maintain into oyster during winter, in a non-detectable quantity with our diagnostic tool (= less than 10^2).

Cycle de *V. aestuarianus* dans les huîtres creuses

V. aestuarianus – *Crassostrea gigas* in oyster pond





Mortalities observed on 11 weeks out of the 15 weeks, up to temperature < 10°C (45 % cumulative mortality)

Very few detection in environnement (water mainly, free-living fraction)
max 10^3 bacteria/L
(only 3 Va strain isolated / 600)

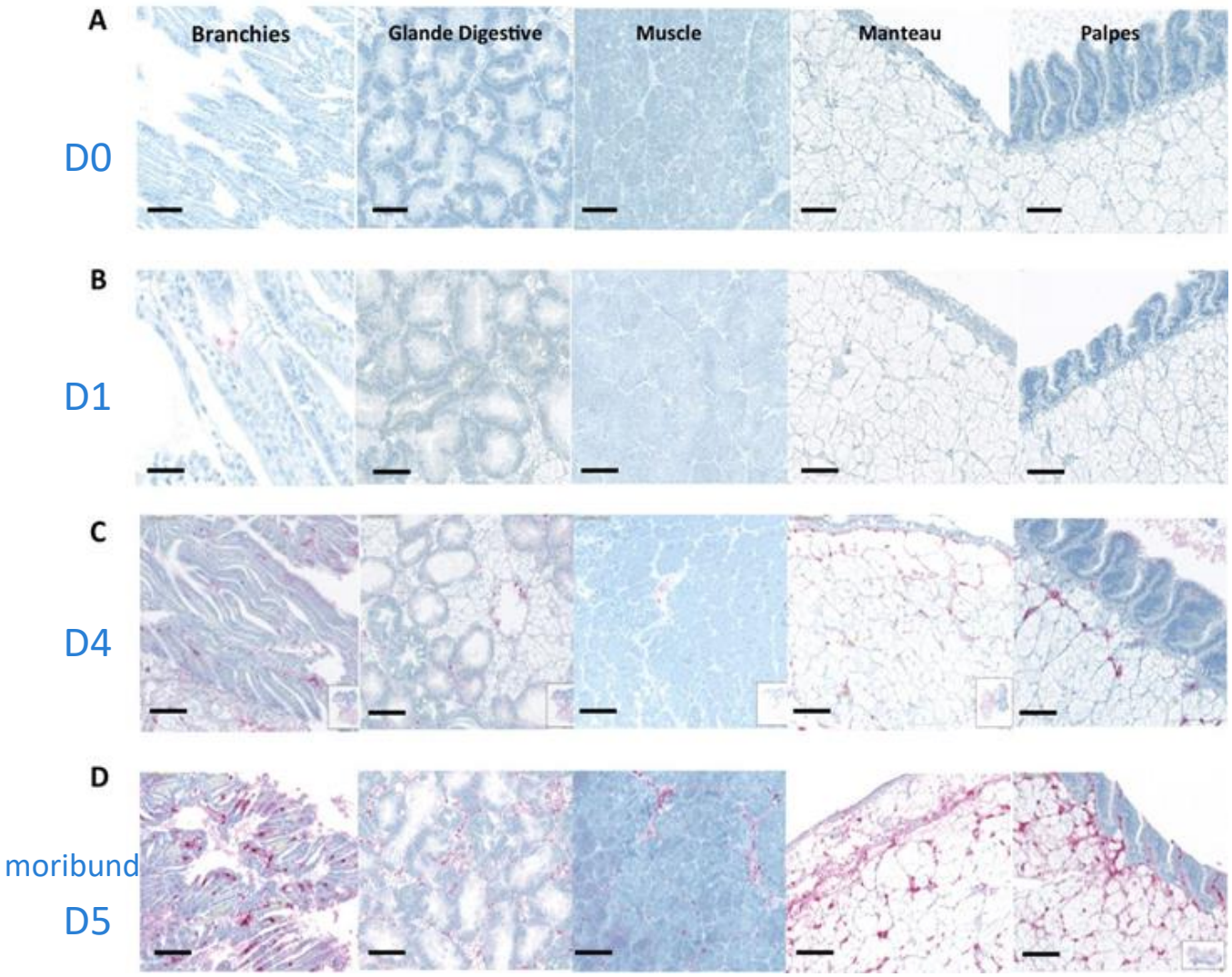
Detection in all moribund oysters and in some healthy oysters
(212 Va isolated strains /~ 600)

At 10°C, mortalities stopped and the bacteria was not detected in oysters, but the presence of the bacteria was revealed in 25% of the oysters after thermic choc.
=> some bacteria can maintain into oyster during winter, in a non-detectable quantity with our diagnostic tool

Parizadeh et al. Env Mic 2018

Infection in Pacific oysters

Experimental infection => follow-up with histological analyses + immunohistochemistry to localise Va (red)



24 h contact

▼

initial colonization
Gills and hemolymph

▼

Conjonctive tissues
(mantle and digestive diverticula)

▼

Septicemia at D5

Bacterial colonization observed in individuals infected with virulent *V. aestuarianus* strain. (experimental infection)

Parizadeh et al. Env Mic 2018

Factors influencing *V. aestuarianus* dynamics

- T°C / Salinity
- Age of oysters
- Genetics

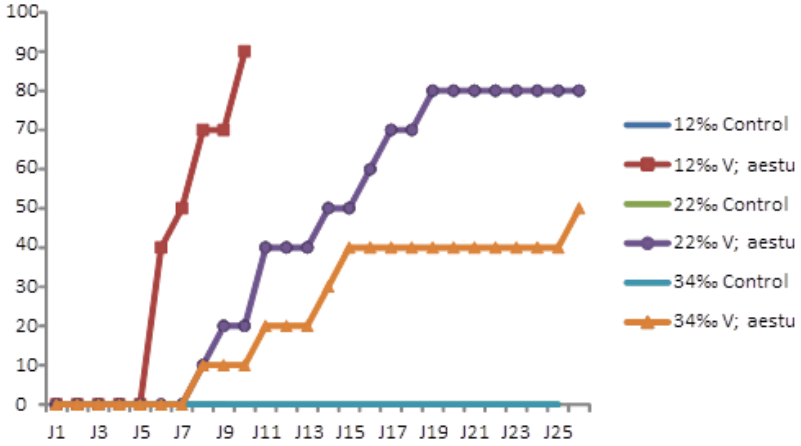
1/ Temperature and salinity

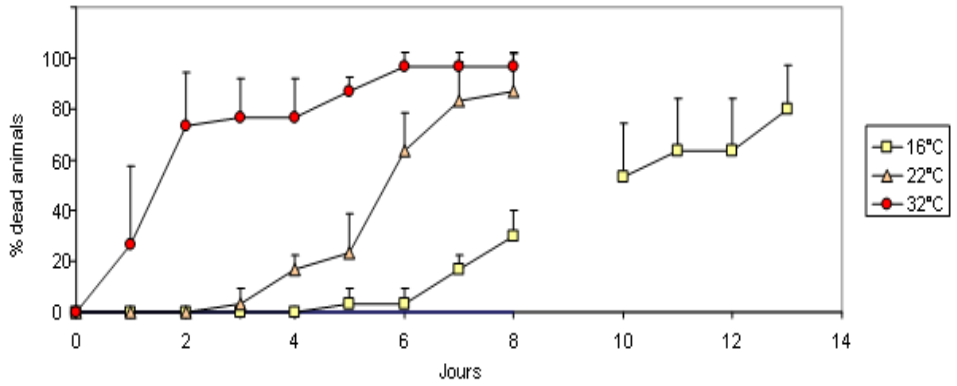
Abiotic factors (Temperature & Salinity) control the development of the disease

Balneation experiments (bacteria suspension added in seawater tanks containing oysters),

Tests with 3 differents **salinities** (12, 22, 34‰)

Tests with 3 differents **temperatures**(16, 22, 32°C)





The disease develops faster with low salinity and high temperature

(experiments are currently being repeated with cohabitation protocols)

Correlation with salinity also
observed on the field (Thau lagoon)

Travers et al in prep

2/ Age of oysters

Azéma *et al. Genet Sel Evol (2017) 49:23*
DOI 10.1186/s12711-017-0297-2

GSE Genetics Selection Evolution

RESEARCH ARTICLE Open Access

Genetic parameters of resistance to *Vibrio aestuarianus*, and OsHV-1 infections in the Pacific oyster, *Crassostrea gigas*, at three different life stages

Patrick Azéma[1], Jean-Baptiste Lamy[1], Pierre Boudry[2], Tristan Renault[3], Marie-Agnès Travers[1†] and Lionel Dégremont[1*†]

Objectives :

(1) to investigate the susceptibility to *V. aestuarianus* and OsHV-1 infections at successive life stages;

(2) to estimate the heritability of resistance to these infections at each life stage

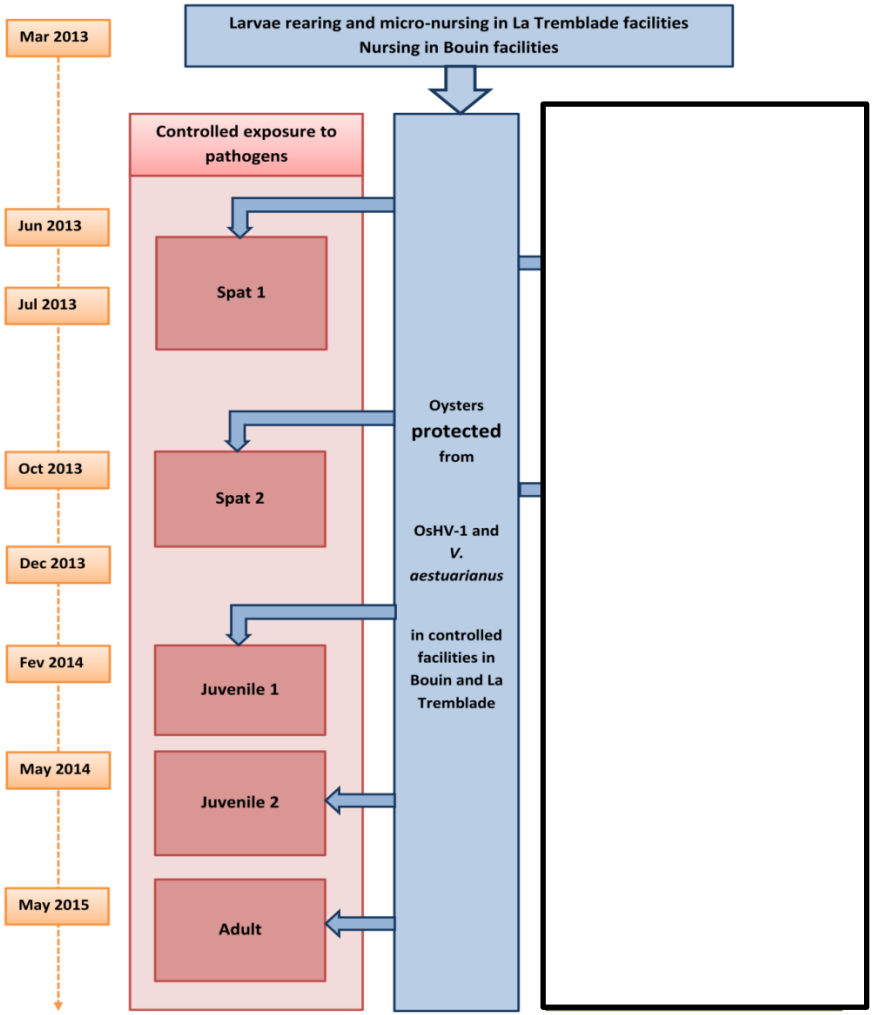
(3) to determine the genetic correlations between resistance to *V. aestuarianus* and OsHV-1 infections during a given life stage and between life stages.

2/ Age of oysters

40 two-parents families

Experimental infections (cohabitation) with OsHV-1 and *V. aestuarianus* at different ages (3, 6, 11, 15, 26 months)

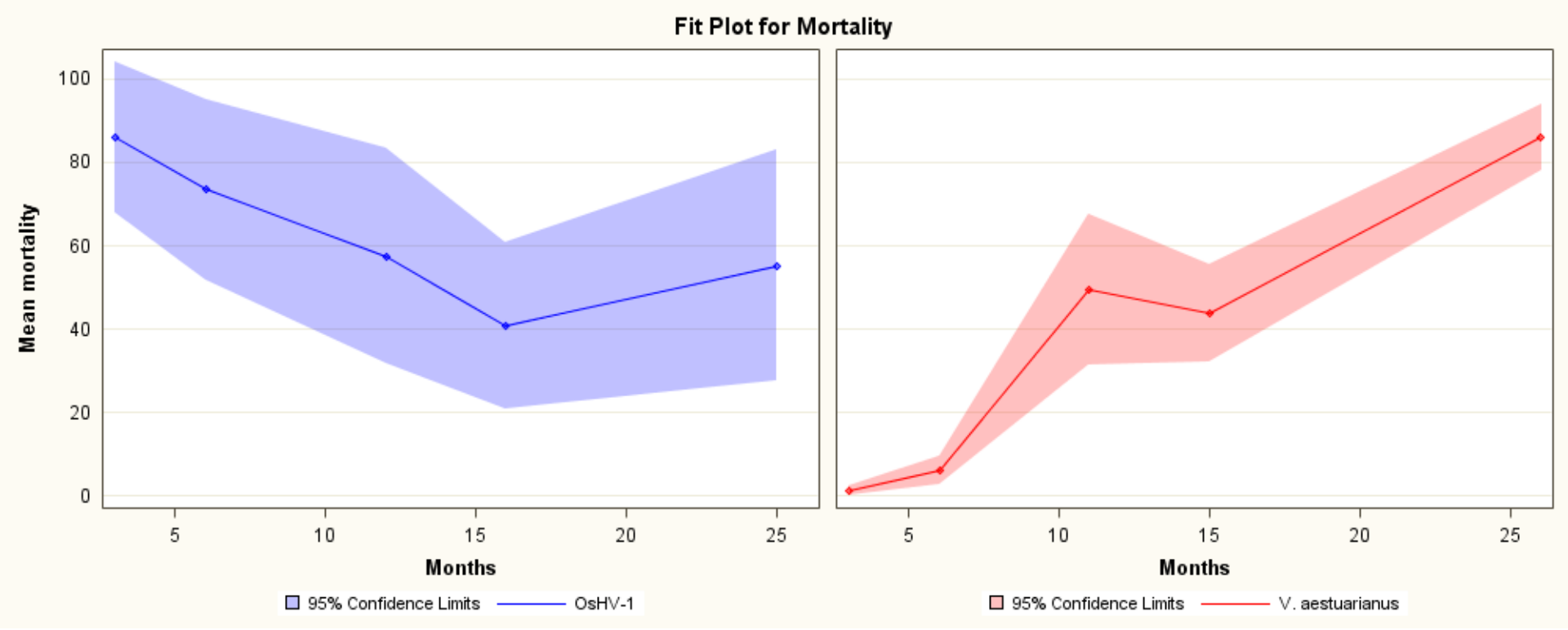
Mortality monitoring and screening for pathogens



2/ Age of oysters

OsHV-1

V. aestuarianus 02/041



- High mortality at the spat stage (70-85%) and moderate at the juvenile (40-60%) and adult stages: resistance to OsHV-1 infection increases with age and weight

- Low mortality at the spat stage (<10%), moderate at the juvenile stage (40-50%), important at the adult stage: the susceptibility to infections by Vibrio aestuarianus increases with age and weight

3/ Genetics

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Conclusions

- lack of genetic correlations between resistance to OsHV-1 infection and resistance to *V. aestuarianus* infection (but small sample size) => selection to improve resistance to OsHV-1 infection should neither increase nor decrease the resistance to *V. aestuarianus* infection
- Selection for dual resistance to OsHV-1 and *V. aestuarianus* infections in *C. gigas* may be possible
- Lower heritability for Va resistance than for OsHV-1

3/ Genetics: diploids VS triploids

- Triploid oysters are said in the field to be more sensitive than diploid oysters
- What is the effect of ploidy on mortalities caused by *Vibrio aestuarianus*?

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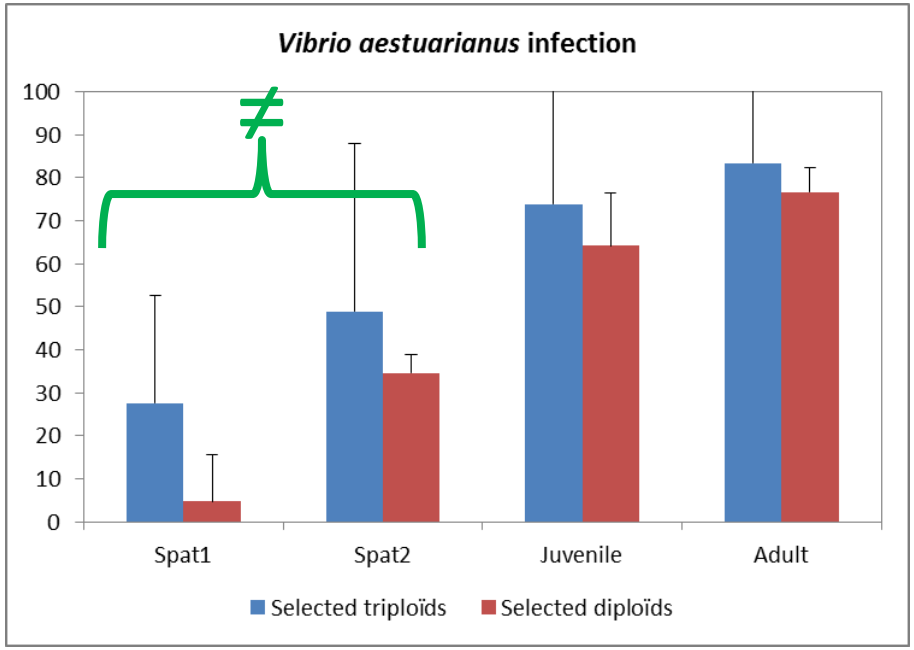

Triploids more susceptible at spat age
Final cumulative mortality was not significantly different between triploids and diploids in Juveniles and Adults

Experimental infections with Va

Oysters with same genetic background => **diploids** and **triploids** (chemically induced)

Lines selected for OsHV-1 resistance

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Contents lists available at ScienceDirect
Journal of Invertebrate Pathology
journal homepage: www.elsevier.com/locate/jip

Single or dual experimental infections with *Vibrio aestuarianus* and OsHV-1 in diploid and triploid *Crassostrea gigas* at the spat, juvenile and adult stages

Patrick Azéma, Marie-Agnès Travers, Abdellah Benabdelmouna, Lionel Dégremont *
Ifremer, RBE-SG2M-LGPMM, station de la Tremblade, F-17390, France

Mitigation measures?

- Selection of Va & OsHV-1 resistant lines should be possible, but lower heritability for Va resistance than for OsHV-1
- Cultivation, handling and harvesting pratices to reduce mortality in older oysters in summer

Avoid handling (turning & grading) of oysters in periods of extreme heat;

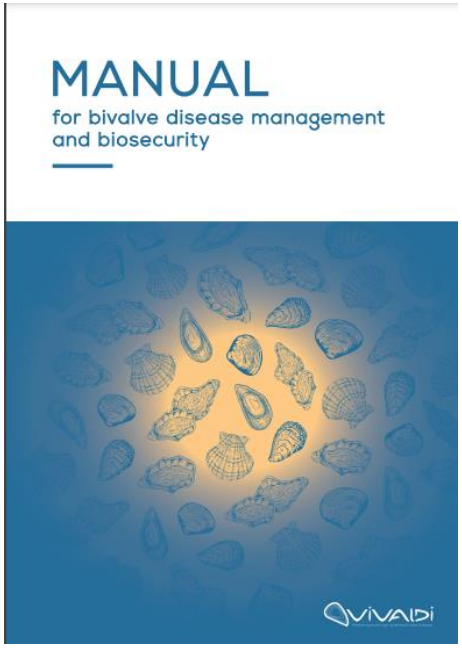
Consider hand grading where and when feasible;

Use water graders instead of traditional graders or use water bins for the oysters to fall into at high impact points in traditional graders;

Return stock to the water as soon as possible after grading;

Following grading & hardening immerse oysters in cold water tanks prior to shipping;

Differences between harvesting, transport and immersion temperatures should be managed to minimise stress on the oysters especially for movements of oysters during periods of warm weather when the disease is active.



- Low salinity and high temperature favour Va
- Possibility to identify zone that are favourable to Va based on abiotic parameters

Thank you !