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Killing or Cheating: two strategies to colonize an oyster

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High prevalence of the *Vibrio Harveyi* clade during a Pacific Oyster Mortality Syndrome episode in the Thau Lagoon

Since **2008**, the worldwide production of *Crassostrea gigas* oysters has been dramatically affected by the Pacific Oyster Mortality Syndrome (POMS)¹. This polymicrobial disease is caused by a Ostreid herpesvirus, **OsHV-1 μ Var**, and opportunistic bacteria, among which members of the **Vibrionaceae** family are the best characterized².

Oysters **susceptible** to POMS present an overall lower basal expression of immune related genes and an inefficient transcriptomic response against OsHV-1 μ Var infection. **Viral replication** in susceptible oysters leads to the host entering an **immune-compromised state**, causing a dysbiosis that evolves towards **bacteremia**³.

Here, we investigated the *Vibrio* community associated with the disease in the Thau lagoon (south of France), where we found a strong prevalence of the Harveyi clade in diseased oysters.

Mortality outbreak with oysters **positive** for OsHV-1 μ var-1



Thau Lagoon, France
2015

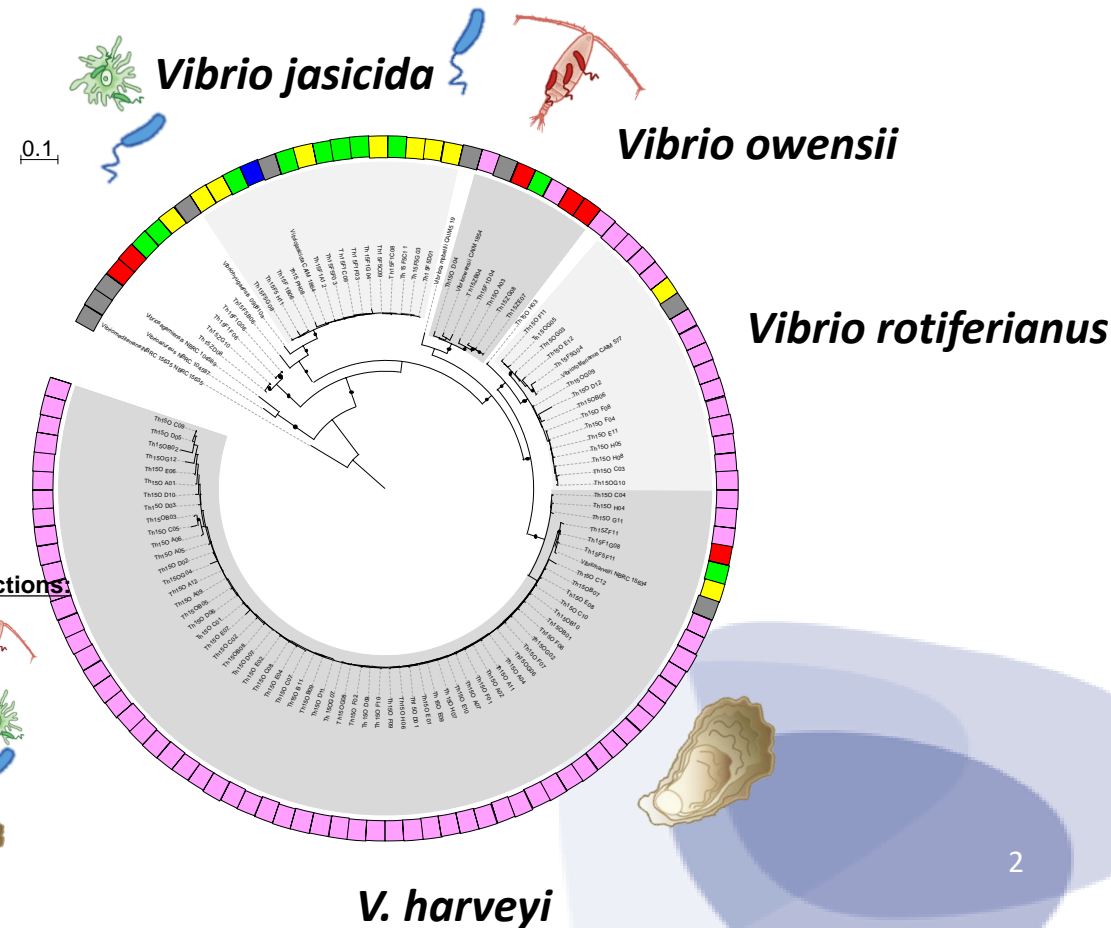
MORTALITIES

01/10/2015; 18,6°C

Oysters and seawater were sampled for **vibrio isolation**

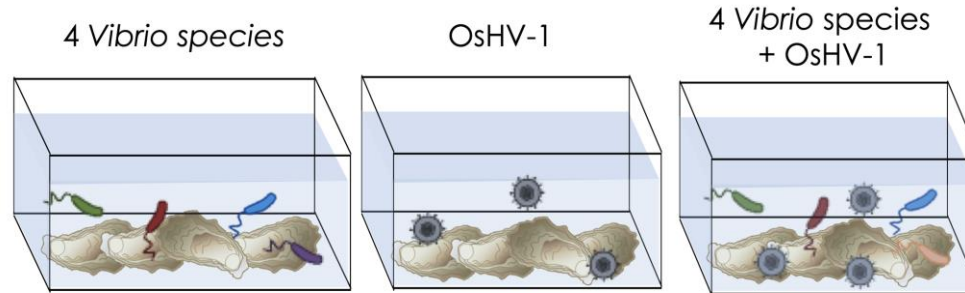
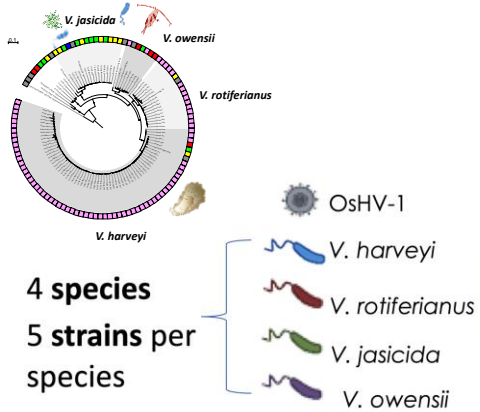
Seawater size fractions:

- > 60 μ m
- 60-5 μ m
- 5 - 1 μ m
- < 1 μ m
- Oysters



Can we reproduce the association of *V. harveyi* and *V. rotiferianus* found in the field?

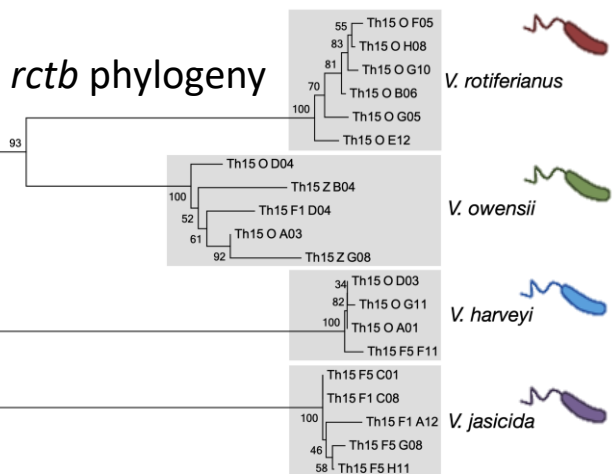
Polymicrobial infection in a mesocosm



Tanks with 2L of seawater and 10 oysters were prepared for **sampling** at different time points and tanks with 3L of seawater with 15 oysters to **track the daily mortalities** on each condition.

Seawater containing **OsHV-1** μ Var virions was produced through **donor oyster**. **Donors** were injected with the **OsHV-1** virus and released virions into the seawater through a **24hr** period. This **contaminated seawater** was used to fill the tanks for the different experimental conditions.

rctB gene polymorphism allows discrimination at the species level



- Monitoring of mortalities
- Viral replication
- Vibrio* colonization
- MiSeq metabarcoding for species discrimination

After placing the oysters in the tanks, bacterial suspensions in seawater adjusted to OD600 = 1 (10^9 CFU/mL) were added to each tank to reach a final concentration of 10^7 CFU/mL.

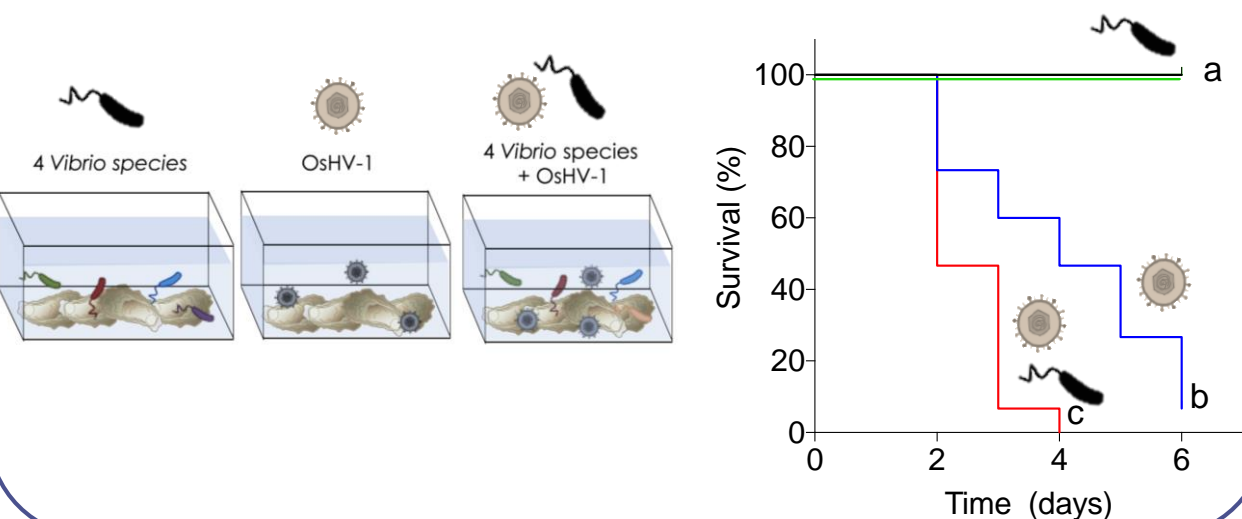
10 oysters at each time point and for each condition were sampled for analysis

OsHV-1 favors colonization by vibrios and synergizes with members of the Harveyi clade to kill oysters

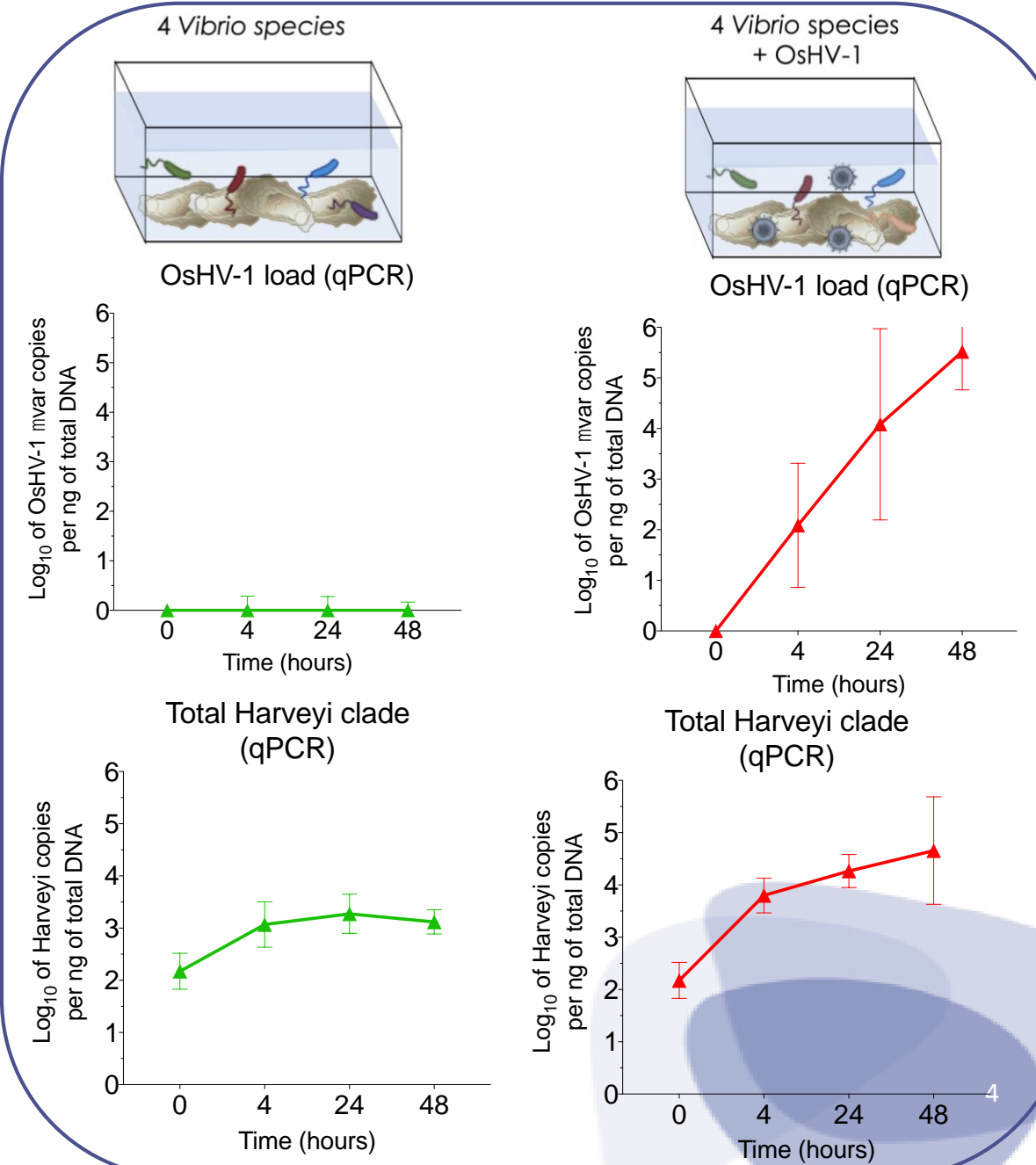
No mortalities were observed when oysters were immersed in seawater containing **only the Harveyi community**. Consistently, a transient increase in total Harveyi load was observed from 4h to 24h followed by a decreased at 48h, revealing a **control of *Vibrio* colonization**

Mortalities were **significantly enhanced (100% at day 4)** when the Harveyi community and OsHV-1 μ var were **combined into the mesocosm**. In this condition the total Harveyi load in tissues of live oysters was **1-2 log higher** than in other conditions at 2 day

Mortalities



Pathogen load

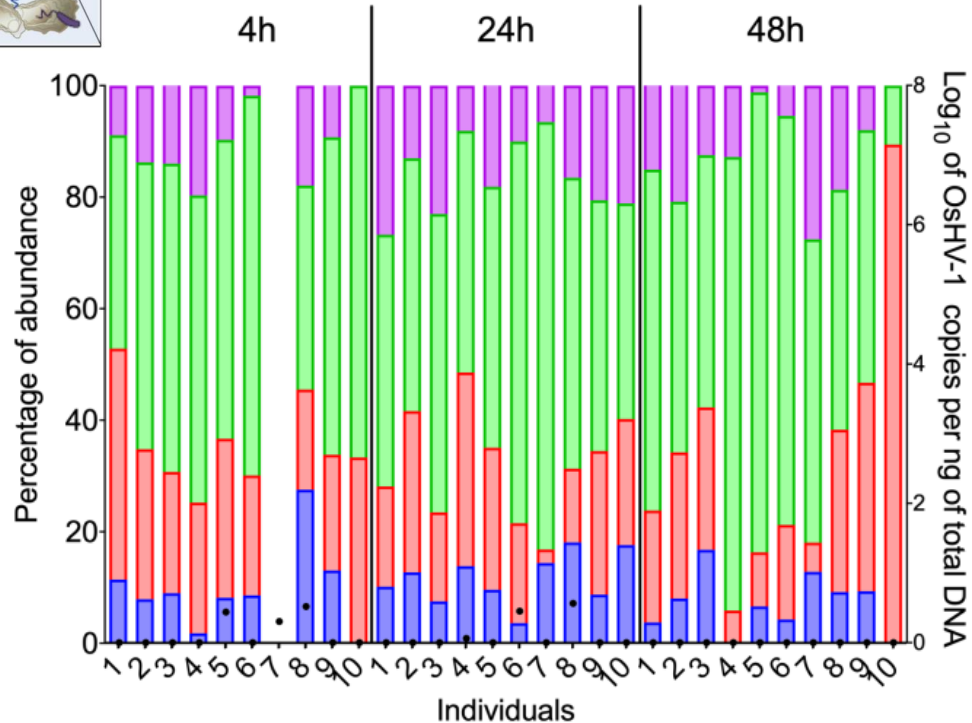
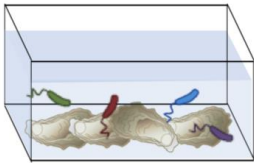


OsHV-1 μ Var favors colonization by *V. harveyi* and *V. rotiferianus* only

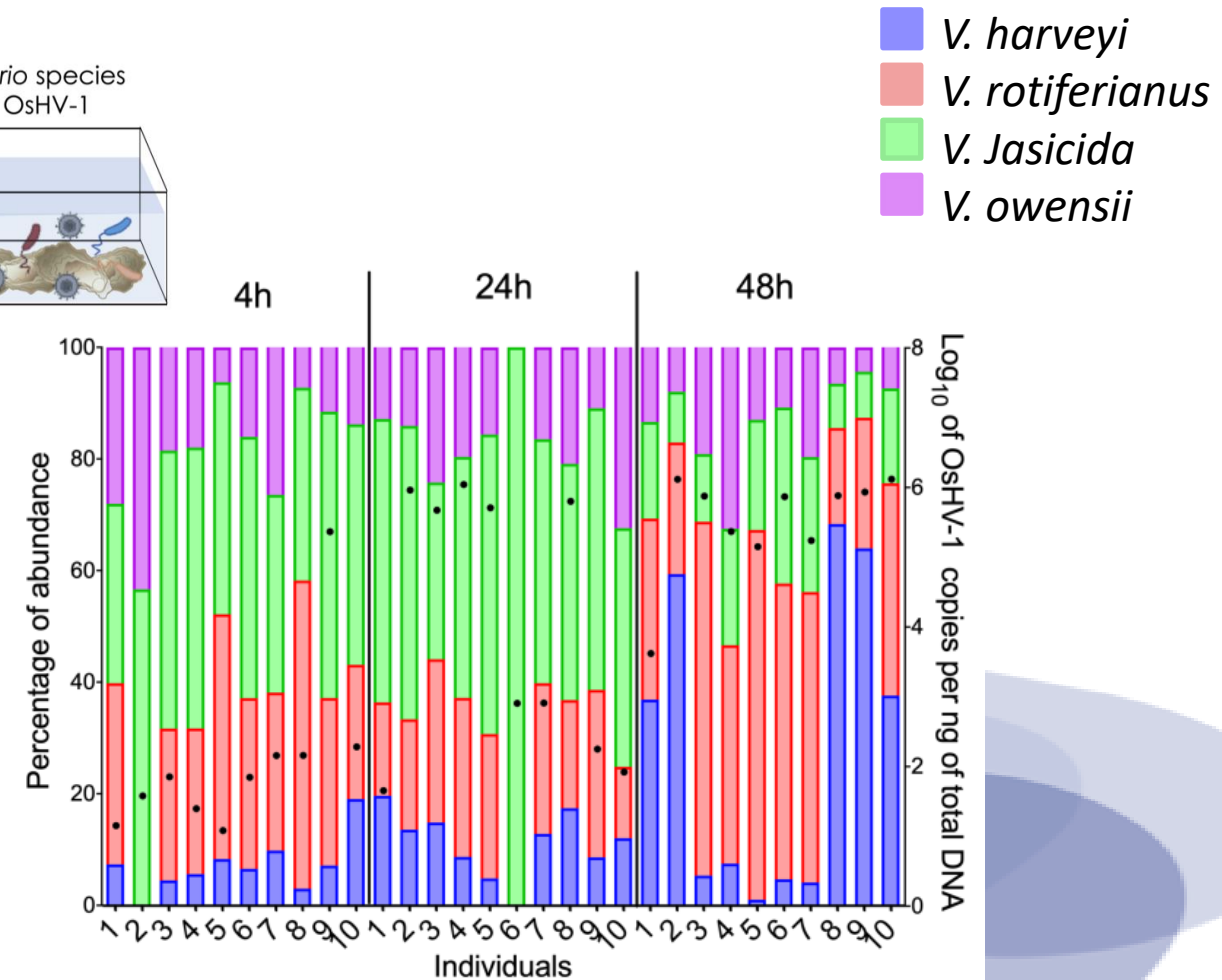
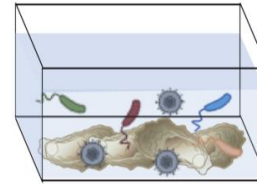
Individuals collected during the time course of the infection were therefore subjected to illumina Miseq sequencing of the *rctB* polymorphic region which discriminated the four species introduced into the mesocosm. **The relative abundance of the 4 species remained stable in the oysters only exposed to the Harveyi community during 48h, as revealed by *rctB* barcoding analysis**

On the contrary, **in the presence of OsHV-1 μ var**, not only the total Harveyi load increased over time but there was also a **significant enrichment of the species *V. harveyi* and *V. rotiferianus*** at 48 h, after viral replication occurred (24h)

4 *Vibrio* species



4 *Vibrio* species + OsHV-1

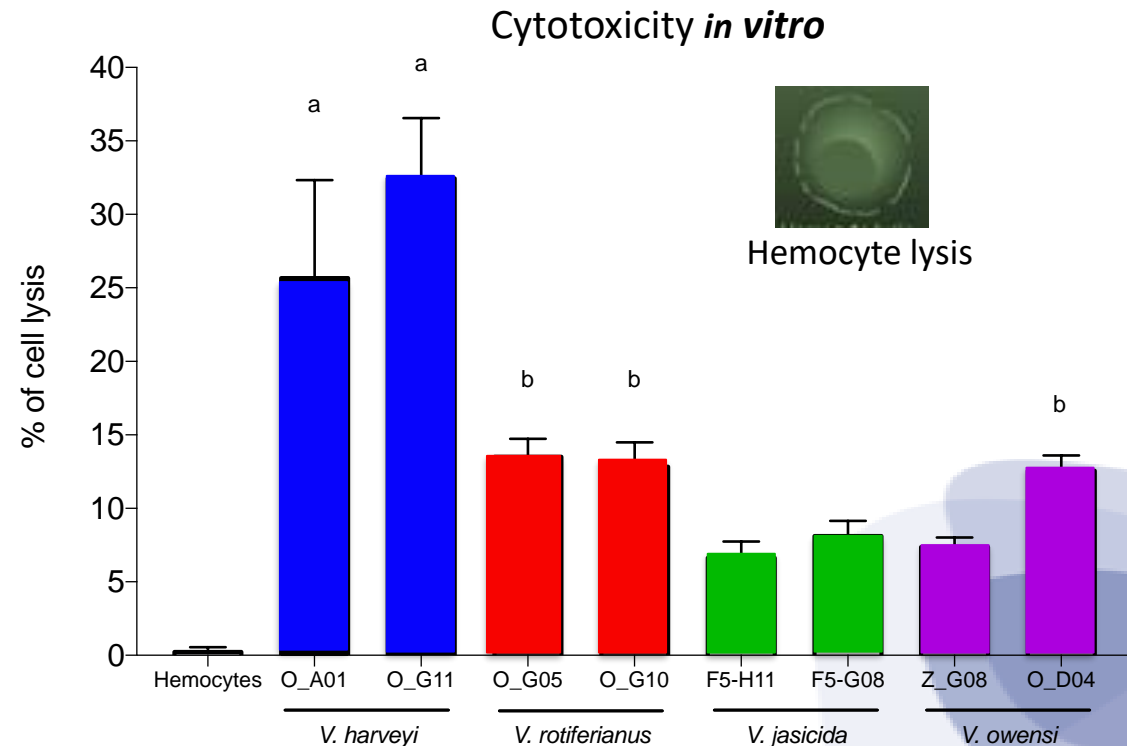
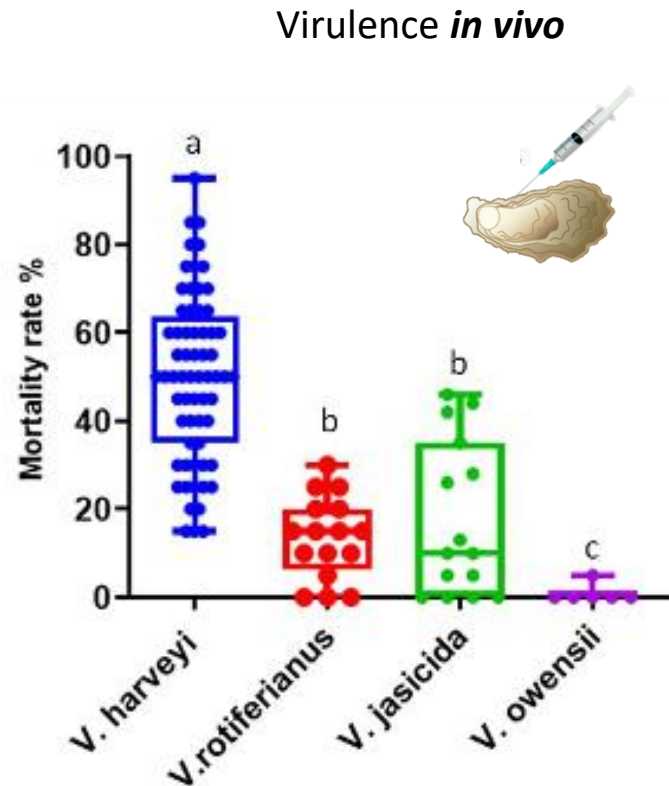


What makes *V. harveyi* and *V. rotiferianus* successful colonizers?

V. harveyi, the strategy of a killer

In experimental infection by injection, *V. harveyi* isolates were by far the most virulent with $\geq 50\%$ of *V. harveyi* isolates inducing $\geq 50\%$ mortality after 1 day. The cytotoxic potential of *V. harveyi* was confirmed experimentally *in vitro* by comparing two strains representative of the four Harveyei species from our study

We found that *V. harveyi* strains exerted a potent cytotoxicity toward hemocytes, the oyster immune cells, 25-35% of which were lysed upon incubation with *V. harveyi* strains. *V. harveyi* harbored a type 3 secretion system homologous to the *V. parahaemolyticus* T3SS1 that could contribute to its virulence and cytotoxicity.



What makes *V. harveyi* and *V. rotiferianus* successful colonizers?

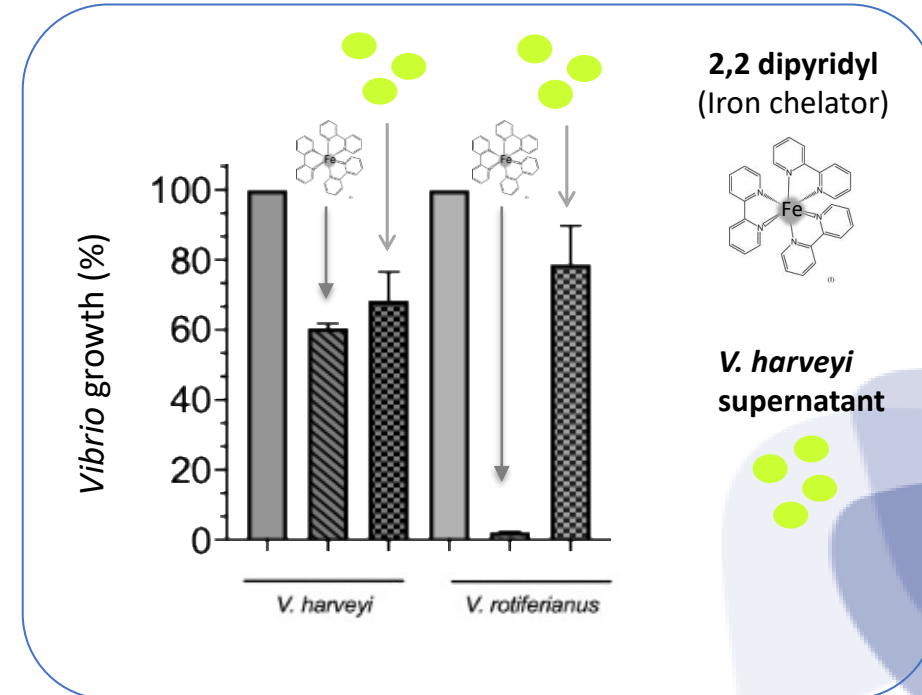
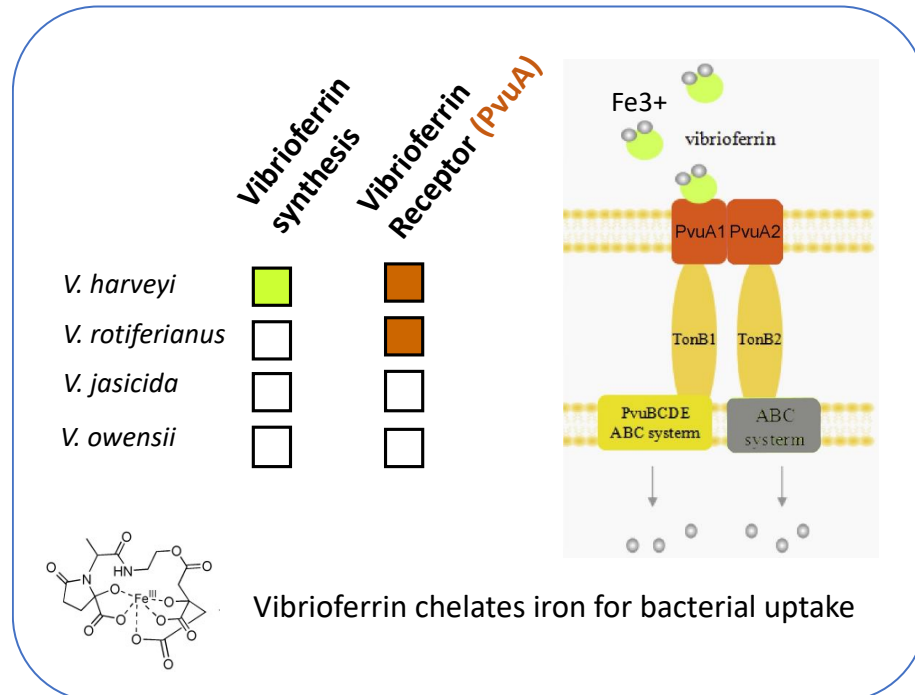
V. rotiferianus, the strategy of a cheater

Indeed, *V. harveyi* was found to harbor a conserved set of genes responsible for the synthesis of vibrioferrin, a tricarboxylic acid siderophore. In contrast, *V. rotiferianus* does not exhibit known *Vibrionaceae* siderophore biosynthetic proteins **but it carries the vibrioferrin receptor, present in both species, necessary for the siderophore uptake.**

***V. harveyi* growth was not impaired in iron depleted media** (EDDA used as iron chelator). On the contrary, **iron depletion significantly affected the growth of *V. rotiferianus*.** This growth defect was rescued by the addition of cell-free culture supernatant from *V. harveyi*. This demonstrates that ***V. rotiferianus* is able to acquire important resources (most likely vibrioferrin) produced by *V. harveyi*,** which are needed to grow in iron-poor environments

Comparative genomics identifies a **siderophore receptor** present in *V. harveyi* and *V. rotiferianus* only

V. rotiferianus uses *V. harveyi* secretion products to grow in iron-poor conditions



Conclusions



- ❑ *V. harveyi* acts as a **killer**: it uses **virulence** and **cytotoxicity** to colonize its **immunocompromised** host



- ❑ *V. rotiferianus* acts as a **cheater**: it benefits from the **virulence/cytotoxicity** and the **siderophore** production of *V. harveyi*

References

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