Modulations of the interactions between pacific cupped oyster Crassostrea gigas and Vibrio according to bacterial virulence and to genetic and physiological status of the host.

## Methodology and first results.

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Rearing of Crassostrea gigas is the most economically important aquaculture activity in France. If Vibrio are often documented as pathogenic of farmed marine species (Paillard, 2004), it represents about 30 % of the oyster natural flora (Vasconcelos, 1972). Since several decades, two Vibrio species, V. splendidus and V. aestuarianus, were associated with many cases of mortality events in reared C. gigas spat and juvenile oysters, frequently during summer (Gay, 2004; Garnier, 2007). This summer mortality syndrome has been well documented as the result of complex interactions between pathogens, host and environmental conditions. This work aims to study the Vibrio-oyster interactions and their modulations according to the virulence of pathogens and the genetic and/or physiological parameters of the host.

## Methodological approach

### Vibrio,

broad collection of *V. splendidus* and V. aestuarianus strains:

-Wild strains : virulent & avirulent

marker genes

-Mutated strains (Le Roux et al. 2007) on gene candidates to virulence and/or harboring Virulence Factors

1. Searching for contrasted responses to experimental infection with virulent Vibrio, in different populations of oysters, which are genetically and/or physiologically characterized



Characterization of pathogenesis to dicument the variability of virulence phenotype

## Oysters,

Produced by controlled crossing, resulting in:

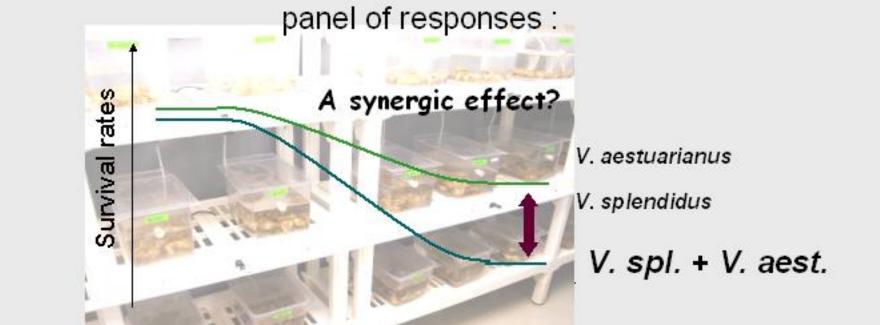
- half-sib families of diploid and triploid oysters
- families presenting high level of inbreeding

Physiological and genetic parameters

### Technical tools

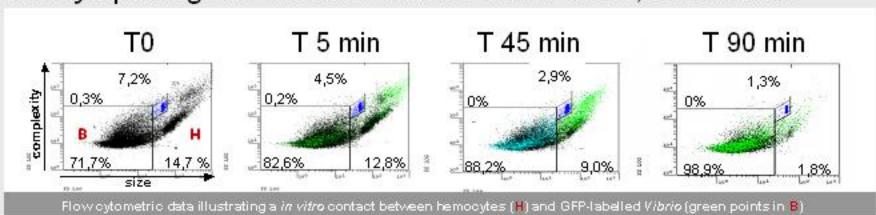
#### *In vivo*, by experimental infection

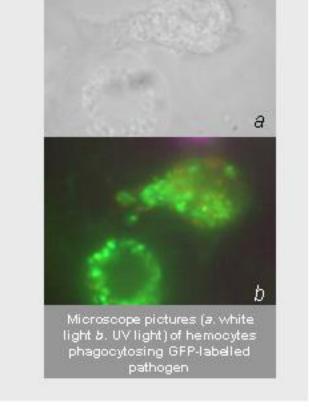
Obtention of a reproducible experimental co-infection model using a mix of V. splendidus and V. aestuarianus bacterial strains to get a large



### Characterization phagocytic ability of oyters hemocytes by using GFP-labelled Vibrio because

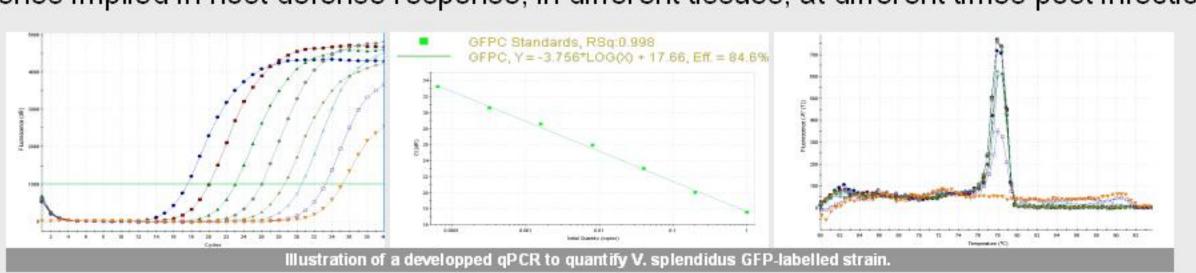
- hemocytes are the only-known cells implied in defense mechanisms
- a great variation of phagocytic responses is observed in wild and genetically divergent oysters
- of a possible cytopathogenic effect of virulent Vibrio strain, to confirm:





### Quantification of hemocyte-Vibrio interactions by qPCR

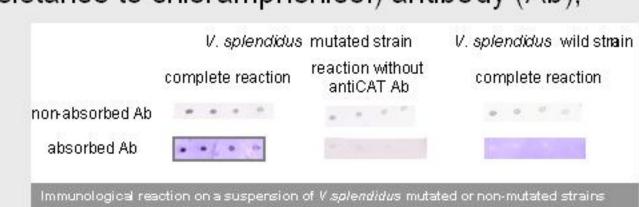
like pathogen-marker gene (GFP), virulence genes (metalloprotease), candidate immune genes implied in host defense response, in different tissues, at different times post infection



# Immuno-histology to localize pathogen in host

tissues

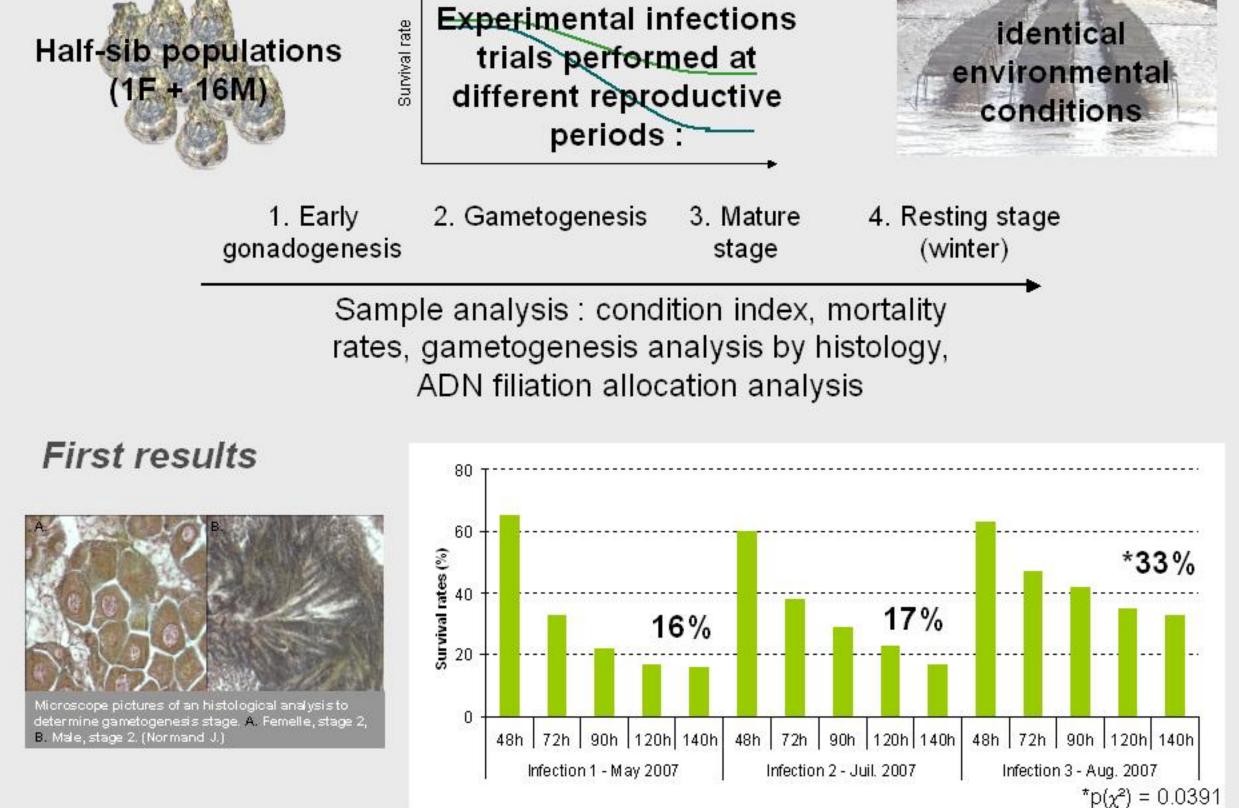
Targeting specifically mutated strain, using a polyclonal anti-CAT (resistance to chloramphenicol) antibody (Ab),





# First results and perspectives

### Effect of gonadogenesis on oyster susceptibility to vibriosis



### Perspectives

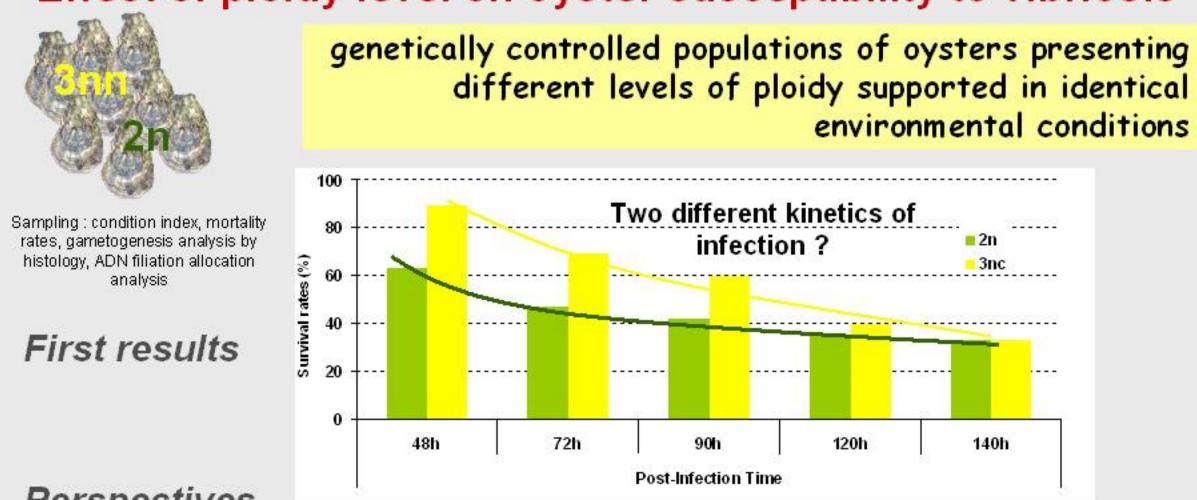
- → A possible effect of gonadogenesis on oyster susceptibility as soon as the beginning of gonad development, to confirm thanks to the 4th experimental infection
- → Histological analysis of gametogenesis stage to find out this possible effect of gonadogenesis

### References:

Garnier M., Labreuche Y., Garcia C. Robert M. Nicolas J. L. (2007). Evidence for the Involvement of Pathogenic Bacteria in Summer Mortalities of the Pacific Oyster Crassostrea gigas. Microb Ecol. 53 (187-196.

Gay M., Berthe F. C., Le Roux F. (2004). Screening of Vibrio isolates to develop an experimental infection model in the Pacific oyster Crassostrea gigas. Dis Aquat Organ 59(1): 49-56. Gay M. (2004) Experimental infection in Crassostrea gigas: study of two pathogenic strains related to Vibrio splendidus. PhD thesis.

## Effect of ploïdy level on oyster susceptibility to vibriosis



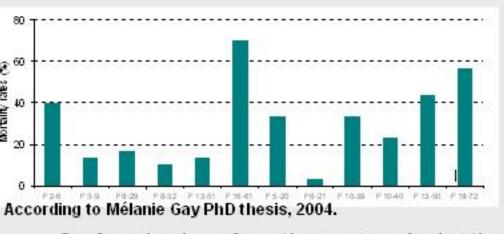
Perspectives

477-498.

→ Intersect data about the effect of gonadogenesis with future ones from samples analysis and try to emerge patterns correlated to susceptibility to vibriosis

#### Analysis of a genetic base in oyster susceptibility to vibriosis

high level of inbreeding families, obtained by divergent selection criteria based on summer survival



1. Screening of 6 groups of bi-parental families by several experimental infections and selection of families presenting contrasted and stable responses to vibriosis

2. Analysis of pathogenesis in these families and measurement of heritabilities of two characters « Resistant » or « Susceptible » on the next generation.

Le Roux F., Binesse J., Saulnier D. and Mazel D. (2007) Construction of a Vibrio splendidus Mutant Lacking the Metalloprotease Gene vsm by Use of a Novel Counterselectable Suicide Vector. Applied and Environmental Microbiology p. 777-784, Vol. 73, No. 3. Paillard P., Le Roux F., Borrego J.J. (2004). Bacterial disease in marine bivalves, a review of recent studies: trends and evolution. Aquat. Living Resour. 17 (2004).

Vasconcelos G. J. & Lee J. S. (1972). Microbial flora of Pacific Oysters (Crassostrea gigas) subjected to ultraviolet-irradiated seawater. Applied Microbiology 23

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