World Aquaculture Society
Busan, South Korea,20-23 May 2008

Antiviral immunity in the Pacific oyster, *Crassotrea gigas*: last development and perspective

T. Renault*, N. Faury, V. Barbosa Solomieu, K. Moreau, P. Haffner and J.-F. Pepin

IFREMER, Laboratory of Genetics and Pathology, 17390 La Tremblade, FRANCE

Ifremer

Viruses infecting molluscs

The discovery of viruses in marine molluscs is fairly recent. Several massive mortality outbreaks have been correlated to viral infections.

Irido-like virus infections led to the almost total extermination of the Portuguese oyster, *Crassostrea angulata*, in French and European Atlantic waters in 1973.

Viruses morphologically similar to members of *Herpesviridae* family have been identified in various marine mollusc species around the world.

Control of viral diseases in mollucs

Despite the impact that viral diseases have on aquatic organisms, we know relatively little about what farmers can do to prevent and treat viral infections and how shellfish fight viral diseases. Difficulties for control of viral infections in aquaculture come mainly from the absence of specific therapeutic agents.

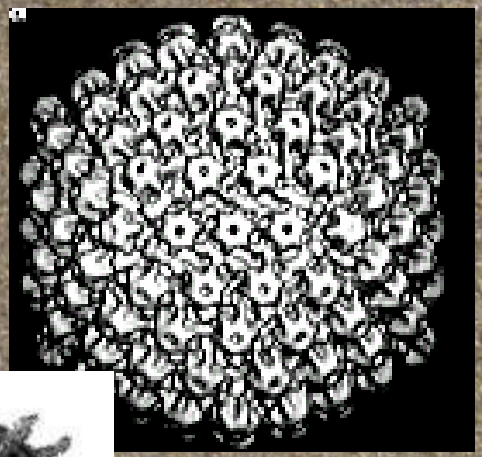
Aternative treatments using anti-viral drugs may be developed and the most effective way for sustainable aquaculture production will certainly rely on the production of selected animals for disease resistance.

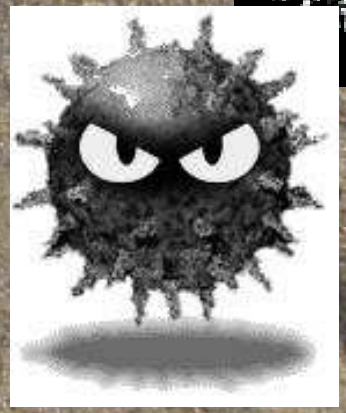
Antiviral innate immunity in mollucs

Anti-viral non-specific defence mechanisms (innate immunity) are important because they constitute the first line of defence in vertebrates, and the only one in invertebrates.

Antiviral innate immunity must be investigated in molluscs.

Antiviral innate immunity in molluscs





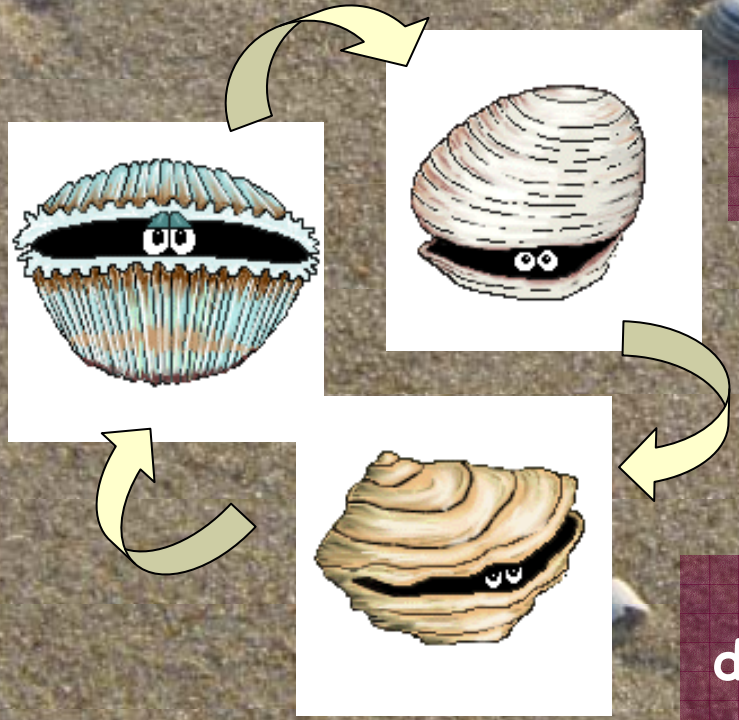
FOCUS

on Ostreid Herpes virus 1 (OsHV-1) and Pacific oyster interactions

OsHV-1, a virus infecting bivalves in France

Description in the French Pacific oyster
(diseased larvae and seed)

Pathogenicity and transmissibility
(experimental infections)



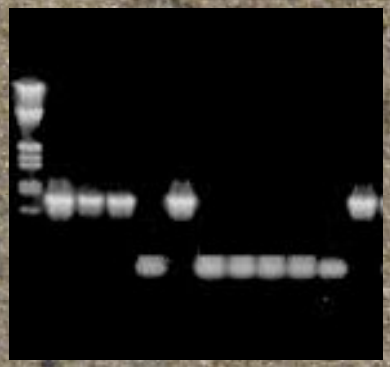
Viral purification
(DNA extraction, cloning, sequencing…)

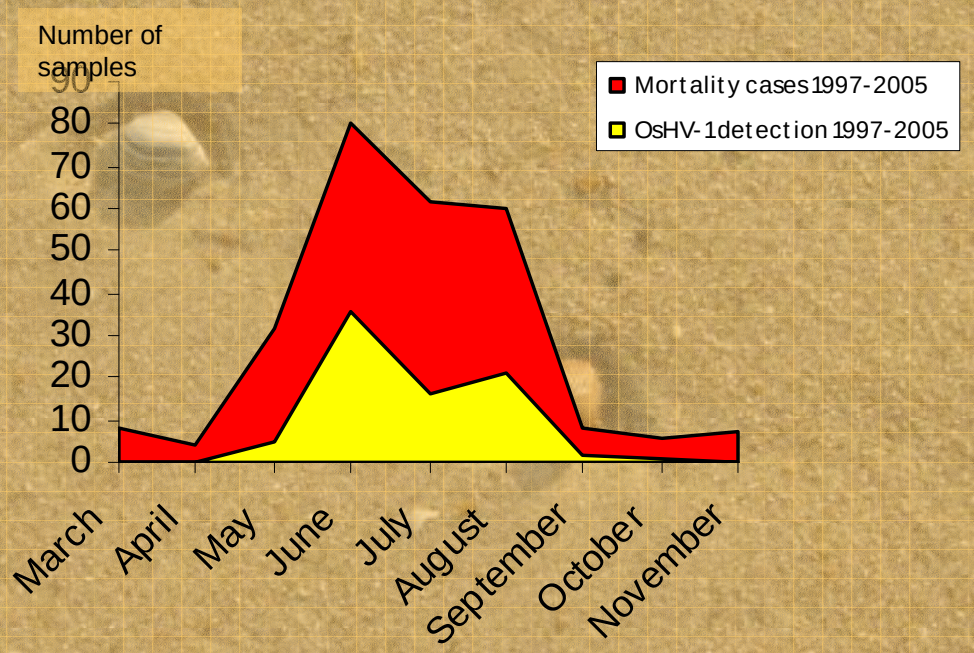
Various detection tools developed

Characteristics of the viral genome

Ostreid Herpes virus 1

OsHV-1, a virus associated with high mortality outbreaks in France





An epidemiological survey using PCR in France from 1997 to 2005

Identification of OsHV-1 induced genes in the Pacific oyster by SSH

Principle: obtain uninfected and OsHV-1 infected individuals to perform subtractive suppressive hybridisation (SSH)

Use of oyster haemocytes maintained in vitro in contact with OsHV-1 (ground infected larvae after filtration through 0.22µm filter)

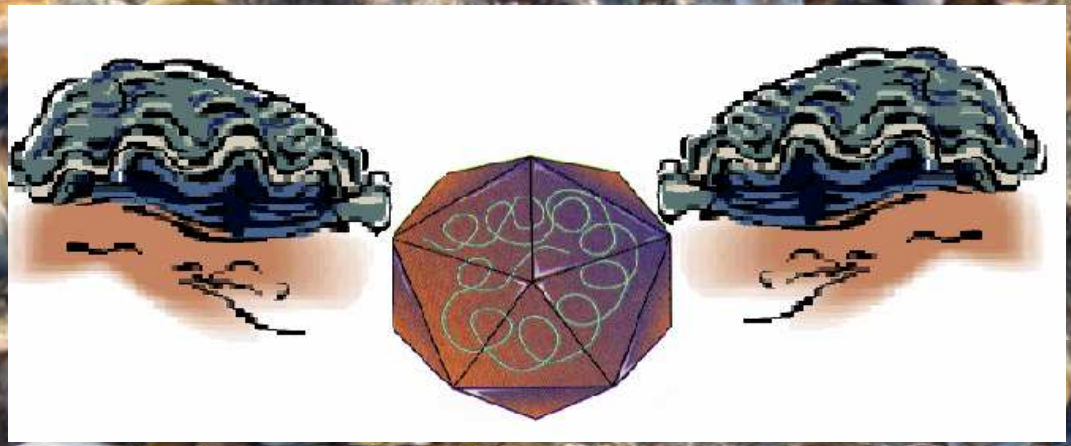
OsHV-1 induced genes

Identification of OsHV-1 induced genes in the Pacific oyster

- Molluscan Defence Molecule (*Lymnaea stagnalis*, 2.00E^{-10}, Ig superfamily member similar to *Hemolin* from insects)
- Macrophage expressed protein (*Halotis rufescens*, 4.00E^{-95}, Perforin-like protein)
- Laccase (*Tribolium*, 1.00E^{-31}, Phenoloxidase or multi-copper oxidase)
- Glypican 6 (*Homo sapiens*, 3.00E^{-105}, Cell receptor for viruses)
- IK Cytokine (*Danio rerio*, 5.00E^{-85}, Down regulation of HLA II)
- Myeloid differentiation factor 88 (*Oncorhynkus mykiss*, 1.00E^{-07}, Toll-like receptor activation)

OsHV-1 induced genes

Expression of immune related genes in adult oysters after a immune challenge



Expression of immune relevant genes

What does the experimental scheme look like?

❶ Tissue extracts from OsHV-1 infected spat

❷ Injection of tissue extracts in oysters

❸ Withdraw haemolymph from oysters after injection

❹ Extraction of RNA from oyster haemocytes

❺ Analysis of gene expression by real-time RT PCR

Experimental scheme

Experimental scheme

- Pacific oysters (adults)
- OsHV-1 infected spat (Pacific oyster) kept frozen (-20°C) as the viral source
- Exposition to OsHV-1 by injection of tissue extract after filtration through a 0.22 µm filter (50 µl) in pericardic cavity (extracts from OsHV-1 infected spat versus non-infected spat)

First Experiment

- Oyster (2 year old) mortality recorded daily during a seven day period
- RNA extraction of hemocytes 24h and 48h post-injection
- Analysis by real-time RT PCR (6 oyster genes selected)

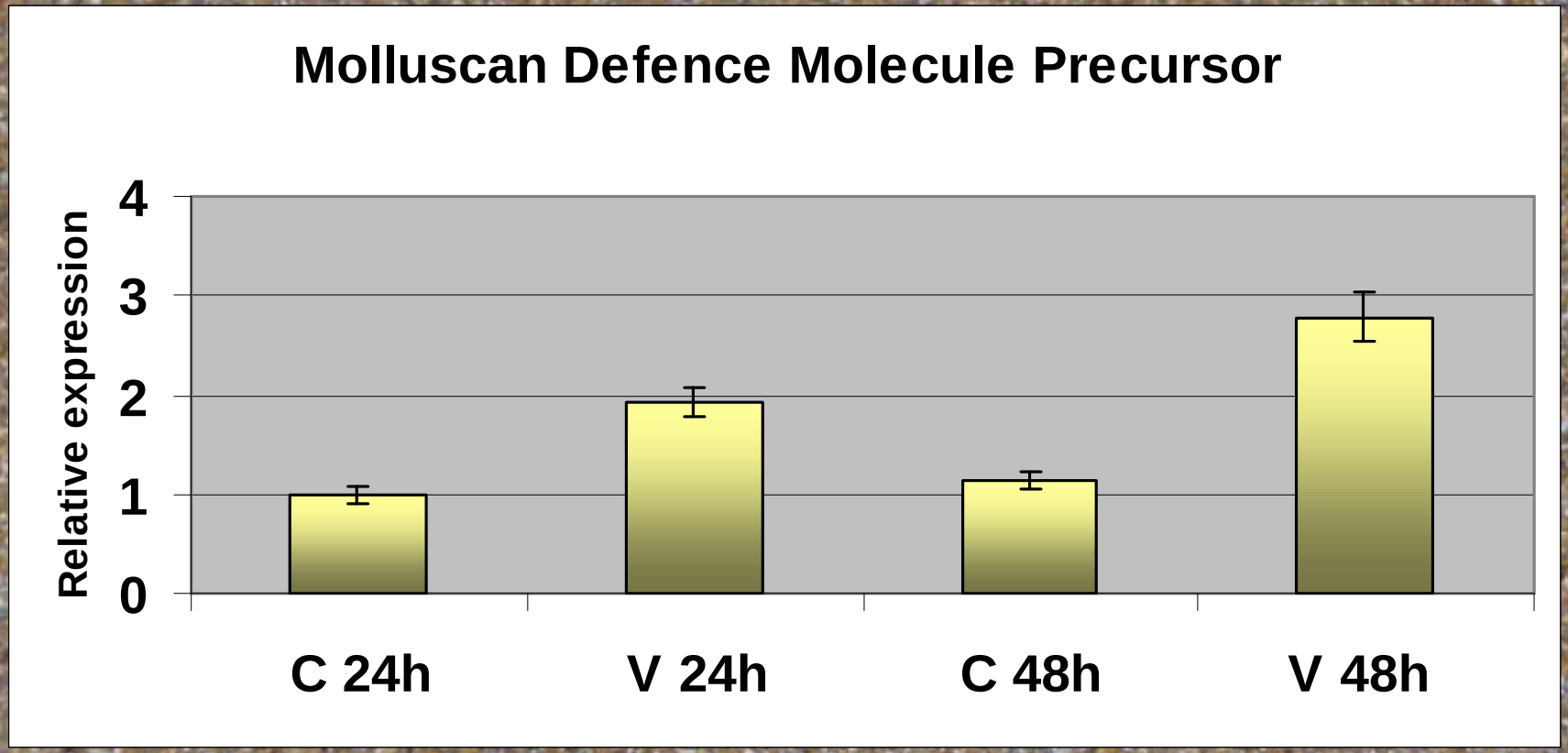
First experiment

First Experiment

- Expression at 24h and 48 h post-injection (V vs C) using real-time RT PCR (elongation factor I as internal standard for gene expression)
- Up-regulation of 2 genes (Molluscan Defence Molecule and multicopper oxidase) in exposed oysters at 24h and 48h post injection in comparison to non-exposed animals

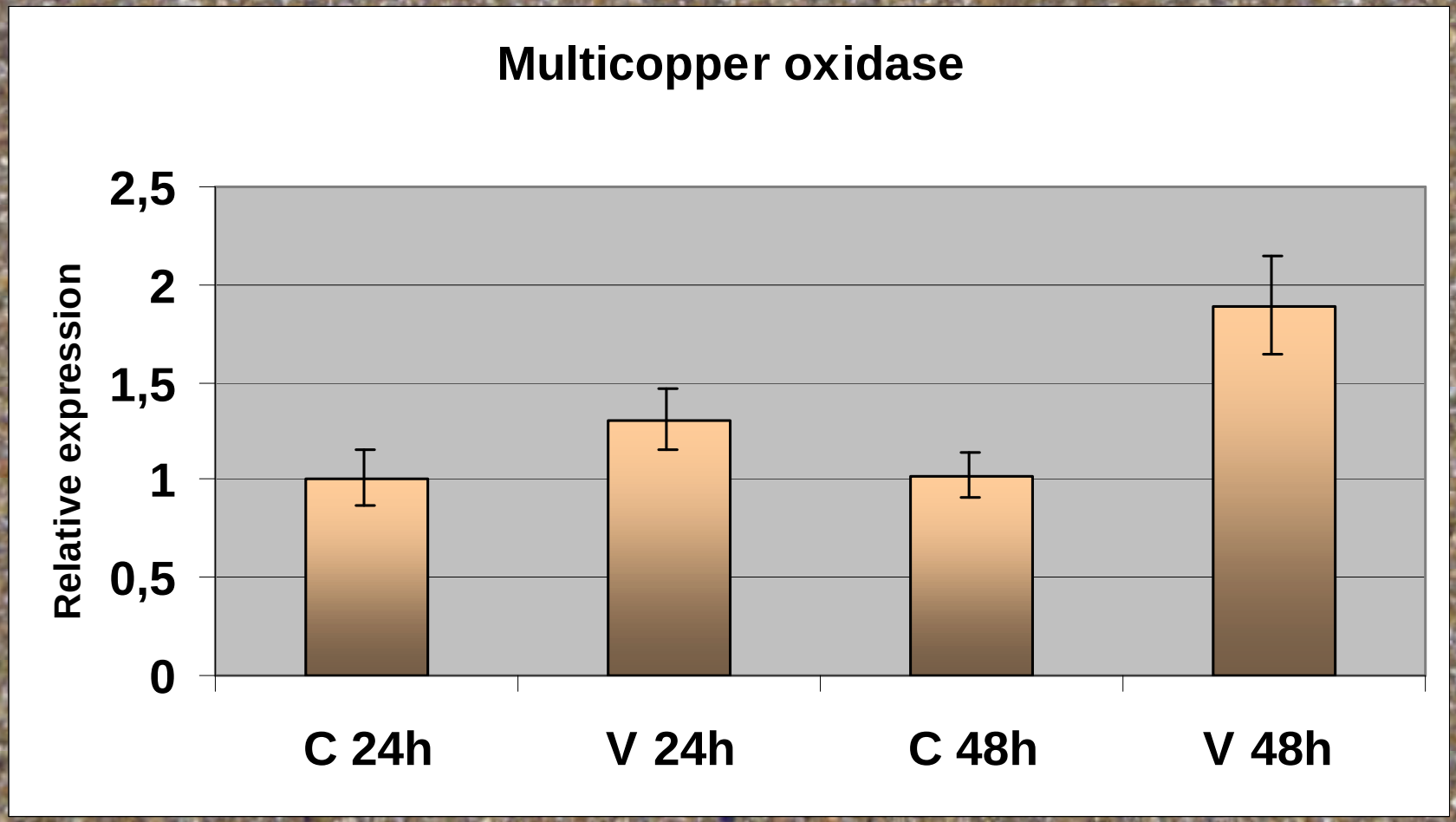
First experiment

First Experiment



Significant increase in mRNA levels (relative to elongation factor I gene) in exposed oysters (V) compared to non-exposed oysters (C) at 24h and 48h

First Experiment



V > C at 24h and 48h

First Experiment

Up-regulation of two genes involved in innate immune defence in exposed oysters (Molluscan Defense Molecule, similar to *Hemolin* and multi-copper oxidase)

- *Hemolin*: the most strongly induced immune gene known in Lepidoptera (by bacteria and, in contrast to anti-microbial peptides, by viruses)
- Ability of *Hemolin* to bind to hemocytes: mediator between microorganisms and hemocytes
- Link between *Hemolin* and the prophenoloxidase system (Terenius et al, 2007) : *Hemolin* is a pattern recognition protein with the ability to bind to viruses and act through the prophenoloxidase activating pathway.

Second Experiment

- Oysters (14 month old) maintained in tanks 48 hours after injection
- RNA extraction of hemocytes at 0, 12, 24 and 48h post-injection
- Analysis by real-time RT PCR (6 immune relevant genes and one viral gene using the C9/C10 primer pair)

Second Experiment

	C 0h	C 12h	V 12h	C 24h	V 24h	C 48h	V 48h
DNase treated RNA	–	–	–	–	–	–	–
cDNA	–	–	–	–	–	–	+

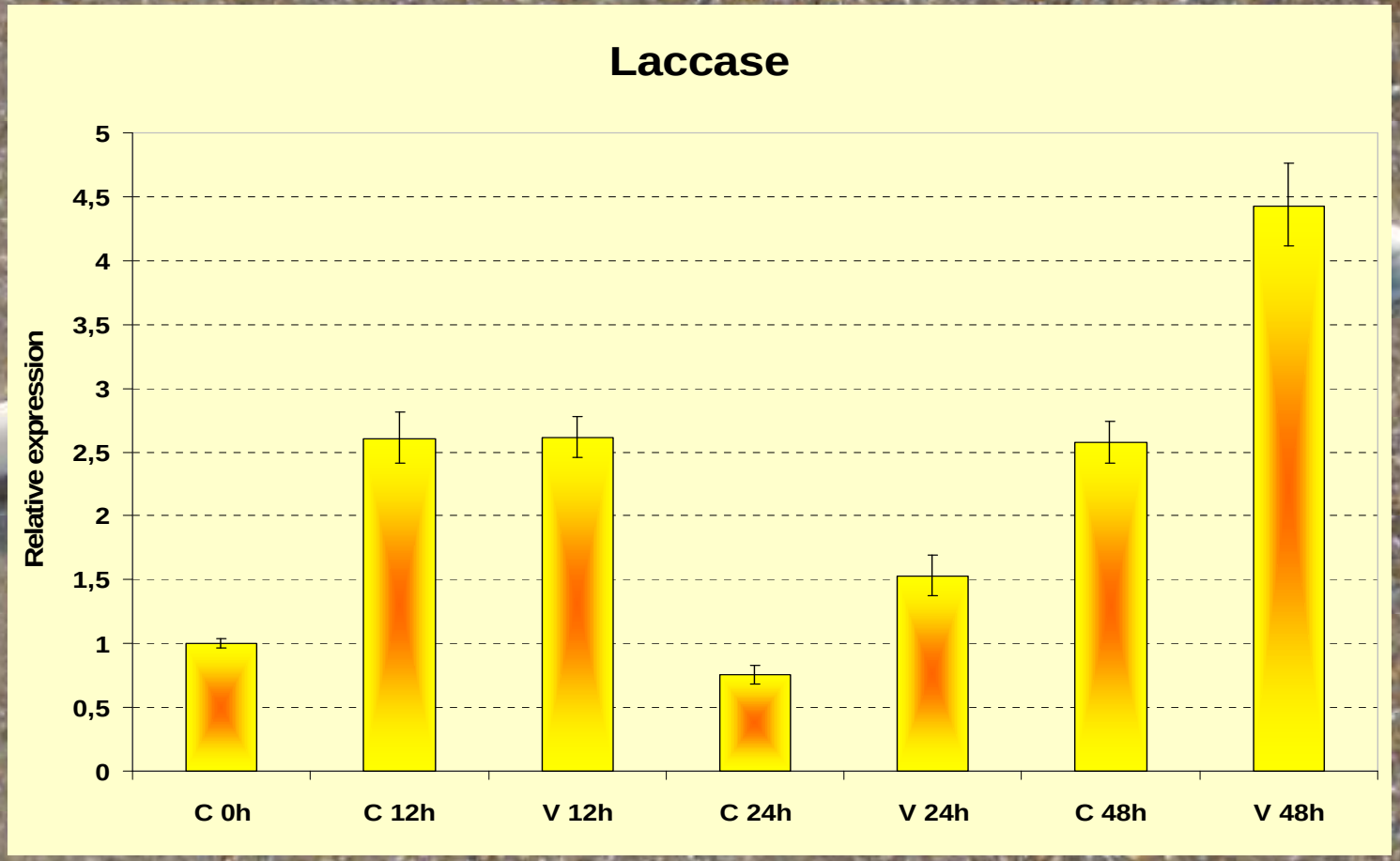
- Analysis of viral expression (RNA) at 0, 12, 24 and 48h post-injection (V: OsHV-1 exposed vs C: non-exposed) using real-time PCR
- Detection only in OsHV-1 exposed oysters (V) 48h post-injection

Second Experiment

- Expression of immune relevant genes at 0, 12, 24 and 48h post-injection (V vs C) using real-time RT PCR (elongation factor I as internal standard for gene expression)
- Up-regulation of 3 genes (MyD88, Macrophage expressed protein and laccase) in OsHV-1 exposed oysters post injection in comparison to non-exposed animals

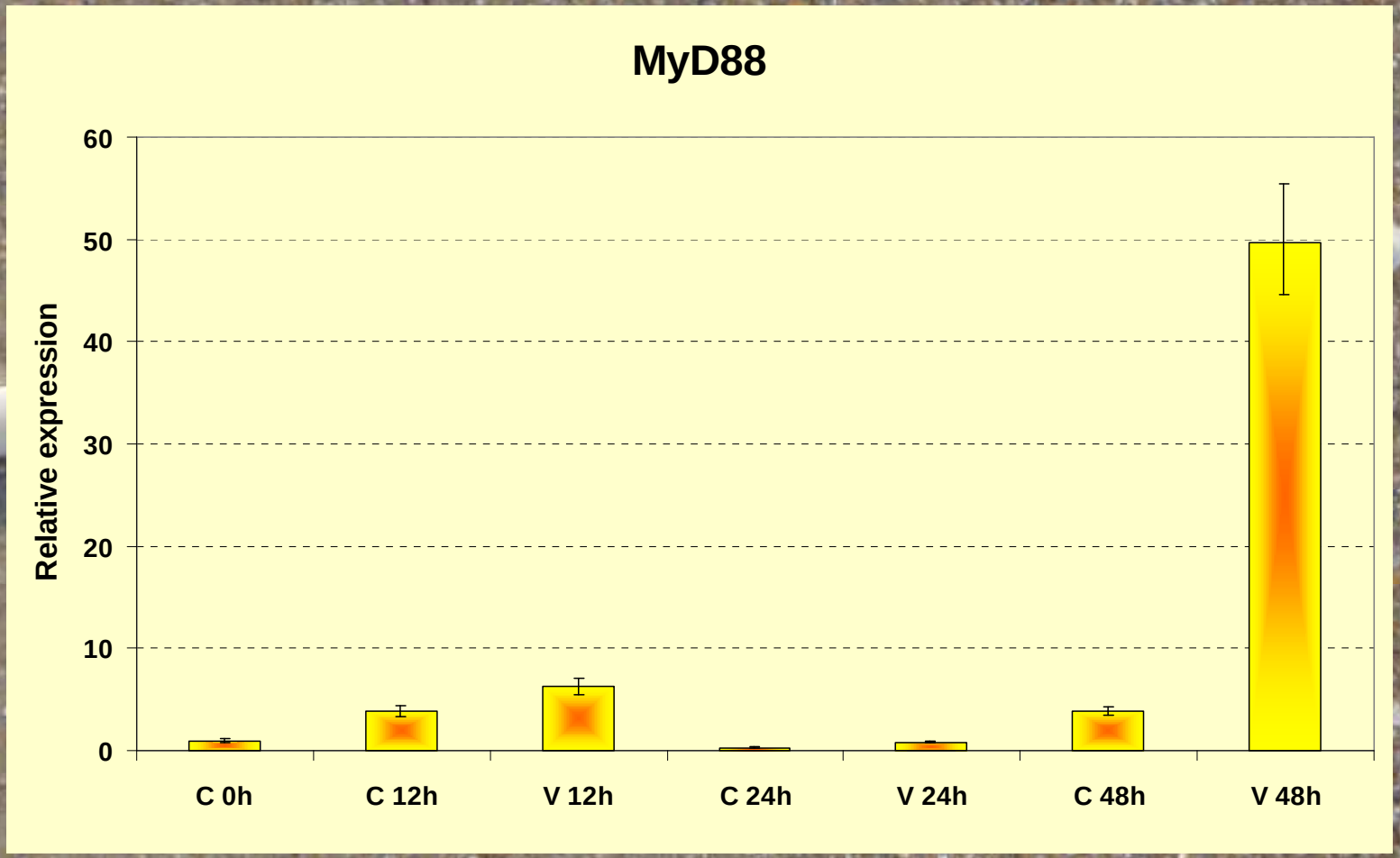
Second experiment

Second Experiment



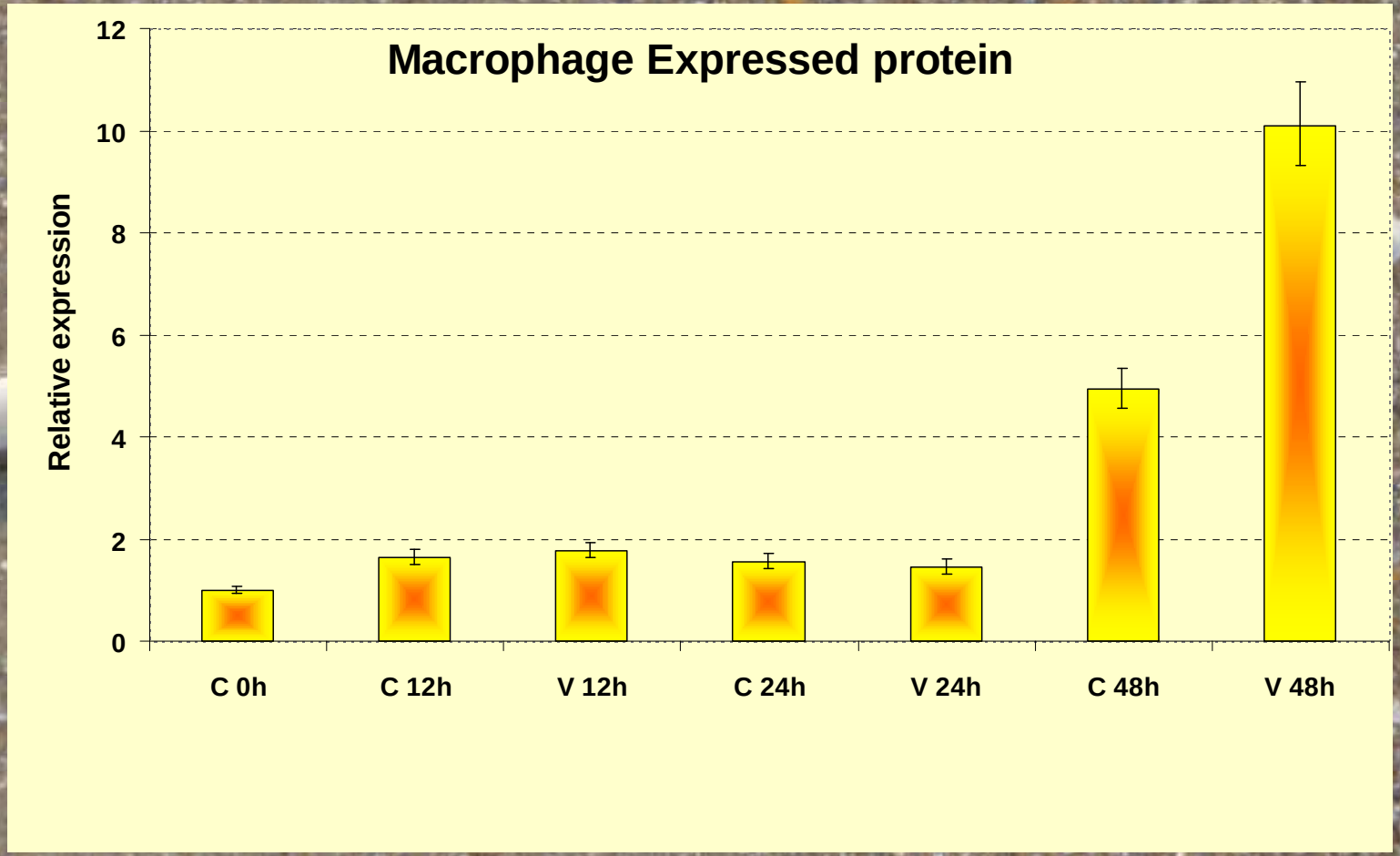
Significant increase in mRNA levels in OsHV-1 exposed oysters (V) compared to non-exposed oysters (C) at 24 and 48h

Second Experiment



V > C at 48h

Second Experiment



V > C at 48h

Second Experiment

Up-regulation of 3 genes interpreted as involved in innate immunity in OsHV-1 exposed oysters (Laccase, MyD88 and Macrophage-expressed protein)

- Phenoloxidase has been shown to be important for immune defence in invertebrates including bivalves (Nelson et al., 2004)
- The macrophage-expressed protein, a perforin-like protein, identified as an executing molecule of a MyD88-dependent signaling pathway in the sponge *Suberites domuncula* (Wienst et al., 2005)
- Perforin-like proteins are also reported in NK cells and are key actors in anti-viral defence (Young et al., 1986)

Second experiment

As a conclusion

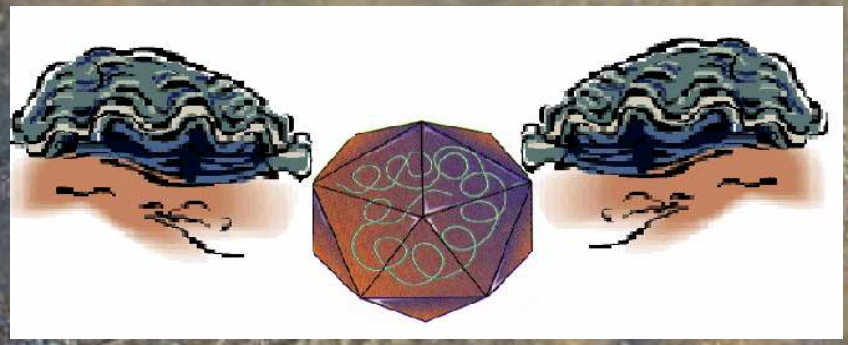
Data may indicate that the Pacific oyster, *C. gigas,* is provided with an innate immune system against OsHV-1.

The virus recognition through a Hemolin like protein may induce cell activation. A signal transduction may result in an elevated expression of MyD88 as well as of the macrophage-expressed protein as an executing protein.

Expression of immune relevant genes

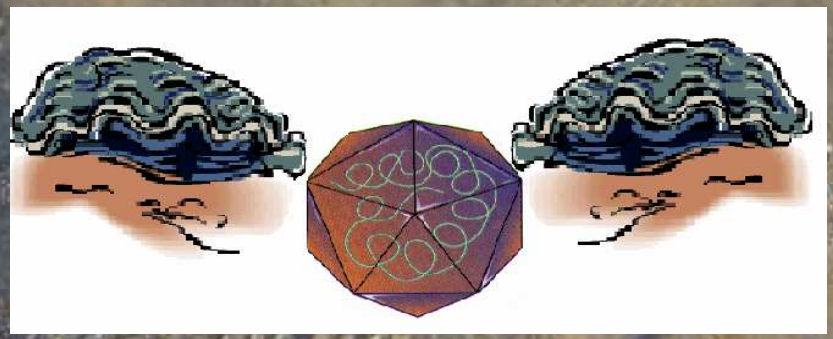
Go further

- Repeat experiments to confirm results (more than 48 hours)
- Expression analysis for other oyster genes using real-time RT PCR (α2 macroglobuline, phosphatidylinositol 4-kinase, …)



Go further

- Production of recombinant proteins for candidate genes and analysis of their biological activities
- Selection of oyster families presenting OsHV-1 resistance based on QTL markers




Thank you for your attention