Novel biotechnological strategies for the detection of ostreid herpesvirus

Mònica Campàs, Anna Toldrà, Karl B. Andree, Edgar Bertomeu, Ana Roque, Noèlia Carrasco, Ignasi Gairín, Dolors Furones

VIVALDI final meeting – Brest 28/11/19

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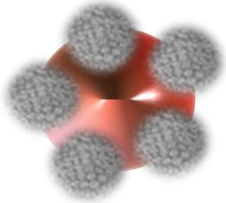
VIVALDI
Preventing and mitigating farmed bivalve diseases

The objective

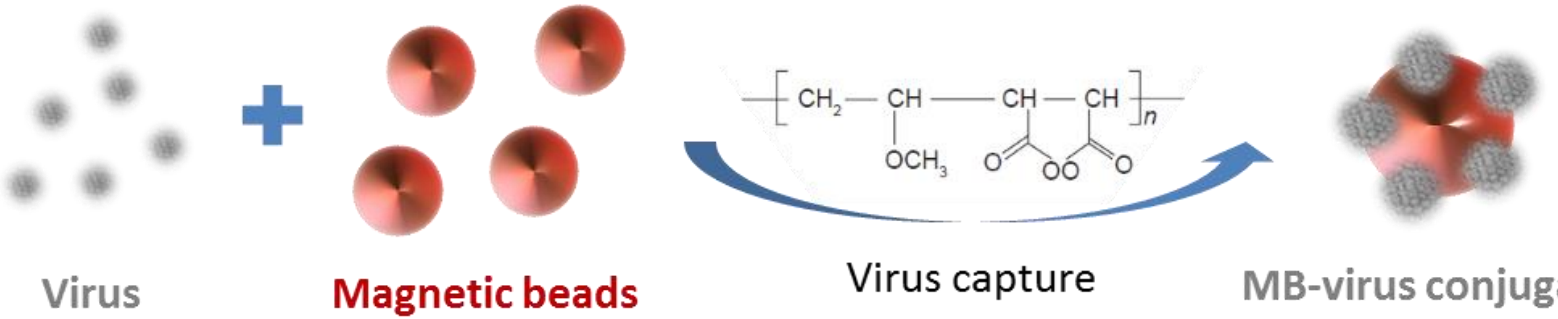


To exploit magnetic beads for the capture and pre-concentration of ostreid herpesvirus

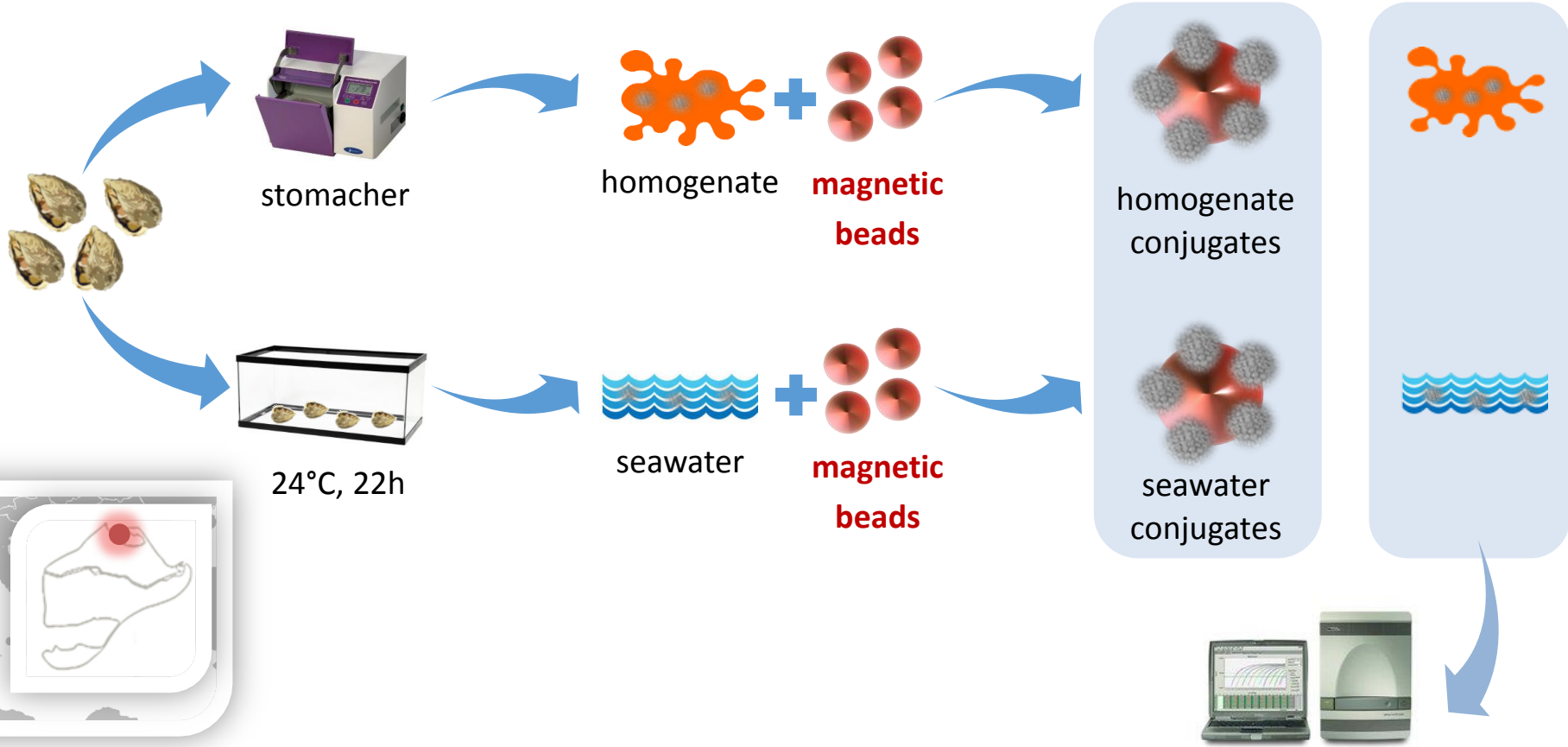
To develop an electrochemical biosensor for the detection of ostreid herpesvirus

Magnetic beads to capture the virus: the strategy

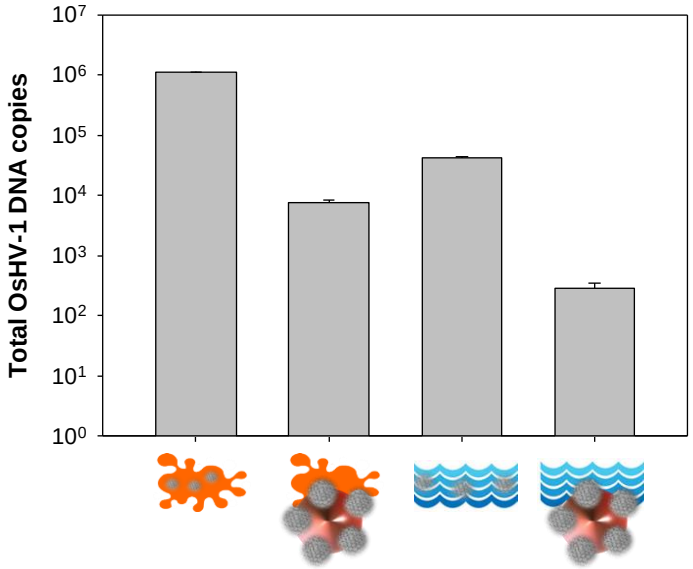
Rapid, simple and cost-effective MB-based viable OsHV-1 capture strategy



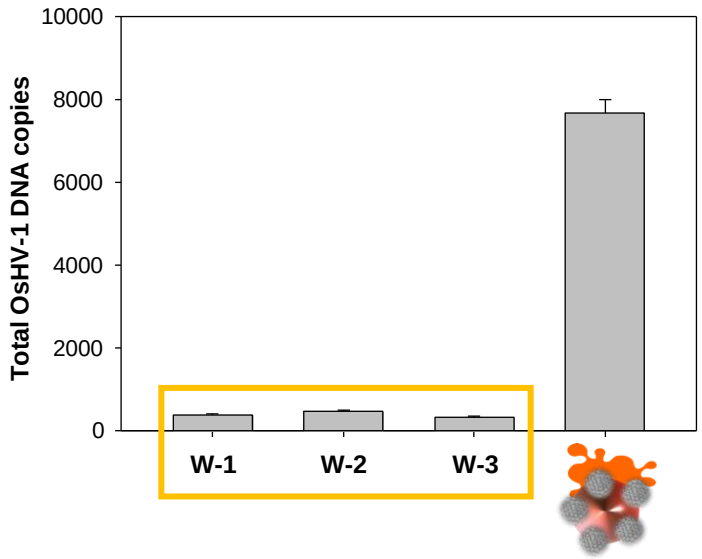
Experimental design



Virus capture and detection



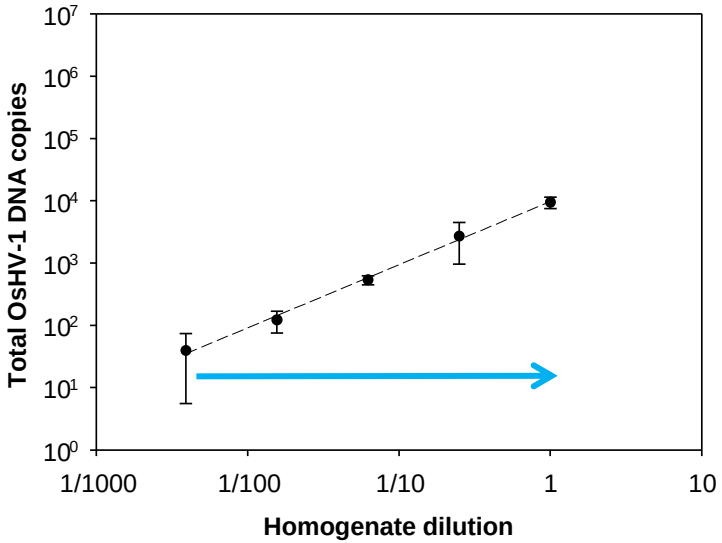
MBs are able to capture the virus from both the homogenate and the seawater



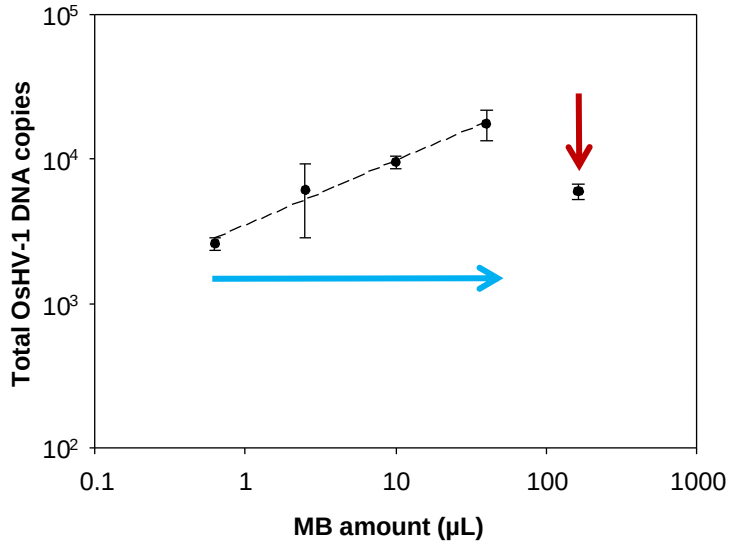
The viral DNA detected in the conjugates came from virus particles captured by the MBs

Virus capture and detection

Homogenate dilutions



MBs dilutions



MBs were not saturated

Steric impediments may decrease capture efficiency

Experimental infections

intramuscular injection

~30 oysters/aquarium

MB-conjugates

Positive controls

Negative controls

homogenate

seawater

sterile water

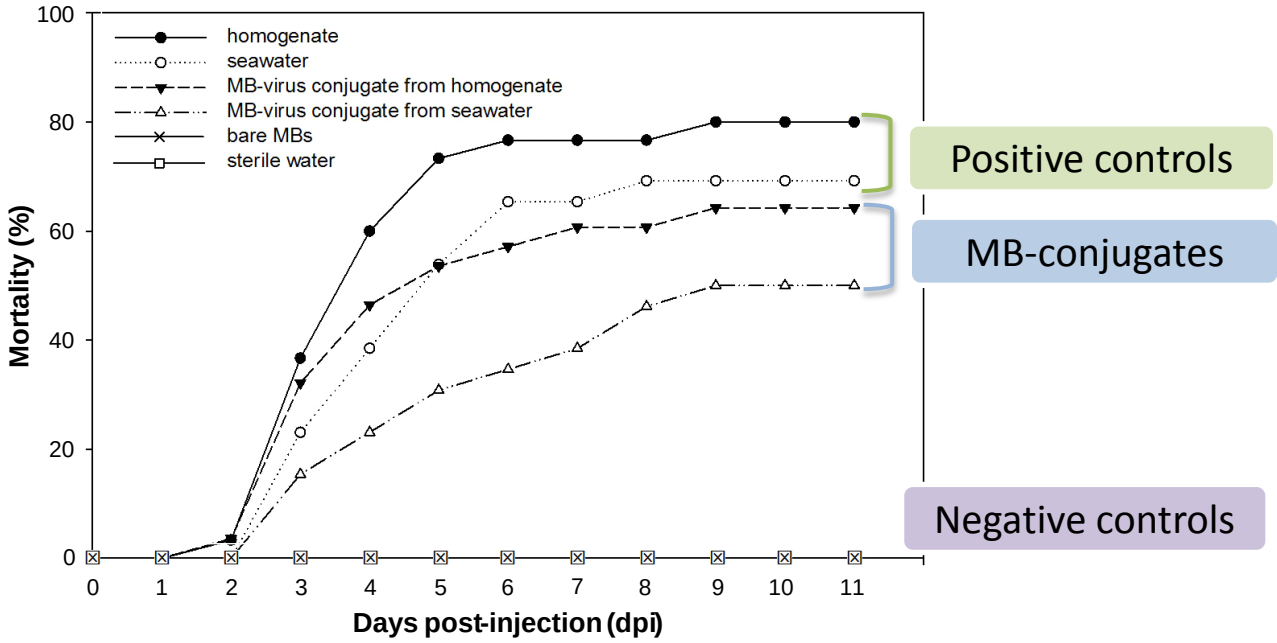
homogenate MBs

seawater MBs

bare MBs

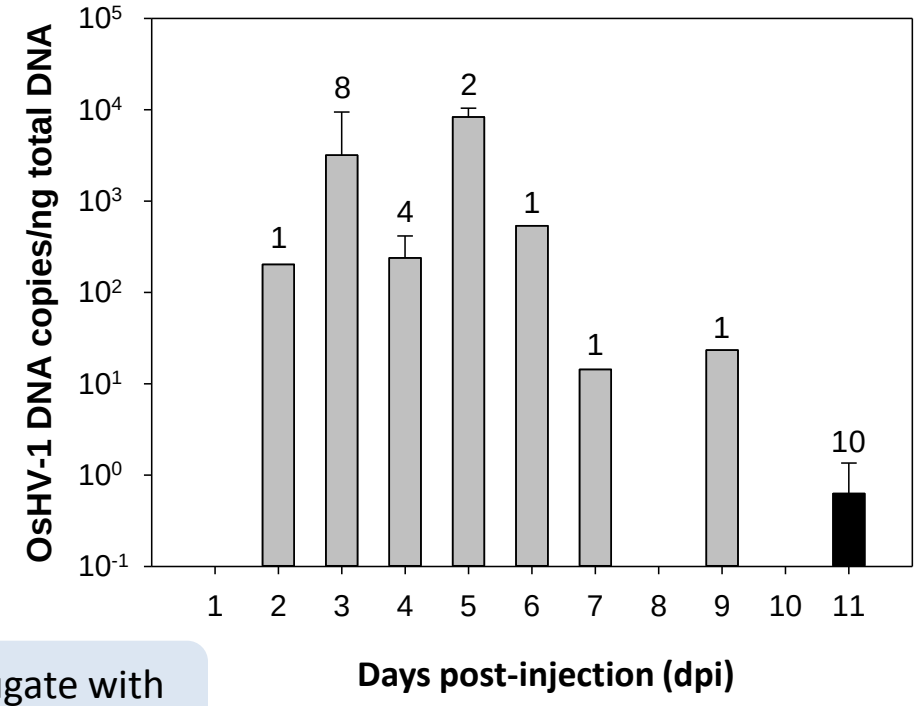
Mortality monitoring, DNA analyses and RNA analyses

Mortality monitoring



MB-conjugates are able to infect oysters

DNA analyses



dead/moribund oysters

living oysters

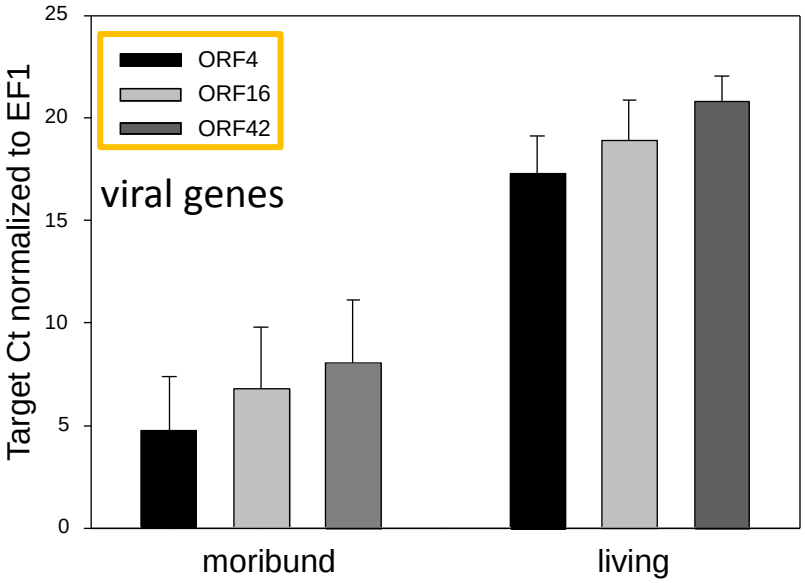
viral DNA loads:

moribund/dead oysters > living oysters

MB-conjugate with the homogenate

MB-conjugates are able to infect oysters

RNA analyses



no differences among ORFs

viral gene expression:

moribund oysters > living oysters

Active replication of the virus

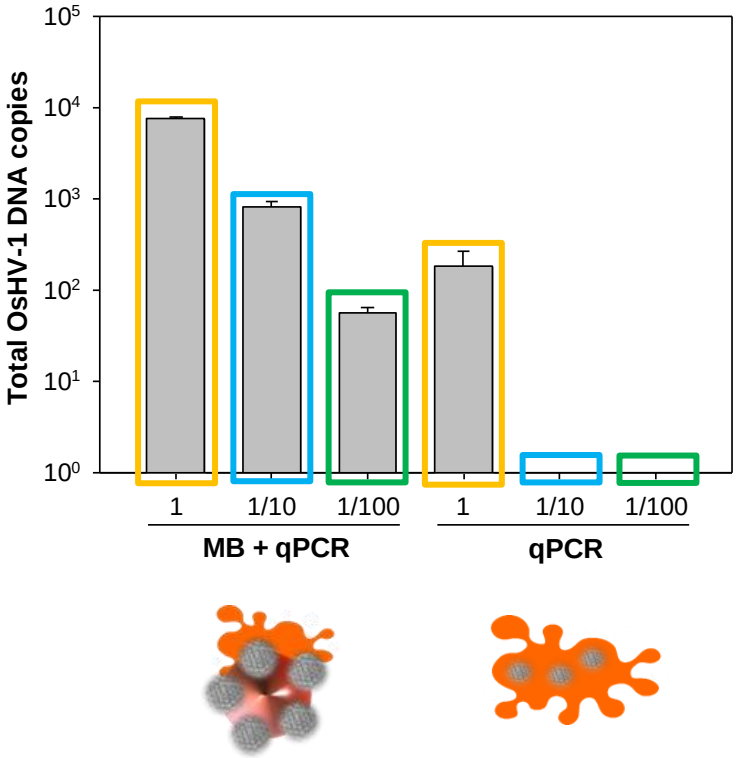
↓

MB-conjugates are able to infect oysters

↓

MBs are able to capture viable virus particles

Pre-concentrating agents: homogenate



Experimental conditions:

MBs volume

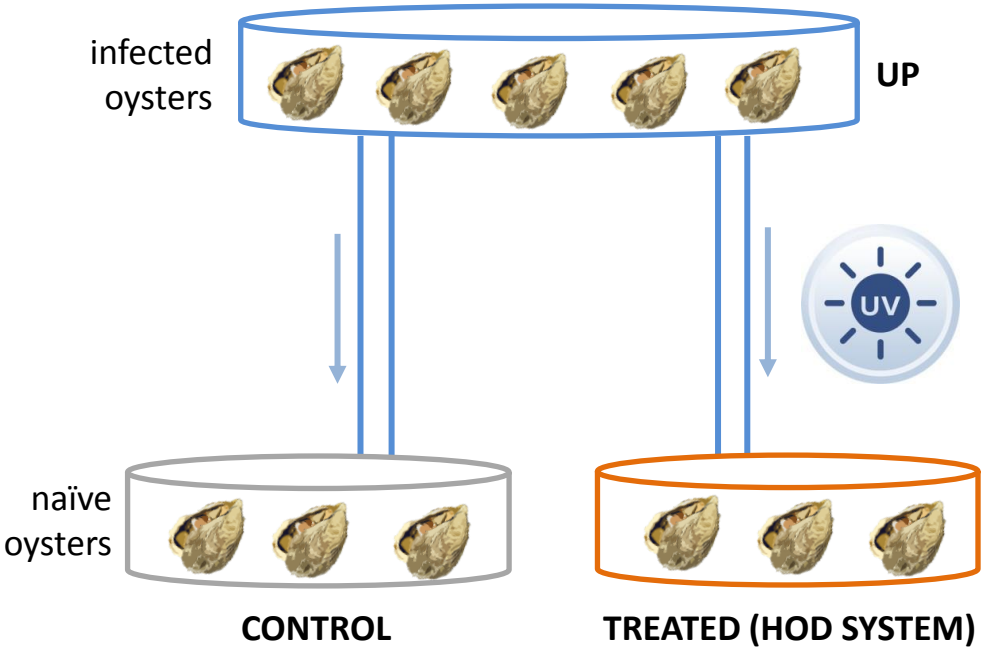
Elution volume

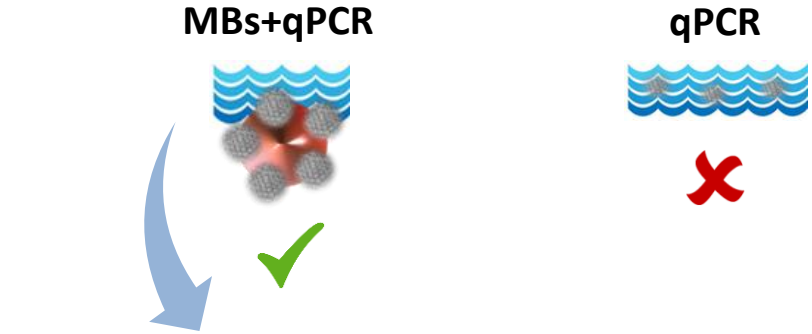
Sample volume

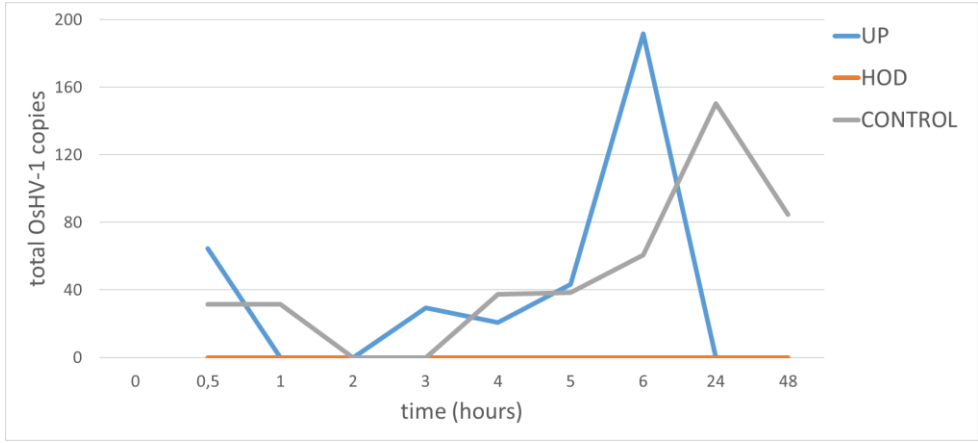
MBs are able to pre-concentrate OsHV-1 at least 100 times

Pre-concentrating agents: seawater

Application to seawater from a depuration experiment (April 2019)



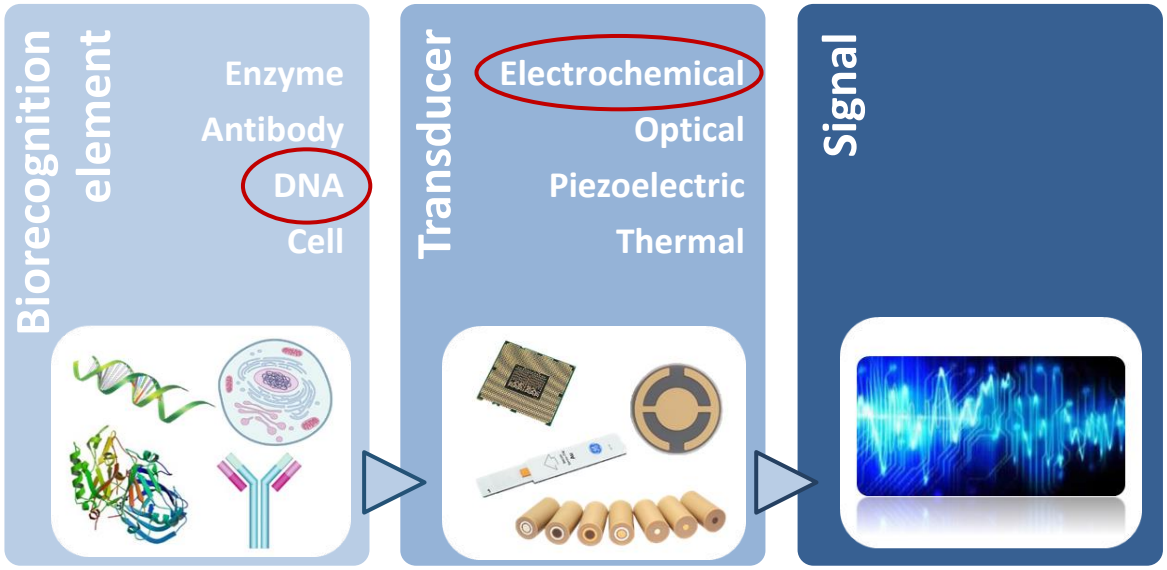




- no oyster mortality
- no OsHV-1 in oysters

DNA-based biosensor: the strategy

Fast
Low-cost
Simple



Amplification technique

PCR

thermal cycling (↑ energy)

✘ miniaturised devices

✘ *in situ* analysis

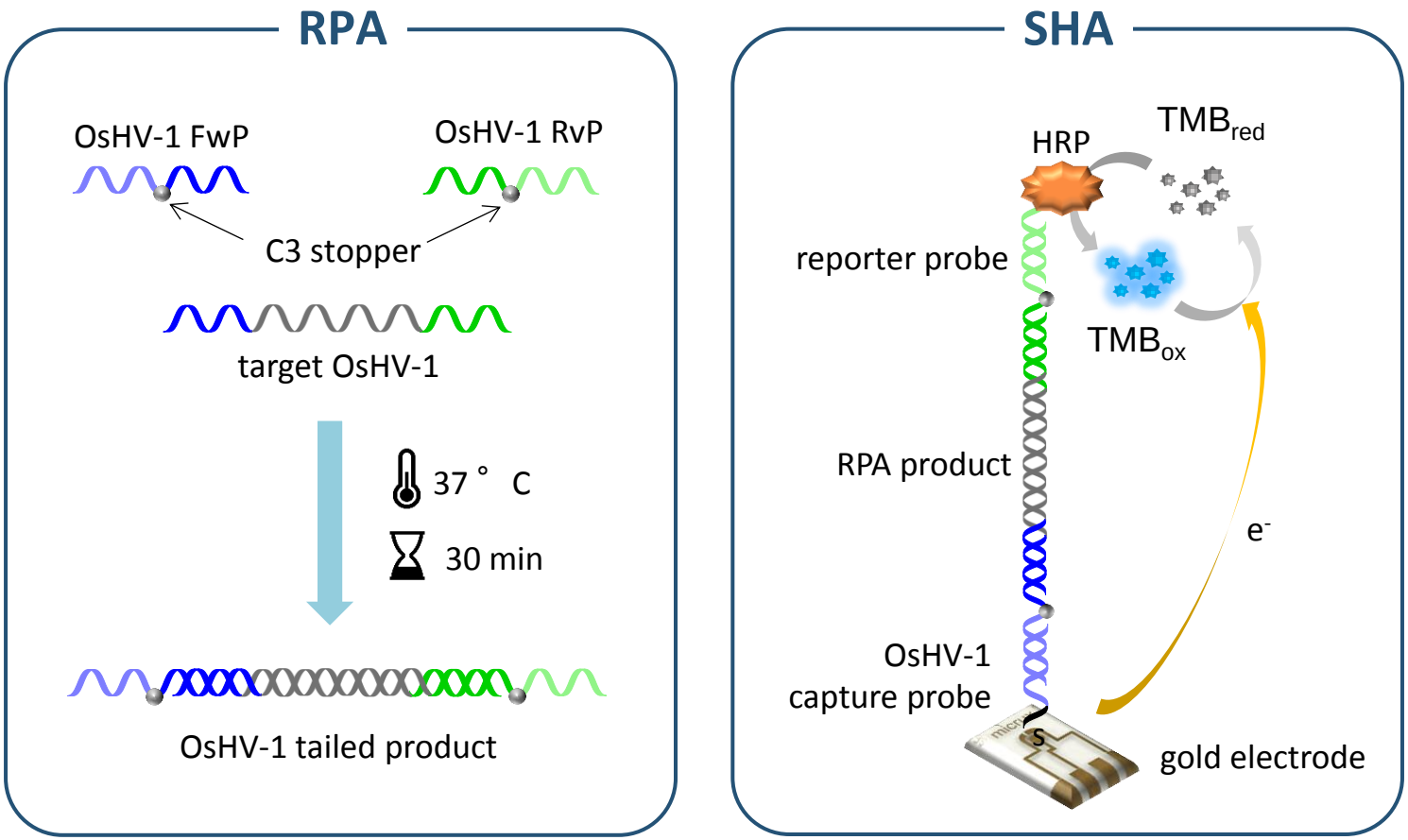
Isothermal amplification

constant temperature (↓ energy)

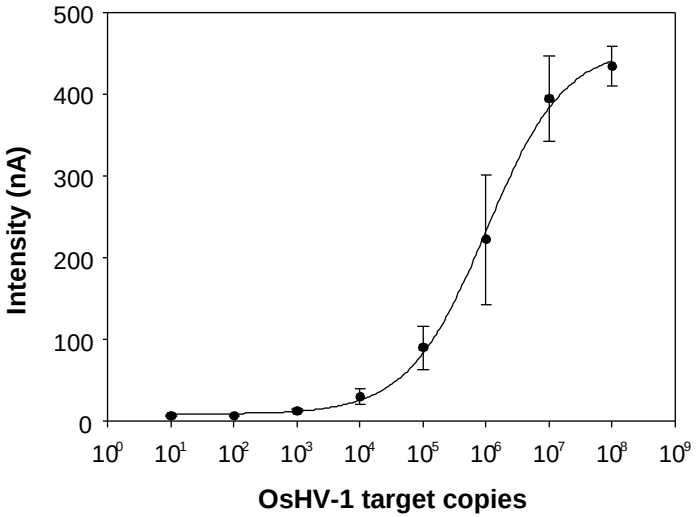
✓ miniaturised devices

✓ *in situ* analysis

Experimental design

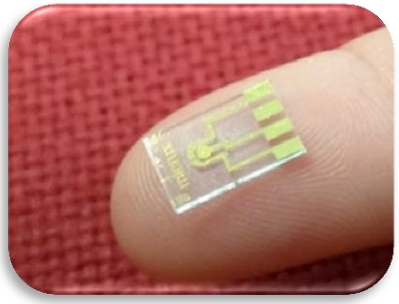


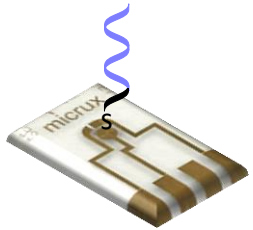
Calibration curve and storage stability



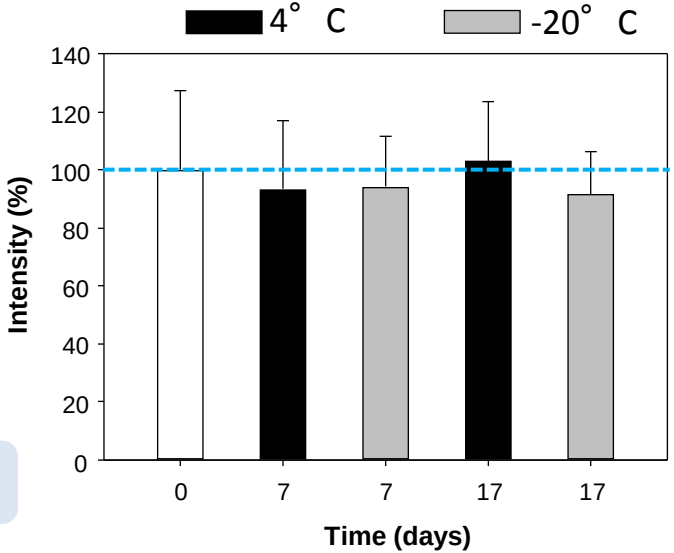
LOD biosensor = 207 copies

LOD qPCR = 4 copies





Overnight



Predicted stability of **3 months** at -20 ° C

Ready-to-use functionalised electrodes

Analysis of oysters

Sample	Physical state	Aquarium	OsHV copies/50 ng total DNA	
			Biosensor	qPCR
1	dead	treated	3.34×10^5	7.13×10^5
2	dead	treated	4.78×10^5	4.81×10^5
3	dead	treated	7.26×10^4	1.79×10^5
4	dead	treated	6.10×10^3	6.52×10^3
5	dead	treated	5.21×10^3	4.38×10^3
6	dead	treated	2.75×10^3	1.98×10^3
7	alive	treated	1.97×10^2	1.21×10^2
8	alive	treated	5.27×10^2	7.83×10
9	alive	treated	1.50×10^2	3.24×10
10	alive	treated	n.d.	1.04×10
11	alive	treated	n.d.	7.93
12	alive	control	n.d.	n.d.
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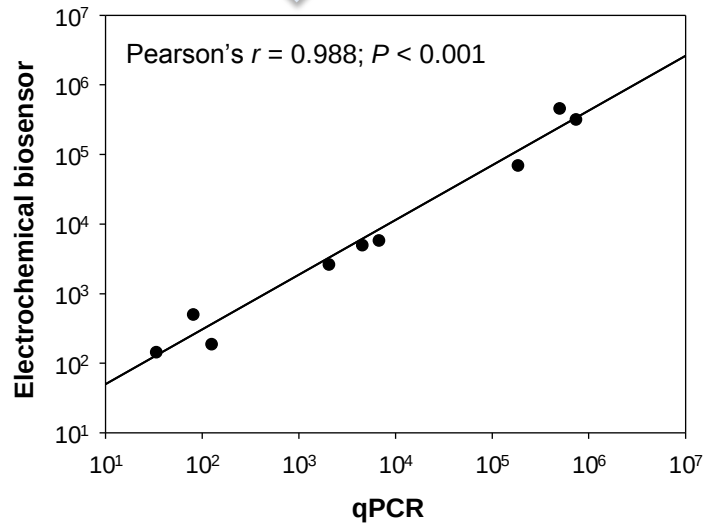
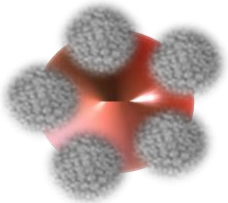
excellent agreement



Summarising

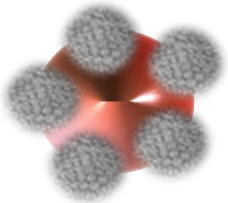


- ✓ MBs are able to **capture OsHV-1** from both the homogenate and seawater.
- ✓ Both homogenate and seawater conjugates have the ability to **infect** oysters.
- ✓ MBs are able to **pre-concentrate** virus particles at least 100 times.
- ✓ MBs are able to pre-concentrate viruses from **seawater**, being closer to an early warning system.

- ✓ An **electrochemical biosensor** for the detection of OsHV-1 has been developed.
- ✓ The biosensor exhibits **good analytical performance**, specificity, sensitivity and storage stability.

Publications

PLOS ONE



RESEARCH ARTICLE

Rapid capture and detection of ostreid herpesvirus-1 from Pacific oyster *Crassostrea gigas* and seawater using magnetic beads

Anna Toldrà, Karl B. Andree, Edgar Bertomeu, Ana Roque, Noèlia Carrasco, Ignasi Gairín, M. Dolores Furones, Mònica Campàs*

IRTA, Ctra., Sant Carles de la Ràpita, Tarragona, Spain

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Detection of isothermally amplified ostreid herpesvirus 1 DNA in Pacific oyster (*Crassostrea gigas*) using a miniaturised electrochemical biosensor

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Preventing and mitigating farmed bivalve diseases

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