

Combining magnetic beads with qPCR for the detection of ostreid herpesvirus

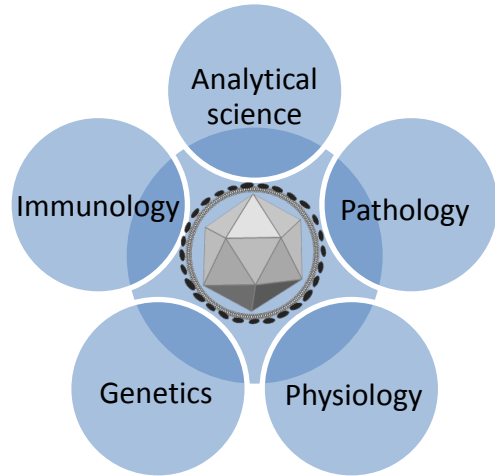
Anna Toldrà, Karl B. Andree, Edgar Bertomeu, Ana Roque, Noèlia Carrasco, Ignasi Gairín, Dolors Furones, Mònica Campàs
IRTA, Spain

VIVALDI WORKSHOP – Porto 09/09/19

“Rapid capture and detection of ostreid herpesvirus-1 from Pacific oyster *Crassostrea gigas* and seawater using magnetic beads”, Toldrà et al., 2018, PLOS ONE



The strategy



OsHV-1



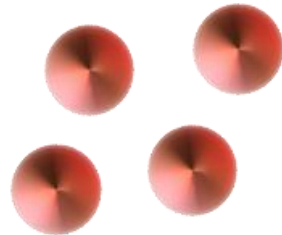
infected material



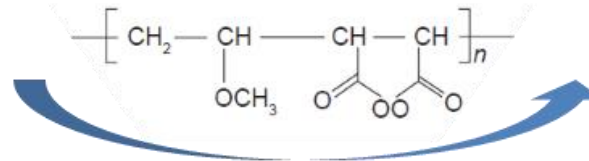
Rapid, simple and cost-effective MB-based viable OsHV-1 capture strategy



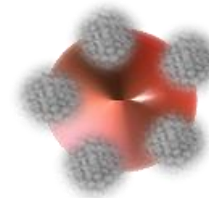
Virus



Magnetic beads

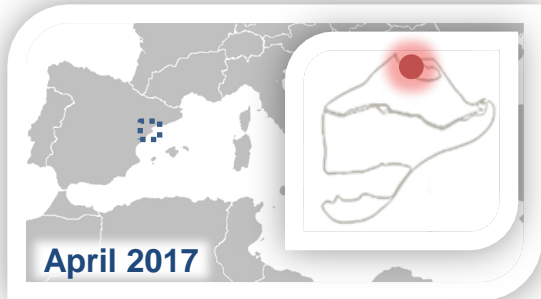
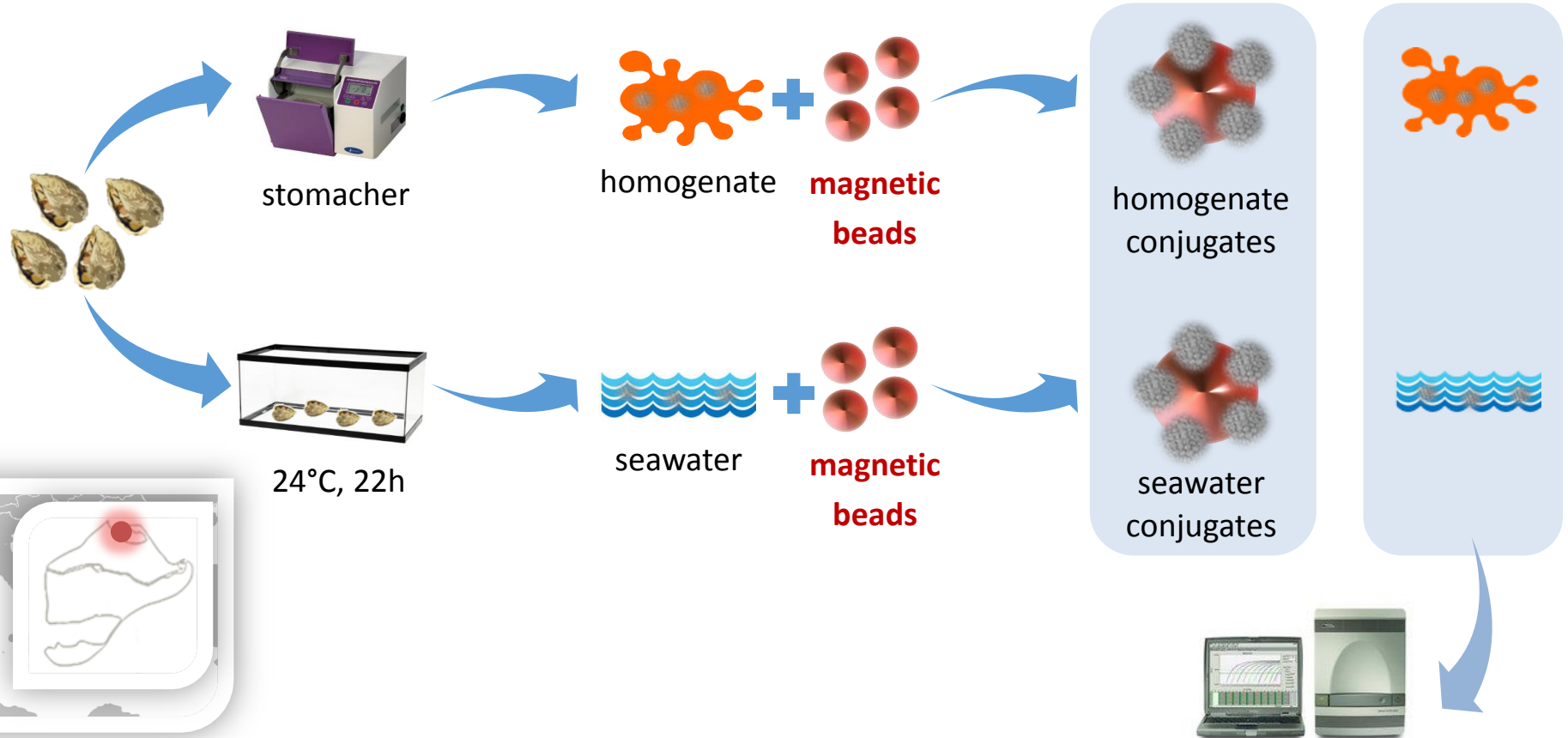


Virus capture

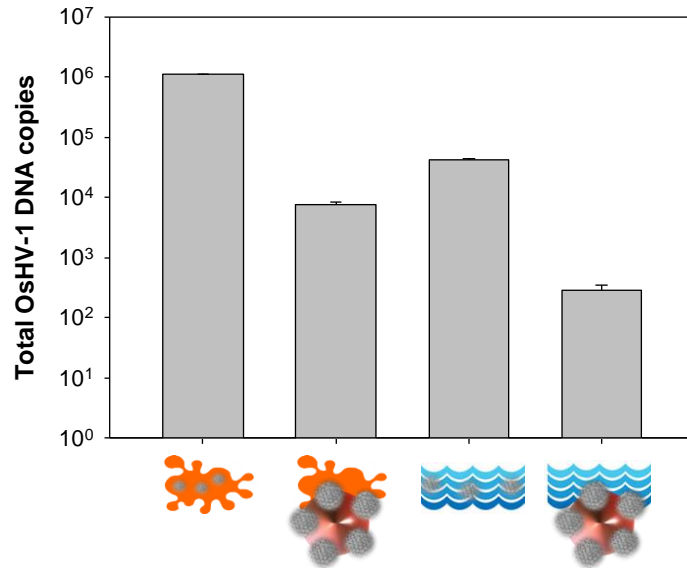


MB-virus conjugates

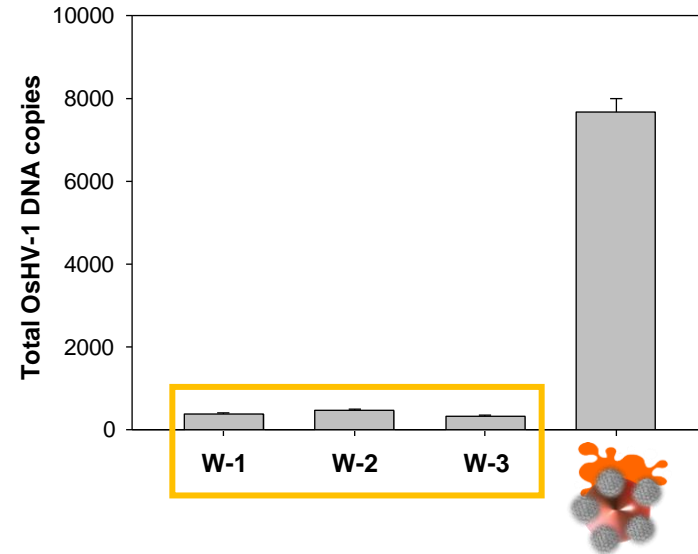
Virus capture



Virus detection



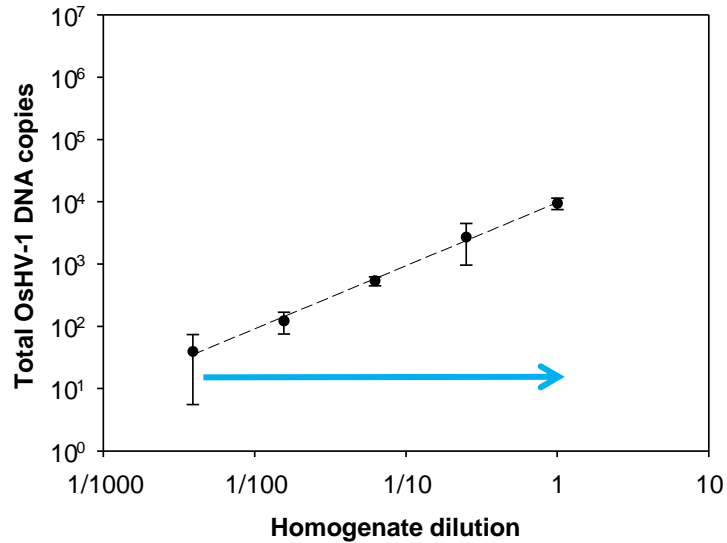
MBs: able to capture the virus from both the homogenate and seawater



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

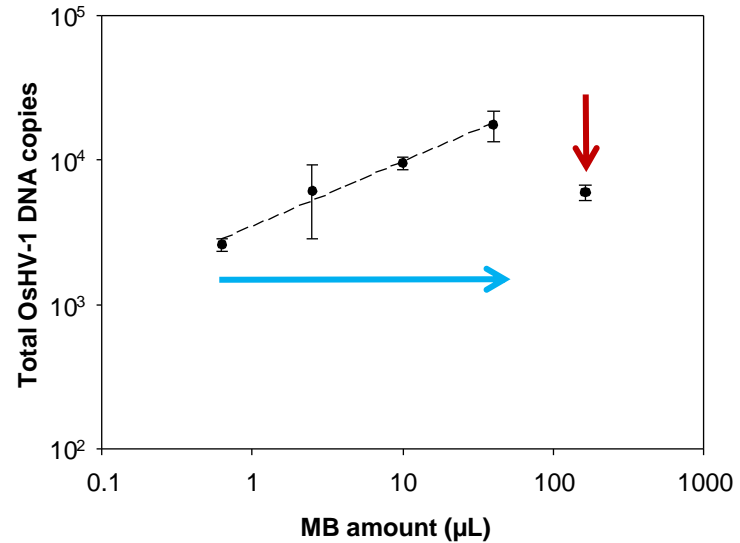
Virus capture

1) Homogenate dilutions



MBs were not saturated

2) MBs dilutions



Steric impediments may decrease MBs capture efficiency

Experimental infections



intramuscular injection
~30 oysters/aquarium

MB-conjugates

Positive controls

Negative controls



homogenate



seawater



sterile water



homogenate MBs



seawater MBs



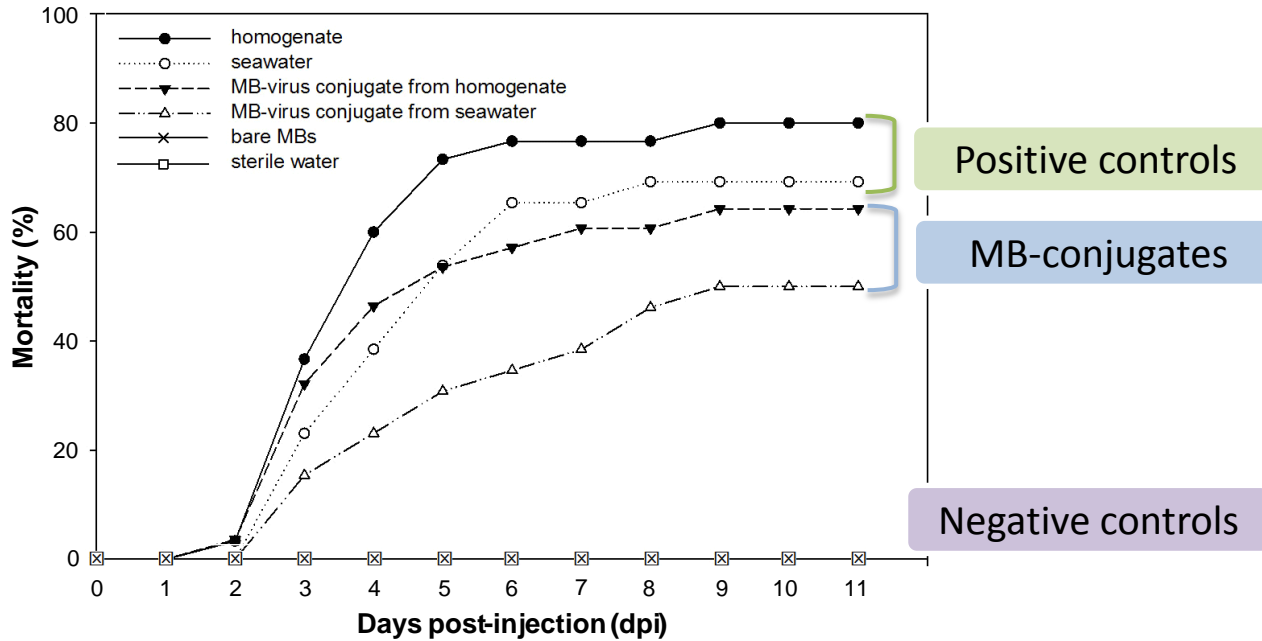
bare MBs



A. Mortality monitoring; B. DNA analyses; C. RNA analyses



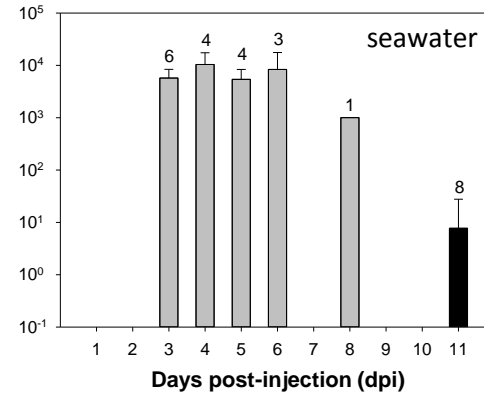
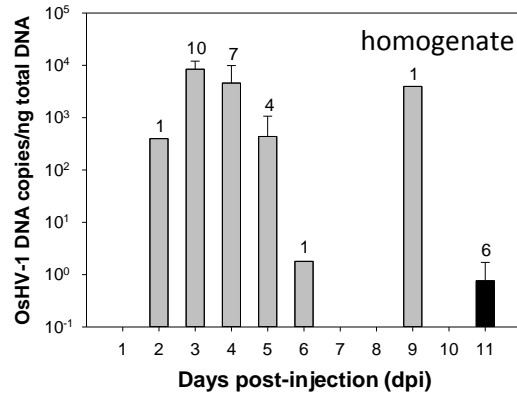
A. Mortality monitoring



MB-conjugates: able to infect oysters

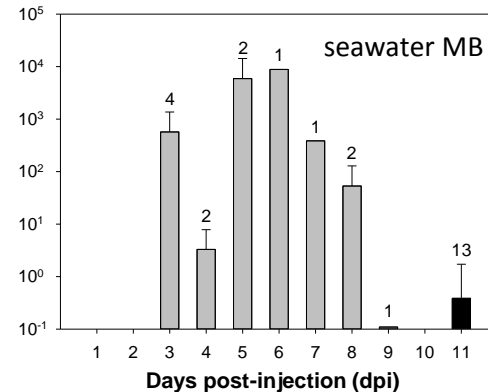
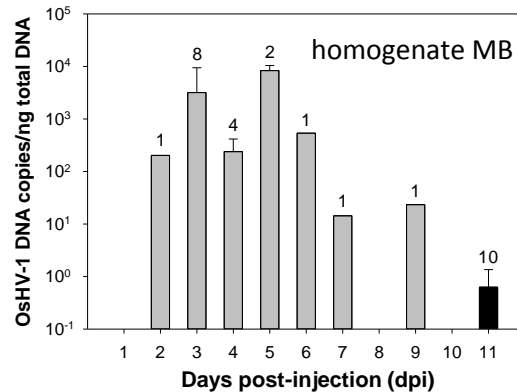
B. DNA analyses

Positive controls



dead/moribund oysters
 living oysters

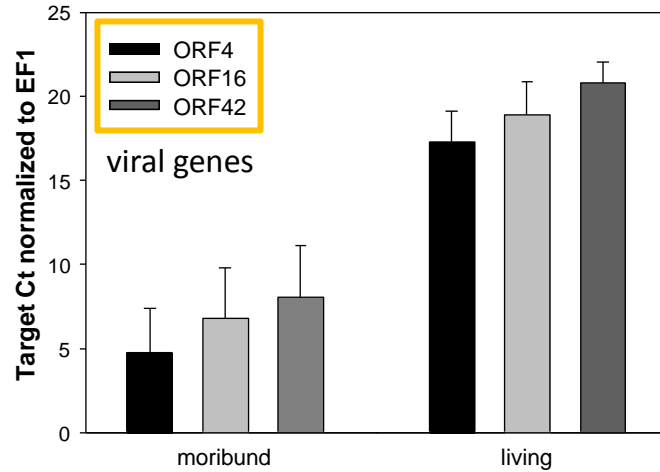
MB-conjugates



viral DNA loads:
 Moribund/dead oysters > living oysters

MB-conjugates: able to infect oysters

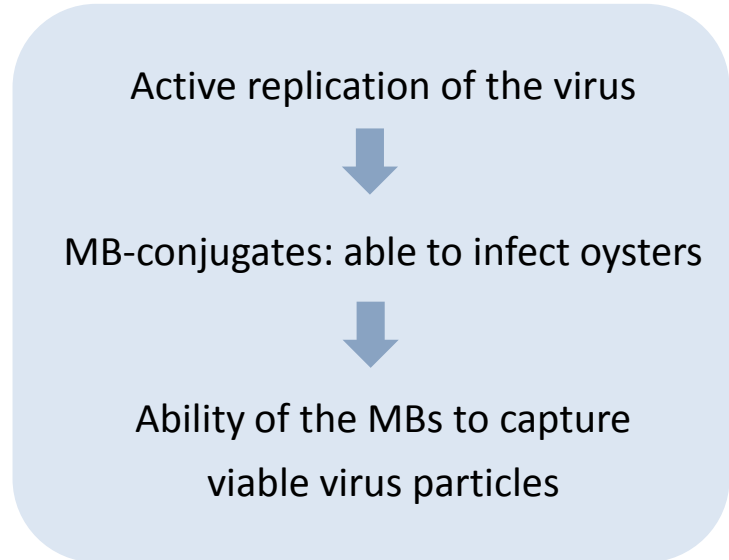
C. RNA analyses



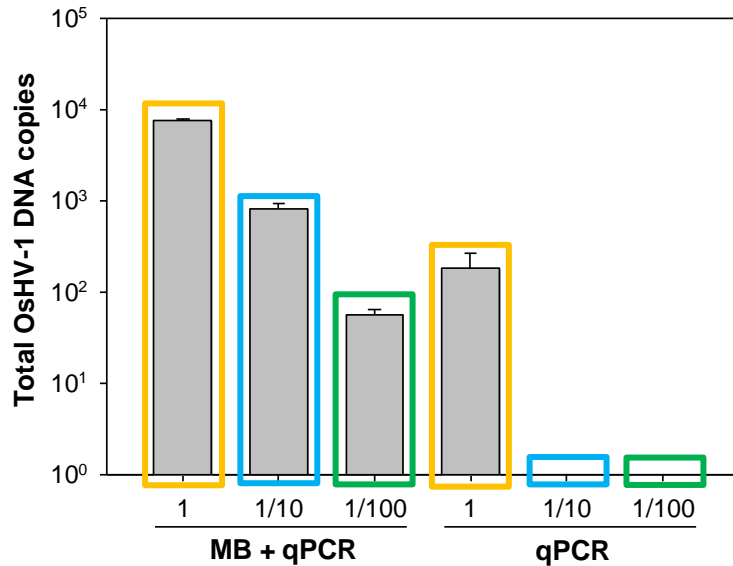
no differences among ORFs

↓ Ct → ↑ gene expression

viral gene expression:
moribund oysters > living oysters



Pre-concentrating agents: homogenatae



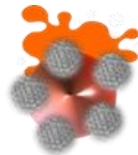
Experimental conditions:

MBs volume

Elution volume

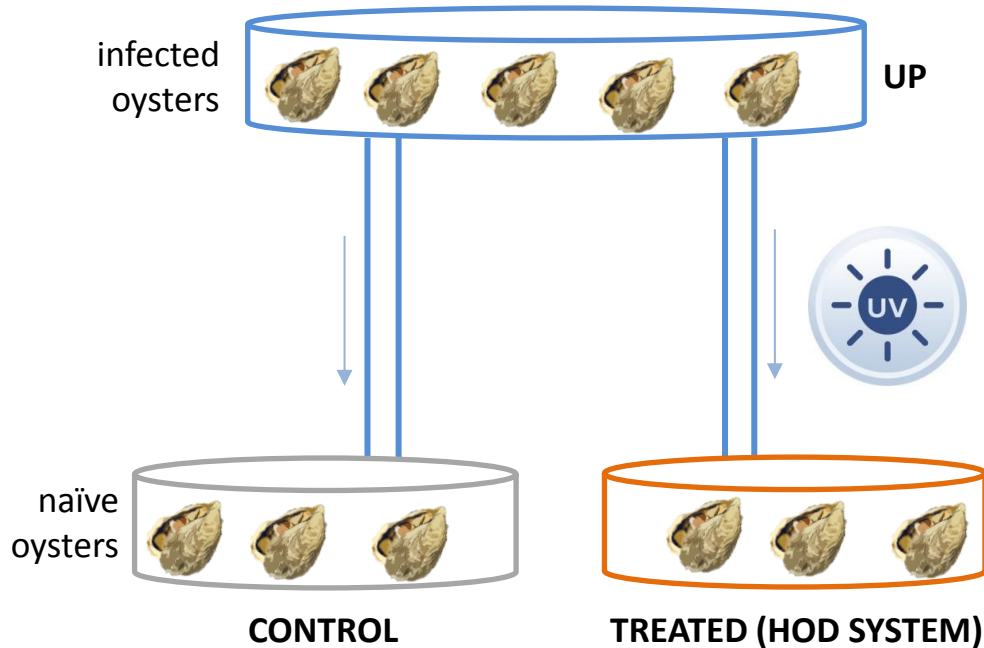
Sample volume

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

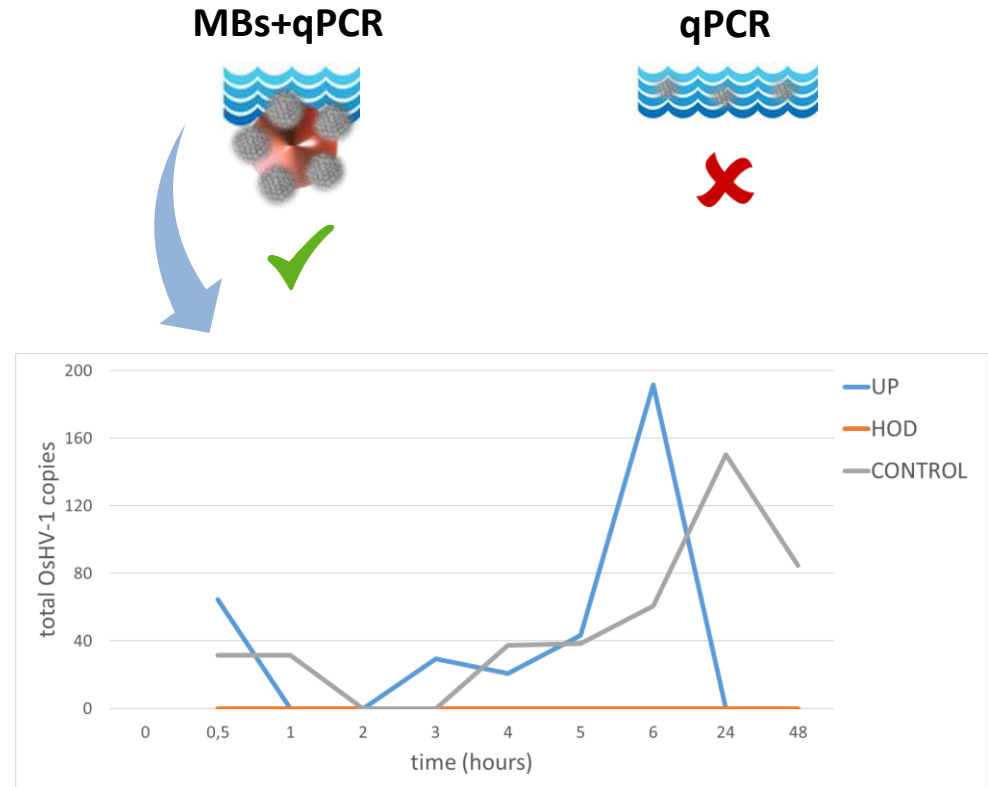


Pre-concentrating agents: seawater

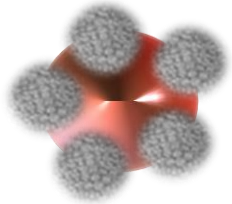
Application to seawater from a depuration experiment (April 2019)



- no oyster mortality
- no OsHV-1 in oysters



Summarising



- MBs are able to **capture OsHV-1** from both the homogenate and seawater matrices
- 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.



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