Session 1 : Preventing the entry of diseases

Deborah Cheslett (Marine Institute, IE)
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VIVALDI
Preventing and mitigating farmed bivalve diseases

Preventing the entry of diseases

Brief presentation of the main results of VIVALDI : Pathogen diversity, early warning systems and water treatment by UV irradiation

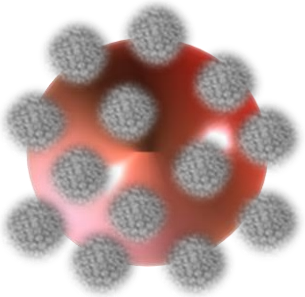
Works of interest to establish measures for the prevention of mollusc disease entry

- *Pathogenesis and molecular epidemiology of Ostreid herpesvirus 1 in China,* by Changming Bai (Chinese Academy of Fishery Sciences)
- *Ensuring the safety of shellfish for human consumption*, by Corinne Audemard (Virginia Institute of Marine Science, USA)
- *An overview of mollusc animal health controls in the EU,* by Niall Gerlitz (Directorate-General for Health and Food Safety, European Commission)

Round Table

Discussion about the expectations of experts and the tools studied in the VIVALDI project

Preventing the entry of diseases



Requires a knowledge / understanding of :

- the diversity & life cycles of pathogens present in the environment
- the potential routes of pathogen entry
- measures to mitigate against entry: early detection / inactivation
- A legislative framework to achieve control

VIVALDI's Approach:

→ To gain a better understanding of pathogen diversity, distribution & life cycles to allow the development of better tools

→ Early warning systems: development of biosensors to allow early detection of pathogens in the environment

→ Inactivation of pathogens: treatment of water supplying the farms as a tool to inactivate the pathogens and contribute to a better biosecurity (session 3)

Pathogen Diversity

Marteilia refringens and *Marteilia pararefringens* sp. nov. are distinct parasites of bivalves and have different European distributions

R. Kerr[1,2], G. M. Ward[1,2,3], G. D. Stentiford[1], A. Alfjorden[4], S. Mortensen[5], J. P. Bignell[1], S. W. Feist[1], A. Villalba[6,7], M. J. Carballal[6], A. Cao[6], I. Arzul[8], D. Ryder[1] and D. Bass[1,3]

High resolution molecular taxonomy for discriminatory diagnostics and monitoring

1. *Marteilia refringens* is split into ***M. refringens*** (previously O-type) and ***M. pararefringens*** n. sp. (previously M-type).

2. ***M. refringens*** was **not detected** in mussels, oysters, or environmental samples in the **UK, Sweden, or Norway**.

3. In the UK, Sweden, or Norway **only mussels** were found with ***M. pararefringens*** infections.

4. New *Marteilia*-specific 18S - 5.8S rRNA gene primers designed in this study amplifies M. refringens and M. pararefringens from animal-associated and environmental samples. No non-target sequences amplified.

5. *M. pararefringens* detected in filtered **water samples** near infected mussels; also in other **co-occurring invertebrates**.

Pathogen diversity

Group-specific studies of parasitic groups: discovery of novel parasites, lifecycle and ecological insight

Importance of taxonomic & expert knowledge of the organisms involved

Building a published information resource on all parasite groups of relevance to aquatic animal health

Contents lists available at ScienceDirect

International Journal for Parasitology

journal homepage: www.elsevier.com/locate/ijpara

A new phylogeny and environmental DNA insight into paramyxids: an increasingly important but enigmatic clade of protistan parasites of marine invertebrates ☆

Georgia M. Ward [a,b], Martyn Bennett [a,c], Kelly Bateman [a], Grant D. Stentiford [a,c], Rose Kerr [a], Stephen W. Feist [a], Suzanne T. Williams [b], Cedric Berney [d], David Bass [a,b,*]

The ISME Journal (2013), 1–10

www.nature.com/ismej

ORIGINAL ARTICLE

Lineage-specific molecular probing reveals novel diversity and ecological partitioning of haplosporidians

Hanna Hartikainen[1], Oliver S Ashford[1], Cédric Berney[1], Beth Okamura[1], Stephen W Feist[2], Craig Baker-Austin[2], Grant D Stentiford[2,3] and David Bass[1]

[1]*Department of Life Sciences, The Natural History Museum, London, UK;* [2]*Centre for Environment, Fisheries and Aquaculture Science (Cefas), The Nothe, UK and* [3]*European Union Reference Laboratory for Crustacean Diseases, Centre for Environment, Fisheries and Aquaculture Science (Cefas), The Nothe, UK*

Current Biology

Volume 24, Issue 7, 31 March 2014, Pages 807-812

Report

Mikrocytids Are a Broadly Distributed and Divergent Radiation of Parasites in Aquatic Invertebrates

Hanna Hartikainen [1, 4], Grant D. Stentiford [2, 3], Kelly S. Bateman [2, 3], Cédric Berney [1], Stephen W. Feist [2], Matt Longshaw [2], Beth Okamura [1], David Stone [2], Georgia Ward [1], Charlotte Wood [1], David Bass [1]

Follow-on mikrocytid paper in prep, including VIVALDI results

environmental microbiology reports

Brief report

Group-specific environmental sequencing reveals high levels of ecological heterogeneity across the microsporidian radiation

Bryony A. P. Williams, Kristina M. Hamilton, Meredith D. Jones, David Bass

First published: 26 March 2018 | https://doi.org/10.1111/1758-2229.12642

Pathogen Diversity

Development of a target enrichment NGS-based protocol to study "VIBRIOME" and "PATHOBIOME" in environmental and bivalve samples (TEBP protocol)

Marker selection (n=958)

BIVALVE DISEASE=Travers et al., 2015 Journal of Invertebrate pathology 131:11-31

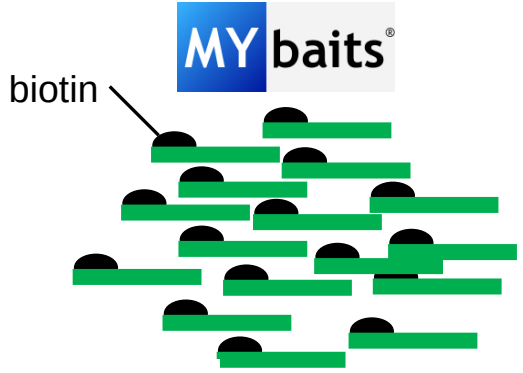
CORAL DISEASE=Rosenberg et al 2007., Nature Review Microbiology 5:355-362

Target enrichment protocol

(Vezzulli et al 2017)

Whole-Genome Enrichment Provides Deep Insights into *Vibrio cholerae* Metagenome from an African River

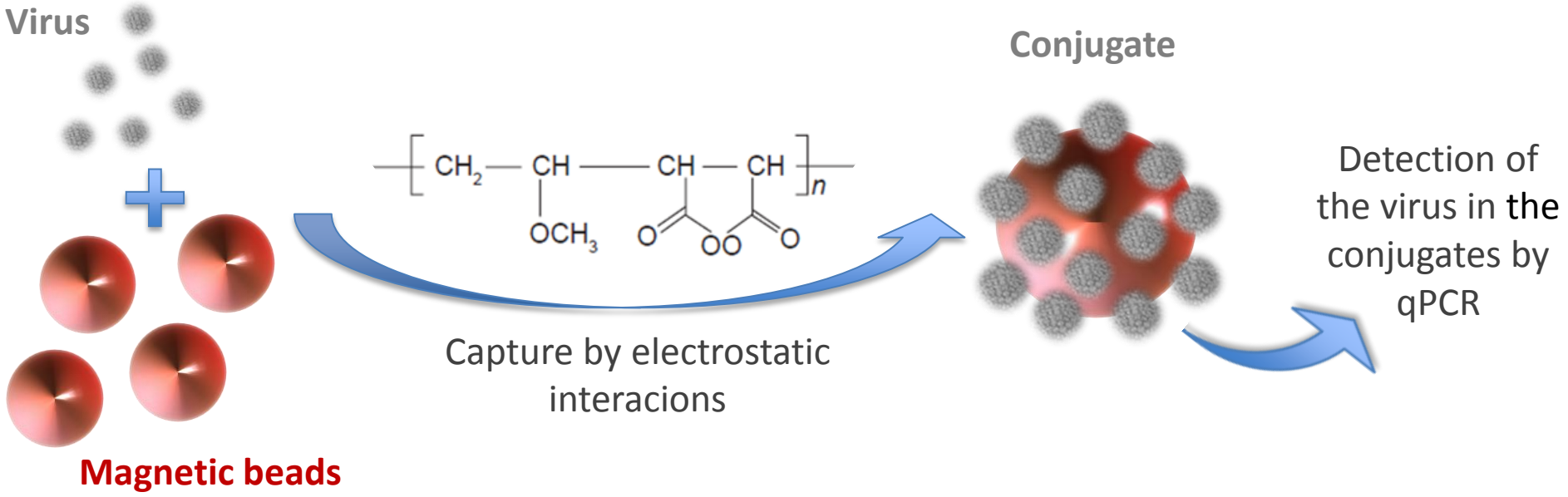
L. Vezzulli[1] · C. Grande[1] · G. Tassistro[1] · I. Brettar[2] · M. G. Höfle[2] · R. P. A. Pereira[2] · D. Mushi[2] · A. Pallavicini[3] · P. Vassallo[1] · C. Pruzzo[1]



Group	Strain	Main target host	marker
VIBRIOMA	Vibrio spp		gyrB (+consensus)
	Vibrio spp.		recA (+consensus)
	Vibrio spp.		atpA (+ consensus)
	Vibrio spp.		dnaJ (+consensus)
	Vibrio spp.		pyrH (+consensus)
	LGP32 probes		
VIBRIO virulence factors			
V cholerae	Vc O1 el Tor	Human	ctxA
	Vc O1 el Tor		ctxB
	Vc O139		ctxA-B
	Vc O1 el Tor		tcpA
	Vc O1 classical		tcpA
	Vc O1 el Tor		rstR
	Vc O1 classical		rstR
	Vc O139		wbf (antigen)
	Vc O1 el Tor		gbpa
V parahaemolyticus		Human	toxR
			tdh and trh
V vulnificus		Human	vvhA
			rtxA1
V tasmaniensis		Crassostrea gigas	vsm (LGP32 strain)
			ompU (LGP32 strain)
V aestuarianus		Human	vam
V tapetis		Ruditapes philippinarum	djlA protein
V coralliilyticus		Paramuricea clavata	vcpA
V harveyi		Stony corals	vhhA
V crassostreae		Crassostrea gigas	R-5.7
V tubiashii		Crassostrea gigas	metalloprotease
V cholerae, V vulnificus, V parahamolyticus ..etc			MSHA
ARCOBACTER	Arcobacter spp	Crassostrea gigas ?	gyrB
NOCARDIA	Nocardia crassostrea	Crassostrea gigas, Ostrea edulis	rpoB, hsp65, gyrB
			16S probe1
MARTELIA	Martelia refringens	Ostrea edulis, Mytilus edulis, M. galloprovinc	18-28S rDNA, ITS1O, ITS1M, probe, 18SrDI
BONAMIA	Bonamia ostrea	Ostrea edulis	5.8S-ITS rDNA, hsp90, act1
			18S probe1
			18S probe2
OsHV-1	Ostreid herpesvirus 1	Crassostrea gigas	C2/C6 (2), IA1-IA2, orf4, Hyp. Protein (2),
			ORF100
			C9-C10
			B3-B4
ENTEROCOCCUS SPP	E. faecalis,E faecium, E avium, E gallinarum, E casseliflavus, E durans, E raff		atpA
ROSEOVARIUS	Roseovarius crassostrea	Crassostrea virginica	16-23S IS rDNA, dnaJ, PyrH
ESCHERICHIA	Escherichia coli		dnaJ, PyrH, atpA, gyrB
ASPERGILLUS	Aspergillus sydowii	Gorgonia ventalina, Human	TUB2,trpC,ITS,calmodulin gene, 18SrDNA
RICKETTSIA LIKE ORGANIS	Rickettsia like organism	Aquatic bivalves	16rDNA
AURANTIMONAS	Aurantimonas coralicida	Corals (White plague in the Caribbean)	atpD, gyrB, recA, rpoB
SERRATIA	Serratia marcescens	Acropora palmata (White pox in the caribbean)	gyrB, recA, dnaJ
PSEUDOALTEROMONAS	Pseudoalteromonas sp. N	Rhopaloeides odorabile	gyrB

Bio sensors : goal and strategy adopted by IRTA

Investigate the use of MBs to capture viable OsHV-1 particles from naturally infected matrices (to assess the presence of infectious viruses and to lower the limits of detection).



Bio sensors : protocol

Oysters from a mortality event, Fangar bay, April 2017

«homogenate»

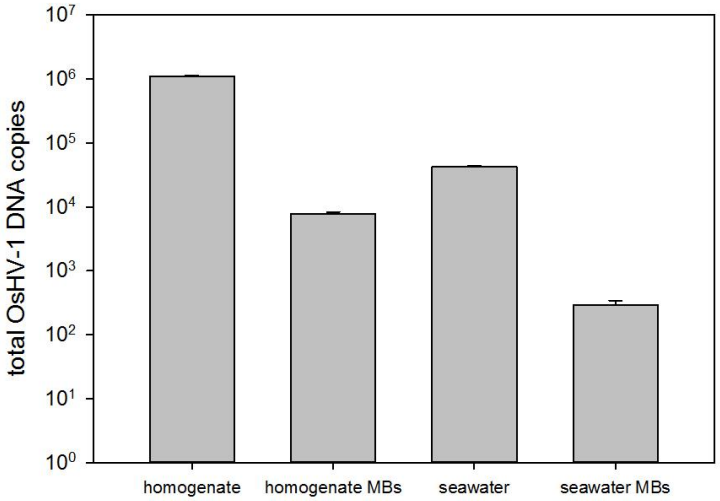
Magnetic beads

C

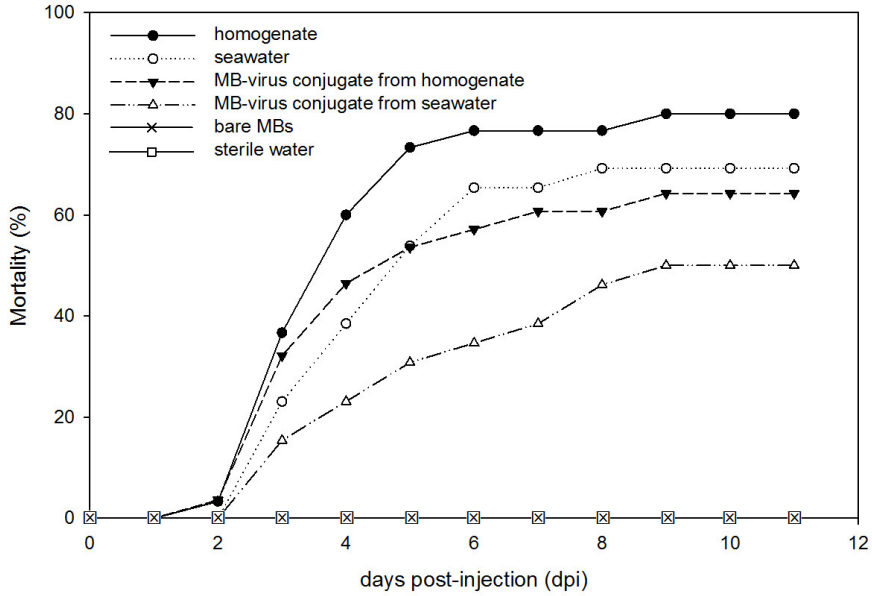
«seawater»

Magnetic beads

Bio sensors : first results with qPCR analysis and mortality tracking



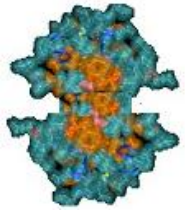
Magnetic beads are able to capture the virus from both the homogenate and seawater.



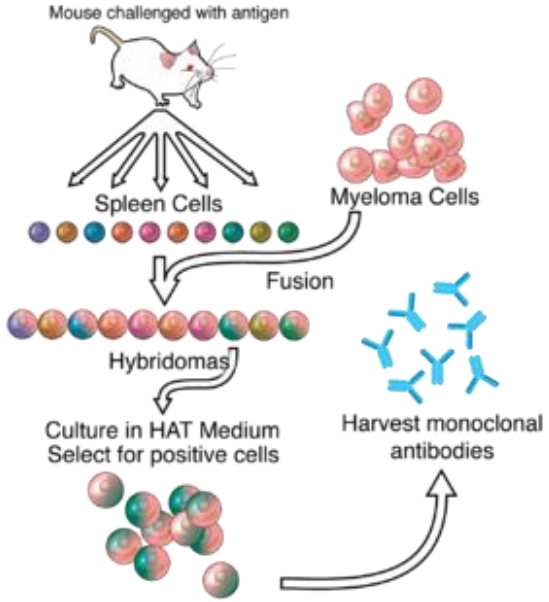
Both conjugates have the ability to infect oysters.
Mortality trend: homogenate seawater
«homogenate» conjugate «seawater» conjugate
No mortality with bare magnetic beads
(and with «nothing»).

Biosensors: QUB

Viral Protein Purification



Immunisation



Based on the literature & materials received

Immunisation strategy developed
using Balbc mice for antibody production

- Dose
- Frequency of dose
- DNA material
- Crude extracts

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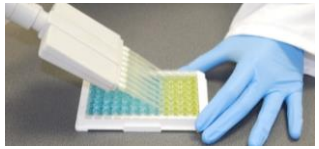
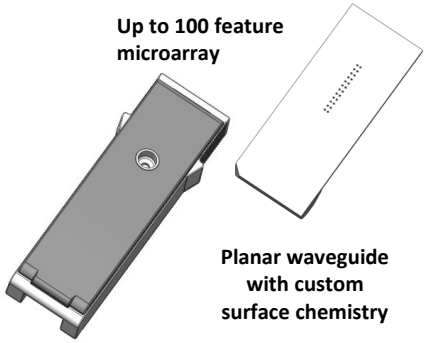
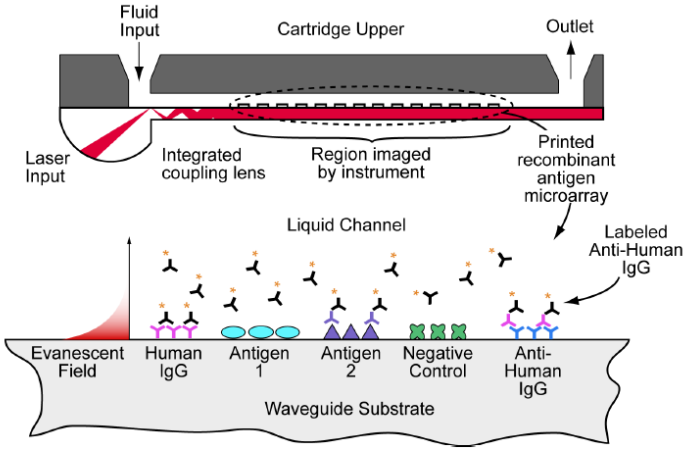
Challenge is titre for sensitivity & specificity



Immunisation : rapid multiplex Mbio diagnostics





Aim to produce platform offering

- Low cost analysis
- Simplicity in use
- Highly specific single target analysis
- Multiplexing – multiple target analysis
- Bespoke sensitivity
- Robust – high performance
- Field deployable

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- **Molecular level – DNA / RNA for pathogen testing**
- **Protein level – Allergens / Biomarkers**
- **Residual level – Low molecular weight toxins & contaminants**

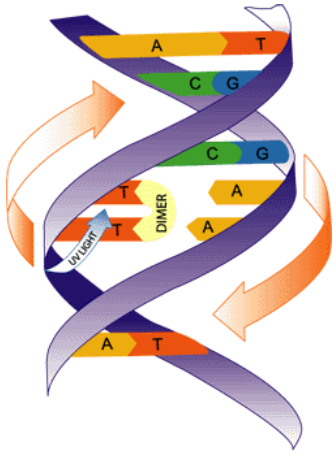
Inactivation of pathogens : Atlantium's Medium Pressure UV

- Applications represent critical points of control evaluated in VIVALDI in several experimental sites :

- **Intake:** protecting the hatchery for all incoming water (especially in the more "sensitive" and vulnerable stages of production) → Ifremer station of Bouin (France)
- **Outflow:** safeguarding the environment from contamination created during production as well as ensuring no invasive species reach the environment (larva also sensitive to UV light) → Ifremer station of Bouin
- **Depuration:** ensuring high quality / purity water are used during this critical stage of production where batch losses are of the greatest economic ramifications → IRTA

Inactivation of pathogens: Atlantium's Medium Pressure UV

How does Atlantium Medium Pressure UV light work?



- Medium Pressure UV light inactivates cells by damaging their DNA and their repair mechanism
- It also has the ability to decompose trace contaminants with or without oxidizing agents.
- **The UV dose equation:** a fundamental tool to match operational conditions and needs on site with the adequate UV system.

UV DOSE	=	FLOW RATE	+	LAMP POWER	+	WATER UVT	+	UV system design
The minimum UV dose required for bio-security against the target pathogens		The higher the flow rate, the less UV dose per given amount of energy		The higher kW ratio per water volume the higher the UV dose		Higher UV transmittance yields higher UV dose per given kW consumption		The design and built of the UV system determines how effective the UV light is used in the disinfection chamber

Inactivation of pathogens: Atlantium's Medium Pressure UV

Determining the required UV dose

Pathogen	Log Inactivation
Nocardia Crassostreae	>3 (LP) >4 (MP)
OsHv-1	>6
V. aesturianus	>6
V. splendidus	>6
V. harveyi	>6
V. tapetis	>6

- Required UV dose is determined according to the defined target microorganism/s.
- Medium pressure lamps exhibit clear advantage over low pressure lamps in terms of:
  - Required UV dose for inactivation (less UV dose is needed to achieve same log reduction)
  - Bacteria re-generation: with MP lamps little or no regeneration was observed.
- The table on the left lists some of the tested microorganisms with the respective log reduction achieved by the Atlantium medium pressure UV lamps.

Inactivation of pathogens: HOD application in IRTA

Water treatments to prevent spread of pathogen and active mollusc stages (gametes and spat) including in shellfish depuration plants (SDP)

IRTA

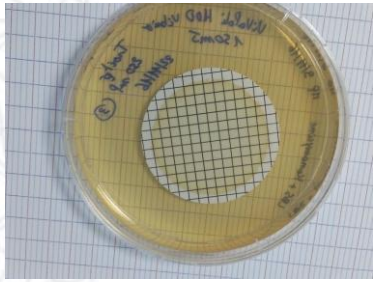
- After meeting with representatives from Antlantium lead to the installation of the HOD system, the device is now ready for performing experimental trials on depuration facility to evaluate such facilities contributions to kinetics of spread of OsHV, and amelioration of same using HOD systems.

- Local water quality parameters for UV transmission were evaluated and found to be well within operational guidelines for proper functioning of the HOD system

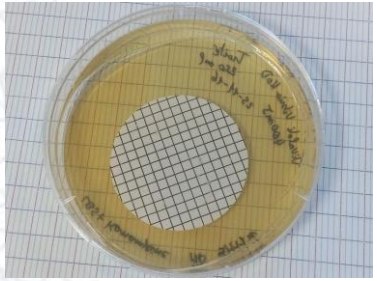
Inactivation of pathogens: HOD application in Ifremer

Water treatments to prevent spread of pathogen and active mollusc stages (gametes and spat) including in shellfish depuration plants (SDP)

Ifremer Bouin

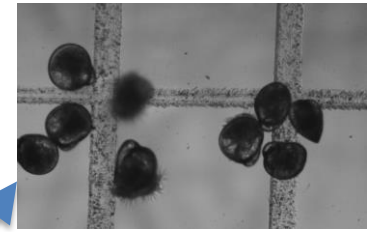


***V. Aestuarianus* analysis on a specific culture media**

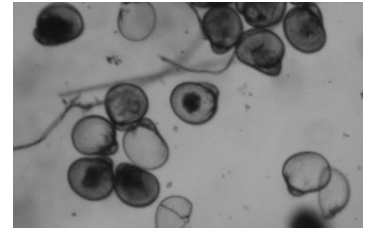


Analysis of treated water with HOD

- HOD system was tested to inactivate *Crassostrea gigas* gametes and larvae, *V. aestuarianus* pathogen and microalgae.
- Experimental conditions could only allow the use of high UV doses (>300 mJ/cm2) leading to the death of larvae (200 µm) and gametes
- UV doses between 40 and 100 mJ/cm2 are sufficient to obtain a 6 log inactivation of pathogens (including OsHV-1) and microalgae
- Additionals experiments are planned to determine the minimal UV dose required to inactivate all the targets cells in real conditions of treatment



Before HOD …



… after HOD

Conclusions

- Study of pathogen diversity, distribution and life cycles:
  - development of better diagnostic tools
  - Better understanding of disease processes / markers of disease
  - More accurate / rapid diagnosis

- Biosensors provide the potential for early warning systems
  - Allowing producers time to respond to threat

- Development of effective UV water treatment systems against known pathogens under "real" conditions
  - More effective prevention of disease entry into controlled systems
  - Reduce the likelihood of dissemination of pathogens to ongrowing sites and increase the efficiency of shellfish depuration plants
  - Allow for the establishment of disease free hatcheries in infected compartments

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