



Australian Government  
Fisheries Research and  
Development Corporation



# FINAL REPORT

## OsHV-1 $\mu$ Var

Ostreid herpesvirus-1  $\mu$ Var

# INTERNATIONAL WORKSHOP

Cairns, Queensland Australia

9-10 July 2011

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## 1. SUMMARY OF MAJOR FINDINGS

### THE DISEASE

- Ostreid herpesvirus-1 microvariant (OsHV-1  $\mu$ Var) has been associated with high mortality events involving the Pacific oyster (*Crassostrea gigas*) in Europe, Australia and New Zealand.
  - In France, these higher mortalities started in 2008 and have continued in 2009 and 2010
  - UK, Jersey, Ireland and the Netherlands have all experienced mortalities
  - In Australia, mortalities occurred in late 2010 and, to date, appear to be limited to two estuaries
  - In New Zealand, mortalities may have started in early 2010 and OsHV-1  $\mu$ Var was confirmed with a second round of mortalities in late 2010. The virus appears to be widespread in the northern part of the North Island.
- The disease is characterised by high mortalities in Pacific oysters. All ages may be affected, but spat and juvenile oysters often suffer higher mortalities. Other species may become infected but have not exhibited mortalities associated with the virus.
- There is no evidence that the virus can infect humans.

### THE VIRUS

- The virus is characterised primarily by a 13 base-pair deletion in the C region of ORF4.
- Phylogenetic studies indicate that the New Zealand strain shared a 13bp deletion in the non-coding area of the C-region, although minor other point mutations in the coding area of the C-region were not present in the NZ isolate as compared with the French strain of OsHV-1  $\mu$ Var. The Japanese and Chinese isolates (despite absence of reports of large scale mortalities) are also more closely related to OsHV-1  $\mu$ Var than the reference strain of OsHV-1.
- The OsHV-1  $\mu$ Var has completely replaced classical OsHV-1 as the dominant strain isolated during mortality events from oysters in France since mid-2008.

## DIAGNOSIS

- Increased mortality rate
- Histopathology is generally non-specific, but recent work indicates that it may be possible to identify typical lesions. Histological examination continues to play an important role to exclude other possible causes of mortalities such as infection with protozoan parasites.
- PCR is used for surveillance and for confirmation of suspect cases.
  - A variety of different PCR tests are being used, with different primers and formats.
  - This lack of standardisation may pose a potential problem. Current work to draft a chapter on OsHV-1  $\mu$ Var for the *OIE Manual of Diagnostic Tests for Aquatic Animals* is likely to help resolve this lack of standardisation.
  - Existing tests have not been adequately validated.

## TRANSMISSION AND SPREAD

- Transmission is horizontal and is likely to occur through the water body.
- There is some evidence from New Zealand that uninfected larvae may be able to be produced from infected brood stock.
- While sub-clinical infections may occur, it is not known if a true latent infection occurs.
- Spread is most likely through the movement of live oysters, although spread by movement of equipment is also possible.
- It was hypothesised that international spread may have taken place in association with biofouling (i.e. oysters attached to the hulls of ships).

## SURVEILLANCE

- Passive farmer reporting is the main system used to identify new outbreaks in all countries.
  - Currently, on-farm mortality events occur relatively frequently for a variety of reasons. Many of these events are not reported.
  - Current reporting pathways are not clear to all growers
  - Collation, analysis and response may need improvement
  - An industry benchmarking project may offer a mechanism for improving passive reporting

- Structured surveys are underway in Australia and the UK to demonstrate freedom from infection in the non-affected areas.
- Few mechanisms exist to routinely collect risk factor data (other than data loggers for water temperature and water quality).

## RISK FACTORS

- The disease is multifactorial. Factors that are likely to be associated with the disease include:
  - Host
    - Pacific oyster
    - Spat and juveniles appear to be more susceptible
    - Rapidly growing oysters may be more susceptible
    - Genetics: There is evidence of decreased mortalities in certain Pacific oyster families in challenge trials
  - Pathogen
    - OsHV-1  $\mu$ Var
    - Evidence, especially from France, of co-infection with other pathogens including *Vibrio* spp. and various parasites.
  - Environment
    - Water temperature: Outbreaks are rare below 16°C or 18°C
    - Water quality
    - Depth: Intertidal oysters may be less affected
    - Proximity to other outbreaks: Remote oysters are less affected.
- The role of the following risk factors is inconsistent:
  - Wild vs. hatchery spat
  - Triploid vs. diploid oysters
- The following factors appear to play no important role
  - Salinity

## REGULATIONS

- Current control measures are based on restrictions of movements of oysters and, in some countries, infrastructure out of affected estuaries.
  - Legislative controls in different countries vary, as do the strength of the control measures.

- OsHV-1  $\mu$ Var appears to meet the criteria for being listed by the OIE, but no request for listing has yet been made by an OIE member state.
  - Listing would provide increased information about the global distribution of the virus, help limit the spread, and may assist in obtaining funding for research and surveillance. On the other hand, listing would limit exports from affected countries.

## CONTROL MEASURES

- Strategies currently being used, or under research, for controlling the disease when it is already present include:
  - Breeding Pacific oysters for resistance
  - Use of alternative, resistant species
  - Growing hatchery spat to a larger size before stocking
  - Avoidance of stocking susceptible animals during periods of warmer water temperature
  - Stocking larger numbers of spat to compensate for the expected losses
  - Emergency harvest in the face of possible outbreaks

## 2. RECOMMENDATIONS

### COORDINATION AND COLLABORATION

1. Establish an international OsHV-1  $\mu$ Var expert **technical advisory and coordination group** to promote and coordinate collaborative work in the following areas:
  - Phylogenetic studies
  - Histology
  - Test validation
  - Global surveillance

It is proposed that the FRDC Aquatic Animal Health Subprogram (AAHS) may initially form the basis of this group. In order for AAHS to take on the role of the technical advisory and coordination group it may wish to consider:

- Industry participation to include oyster industry representative/s
- Inclusion of the Australian state jurisdictions involved in oyster production

- Establishment of international links and collaboration, in particular with IFREMER, OIE Reference Laboratories, and industry and researchers from not only affected countries but also unaffected countries with large oyster industries.
  - Pursuing active engagement with **Asian industry and researchers** to promote collaborative research and sharing of surveillance information. Asia has the world's largest Pacific oyster industries. Sharing any experience Asian producers may have with similar diseases, and collaborating with them to develop measures for early detection and control, would be mutually beneficial.
  - Actively engaging **major oyster-producing countries** (affected and unaffected) in collaborative research and collaborative funding of research. Unaffected major oyster-producing countries (such as, presumably, China, Japan, Korea and the USA) have a vested interest in improving our understanding of OsHV-1  $\mu$ Var in order to improve quarantine, preparedness and rapid response capabilities. Funding offshore research may be seen as an excellent investment.
  - Membership should therefore represent the following groups (from both within Australia and internationally):
    - Laboratory scientists
    - Epidemiologists
    - Regulators from national and state jurisdictions
    - Farmers
    - Hatchery managers
2. **Mechanisms for the continuation of the dialogue** that has been promoted by this workshop between researchers, regulators and industry should be considered. This may involve a similar or smaller group meeting every year or more frequently depending on the evolution of the disease.
3. Seek greater cooperation with organisations responsible for **shipping**, environmental management and quarantine. Biofouling has been identified as a possible route of introduction of OsHV-1  $\mu$ Var as well as other diseases. Current attention appears to be focused solely on protection against pest species, but the risk of introduction of microorganisms needs to be considered. Measures to address the risk of consumers spreading the disease in oysters for consumption should also be considered.

## DIAGNOSTICS

4. Support processes to **standardise diagnostic tests**, in particular PCR. Without limiting the active development of new and improved tests, researchers should support the current initiatives in the development of a chapter on OsHV-1  $\mu$ Var for the *OIE Manual of Diagnostic Tests for Aquatic Animals*. This chapter will provide guidance on diagnostic testing options and may have legal status for international trade if the disease is listed by OIE. Scientists working on the development and application of OsHV-1  $\mu$ Var diagnostic PCR techniques should ensure that they have input into the draft chapter when it is circulated to member countries for comment before it is considered for adoption (probably in May 2012).
5. Continue current efforts and exploit available opportunities to **validate currently available tests** where possible. If appropriate, utilise the results of large-scale surveillance (such as that currently being undertaken in Australia and UK) to better characterise the performance (diagnostic sensitivity and specificity) of key tests, using techniques that do not require a gold standard.
6. AAHL should consider preparation and distribution of **standard positive controls** for PCRs to assist in test standardisation and quality control. This must be done in response to requests from laboratories (Tasmania has agreed to pursue this issue).

## RESEARCH

7. Undertake further **phylogenetic studies** to better understand the relationship between geographical isolates and thus possibly inform paths of spread of OsHV-1  $\mu$ Var.
  - Continue work on **full genome sequencing** currently being undertaken at different laboratories, to assist better understanding of possible virulence and pathogenicity mechanisms.
  - Include sequences from Australian and New Zealand isolates (amongst others) in **phylogenetic analysis** being conducted in France, to determine the relationship between the viruses and possible paths of spread.
  - Investigate the use of other sequencing techniques to provide **higher resolution analysis** of molecular differences between isolates of OsHV-1  $\mu$ Var.

8. Promote **collaboration between histologists** in France, Australia and elsewhere to more precisely characterise histopathology associated with OsHV-1  $\mu$ Var infection.
9. Noting that France has developed an experimental infection model, establish a standardised **experimental infection model** to facilitate research into the effect of different risk factors. Several States and organisations have the capacity to undertake this work in Australia with SARDI, EMAI and USyd expressing interest to do so.
10. Undertake a range of specific studies to better understand **disease spread and transmission**.
  - Studies to investigate whether the virus is **transmitted vertically**, and, as has been suggested, whether methods are available to produce uninfected larvae from infected brood stock.
  - Studies into the **role of different species** (other than the Pacific oyster) in maintaining and spreading the infection. This should address the issues of (a) whether other species are able to become infected (with replicating virus) or merely become passive carriers (mechanical vectors of the virus), (b) the duration of infection or carriage, (c) their ability to spread the infection, and any possible role they may play in decreasing the viral load in the environment.
  - Studies into the **persistence of infection** including the existence of subclinical carriers.
11. Undertake research into the possibility of **inducing immunity** to viruses in oysters.
12. Continue the analysis of possible **entry pathways** currently being undertaken by DAFF.
13. Support epidemiological analysis of the role of different purported **risk factors** in causing the disease, with the aim of identifying possible control mechanisms or predicting periods of high risk for disease outbreaks.

## SURVEILLANCE

14. Consider effectiveness of the **passive farmer reporting** system in preparation for the 2011-12 southern hemisphere summer risk period. The system should aim to provide rapid reporting of mortalities that may be associated with OsHV-1  $\mu$ Var.

- Establish a simple unambiguous **definition of when reporting is required**. Current regulations generally include a mortality threshold (e.g. 5%) and the phrase ‘unexplained mortalities’. Assessing mortality rates is often difficult.
  - Consider ways to determine what constitutes **normal and abnormal mortalities**, and prioritise response and investigation on this basis.
  - Clarify **disease reporting pathways** and responsibilities so that all stakeholders are aware of how, when and to whom to report, and what to do with any reports received.
  - If possible, minimise any **negative consequences** of reporting. When a significant event, e.g. suspect exotic disease outbreak, is reported (and requiring immediate movement standstill), ensure that communication and mechanisms are in place to achieve rapid laboratory confirmation or exclusion, so the standstill does not cause unnecessary hardship to the industry.
15. With agreement between industry and regulators, and on a cost/benefit basis, consider development of a comprehensive system for reporting, managing and analysing data on **transfers of oysters** within and between estuaries, including movements of hatchery and wild spat. These data will provide a valuable resource for understanding the spread of OsHV-1  $\mu$ Var, for planning appropriate emergency control strategies to limit disease spread while minimising the impact on industry, and provide a basis for management of possible future diseases.
16. Consider development of systems to capture **data on risk factors** possibly associated with OsHV-1  $\mu$ Var infection and other oyster diseases. Data on risk factors can support epidemiological studies which aim to:
- better evaluate the role of different risk factors
  - develop improved control measures
  - predict the risk of disease occurrence

Risk factors of interest relate to management and environmental factors as well as the host population at risk. To understand the role of possible risk factors, they must be measured in both affected and unaffected areas. Ideally, such a system would be in place before the southern hemisphere summer risk period.

Links should be established to existing data sources of interest, for example, databases of environmental data derived from data loggers.

17. Explore the possibility of establishing a **national industry-run, event-based database** to achieve the objectives of recommendations 144, 155 and 166. The database would capture data on:

- Population distribution
- Movements
- Mortalities
- Management and environmental risk factors

Any system should capitalise on existing activities such as the benchmarking project, and be designed to minimise the data collection burden on farmers (for example, using telephone or a digital diary for reporting) and minimise the data analysis and reporting burden (through automated analysis and alerts).

18. Establish the global distribution of OsHV-1  $\mu$ Var. There is little information available about mortalities or testing in the major oyster-producing countries of Asia (in particular) or other parts of the world. This should be an initiative of the OIE Reference Laboratory, through collaboration with scientists and regulators in the major oyster-producing countries.

19. Investigate any shellfish mortality to exclude OsHV-1  $\mu$ Var as a possible cause. Retrospective studies on previous scallop mortalities for OsHV-1  $\mu$ Var should be undertaken.

## PREPAREDNESS

20. Further develop industry, state and national **contingency plans** to allow rapid response to mortality events that may be due to OsHV-1  $\mu$ Var.

Components of the contingency plan should include:

- Restricting movements of oysters, equipment and other shellfish out of affected estuaries
- Establishing systems for rapid diagnostics (see recommendation 144)
- Development of standard biosecurity guidelines for farmers and provide training in the application of the guidelines
- Development of documented Good Management Practices for data collection, response, control and surveillance
- Training of stakeholders in responsibilities and responses during an outbreak, including industry training in sample collection
- Providing advice to industry on testing protocols and costs

- Providing advice to industry on management measures as well as technical, financial and personal resources available should the disease become established.
21. Develop effective communication with media outlets and the public, relating to disease outbreaks, that emphasises that the disease poses no threat to human health.

## CONTROL

22. Continue and support research on selective breeding to develop oysters that are resistant to the virus.
23. In Australia, industry and regulators should support all measures to contain infection to the current two affected estuaries in NSW (Georges River and Parramatta River).
24. Consider potential management strategies to minimise the impact of the disease in affected areas:
- Minimise the movement and handling of oysters to minimise stress during outbreaks or risk periods
  - Grow spat to a larger size before stocking after the end of the risk period, and attempt to grow them to marketable size before the start of the next risk period
  - Explore the use of alternative species that are not susceptible to the virus (for example, Sydney rock oysters (*Saccostrea glomerata*), flat oysters (*Ostrea angasi*) or pipis (*Paphies australis*)), either as replacement product, or to decrease the viral load in the water column and limit the impact on Pacific oysters (*Crassostrea gigas*).

## 3. INTRODUCTION

### OBJECTIVES

The objectives of the workshop were to:

- Review current knowledge about OsHV-1  $\mu$ Var, in particular in relation to its detection, epidemiology and current global distribution
- Assess current practices for surveillance, prevention and control of the associated disease and provide recommendations to national, regional and global authorities for their improvement

- Identify priorities for further research, and plan and coordinate research activities between international partners

## VENUE

The workshop took place at the Pullman Reef Hotel, 35-41 Wharf Street, Cairns, Queensland, Australia which was the same venue as the First Australasian Scientific Conference on Aquatic Animal Health held during the preceding week. The program commenced at 9:00 am on Saturday 9 July and ran through to 5:00 pm on Sunday 10 July.

## PARTICIPANTS

See [appendix](#).

## PROGRAM

See [appendix](#).

## FORMAT

The workshop did not consist of formal presentations by different participants, but instead focused on structured discussion of a series of topics and brief informal presentations from those with knowledge and experience of the topic.

Participants were invited to contribute to the discussion for those topics in which they had experience or expertise. Participants were requested to prepare brief materials to assist the discussion and documentation, either as a MS Word document or a few MS PowerPoint slides.

A great deal of material was covered and a relatively large number of participants (over 30) attended. The facilitators therefore limited discussion on some topics in the interest of time. It was thought likely that a disproportionate amount of time would be given for contributions from those participants with the most experience of the disease, but questions and interactions were received from all participants.

## FACILITATORS

The workshop was facilitated by Drs Angus Cameron (Director, AusVet Animal Health Services) and Mark Crane (FRDC Aquatic Animal Health Subprogram Leader and Research Team Leader, AAHL Fish Diseases Laboratory, CSIRO Livestock Industries).

## 4. THE DISEASE

### WHAT IS THE DISEASE? OsHV-1 $\mu$ VAR

The workshop focussed its interest on the detection and identification of the Ostreid herpesvirus-1 microvariant (OsHV-1  $\mu$ Var) in the presence of high mortalities amongst Pacific oyster (*C. gigas*) populations, whether farmed or wild.

The disease associated with infection by OsHV-1  $\mu$ Var has been given several names, for example, in New Zealand it was initially referred to as a herpes virus but after consideration of the possible negative effect this might have on market perceptions it was changed to Juvenile Oyster Mortality Syndrome (JOMS). First reports from NZ suggested that some supermarket chains ceased oyster purchases, but more recent information noted that the initial public reaction was motivated by an initial “giggle factor” association with human Herpes Simplex but that little lasting impact has been seen in the marketplace. In New South Wales, the first outbreak occurred just prior to the Christmas market season and it was therefore decided to refer to the disease as Pacific Oyster Mortality Syndrome (POMS) in an attempt to reduce any negative impact to the industry.

For the purpose of the workshop it was agreed that the disease under discussion was high mortality in Pacific oysters associated with the presence of OsHV-1  $\mu$ Var. This excludes “summer mortality” not associated with OsHV-1  $\mu$ Var, and disease caused by strains of OsHV-1 other than OsHV-1  $\mu$ Var.

## 5. EMERGENCE AND DISTRIBUTION

### WHERE IS IT?

Infection with “classical herpes” viruses is known to occur in a large number of mollusc species and is found widely, however OsHV-1  $\mu$ Var has thus far been declared in the EU, Australia and New Zealand.

### EMERGENCE AND DISTRIBUTION IN EUROPE

#### France

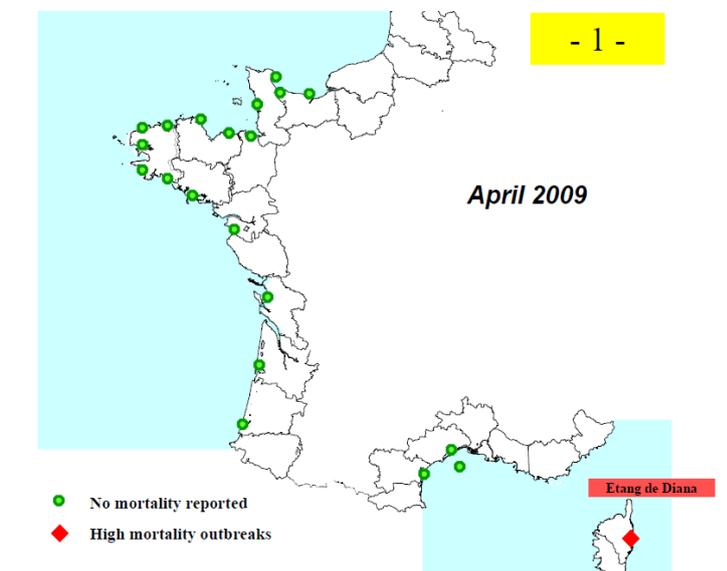
France reported high mortalities in Pacific oysters (*C. gigas*) associated with Oyster Herpes Virus in the presence of *Vibrio splendidus* in April-May 2008<sup>1</sup> during

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<sup>1</sup> [http://web.oie.int/wahis/public.php?page=single\\_report&pop=1&reportid=7288](http://web.oie.int/wahis/public.php?page=single_report&pop=1&reportid=7288)

investigations into widespread mortalities along the French coasts. Mortality rates in spat (up to 12 months old) and in juveniles (up to 18 months old) between 40% and 100% were reported. The pattern of occurrence was not a smooth progression from south to north as has been observed in outbreaks of classical OsHV-1, but rather outbreaks occurred at scattered sites along the coastline prompting the hypothesis that the disease was spread by the movement of animals.

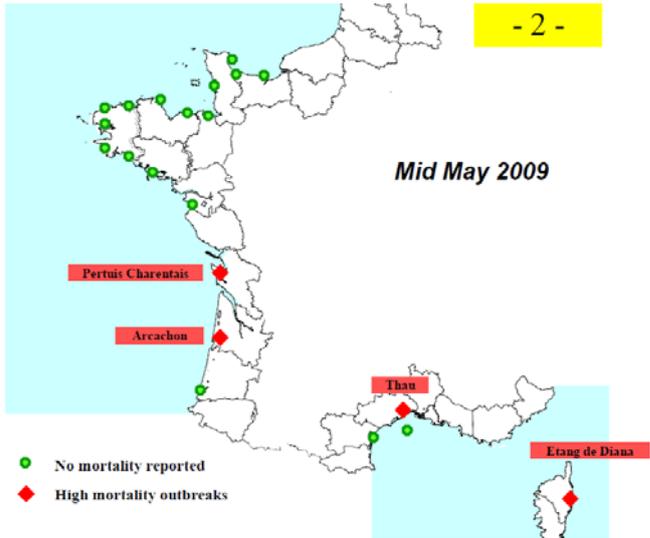
In 2009<sup>2</sup> the disease progressed more smoothly from the south to the north as water temperatures increased. This suggested that the virus may have already been widely distributed by this stage and outbreaks were initiated by an increase in temperature.



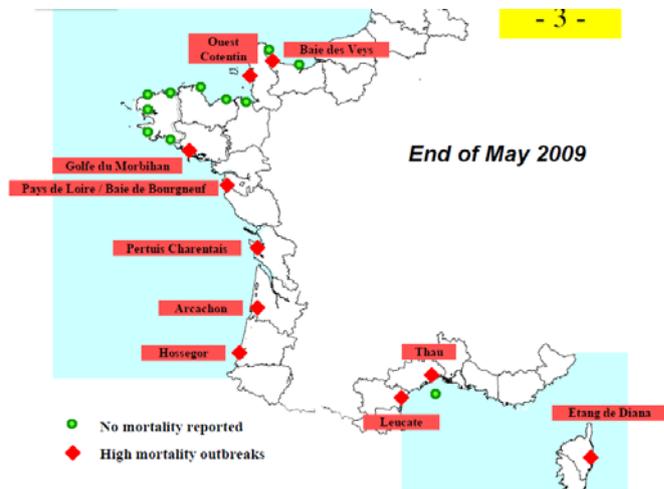
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<sup>2</sup> [http://web.oie.int/wahis/public.php?page=single\\_report&pop=1&reportid=8265](http://web.oie.int/wahis/public.php?page=single_report&pop=1&reportid=8265)

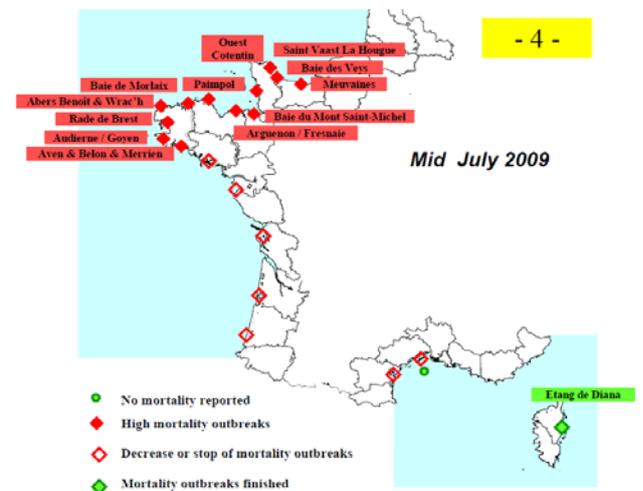
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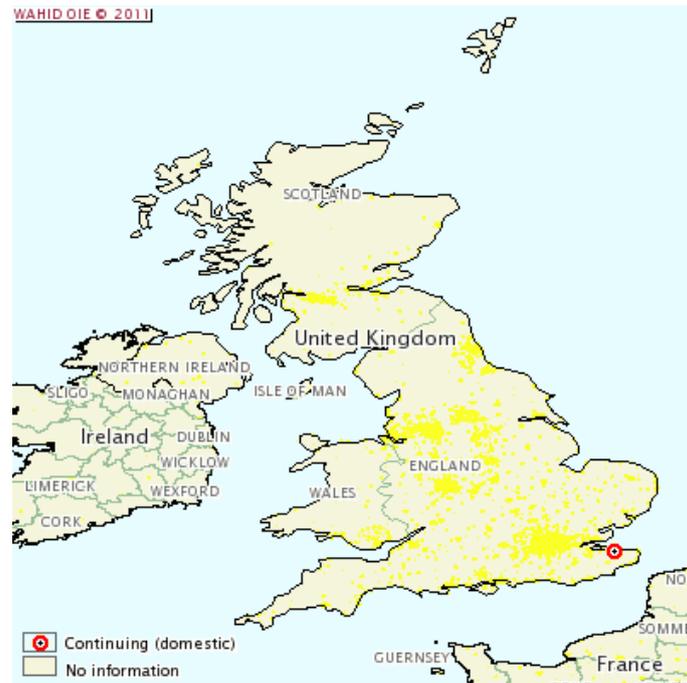


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## UK

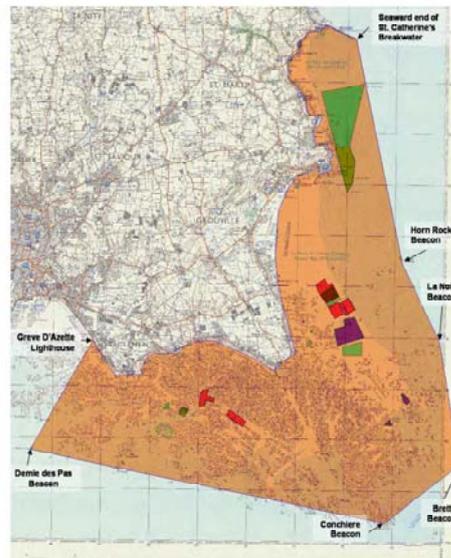
UK experienced high mortalities in 2008 and 2009. In July 2010<sup>3</sup>, the UK reported significant mortalities associated with OsHV-1  $\mu$ Var in [Whitstable Bay](#) in the Thames estuary and in Grouville Bay, South-East Jersey<sup>4</sup>. The UK has managed to contain the virus to the outbreak sites after immediate containment measures were established at the time of detection.



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<sup>3</sup> [http://web.oie.int/wahis/public.php?page=single\\_report&pop=1&reportid=9527](http://web.oie.int/wahis/public.php?page=single_report&pop=1&reportid=9527)

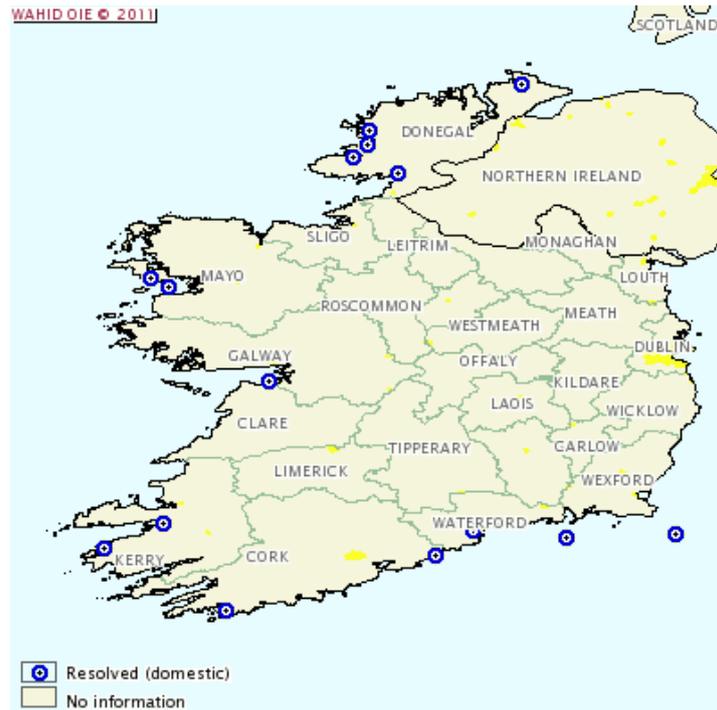
<sup>4</sup> [http://web.oie.int/wahis/public.php?page=single\\_report&pop=1&reportid=9571](http://web.oie.int/wahis/public.php?page=single_report&pop=1&reportid=9571)



## Republic of Ireland

The presence of OsHV-1  $\mu$ Var was confirmed in 2009<sup>5</sup> in samples from farms experiencing high mortalities from three bays in the Republic of Ireland (RoI). Approximately 16 bays were affected the following year. All sites found positive for OsHV-1  $\mu$ Var had been stocked with spat from France. Sites which had not received shellfish from France all tested negative for OsHV-1  $\mu$ Var. The RoI has since been able to declare a number of areas disease free but the disease remains widespread.

<sup>5</sup> [http://web.oie.int/wahis/public.php?page=single\\_report&pop=1&reportid=8503](http://web.oie.int/wahis/public.php?page=single_report&pop=1&reportid=8503)



### Other European countries

Spain, the Netherlands and Italy<sup>6</sup> have detected OsHV-1  $\mu$ Var but have experienced no mortalities associated with the disease.

Most recently, in June 28 2011<sup>7</sup>, the Netherlands reported high mortalities in the presence of OsHV-1  $\mu$ Var.

### EMERGENCE AND DISTRIBUTION IN AUSTRALIA AND NEW ZEALAND

#### Australia

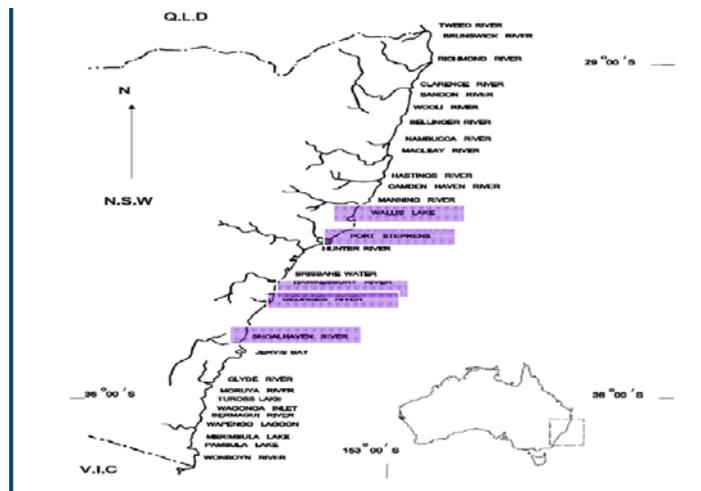
The Sydney rock oyster (*Saccostrea glomerata*) is the predominant oyster species farmed in New South Wales (NSW). There are presently 5 estuaries in NSW where triploid Pacific oysters are farmed (Wallis Lake, Port Stephens, Hawkesbury River, Georges River and Shoalhaven River). Diploid Pacific oysters are also farmed in Port Stephens. Excellent maps of these farmed areas and details of lease holdings

<sup>6</sup> Detection of OsHV-1  $\mu$ var and *Bonamia exitiosa* in farmed oysters in Italy during 2010.

[http://ec.europa.eu/food/committees/regulatory/scfcah/animal\\_health/presentations/ostreid\\_herpesvirus\\_bonamia\\_exitiosa\\_italy.pdf](http://ec.europa.eu/food/committees/regulatory/scfcah/animal_health/presentations/ostreid_herpesvirus_bonamia_exitiosa_italy.pdf)

<sup>7</sup> [http://web.oie.int/wahis/public.php?page=single\\_report&pop=1&reportid=10749](http://web.oie.int/wahis/public.php?page=single_report&pop=1&reportid=10749)

exist. There are also wild populations of Pacific oysters in estuaries south of Hastings River, however the full extent of these populations is much less well understood.



Reports of high mortalities in 4mm–15cm farmed spat and wild Pacific oysters were received from several farmers in Woollooware Bay, Georges River estuary in late November 2010. Poor water quality nearby had also been reported just prior to the reports of mortalities. The mortalities were investigated and a response under the NSW Fish Kill Protocol was initiated. Water quality samples showed no significant contamination. Initial histopathology was inconclusive. OsHV-1  $\mu$ Var was confirmed in December 2010 at AAHL and an immediate notification was made to OIE. Movements of stock and farming infrastructure were controlled between estuaries but not within the estuary. Sales of healthy oysters from affected areas for human consumption were permitted.

During population distribution surveys in preparation for testing wild oysters in Sydney Harbour, mortalities of wild Pacific oysters were observed in the Parramatta River in January 2011 and OsHV-1  $\mu$ Var was identified<sup>8</sup>. Anecdotal reports from the public suggested that the mortalities may have started four months earlier.

Sites in the upper reaches of the Georges River showed mortalities in wild Pacific oysters in early 2011 but cohabiting populations of Sydney rock oysters appeared unaffected. Previous mortalities in wild populations would probably not have been reported therefore it is impossible to say that Woollooware Bay was the first site where the disease occurred.

<sup>8</sup> [http://web.oie.int/wahis/public.php?page=single\\_report&pop=1&reportid=10136](http://web.oie.int/wahis/public.php?page=single_report&pop=1&reportid=10136)



The oyster growing industry in Australia, but particularly in NSW, has a heightened state of awareness. Unexplained mortalities in Pacific oysters in other NSW estuaries during the first six months of 2011 were reported, samples submitted and OsHV-1  $\mu$ Var was not identified. This provides good evidence that the disease is not present in farmed Pacific oysters elsewhere.

#### New Zealand

New Zealand investigated mortalities on a farm in the Coramandel Peninsula, North Island in March 2010. Other reports of high mortalities were received from around Auckland and the Bay of Islands. An environmental problem was initially suspected. Samples were sent to a private laboratory – MAF was not informed. Deaths stopped after 7 days, winter came, and notifiable diseases were ruled out by the private laboratory.

In late November 2010<sup>9</sup>, simultaneous mortalities were reported in the same areas and had been preceded by a rapid rise in water temperatures (3°C in one week). MAF was informed, investigated and determined that OsHV-1 was associated with the mortalities. Sequence analysis indicated that the isolates shared a 13bp deletion in the non-coding area of the C-region, although other minor point mutations in the coding area of the C-region were not present in the NZ isolate as compared with the European isolate of OsHV-1  $\mu$ Var. Areas affected in March appeared less affected in

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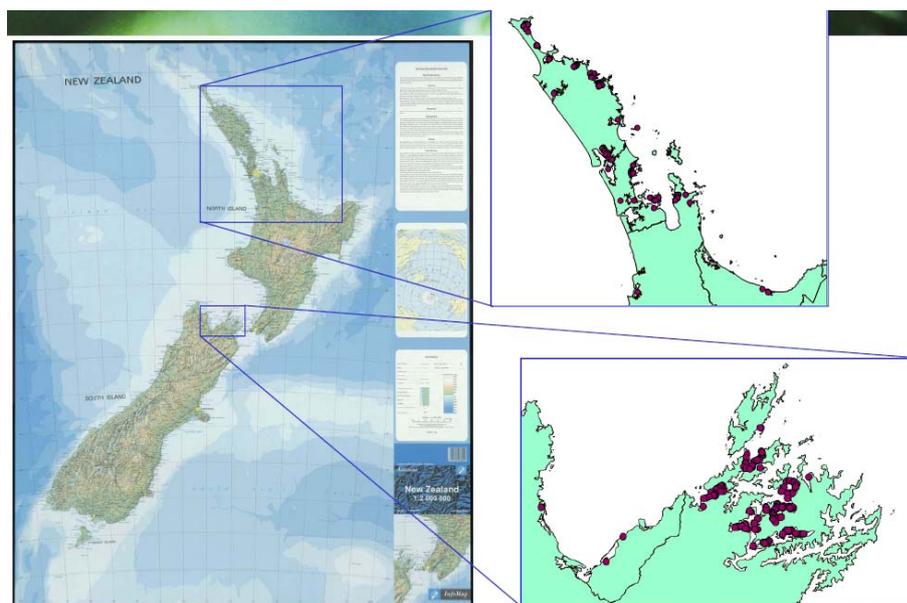
<sup>9</sup> [http://web.oie.int/wahis/public.php?page=single\\_report&pop=1&reportid=10013](http://web.oie.int/wahis/public.php?page=single_report&pop=1&reportid=10013)

November. Mortalities continued to mid-December. There are two areas in the North Island where there have been no introductions through infected spat or animal movement and these have remained OsHV-1  $\mu$ Var free. No mortalities were reported in the South Island.

New Zealand uses approximately 90% wild spat in farming. Most spat are caught on sticks in harbours on the west coast of the North Island and are moved to other areas. Half a million sticks are taken into the harbours to capture spat and then taken away and returned the next year. These sticks are often not cleaned.

Wild spat showed 50% mortality. Larger oysters seemed to be greatly affected. Hatchery spat experienced very high mortality, up to 100%, and it was speculated that this was due to their higher growth rate. Spat were seen to die within 48 hours of being put out to sea, although a period of 6 days is more likely.

A hatchery in the South Island, which had larval crashes, detected the presence of OsHV-1  $\mu$ Var. Movements of oysters between the North and South Islands are infrequent. Brood stock however was moved from North to South.



#### EVIDENCE FROM OTHER PARTS OF THE WORLD

Recent high mortalities in the US are not known to be associated with OsHV-1  $\mu$ Var at this stage. There is some question regarding the PCR test the US has used during investigations of high mortalities and whether they would have detected OsHV-1  $\mu$ Var had it been present.

China, Japan and Korea are major producers of Pacific oysters. Investigations involving oyster samples from these countries allowed detection of oyster herpes virus, OsHV-1, but not the new  $\mu$ Var. Recent large scale mortalities from these countries have not been reported. The genetic similarity between OsHV-1 $\mu$ Var and east Asian herpes isolates (see phylogenetic tree on page 30) raises the question of whether the disease could be present in this region despite the absence of reports.

## 6. IMPACT

Estimating impact is difficult and complex and can include sociological, economic, regulatory and institutional factors amongst others.

### SUMMARY OF IMPACT IN DIFFERENT LOCATIONS

	FRANCE	UK AND EUROPE	AUSTRALIA	NEW ZEALAND	REST OF THE WORLD
<b>First outbreak</b>	April 2008	UK: July 2009 IRE: 2009 NL: June 2011	Nov 2010	March 2010	None reported
<b>Growing areas affected</b>	100%	66% Ireland 3% England	20% in NSW* 1% nationally by lease area	73%	
<b>Spat mortality &lt;12mths</b>	High	High	Highest	80-100%	
<b>Juvenile mortality 12-18mths</b>	Medium	Medium	Higher	25-42%	
<b>Adult mortality &gt;18 mths</b>	Low	Low	High	8-20%	
<b>Is there a decrease in the impact of disease over time?</b>	No (same)	No (same)	Not applicable	Exposed populations less affected than naïve	
<b>Economic Impact to date</b>	Not apparent but little available data		10% estimated loss for NSW Pacific oyster production	Farm production fell by 25%	
<b>Potential future impact</b>			SA and Tas: Very high due to SA reliance on one hatchery in Tasmania and plan to expand export markets	Current 30% export to AUS, 30% to SEA, 30% to Pacific. Listing of disease may affect exports	

\*Refers only to the 5 estuaries currently used for Pacific oyster culture

## France

Significant widespread mortalities have been reported since 2008. The economic losses caused by the disease have not been estimated because of a lack of data. Subsidies and compensation schemes may lessen the impact to producers. The slow growth of Pacific oysters and lower mortality in adults in Europe may mean that the impact on marketable product is yet to be seen. French producers are reported to be increasing the number of spat stocked to compensate for expected mortalities. This strategy may also be masking the effect of disease on total production.

## UK

The disease has been successfully contained to one small bay in the Thames estuary and in Jersey thus far, so the economic impact to the oyster growing industry has been limited.

## The Republic of Ireland

Widespread disease has been detected (16 bays) however some areas have been able to be declared free from the disease, permitting movement and trade from and within these areas.

## Other countries in Europe

Impacts in other European countries are thought to be minimal given the lack of mortalities attributable to the disease. The impact in the Netherlands following a recent report of mortalities is as yet unknown.

## Australia

The triploid Pacific oyster industry in NSW was worth an estimated AUD \$4.5 million in 2009/10 financial year. Significant economic losses were experienced by affected farms in Georges River. There is a potential high future impact in Australia because of the reliance on hatchery (triploid and diploid) spat. According to the New Zealand experience, mortalities in hatchery spat are higher than in wild spat, although this difference has not been demonstrated in France. If the French strategy of increasing stocking of spat was adopted in Australia the costs for production would increase due to the cost of the extra hatchery spat.

There is an unknown potential impact on Sydney rock oysters, although no mortalities have been reported (despite their close proximity to affected Pacific oysters).

Pacific oysters are not native to Australia and in some areas they are considered a pest. This is the reason for the requirement in NSW to only farm triploids in all estuaries except in Port Stephens.

### New Zealand

A lower impact has been perceived in New Zealand because the industry is mostly reliant on wild spat which have experienced lower mortalities. Nevertheless production has been reduced by between 25–33%. New Zealand exports oysters to Australia (30%), Asia (30%), the United States, Japan and the Pacific Islands.

Lower overall water temperatures are also seen as an advantage as this may reduce the likelihood or extent of disease outbreaks. Overstocking is seen as a more viable strategy due to the use of wild spat.

### Other countries

The major producers of Pacific oysters are China, Korea, Japan, France, US, Canada and South America (in order of importance). The potential impact of OsHV-1  $\mu$ Var on production in these countries is enormous, and their collaboration could be sought for test development or surveillance activities.

## ZOONOTIC POTENTIAL

Herpes viruses are normally highly host specific. Despite the widespread presence of Oyster Herpes Virus and OsHV-1  $\mu$ Var in France there is no evidence of human infection or impact. It is considered to pose no threat to human health.

## 7. CHARACTERISATION

### DEFINITION OF OSHV-1 $\mu$ VAR

During the initial outbreaks in France, OsHV-1 was detected using conventional PCR in 2008, and qPCR in 2009 and 2010. The isolates were sequenced and comparisons made with the OsHV-1 reference strain (GenBank # AY509253<sup>10 11</sup>) using open reading frames 4 and 43 (ORF4 and ORF43). The C region (corresponding to the internal and terminal long repeats and which includes ORF4)

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<sup>10</sup> <http://www.ncbi.nlm.nih.gov/nucleotide/48696722?>

<sup>11</sup> <http://www.ncbi.nlm.nih.gov/nucleotide/41352386?>

is widely used to diagnose classical herpes virus (OsHV-1) and this is why it was first targeted for study. A number of differences were systematically detected:

- In the microsatellite zone of the non-coding part of ORF4, there is a 13 base pair deletion;
- There are two mutations in the coding region of ORF4;
- A number of other differences in the non-coding zone of ORF4; and
- Differences in the IA1-IA2 fragment of ORF43

More detailed genetic analysis was then conducted in France using up to 8 different ORFs. ORF4, ORF37 and ORF43 were identified for more detailed analysis using 79 French isolates, which showed that there was a further systematic 604 base pair deletion corresponding to the total lack of 2 ORFs (36 and 37) and a partial lack of ORF38. The deleted genes code for membrane proteins.

On the basis of these changes, the new strain was named OsHV-1 microvariant ( $\mu$ Var). While changes may appear in various areas of the genome, it was agreed that the 13 base pair deletion in the microsatellite zone of the non-coding region of ORF4 should be used to define OsHV-1  $\mu$ Var for diagnostic purposes. This definition is consistent with that proposed in the EFSA opinion<sup>12</sup>

“According to the Council Regulation 175/2010/EC, OsHV-1  $\mu$ var means a genotype of the virus Ostreid herpesvirus-1 (OsHV-1) which is defined on the basis of partial sequence data exhibiting a systematic deletion of 12 base pairs in a microsatellite zone of the ORF 4 in comparison with OsHV-1 (GenBank # AY509253). OsHV-1  $\mu$ var was later defined by Segarra et al (2010) on the basis of numerous mutations in comparison with the sequence of the reference virus (Davison et al; , 2005) in two different ORFs, the C region (ORF4) and the IA region (ORF43). The number of deletions in ORF4 is contentious. Council Regulation 175/2010/EC states there are 12 deletions, which is also stated in the text of the Segarra et al., (2010) publication, although Fig 4 of the same publication shows 13 deletions.”

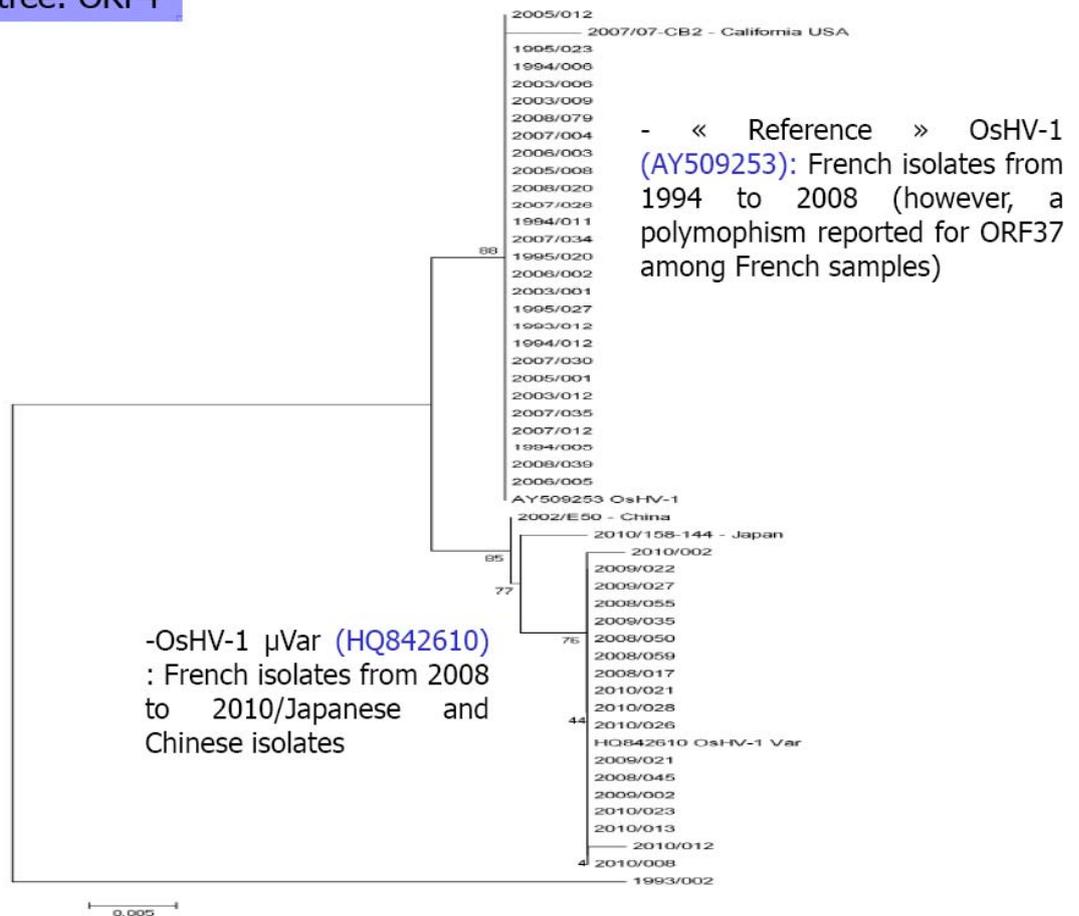
The ORF4 of 52 isolates was used to generate a phylogenetic tree. Analysis included classical OsHV-1 isolates from France from between 1993 and 2008, isolates of OsHV-1  $\mu$ Var from France between 2008 and 2010, and further isolates of oyster

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<sup>12</sup> Scientific Opinion on the increased mortality events in Pacific oysters, *Crassostrea gigas*, EFSA Journal 2010;8(11):1894 <http://www.efsa.europa.eu/en/scdocs/doc/1894.pdf>

herpes viruses from USA, Japan and China. The result of the analysis is shown below. Within ORF4, there is clear differentiation between the classical OsHV-1 and OsHV-1  $\mu$ Var isolates identified in France, but virtually no differentiation between isolates within these two groups. The US strain groups closely with the classical OsHV-1, while the Chinese (2002) and Japanese (2010) strains are much more closely related to the French OsHV-1  $\mu$ Var isolates.

Phylogenetic tree: ORF4



No sequence data is yet available from Australia for comparison with the French OsHV-1  $\mu$ Var or classical OsHV-1 isolates, although this work is currently underway. In New Zealand, at least 3 isolates from separate areas have been sequenced and compared to both the OsHV-1 reference strain and the published OsHV-1  $\mu$ Var sequence, for ORF4. They were all found to be almost identical (two bp differences and other minor point mutations in the coding area of the C-region were not present in the NZ isolate as compared with the European isolate of OsHV-1  $\mu$ Var) to OsHV-1  $\mu$ Var in the C2/C6 region.

		1	10	20	30	40	50	60
NZ-81	C2:C6	ATAGATGTGATGTGCGGCCAAGATGAATGGCAAGATACACAATGAGCTATTACCCGACCAC						
OsHV-1	C2-C6	ATAGATGTGATGTGCGGCCAAGATGAATGGCAAGATACACAATGAGCTATTCCCGACCAC						
<u>μVar</u>	C2:C6	ATAGATGTGATGTGCGGCCAAGATGAATGGCAAGATACACAATGAGCTATTACCCGACCAC						
NZ-81	C2:C6	AAACCTAACGTTGTATTTCGATTACGGATTAAGAAAATGGGTTCCACAATCTAAAATTTAAA						
OsHV-1	C2-C6	AAACCTAACGTTGTATTTCGATTACGGATTAAGAAAATGGGTTCCACAATCTAAAATTTAAA						
<u>μVar</u>	C2:C6	AAACCTAACGTTGTATTTCGATTACGGATTAAGAAAATGGGTTCCACAATCTAAAATTTAAA						
NZ-81	C2:C6	-AACCCACATGGGGGCCAAGGAATTTAAAGCCCCGGGGAAAAAGTATAAATAGGCGCGA						
OsHV-1	C2-C6	AAAACCCACATGGGGGCCAAGGAATTTAAAGCCCCGGGGAAAAAGTATAAATAGGCGCGA						
<u>μVar</u>	C2:C6	-AACCCACATGGGGGCCAAGGAATTTAAAGCCCCGGGGAAAAAGTATAAATAGGCGCGA						
NZ-81	C2:C6	TTTGTTCAGTTTAGAATCATACC--CACACTCAATCTCGAGTATACCACAACACTGCTAAATT						
OsHV-1	C2-C6	TTTGTTCAGTTTAGAATCATACCCAACACTCAATCTCGAGTATACCACAACACTGCTAAATT						
<u>μVar</u>	C2:C6	TTTGTTCAGTTTAGAATCATACC--CACACTCAATCTCGAGTATACCACAACACTGCTAAATT						
		< <b>Microsatellite zone</b> >						
NZ-81	C2:C6	AACAGCATCTACTACTACTACTG-----AAAAATGCAGCCTTTCACAGAATT						
OsHV-1	C2-C6	AACAGCATCTACTACTACTACTACTACTACTGAAAAATGCAGCCTTTCACAGAATT						
<u>μVar</u>	C2:C6	AACAGCATCTACTACTACTACTG-----AAAAATGCAGCCTTTCACAGAATT						
NZ-81	C2:C6	TTGCACCTTGACCAAAGCCATCACATCAGCCAGCAACGACTTTTTTCATCAACCAGACGAG						
OsHV-1	C2-C6	TTGCACCTTGACCAAAGCCATCACATCAGCCAGCAACGACTTTTTTCATCAACCAGACGAG						
<u>μVar</u>	C2:C6	TTGCACCTTGACCAAAGCCATCACATCAGCCAGCAACGACTTTTTTCATCAACCAGACGAG						
NZ-81	C2:C6	GTTAACATGCGACATTTGTAAAGAGCTCGTCTCTTTTCGATTGCGAAGATAAAGTCGTGGC						
OsHV-1	C2-C6	GTTAACATGCGACATTTGTAAAGAGCTCGTCTCTTTTCGATTGCGAAGATAAAGTCGTGGC						
<u>μVar</u>	C2:C6	GTTAACATGCGACATTTGTAAAGAGCTCGTCTCTTTTCATTGCGAAGATAAAGTCGTGGC						
NZ-81	C2:C6	ATCATTGGCTGCAGTCAGATCTGACATACCCATAGAAGTCACGGAACGCAAAGACCTGAA						
OsHV-1	C2-C6	ATCATTGGCTGCAGTCAGATCTGACATACCCATAGAAGTCACGGAACGCAAAGACCTGAA						
<u>μVar</u>	C2:C6	ATCATTGGCTGCAGTCAGATCTGACATACCCATAGAAGTCACGGAACGCAAAGACCTGAA						

It was agreed that genetic studies were valuable to understand both the origin and possible mode of spread of the virus, as well as understanding the mechanisms of pathogenicity. Inclusion of both Australian and New Zealand isolates, as well as those from other European countries in the phylogenetic analysis was seen as a priority, requiring collaboration between the different countries.

Full genome sequencing has not yet been completed although it is anticipated that a sequence will soon be available. This should provide insight into the differences between classical OsHV-1 and OsHV-1 μVar that may assist explain the apparent increase in virulence.

Using ORF4 failed to provide adequately fine resolution to distinguish between the French isolates or the New Zealand and the French strain. In order to understand the relationships between these viruses, it was suggested that more or different sequences need to be examined.

## VIRAL POPULATION SHIFTS

OsHV-1  $\mu$ Var was first identified in France in 2008. In that year, 42% of herpesvirus isolates identified during mortality events were OsHV-1  $\mu$ Var while the remainder were classical OsHV-1. In 2009 and 2010, 100% of herpesvirus isolates detected during mortality events were OsHV-1  $\mu$ Var. This suggests that the new strain had successfully replaced the classical strain as a cause of morbidity in oysters within the space of one year.

The implications of this observation in France are that OsHV-1  $\mu$ Var may be able to compete well with the classical strain, and that it appears to have been rapidly disseminated throughout virtually the entire French Pacific oyster population.

## STABILITY OF THE VIRUS

The transcription mechanism in the replication of double-stranded DNA viruses, such as Herpes viruses, has built-in quality checks, significantly reducing the error-rate compared to RNA viruses such as influenza. Herpes viruses are therefore relatively genetically stable.

The stability of OsHV-1 viruses is nevertheless uncertain, as little work has been done in this area. It was noted that in cases where there is heavy infection in large populations (as may be the case in intensive oyster growing areas), there is greater opportunity for mutation because of the rapid replication and large number of viral generations possible.

## 8. DIAGNOSTICS

A number of diagnostic tools are available for detecting suspect cases of OsHV-1 infection, including histology and *in situ* hybridisation. Molecular techniques (PCR) are used for confirmatory diagnosis of infection with OsHV-1  $\mu$ Var, and surveillance for subclinical infections.

## CLINICAL SIGNS

Affected oysters are usually found dead with decomposing material or empty shells. New Zealand reports that early cases show gaping and weak closure reflex. No characteristic clinical or gross pathological changes have been noted.

## HISTOLOGY

Histology is an important tool in the diagnosis of OsHV-1  $\mu$ Var, primarily due to its use in excluding other possible causes of mortality, including bacterial and parasitic diseases.

Histology in oysters affected with OsHV-1  $\mu$ Var indicates non-specific histopathology (inflammation), and to date, no characteristic histopathological lesions have been identified. Experiments are underway in Australia involving the serial sampling of naïve oysters introduced into an environment that was previously habitat for infected oysters, and histologists indicate that they are beginning to get a picture of the progression of the infection that may be useful for diagnosis. Histologists in France also indicate that they are able to identify pathological changes associated with OsHV-1 infection. It was agreed that these new results offer significant promise and that collaboration between histologists in affected countries should be encouraged. It was suggested that web-based tools developed by ABIN could represent a useful resource for histopathologists to collaborate using real-time discussion and shared viewing of high quality histopathology images.

In the past, inclusion bodies have been often recognised as a feature of herpes virus infections. In Australia, histologists have noted that inclusion bodies have not been identified in OsHV-1  $\mu$ Var samples. In France, inclusion bodies have not been seen with either classical OsHV-1 or OsHV-1  $\mu$ Var infections in *C. gigas*.

## ELECTRON MICROSCOPY

Electron microscopy has been used in France and is underway at EMAI in Australia. There is interest in the possibility of using electron microscopy to understand the way in which the virus may acquire its coat and make its way through the cell membrane, given that genetic deletions have occurred in areas that code for membrane proteins. To date, in France, no differences have been noted between classical OsHV-1 and OsHV-1  $\mu$ Var by electron microscopy.

## POLYMERASE CHAIN REACTION (PCR) TESTS

Polymerase chain reaction (PCR) tests are used to identify specific genetic sequences in samples. PCR tests are very sensitive because very small amounts of genetic material are repeatedly replicated to levels that allow detection. The sequence to be replicated is determined by primers based on specific nucleic acid sequences that

are designed to match only a small part of the target organism's genome, making the tests very specific.

If sufficient genetic material matching the primers is present in the sample, a PCR test will give a positive result. This doesn't necessarily mean that the oyster was infected, or that the virus was causing the disease. Passive carriage of virus, or the presence of non-infectious fragments of viral DNA may both result in a positive test result. The extreme sensitivity of the test means that cross contamination due to poor sampling technique and/or poor laboratory technique can also cause false positive results.

In order to provide a positive reaction, the primer must exactly match a small portion of the virus' DNA (n.b. for some PCR tests a small degree of mismatch may be tolerable but is likely to reduce the test's sensitivity and specificity). If a mutation has occurred in just a single base pair in the region targeted by the primer, then the test may be negative, even though the rest of the viral sequence may be identical.

A number of different PCR tests for OsHV-1 and OsHV-1  $\mu$ Var have been developed and are being used in different laboratories. These different tests are distinguished either by the primers they are using or the technology used to implement the test (conventional PCR, real-time PCR, TaqMan PCR).

Currently PCR tests used to identify OsHV-1 include real-time PCR using SYBR green and a TaqMan formats. These tests are not able to differentiate classical OsHV-1 from OsHV-1  $\mu$ Var.

Characterisation of OsHV-1  $\mu$ Var is currently being done using conventional or nested PCR using primers targeting the C2/C6 segment (the location of the 13 base pair deletion). A number of laboratories have developed different primers targeting this segment. A TaqMan real-time assay to directly identify OsHV-1  $\mu$ Var is currently being developed in France to support EU surveillance requirements.

The development of multiple new and subtly different PCR tests in response to the recognition of a new disease-causing agent is not surprising, and provides an opportunity for improved tests to be developed. However, this process results in a lack of standardisation (different tests may give different results on the same sample), potentially undermining confidence in test results. In addition, the process of determining which test is appropriate in a particular situation normally involves comparison of candidate tests to an accepted 'standard' test with known performance. In the case of OsHV-1  $\mu$ Var, all tests are new and none have yet been

adequately validated to provide reliable information about their performance. This issue is discussed in the next section.

A summary of different published PCR tests is provided in Appendix B of the EFSA report<sup>13</sup>. In addition to published methods, some laboratories (including EMAI) are using, as yet, unpublished modifications in an attempt to improve the tests' performance.

Tristan Renault from IFREMER is currently drafting a chapter on diagnostic tests for OsHV-1  $\mu$ Var for the OIE Manual of Diagnostic Tests for Aquatic Animals. This is seen as an important step in achieving improved test standardisation. OIE member states will have an opportunity to comment on the Manual chapter before its expected adoption in May 2012. The OIE Manual normally only contains information on tests for listed diseases (those reportable to the OIE), so the inclusion of this chapter is seen as an unusual step.

#### IN SITU HYBRIDISATION

This test uses a labelled nucleic acid probe to bind to specific genetic material in histological slides. RNA probes are used to bind to messenger RNA produced by the virus during replication. This technique can distinguish between virus that is being passively carried by the oyster and virus that is actively replicating in the tissues. Furthermore, it allows visualisation of the location of the virus in specific tissues, identifying the preferred sites of infection and replication.

This approach is being used to study OsHV-1  $\mu$ Var in a longitudinal study in New Zealand. Currently, it is being used primarily as a research tool rather than a diagnostic tool.

#### PROTEOMICS

Proteomics involves testing for protein biomarkers (products of infection) that may be switched on before PCR testing is able to reliably detect the infection. This option was raised as a possibility, but no work has been undertaken in this area.

#### RAPID FIELD TEST

Effective prevention of spread of the disease from new outbreaks is likely to depend on rapid recognition, rapid reporting and introduction of a movement standstill

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<sup>13</sup> Scientific Opinion on the increased mortality events in Pacific oysters, *Crassostrea gigas*, EFSA Journal (2010), 8(11):1894, 54-55.

until diagnostic tests are able to determine whether or not the mortalities are caused by OsHV-1  $\mu$ Var. Controls on the movement of oysters out of an estuary while waiting for test results are likely to cause hardship to producers, and if most cases prove not to be due to a new disease requiring movement restrictions, compliance and reporting rates are likely to drop rapidly. This issue emphasised the need for rapid diagnosis to minimise the negative impact of temporary movement restrictions on producers.

The potential value of developing a rapid field test was raised and discussed. It was agreed that laboratory tests are more reliable than is possible with rapid field tests (especially when results are negative) and that the speed with which laboratory tests can be conducted was adequate in most situations. However, it is important for laboratories and disease control authorities to know when rapid field testing is important and when it isn't. For routine testing, or surveillance work, rapid results are not critical. However, whenever a new incursion of OsHV-1  $\mu$ Var into a previously uninfected area is suspected (and therefore results in a movement ban), laboratories should be clearly informed that urgent test results are required.

The conclusion was that there is no real need for the development of a rapid field test (considering the time and cost that would be involved in such development) and that current laboratory tests are capable of providing adequate test turnaround if communication and transport systems work as they should.

**Editor's note:** Post workshop, information on a LAMP assay (Ren et al., 2010) which has potential as a rapid field test was provided.

Currently, OsHV-1  $\mu$ Var PCR testing is only conducted in Australia at EMAI (NSW) and AAHL (in Geelong), and in EU. Both South Australia and Tasmania expressed interest in implementing the PCR in their own state laboratories to further speed exclusion testing. Under agreed arrangements in Australia, testing for suspect exotic disease outbreaks is conducted at AAHL. If OsHV-1  $\mu$ Var was declared to be an endemic disease in Australia, state laboratories would be expected to establish testing capability.

## TEST VALIDATION AND STANDARDISATION

Some aspects of validation and standardisation include:

- Ensuring that when one laboratory identifies a sample as positive for OsHV-1  $\mu$ Var, another laboratory would provide the same conclusion for the same sample. This requires that:

- Laboratories are using the same molecular definition of OsHV-1  $\mu$ Var, which is normally determined by PCR configuration and primers used.
- Ring testing has ensured that the laboratories are meeting the same performance standards
- Understanding the performance and use of the test, including the following aspects:
  - Fitness for intended purpose(s)
  - Optimisation
  - Standardisation
  - Robustness
  - Repeatability
  - Analytical sensitivity
  - Analytical specificity
  - Thresholds (positive and negative cut-offs)
  - Diagnostic sensitivity
  - Diagnostic specificity
  - Reproducibility
  - Ruggedness

Currently, there is no standardisation in the use of tests for OsHV-1  $\mu$ Var, nor are any of the tests in use fully validated according to the OIE guidelines for test validation<sup>14</sup>. While this situation may appear to pose significant problems, it must be considered in context:

- Standardisation
  - The OIE Manual chapter on OsHV-1 tests is in preparation. This should provide guidance on recognised tests. If, in the future, OIE lists OsHV-1  $\mu$ Var, then use of the tests described in the Manual would be a legal requirement for international trade purposes.
  - Most tests are targeting one of two regions, using similar primers. It would therefore be expected that they should give very similar results.
  - For the current national survey in Australia, for which EMAI and AAHL are doing the testing using different tests as part of the same surveillance program, procedures are in place to ensure that results will be consistent. This includes:
    - A comparison of both tests that has been undertaken at AAHL and indicated that the results are consistent, and

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<sup>14</sup> [http://www.oie.int/fileadmin/Home/eng/Health\\_standards/aahm/2010/1.1.2\\_VALID.pdf](http://www.oie.int/fileadmin/Home/eng/Health_standards/aahm/2010/1.1.2_VALID.pdf)

- Follow-up testing of any positive samples detected at either laboratory with a second confirmatory test at the other laboratory.
  - IFREMER conducts inter-laboratory ring tests in Europe. Similar systems are in place in Australia and New Zealand and could be applied to the OsHV-1 PCR.
  - Standard positive control samples are required for test quality assurance. AAHL has undertaken to provide these samples for Australian laboratories but progress is slow. Australian laboratories should continue to exert pressure to ensure that this is done.
- Validation
  - In reality, very few diagnostic tests in current use for any disease are fully validated to meet the OIE standards. Most of the current tests have been evaluated in the laboratory in which they were developed, including an assessment of robustness, repeatability, analytical sensitivity and analytical specificity. Issues of fitness for purpose, diagnostic sensitivity and specificity, and choice of suitable thresholds are often more complex and less well examined.
  - By their nature, it can be confidently expected that the diagnostic sensitivity and specificity of the PCR tests are likely to be very good (in terms of detecting the presence of viral genetic material, rather than as an indicator of active infection).

The current Australian survey will provide valuable information to help characterise the diagnostic specificity of the tests being used. If no virus is detected outside the currently identified estuaries (Georges River and Parramatta River), then it can be assumed that all other samples are from non-infected populations and allow estimation of diagnostic specificity. On the other hand, a small number of positive test results would complicate the matter.

Statistical methods now exist which enable the diagnostic sensitivity and specificity of tests to be estimated, even when a 'gold standard' test, providing the 'true' disease status of animals is not available. These methods require the use of two different and unrelated tests in at least two populations (in which the disease is present at different levels). Surveys and testing in different parts of the world (for instance New Zealand, Australia and France) may provide an opportunity for collaboration to field validate a number of tests.

Fitness for purpose is an important consideration in test validation. Different tests and test combinations will be needed (and possible different cut-offs or thresholds for the same test) for the following situations:

- Diagnosis of the disease as the first occurrence in a previously unaffected area
- Diagnosis of the disease in an area in which it is considered to be endemic
- Surveillance to demonstrate freedom from the virus
- Surveillance to measure the prevalence of infection in an endemic area
- Studies to assess host susceptibility (requiring differentiation of infection from passive carriage of the virus)

## 9. INTERNATIONAL RESPONSE AND REGULATION

### AUSTRALIAN AND NEW ZEALAND REGULATORY RESPONSES

#### Australia

Following the report of mortalities in Woollooware Bay in November 2010 voluntary movement controls were instantaneous and industry cooperation was good. Sales of product to the marketplace for human consumption were permitted. Reporting of mortalities and movements is a long-standing mandatory requirement of oyster farmers in NSW, industry was widely reminded of its importance as soon as the virus was identified, and free testing was provided to the industry.

In general, official quarantine restrictions may not be put in place immediately, for example, if unexplained mortalities are not thought to be infectious disease related. NSW authorities may require confirmation of the disease prior to instigating controls, and diagnosis can take several days. NSW has all oyster leases mapped and in a GIS layer.

South Australia and Tasmania are able to put movement controls in place in the event of a suspected (but not necessarily identified) disease at the discretion of the State Chief Veterinary Officer, however a report of unusual mortalities would not necessarily in itself justify immediate movement restrictions. Fatigue from within the industry in the event of frequent shutdowns due to “unexplained mortalities” would be a likely consequence – and could lead to a reduction in reporting. Movement controls can be difficult to enforce. In certain circumstances when movement restrictions are in place, it might be considered safe for healthy product from affected areas to be sold for human consumption.

Early reporting and diagnosis is important to ensure a rapid response to disease outbreaks which may limit disease spread and increase the opportunities for eradication. The importance of removing disincentives, and increasing incentives, for farmers to report suspect disease or unusual mortalities was recognised. Given the detection of OsHV-1  $\mu$ Var in NSW, in the event of large mortalities, particularly in Pacific oysters elsewhere in Australia, it would be logical to assume that OsHV-1  $\mu$ Var might be the cause. Under these circumstances immediate movement restrictions and testing to confirm or exclude OsHV-1  $\mu$ Var would be appropriate.

The turnaround time for diagnostic results, if slow, could result in unnecessarily long restrictions, impacts on business, and lead to a reduction in reporting mortalities. Diagnostic testing needs to be quick and reporting by laboratories timely. Appropriate specimens, and proper packaging and transport, are necessary to ensure that there are no unnecessary delays in the delivery of specimens to the testing laboratory. Exclusion of OsHV-1  $\mu$ Var can be made initially then exclusions for other diseases completed secondly. Rapid action and response will assist in ensuring greater farmer compliance and cooperation. Under agreed arrangements, testing for suspect exotic diseases should be conducted at AAHL.

Field-based rapid tests do not currently exist but there seems little industry interest in developing such tests. Development of a rapid test would perhaps cost between AUD \$150K to \$450K, take between 1 to 3 years to develop, and would cost approximately AUD \$30 per unit sample. In addition, results from such a test would have to be confirmed at EMAI or AAHL in any event.

**Editor's note:** Post workshop, information on a LAMP assay (Ren et al., 2010) which has potential as a rapid field test was provided.

## New Zealand

Movement in the winter of healthy, but potentially infected, animals occurs throughout sites in the North Island. Only movement of brood stock occurs from the North Island to hatcheries in the South Island.

## OIE, EU, EFSA REGULATORY RESPONSES

The disease associated with OsHV-1  $\mu$ Var infection is not currently listed by the EU or the OIE as a notifiable disease. In 2010, the European Commission commissioned EFSA to prepare a Scientific Opinion document on OsHV-1  $\mu$ Var which included an investigation into causality, an overview of the oyster growing industry, a review of other mortalities and the surveillance activities in place. This paper identified the

significant role French based hatcheries played in supplying spat (both wild caught and hatchery produced) to producers throughout Europe. It also identified key gaps in information including production figures, movement data, health status and husbandry practices. The report discussed environmental factors which might be significant and the possible involvement of several other species. EFSA raised the need for improved biosecurity in hatcheries including the need for certification; improvement in diagnostic methods; the need for a case definition; more data on occurrence and the need for viral strain differentiated epidemiological studies.

In the Council Directives of the European Commission, articles 41 and 43 regulate for emerging and non-listed diseases, respectively. These have recently been reviewed. Commission regulation 175/2010<sup>15</sup> is based on Article 41 and recognises the new genotype associated with increased mortalities. It provides directives for sampling, testing and containment as well as movement restrictions and reporting. A flowchart indicating protocols, in the event of mortalities, for testing, measures to be taken and movement control is also provided.

Decision 2010/221/EU<sup>16</sup> regulates for declarations of freedom from disease and movement restrictions, and amendments to this decision are based on Article 43. As well as providing details on the new genotype, movement restrictions and reporting requirements, this decision offers guidance on the conduct of suitable surveillance programmes. Such guidance will lead to a more harmonised and organised European approach to surveillance for this disease.

The EU is not currently considering listing the disease and is waiting until after April, 2013 to review the question of listing. If the criteria used for listing are consistent with that of other diseases, it is unlikely that OsHV-1  $\mu$ Var will be listed. The OIE has not yet discussed listing however it is currently developing (with Tristan Renault) a chapter for its Manual which is to be tabled at the next OIE Commission meeting in October 2011. This chapter will provide diagnostic information and guidance.

The OIE disease listing process is usually instigated by an affected member country. It could be requested by the October 2011 meeting and then may or may not be officially listed at the May 2012 meeting, for example. The OIE process is often seen as slow. The criteria for listing a disease are stated in Chapter 1.1.1<sup>17</sup> and 1.1.2<sup>18</sup> of

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<sup>15</sup> <http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2010:052:0001:0013:EN:PDF>

<sup>16</sup> <http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2010:098:0007:0011:EN:PDF>

<sup>17</sup> [http://www.oie.int/index.php?id=171&L=0&htmfile=chapitre\\_1.1.1.htm](http://www.oie.int/index.php?id=171&L=0&htmfile=chapitre_1.1.1.htm)

<sup>18</sup> [http://www.oie.int/index.php?id=171&L=0&htmfile=chapitre\\_1.1.2.htm](http://www.oie.int/index.php?id=171&L=0&htmfile=chapitre_1.1.2.htm)

the *OIE Aquatic Animal Health Code*. The chances for successful listing are reasonably high given the timely nature, the emerging disease category and the fact that the OIE is moving towards listing different pathologies or virus genotypes. The goal would be to collect as much data as possible in support of a request made by a member country. It was noted that member countries from the Southern Hemisphere were keen to be involved in the development of the OIE chapter. A draft of the chapter should be circulated for comment in the October report to members.

The EC regulation (Commission Regulation 175/2010) was seen as weak by some because it only recommended testing in the event of mortalities. When the mortalities stop so, too, does the testing which allows movement to recommence even when in reality the disease may still be present. This weakness in regulations had a potential major impact on all EU states given the reliance on spat from France which could in theory be translocated even in the presence of infection (but without mortalities). The economic impact could be catastrophic. The new Decision 2010/221/EU is based on declarations of freedom from disease and movement restrictions from infected zones or zones for which no demonstration of freedom has been achieved.

#### THE IMPLICATIONS OF OIE LISTING

The OIE criteria for listing diseases in the *OIE Aquatic Animal Health Code* are included at Article 1.2.1 of the Code. In summary, the criteria cover the following issues:

- Production loss
- Known causal agent or strong association
- Potential for international spread
- Free areas/zones/countries
- Adequate diagnostic tools

These criteria would likely be fulfilled in the case of OsHV-1  $\mu$ Var. Classical OsHV-1 is not likely to be listed, because it is ubiquitous. It is interesting to note that it took 8 years for koi herpesvirus disease to be listed.

The implications for listing include:

*Positive implications:*

- The OIE processes for collecting information on the disease status of member countries would become more complete and timely reporting would provide

broader, more reliable data. The first occurrence of the disease would have to be made within 24 hours of detection.

- Multilateral framework for safe trade of aquatic animals and aquatic animal products with respect to OsHV-1  $\mu$ Var would be in place - instead of bilateral arrangements between individual trading partners.
- Gives a disease a greater profile for public and industry education purposes and may assist funding for research and surveillance activities.
- Having an OIE Reference Laboratory for a listed disease is seen as an enormous advantage.
- Characterisation and diagnostic standardisation: The OIE chapter (even if not a listed disease) will include guidance on diagnostic techniques.
- OIE standards would provide guidance on the establishment of free zones or compartments within affected countries in accordance with OIE criteria.

*Negative implications:*

- Potential effects on trade of susceptible aquatic animal species and their products. Countries which are disease free may undertake an import risk analysis and make requirements (where there were none previously) of exporting countries for safe trade with respect to OsHV-1  $\mu$ Var. Australia has small but increasing oyster exports, and New Zealand exports most of its production so trade restrictions are of considerable importance.
- Not all trade will be impacted the same way. Australia's oyster export trade, excluding pearls and shell, consists of two commodities: (1) Viable spat and (2) Non-viable product (half-shell and meat) for human consumption. The commodity trade that may be most negatively affected would be the emerging trade in viable product i.e. export of spat.

It was noted that some aquaculture regulatory systems, for example, in Southeast Asia are not well connected to the national veterinary systems and this inevitably leads to underreporting of aquatic diseases by Southeast Asian CVOs.

## 10. PATHOLOGY/NATURAL HISTORY

### SPECIES AFFECTED

Classical Herpesvirus is seen in a broad range of molluscs, however, to date, the OsHV-1  $\mu$ Var has been associated with disease in Pacific oysters (*C. gigas*) exclusively. Evidence of susceptibility of *Ostrea edulis* (European flat oyster), *Pecten maximus* (scallop) and *Ruditapes philippinarum* and *R. decussatus* (clam) has been observed. France has detected low level of OsHV-1  $\mu$ Var DNA in other species of

mollusc, but there are no signs of viral replication. Other species may act only as mechanical vectors for the virus. Gastropods (sea snails, sea slugs, as well as freshwater snails and freshwater limpets) may be susceptible.

It was recognised that high mortalities in all molluscs should be investigated and tested for OsHV-1 $\mu$ Var.

#### DIFFERENCES IN AGE SUSCEPTIBILITY

Data on age susceptibility differ between countries and is perhaps confounded by the rate of oyster growth. In Europe highest mortalities were seen in spat and juvenile oysters, however in Australia and New Zealand adult oysters were also significantly impacted.

#### ORGANS AFFECTED

Oysters are filter feeders, feeding on naturally occurring plankton and algae. French studies have shown that virus could be detected in the haemolymph and digestive gland after six hours of exposure to the virus. However 72 hours post-exposure, levels of virus were highest in the adductor muscle, haemolymph, gills and mantle. A significant increase in the amount of the viral DNA was observed from 72h to 96h post-cohabitation in all analysed tissues, except for the digestive gland.

The dynamics of the virus in oyster tissue will have a major impact on sampling for testing purposes.

#### NATURAL HISTORY

While there is no confirmed evidence that vertical transmission occurs, it has not been studied sufficiently; vertical transmission is very difficult to assess. It is always possible to find infected larvae, and gonads (not in spat) are infected, suggesting that transmission from adults to larvae is a possibility. Improved understanding of transmission pathways will assist in the possible future development of resistant strains.

Further study may be able to establish if true vertical transmission (gametes are infected and the larvae are infected prior to release) occurs or if it is only pseudo vertical transmission (infection occurs at release or directly afterwards). There is some evidence that vertical transmission does not occur. It was noted that eggs pass through ciliated ducts and could become infected at spawning. Anecdotal reports

from New Zealand suggest that it is possible to produce non-infected larvae from infected oysters.

In cohabitation infectivity trials, the virus was first observed in the mantle, gills, gonads and digestive gland, then later in connective tissue and muscular fibres. It is therefore possible to conclude that waste from infected oysters can be infectious. In addition, the adductor muscle is an important carrier of the virus and, following death, the last part to deteriorate which means moving dead oysters could be risky long after the mortalities have ended.

Infectious virus can be released from live oysters before death and following death. Release of high levels of the virus has been recorded from healthy oysters. Huge levels of viral replication have been seen early in infection and this supports a possible hypothesis for differential infectivity of oysters at different stages.

Histopathology results suggest massive excretion of haemocytes which is a classic immunological response in molluscs. More research could be conducted on the question of apoptosis (programmed cell death), particularly whether OsHV-1  $\mu$ Var influences the natural apoptosis response in order to increase its virulence. Examination by transmission electron microscopy (TEM) conducted in France demonstrated apoptosis in cells close to virus-infected cells.

## PERSISTENT INFECTION

We know that the virus is found:

- Free-floating (OsHV-1  $\mu$ Var detected free-floating but may or may not be infectious; it is known that abalone herpesvirus remains infectious for at least 24 hours following release into the water column)
- In survivors of outbreaks (in NSW, survivors show high levels of DNA material a long time after the outbreak)
- In decomposing oyster tissue after death
- In the major organs and tissues of oysters
- In mucus

The virus appears to persist and be able to reinfect in the absence of visible high mortalities which may mean that infected areas may never be able to be cleared of the virus. There is mounting evidence to suggest a latent period where the virus is detectable in healthy oysters. More work needs to be conducted in order to better understand the role of subclinical infection.

## 11. SURVEILLANCE

### WHAT SYSTEMS ARE BEING USED TO LOOK FOR THE DISEASE/VIRUS?

#### Farmer reporting

Passive farmer reporting is used routinely in all countries and is the single most important and affordable surveillance tool. However, it is also notorious for underreporting. Moreover, there is a general lack of standardization in estimating mortality rates and collecting data related to mortality. Improving the level of reporting of mortalities by farmers is vital for early detection. It must be done intelligently by removing any disincentives and by providing incentives to farmers.

A systematic and collaborative improvement of the passive farmer reporting system could include:

- strengthening communication pathways between farmers, scientists and regulators,
- improving public education,
- reducing laboratory response rates and increasing information provided to farmers regarding their results (particularly if inconclusive),
- providing practical assistance and advice to industry in the event of an investigation,
- empowering farmers to improve their on-farm biosecurity practices by providing practical guidelines,
- strengthening cooperation between growers,
- minimising the impacts of movement or trading restrictions where possible in the event of a disease investigation,
- ensuring pathways for reporting a suspected outbreak are understood by all parties,
- providing important information and feed-back to growers regarding collated data and findings,
- free testing in the event of a suspected outbreak
- providing sampling kits to improve the speed and quality of sample collection for early diagnosis.

Systematic reporting of mortalities should be encouraged including delayed discovery of mortalities. All data should be seen as important and useful to the surveillance of disease. The data can be joined with a wide range of available data collected for other reasons such as past weather or environmental events, stock movement and shipping data, for example, to assist in the modelling of the disease

and assessment of causal agents. The more data that are collected provide a more accurate picture of what constitutes normal and abnormal mortalities. It can also produce important benchmarking data for growers to use in assessing their production systems.

A national oyster farming database would be a useful surveillance tool. Such a tool could be purpose built or an existing information system such as the Tasmanian Pacific Oyster Health Program could be adapted to be made national. Such a reporting system could be developed and conducted through the oyster growing industry itself.

France had a system of reporting mortalities and movements but these reports were incomplete due to low participation by farmers and overshadowed by farmer claims for compensation from the state. Together, these two reporting systems provided only a patchy record of disease occurrence, level of mortalities and spread of disease. Some improvements have since been made.

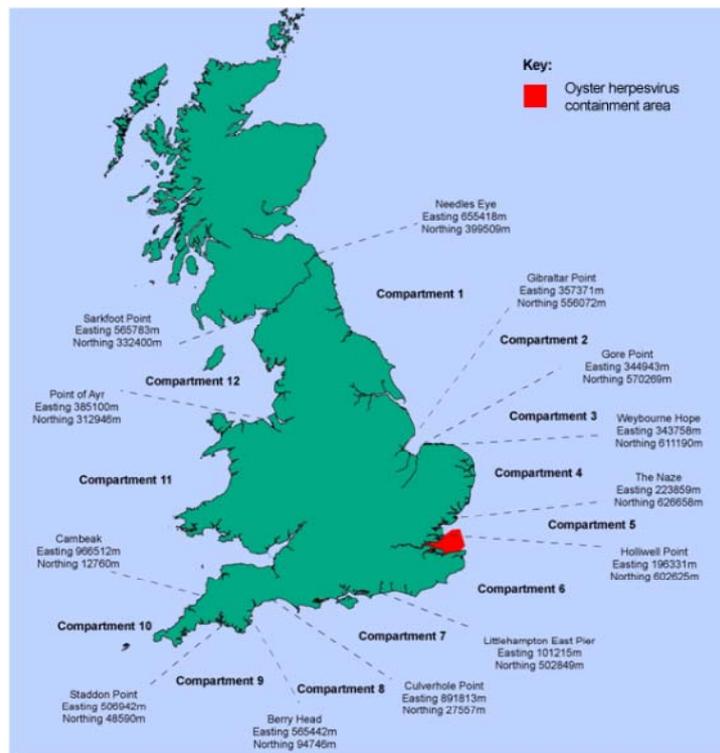
#### Sentinel populations in areas free from the disease ('observatories')

As part of follow-up surveillance activities, NSW instigated a system of sentinel sites in the Georges River. Disease-free oysters were deposited in sites in farmed areas and in areas with wild populations. Frequent visits and testing are required and make the activity labour-intensive, however it is seen as a useful tool in early detection. It also avoids the reliance on passive farmer reporting.

#### Other surveillance activities

The Australian national survey currently being undertaken should provide some much needed information. Pacific oysters only are farmed in South Australia and Tasmania; while Sydney rock oysters, Pacific oysters and Australian flat oysters (*Ostrea angasi*) are farmed in NSW. National surveillance focuses on Pacific oysters only, with farmed populations being a priority (note: wild Pacific oysters are found in NSW & Tasmania). The analysis is to be conducted by AAHL & EMAI using agreed upon methodologies to ensure compatibility.

The UK is currently conducting a surveillance program to demonstrate freedom from disease using a PCR test (the C2/C6 assay) on individual oysters in 13 containment zones. This program is expensive to conduct, however once freedom status is established they will be able to revert to the Passive Farmer Reporting system.



NZ has conducted no surveillance for freedom from disease purposes. They see the costs of attaining and maintaining a free status as prohibitive. The disease is now recognised as being endemic in New Zealand.

#### Surveillance for the virus/subclinical cases?

There is some evidence of a sub-clinical state of the virus. The following questions were raised:

- Are there different mechanisms of latency?
- Can latent infection be detected with PCR?
- Have exhaustive studies been made of latency markers in genomes?
- How is latent state different from low level, sub-clinical infection?
- Can experimental transmission trials confirm the existence of sub-clinically affected “healthy positives”?
- Is it possible that the virus can be found in a carrier host (low DNA levels detected in other molluscs), infected host (i.e. a Pacific oyster, shedding virus or not) or a reservoir host (harbouring virus in an unaffected population)?

The expert technical advisory group could help in directing research and defining sub-clinical infection questions.

## 12. RISK FACTOR DATA

### RISK FACTORS IDENTIFIED

Several risk factors are recognised as significant, however these factors could be considered relevant for any disease affecting Pacific oysters. The overriding feeling is that once an animal is stressed then its susceptibility to disease is often sudden and profound. Animals might be stressed by over-handling, temperature shocks, rapid growth rates, and the presence of other pathogens such as *Vibrio* spp. for example.

A multifactorial approach is needed when examining causal agents or risk factors. There is a complex web of correlation, for example temperature is an important but not definitive trigger for expression of disease. The different effects of temperature on the virus and on the host make the effect of temperature on the virus-host interaction complex.

The following risk factors have been identified as potentially playing important roles in the complex balance which ensures the good health of an oyster:

#### Density of oysters

French studies have not found a correlation between density and mortality.

#### Water temperature

High mortalities associated with the virus have also been associated with higher water temperatures. Patterns of mortalities seen in France and New Zealand have indicated a close connection to a rise in water temperature rather than spread of a pathogen, indicating that the pathogen may have been widespread and disease was expressed due to environmental factors. Simultaneous outbreaks have occurred in various parts of the North Island of New Zealand and in France with no obvious connection between sites. The presence of the disease has been recorded in a range of water temperatures between 13°C and 23°C. Mortalities in New Zealand occurred after a sudden rise in water temperature (3°C in one week). Larvae can stay in the water column for over three weeks and are able to travel over great distances with the prevailing water currents, however the simultaneous occurrence of mortalities in sites around the North Island and not the South Island suggests that infected larvae are not the sole cause of these outbreaks.

The absence of unexplained high mortalities in cooler oyster growing areas, for example, the South Island of New Zealand, and Scotland, may suggest that water temperatures are too cold in these locations for the virus to cause disease even if it is present.

### Salinity

There is no evidence to suggest that salinity plays any important role.

### Water quality

Poor water quality (a brown plume) around Woollooware Bay was reported prior to the outbreak in November 2010. Stock moved from Woollooware Bay to the nearby, more protected Quibray Bay one week after the outbreak did not show mortalities until six weeks after translocation. Water quality testing in Woollooware Bay at the time of the outbreak (but sometime after the report of poor water quality) showed no significant issues.

Oysters are often grown in coastal areas that may have naturally variable water quality. They may also be adjacent to areas of high urban density, shipping harbours, or close to industry or agriculture that could affect water quality.

France found no causal association with poor water quality and infection however agreed that poor water quality may prompt stress and thereby make infected animals more susceptible to disease.

Ireland has anecdotal or scant evidence that suggests differential mortalities in different bays associated with water quality, but differences in mortalities between producers within the same bay were observed. Production techniques and spat source are very similar in France and Ireland.

### Age or Growth rate

In New Zealand, where most oysters are grown from wild spat, the larger oysters from wild stock appeared to die first. However, 90–100% mortalities were seen in hatchery spat. In France, mortalities were seen in all age groups but mostly in spat. In Australia, mortalities were seen in all age groups.

### Management practices

Good management practices can assist in keeping oysters healthy and allow oysters to develop (rather than inhibit) strong generic mechanisms for resisting disease. But what constitutes “good management practices” exactly? Drying equipment, keeping good records, not moving sick stock, reducing stress are some important factors.

### Proximity to infection

Diseases caused by other aquatic Herpesviruses, in pilchards for example, have been modelled and it was shown that close contact was required for transmission to occur. In addition, Ifremer is developing a model to understand spread of infection in a bay including both hydrodynamic and oyster transfer aspects.

### Depth of oysters or tidal location

Research is beginning to suggest that oysters which are in contact with the water column the longest (e.g. sub-tidal) experience the highest mortality rates and oysters in intertidal zones experience lower mortalities. This observation could be confounded by different growth rates, which are higher for animals fully immersed. Oysters out of the water and thus kept closed for longer periods necessarily feed less and grow more slowly. They also display a natural anaerobic resistance to disease. Some evidence suggests that oysters in intertidal zones become adapted to the temperature changes that occur more frequently in shallower areas and are less stressed by temperature shocks than are constantly immersed oysters. New Zealand producers using long-line systems (at depths of 6–8 metres) appear to be as affected as farms at other depths.

### Presence of other pathogens

Work in France on affected oysters found *Vibrio* coexisting with the virus in many cases. Infection with *Vibrio* or other pathogens would probably make oysters more susceptible to other infections.

### Shipping and equipment movements – including biofouling, ballast water etc.

Recently imported, used aquaculture equipment was ruled out as a possible entry point of the virus in the case of the Georges River outbreak. Survival of

infectious virus of outside the host is thought to be low. Movement of Pacific oysters and other potential host organisms on ship hulls (biofouling) was considered a more likely possible mechanism for transmission than ballast water, given the low survival rate of the virus outside its host.

Singapore (a major source of ships to Australia) farms Pacific oysters in their shipping harbour, however there have been no reports of disease. However, it is not known whether an OsHV-1 epizootic would be detected and reported in Asia if it were to occur.

## Genetics

It is not clear whether all *C. gigas* are in fact the same. Studies using selected family lines suggest some lines show marked differences in prevalence of infection. Standardised infection models would assist in determining family susceptibility differences and this would inform any future development of breeding programmes for resistant oyster lines. Several states and organisations in Australia have the capacity to undertake this work, with SARDI, EMAI and USyd expressing an interest to do so.

The development of resistant lines may take a very long time to develop however; selective breeding in the Sydney rock oyster took 12–15 years but the generation times are recognised as being much longer for this species. Between-line and within-line selection and breeding from surviving oysters could be considered.

France has developed a more resistant strain which has shown reduced mortalities.

## SYSTEMS TO MEASURE RISK FACTORS

### Baseline, mortality and environmental data collection and analysis

The routine collection of important baseline data is crucial. Even basic population data are weak and are only estimates. South Australia claims to have very accurate centrally-located data which are readily available. New Zealand has no data on numbers of animals and has only recently collected basic farm data. New Zealand authorities have found movement data very difficult to collect. NSW records how many triploid spat are in the state and on an estuary-by-estuary basis, including what leases they are on and which farmers have them (in NSW, Pacific oysters grow quickly and are replenished regularly - close to annually). Privacy issues and poor

data quality are important and sometimes problematic issues which hamper the sharing or use of any data.

It was suggested that an industry-run national database might avoid some of the privacy issues and may encourage improved data quality. Data could be entered at the lease-side via a digital diary system, for example, collecting data on all routine management activities as well as animal health, water quality and other environmental factors. Data would then be immediately available for analysis and would provide feedback with relevant benchmarking and industry averages to assist farmers in improving management strategies.

- Mortality reports
  - Underreporting of mortalities could weaken any analysis (see Passive Farmer Reporting). A voice recognition system to collect movement data should soon be in operation in NSW. Integration between data sources is important and the benefits of any system need to be demonstrated to producers in order to ensure their cooperation.
- Environmental monitoring, including
  - Hydrodynamic modelling
  - Standardised water quality and temperature measurement
  - Environmental data loggers could be used and would be able to feed information directly into an industry database for real-time epidemiological analysis.
- Measuring husbandry factors
- Movement and traceability

#### Pre-season preparation of risk factor survey

It was suggested that a survey be designed in preparation for summer 2011. This could be put in place in readiness for any possible further outbreaks that might occur in Australia. The survey would provide an opportunity to use current knowledge to measure identified risk factors and the presence of disease. The results could be extremely informative to all stakeholders and may influence management practices, response, husbandry, testing, institutional systems etc. Such a survey could be useful in informing for other diseases as well. Funding limitations were recognised as being a hurdle.

## Experimental Infection Modelling

Development of experimental infection models has been undertaken in France and in Australia. Such models will facilitate further research on the biology of this virus.

### 13. CONTROL

#### WHAT IS BEING DONE? IS IT WORKING? WHAT IS PROPOSED?

It was acknowledged that industry is seeking practical measures which they can rely upon and adopt, however it is probably inappropriate to be too prescriptive because control measures will be site-specific or farm-specific depending on a range of management, environmental and other factors.

The following activities/issues were reported during discussion:

#### France

- Undertaking breeding programmes for oysters with disease resistance.
- Have tried earthen ponds for finishing oysters (claire system where oysters are kept for several days to several weeks for fattening), which is proving useful in reducing mortalities. No mortalities seen.
- Suggesting the production of alternative oyster species which are resistant (may also dilute the virus) for diversification purposes.
- French farmers want to experiment with *C. gigas* from other countries.
- Placing resistant strains in strategic locations in estuaries in order to promote resistance in the wild populations
- Looking at exposing spat to infection in hatcheries in order to make them less susceptible when subsequently re-exposed to OsHV-1  $\mu$ Var in the field (to confirm the observation that naïve oysters are more susceptible to infection than previously exposed oysters).

#### New Zealand

- Some farms stopped moving and grading oysters, for example, to reduce stress caused by handling, particularly during summer. Decreased mortalities were observed, presumably due to the reduced stress levels in animals. The gene expression of noradrenaline suggests stress during handling.
- Conducting small scale experiments on density and water depth

Anecdotal evidence and other suggestions include:

- “Overstocking” to maintain production goals but this is potentially a very expensive option for those farms dependent on expensive spat from hatcheries.
- The seeding of resistant oysters into areas which contribute most to natural recruitment
- Hatchery producing certified OsHV-1  $\mu$ Var free spat. However it was noted that it was not necessarily a good strategy to put naïve spat into infected areas.
- Selling early as an emergency, pre-emptive response.

New South Wales

- Growing other species such as the Sydney rock oyster or others to diminish the infection load.
- Work on 20 family lines indicated marked differences susceptibility. A field trial in Georges River indicated marked differences in infection rate between families. These results indicate that there may be potential for further work. Accessing survivors from infection trials for breeding in a clean hatchery is problematic. However, these are pedigreed lines and full-siblings of each of the lines are available from OsHV-1 free estuaries elsewhere for breeding purposes.
- A standardised infection model may assist with testing different families. However standardising exposure to look at interaction of both genetics and environment was acknowledged as difficult. Several states and organisations in Australia have the capacity to undertake infection trials, with SARDI, EMAI and USyd expressing interest to do so. FRDC is currently considering funding further research in the area.

#### 14. GROUP DISCUSSION

Towards the end of the second day, workshop participants were asked to break into three groups representing the oyster growing industry, scientists, and regulators. Groups were asked to identify the areas of key interest, knowledge gaps and research priorities.

See Annexe 4 for notes on these discussions. [Summaries of key findings, knowledge gaps and research priorities](#)

## 15. SOME PRESENT AND FUTURE RESEARCH ACTIVITIES

### OSTREID HERPESVIRUS-I (OSHV-1) RESEARCH AT THE ELIZABETH MACARTHUR AGRICULTURAL INSTITUTE

Pacific oyster mortality associated with Ostreid herpesvirus-1  $\mu$ Var (POMS) was first diagnosed in Australia in December 2010 at Elizabeth Macarthur Agricultural Institute (EMAI). Since then scientists at EMAI have been engaged in diagnostic activities (virology, histopathology and EM) during investigations of oyster mortality cases. The Virology Laboratory has also provided full laboratory support for a state-wide survey and detailed studies of infected populations. Sample collection was undertaken by NSW DPI staff. Collectively, at the laboratory these activities have involved the collection and testing of samples from more than 4500 individual oysters in a period of just over 6 months. Considerable research has been undertaken to support these activities, as outlined below.

1. Development, optimisation and validation of diagnostic assays: A high-throughput capacity for testing oyster tissues for OsHV-1 using real-time polymerase chain reaction (qPCR) assays was developed. This enabled rapid results to be provided in cases in which POMS was suspected. The procedure included optimised tissue selection, collection and sample preparation methods to provide purified DNA for testing in the qPCR. The qPCR assay was considered by EMAI to be 'fit for purpose' under Australian conditions and was shown to be superior to existing methods, including a published qPCR. A suite of additional molecular techniques targeting multiple portions of the OsHV-1 genome and utilising different PCR platforms were concurrently developed. These additional methods were utilised to distinguish active viral replication from potential environmental contamination and to confirm the OsHV-1 infection status of suspect samples. These assays provided valuable diagnostic and research tools which have already been broadly applied.
2. Investigation of disease outbreaks: Additional resources have been devoted to investigating cases of oyster mortality throughout NSW. In addition to determining the presence of OsHV-1 infection, the roles of other pathogens and environmental conditions have been determined. The aim is to assist the oyster industry by improving general diagnostic capacity and to generate the epidemiological data required to adequately manage the threat of POMS.

3. Pathogen surveillance: As part of the national response to POMS the Aquatic Consultative Committee on Emergency Animal Diseases (AqCCEAD) instigated a survey to determine the distribution of OsHV-1  $\mu$ Var in wild and farmed Pacific oysters in Australia. Testing at EMAI supported this survey and enabled the scope of the survey in NSW to be extended to include Sydney rock oysters and to distinguish the OsHV-1 reference strain from OsHV -1  $\mu$ Var.
4. Pathogenesis and epidemiological studies: In the course of investigating POMS outbreaks in the Georges and Parramatta Rivers, samples have been collected from cohorts of oysters representing different age classes, species and exposure times. These have included survivors of outbreaks as well as newly recruited wild spat and oysters translocated into the area specifically for the purpose of pathogenicity studies. These samples have been subjected to a range of techniques including histopathology, bacteriology, quantification of viral loads by qPCR, viral sequence determination, *in situ* hybridisation and electron microscopy. High quality images of the Australian strain of OsHV-1 were obtained during these studies – the first occasion on which the virus has been visualised. Valuable data are being compiled which will assist in management and control of POMS in the future.
5. Investigation of genetic resistance to POMS: Preliminary research using 20 different Pacific oyster family lines has given promising results, with some lines almost completely resistant to infection and indicates that genetic resistance to OsHV-1 is likely to provide a practical response to the threat of this disease. Additional trials are currently underway in collaboration with NSW Department of Primary Industries, Fisheries staff.

#### FRDC INDUSTRY SUPPORT PROJECT

##### Understanding and planning for potential impacts of OsHV-1 $\mu$ Var for the Australian Pacific oyster industry

1. Collate industry relevant information both published and anecdotal
  - Oyster Herpes Virus Workshop, July 9–10, 2011, Cairns, Queensland
  - International Oyster Symposium Sept 15–18, 2011, Hobart, Tasmania
  - Lessons learned from previous incidents
  - Other sources
2. Field Trip to visit production sites in France and Ireland

- Meet and share experiences with farmers in these affected countries.
  - Probably should be prior to November.
  - Share findings with the Australian industry.
3. Develop and Communicate
- Strategies to minimise risk
  - Response activities and long-term planning
  - Strengthen control measures where disease is established
  - Inform regulators
  - Information sharing

## ANNEXES

### ANNEXE 1: WORKSHOP PARTICIPANTS

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## ANNEXE 2: WORKSHOP PROGRAM

Time	Session	Discussion points
Saturday 9 July 2011		
9:00	Introduction	Introduction of organisers, facilitators and participants Objectives Agenda Outputs
9:30	The disease	What is the disease? What is the cause of the disease? - How strong is the evidence? - Are other pathogens involved? Case definition?
10:00	Emergence and distribution	Where is it? Emergence and distribution in Europe Emergence and distribution in Australia and New Zealand Evidence from other parts of the world?
10:30	Break	
11:00	Emergence and distribution	Continued
12:00	Impact	Impact in different locations Mortality levels observed Scale and importance of the disease Current impact and potential future impact Zoonotic potential?
13:00	Lunch	
14:00	Characterisation	What is it? Relationship to non-variant OsHV-1 - Viral population shifts (proportion of $\mu$ var and traditional viruses isolated over time) - Stability of the virus
15:00	Diagnostics	Current options Case definitions (suspect and confirmed) Tests available - Clinical signs - Gross pathology - Histology - Immunoassays - Molecular assays Validation of tests, estimates of test characteristics (Se and Sp)
15:30	Break	

<b>Time</b>	<b>Session</b>	<b>Discussion points</b>
16:00	International response and regulation	OIE, EU, EFSA, National regulatory responses. Are these appropriate, are they well founded, are they enough? OIE - is this going to become a listed disease? - if so, what are the implications? European, Australian and New Zealand regulatory responses - Reporting requirements? - Internal movement restrictions? - International trade restrictions? Implications for non-affected countries
17:00	End day 1	
Sunday 10 July 2011		
9:00	Pathology / natural history	Species affected Differences in age susceptibility Organs affected Natural history Persistent infection? Transmission - vectors?
10:00	Surveillance	What systems are being used to look for the disease / virus? Surveillance for mortalities - systems used in different countries Farmer reporting - mortalities or claims for compensation? Sentinel populations ('observatories') Surveillance for the virus / subclinical cases?
10:30	Break	
11:00	Epidemiology	Environmental factors - Seasonal effects - Temperature - Water quality - Management factors Transmission and spread - Role of hatcheries - Role of movements
11:30	Risk factor data	Available systems to measure risk factors - Environmental monitoring - Measuring husbandry factors - Movement and traceability
12:00	Control	What is being done? Is it working? What is proposed? Immunity and resistance: vaccination, chemotherapy, immune-stimulation, resistance breeding, restocking with resistant species Sanitary measures: blocking agents, disinfection of eggs and larvae Husbandry practices: stocking density, depth, movements
13:00	Lunch	
14:00	Overview of current knowledge	Summary of key findings

<b>Time</b>	<b>Session</b>	<b>Discussion points</b>
15:00	Identification of key knowledge gaps	Objectives: - Prevent further spread, - Manage the disease in areas where it already exists to minimise impact, - Consider options for eradicating the disease from populations Do we have enough information to achieve these objectives? List of key questions that still need to be answered Key systems and requirements that are not in place
15:30	Break	
16:00	Planning research priorities and opportunities	Review of current research activities and capabilities Identification of priorities and opportunities: - Research - Management - Contingency planning
17:00	Workshop close	

OSHV-1  $\mu$ VAR OUTBREAK IN WHITSTABLE BAY, UK. ED PEELER, CEFAS.

### Descriptive epidemiology of the outbreak

The Fish Health Inspectorate (FHI), Cefas were contacted by Seasalter Foreshore oyster farm on 12 July 2010. The farm reported unexplained mortality in Pacific oysters (*Crassostrea gigas*) (first observed on 8 July 2010). A visit was made by a FH inspector on 13 July. The farm is location in the Thames estuary.

Seasalter Foreshore oyster farm is located on the western edge of Whitstable and is owned by John Bayes who also runs Seasalter hatchery (located approximately 15 km east of the foreshore site at Reculver). The site has been leased to a French company since early this year and stocked with 8 million juvenile oysters from the Seasalter hatchery. The site is largely operated by French staff. Oysters are grown in bags on trestles in the tidal zone.

Mortality varied from 40-90% between batches. All age groups were affected. Oysters higher up the beach (submerged for a shorter period) were reported to be less affected (consistent with observations of higher mortality in oysters submerged for longer periods in the Republic of Ireland). The water temperatures when the mortality occurred were the highest recorded that year ( $>20^{\circ}\text{C}$ ). Sewage had been released into the vicinity of the sites on 8 July which may have resulted in decreased dissolved oxygen levels.

30 oyster samples were taken from both the affected site and Seasalter hatchery. PCR positive results for OsHV-1  $\mu$ var, confirmed by sequencing, were obtained from 26 of the 30 oysters from Seasalter Foreshore site (which will be referred to as the index site). The hatchery sample tested negative. A further 150 oyster sample has been collected from the hatchery but were found to be negative (later sample also tested negative, the hatchery distributed stock to other sites which have all tested negative and there were no reports of mortality).

Cockles and mussels are harvested in the Thames estuary. Native oysters are also present although current levels do not support a significant commercial fishery. Wild beds of *C. gigas* exist in the vicinity of the site. Subsequent testing of wild stocks proved negative except for 3 of 30 wild *C. gigas* which tested PCR positive for the OsHV-1  $\mu$ var.

## Assessment of routes of introduction of OsHV-1 $\mu$ Var to Whitstable Bay

A range of routes were identified. Two routes stood out clearly as the most likely routes of introduction:

### Introduction of materials (e.g. trestles and bags) from France

The company operating the affected site has brought equipment (trestles and bags) from France. The owner of the affected site claims that the bags had been out of the water for 4 years before being shipped to the UK thus no shellfish should have been accidentally transported. The trestles had been stored out of water for longer. The equipment was second hand, and the owner said that as well as being encrusted with acorn barnacles there was empty shell within the bags.

### Oysters from Jersey (via the Whitstable Oyster Company)

The Whitstable Oyster Company operates a fishery for oysters, two quayside restaurants and a small area of trestles for keeping oysters in seawater. It is known that they purchased oysters which had originated from Jersey and had been depurated at Maldon, Essex (a site authorised by Cefas for this trade). These oysters may have been kept in tanks at the purification centre operated by the company from which water had been discharged (untreated) into Whitstable harbour. Secondly, there is a possibility that oysters purchased from Billingsgate or from the purification tanks may have been relaid on trestles opposite one of the restaurants. Relaying depurated oysters is illegal.

## Background

There is an export trade in live Pacific oysters for on-growing from France to other parts of Europe. In 2009 reports of extensive mortalities of oysters were received from the Republic of Ireland (D. Cheslett, pers. comm.) and from Jersey (M. Gubbins, pers. comm.). The presence of OsHV-1  $\mu$ Var1 was confirmed in samples from both the RoI and Jersey, and in both cases, the oysters originated in France. There is an increasing amount of circumstantial evidence from areas where mortalities are occurring that infection can be transmitted from non-clinically affected surviving adult oysters to naive juvenile oysters. (F. Geoghegan, pers. comm.).

In Ireland high mortality and the presence of OsHV-1  $\mu$ Var were reported from oyster growing sites in 16 bays (D. Cheslett pers. comm.). Pacific oysters are cultured in 44 bays in the RoI, of which 21 introduced spat during 2009. Oysters from France had been imported during 2008 or 2009 to all but one of the bays where

OsHV1  $\mu$ Var1 was detected (the other site had introduced oysters from another bay in the RoI which was OsHV-1  $\mu$ Var positive). Anecdotally the level of mortality varied considerably between sites within the same bay.

### Questionnaire study

A retrospective questionnaire survey of 70 oyster farmers was undertaken to investigate the distribution and determinants of the mortality. Based on farmer recall, mortality data at the batch level were recorded: cumulative mortality, duration of the mortality event, age of animals affected, date of introduction. Observable mortality was recorded in 109 of a total of 346 batches from 47 sites, 104 of the 109 batches were located in bays where OsHV-1  $\mu$ Var had been detected. The records from bays where OsHV-1  $\mu$ Var had been detected were analysed to characterise the pattern of mortality and potential risk factors. The mean batch mortality was 37% (18-65% inter-quartile range) but showed a bimodal distribution (half the batches had mortality less than 45%). Mortalities started at the end of May and continued until early August, peaking in early July. On average oysters died over a period of 18 days. There was considerable variation in mortality both between and within bays. Mortality started in batches introduced within the last 12 months and occurred later in the season in established oysters, which is consistent with the introduction of an infectious causative agent. Mortality was significantly higher in spat than other age groups, which supports observations from France. There was a strong association between triploidy and higher batch level mortality: 21% of triploid batches experienced >40% mortality compared with 10% of diploid batches ( $P < 0.01$ ,  $\chi^2 = 10.54$ ,  $n = 293$ ). The apparent susceptibility of triploid stock may be attributable to their increased growth rate, compared with diploid stock. No batch which was out of water for more than 8 hours during the tidal cycle suffered mortality higher than 40%. Again this correlation may be explained by growth rate; oysters which are immersed for longer grow faster. Manual, compared with mechanical handling, of sacks is associated with higher levels of mortality in spat (~80 versus ~50%); the most likely explanation is that handling provides greater opportunity to record mortality. Future studies should develop improved methods to assess oyster mortality and follow stocks over time to better determine the influence of management and environmental factors on mortality.

At Cefas we have compared the sensitivity of three assays available for the detection of OsHV-1 and the OsHV-1  $\mu$ Var. Preliminary results are discussed below.

The four assays compared are:

1. *Conventional*
2. *Nested PCR*

The conventional PCR was performed using the C2 and C6 primers. The nested PCR was performed as above using primers OsHV-1 for and OsHV-1 rev and the C2/C6 reaction product as a template.

3. *Sybr green real-time PCR assay*

The sybr green assay was that described by Webb et al (2007) using primers OsHVDP for (ATTGATGATGTGGATAATCTGTG) and OsHVDPprev (GGTAAATACCATTGGTCTTGTTCC).

4. *Taqman real-time PCR assay*

The Taqman real-time PCR used was that described by Martinot et al (2010) using primers OsHV1BF (GTCGCATCTTTGGATTTAACAA) and B4 (ACTGGGATCCGACTGACAAC) and probe (FAM TGCCCCTGTCATCTTGAGGTATAGACAATC TAMRA).

The Taqman and nested assays proved most sensitive, and were able to detect the virus in the sample when diluted a further 1:10 to 1:100. When using DNA extracted from the low level infections the conventional and SYBR green assays detected the virus in undiluted samples only.

## ASSOCIATION BETWEEN OSHV-1, OSHV-1VAR AND OSHV-1 $\mu$ VAR AND MOLLUSC MORTALITIES. NICK MOODY, AAHL.

Herpes-like viruses have been described in molluscs since the 1990s (see references for general information in the Introduction). The identification of the viral particles in affected oysters as herpes-like viruses was by TEM.

With the advancement of molecular tools, in particular PCR, assays were developed which provided more detail of the genomic characteristics of the viruses which were present. PCR tests were developed to target the A, B and C regions of the ~207kb dsDNA genome (Arzul et al., 2001a). These reported the detection of OsHV-1 associated with mortalities in juvenile *C. gigas*, and *R. descussatus* and from healthy *O. edulis* in France from samples obtained between 1995 and 1999. They also reported the detection of a variant form of OsHV-1 (OsHV-1Var) associated with mortalities in juvenile *C. gigas*, and *R. philippinarum*. The variant produced a smaller amplicon using the C2/C6 primer set and no comparative sequence information was provided on amplicons generated from the A or B genomic regions. The variant contained several single nucleotide substitutions and a deletion of 200bp near the C2 sequence as well as an insertion of 27 bases (Arzul et al., 2001a). Additional PCR testing identified a 2.8kb deletion in OsHV-1Var in the inverted repeat region. In 1991, OsHV-1Var was also reported in larval *P. maximus* associated with mortalities in France (Arzul et al., 2001a). In 2002, OSHV-1 was reported from asymptomatic adult *C. gigas* in France (Arzul et al., 2002).

In the USA, repeated summer mortality events in cultured *C. gigas* occurring during 2002 and 2003 were investigated using the Arzul et al. (2001a) OsHV-1 A, B and C primer sets. Presence of OsHV-1 was confirmed by sequencing of the amplicons however as no amplicons were produced using the C primer set, no discrimination between OsHV-1 and OsHV-1Var was made (Friedman et al., 2005). A review of OsHV-1 in 2007 determined that infection in juvenile bivalves is more likely to result in disease than infection in adult bivalves and that OsHV-1 and OsHV-1Var are considered representatives of a single viral species (Batista et al., 2007).

Investigation of healthy oysters from Asia identified OsHV-1 in *C. ariakensis*, *C. sikamea*, *C. gigas* and *C. hongkongensis* using the Arzul et al. (2001a) OsHV-1 A primer set, however differences in sequences were limited to single nucleotide polymorphisms and detailed sequence comparisons were not presented (Moss et al., 2007). These authors described 2 genetic strains in Japan, 1 in Korea, and 2 in China, and suggested sequencing of additional gene regions to further characterize the differences.

In 2010, an additional variant was reported (Segarra et al., 2010). These authors tested larval *C. gigas* obtained after mortality events in France in 2008 and identified both OsHV-1 and a third genotype, OsHV-1  $\mu$ Var. The OsHV-1  $\mu$ Var differed from OsHV-1 by a single addition, several substitutions and deletions. In one batch of samples both OsHV-1 and OsHV-1 $\mu$ Var were detected. Unfortunately, while sequence comparisons were undertaken between OsHV-1 and OsHV-1  $\mu$ Var, no sequence comparisons were made with OsHV-1Var. Segarra et al. (2010) found that both OsHV-1 and OsHV-1  $\mu$ Var were associated with mortality events in 2008 and there was no relationship between geographical location and virus genotype. These authors proposed additional work to fully investigate the possible infectivity and virulence differences between the OsHV-1 and OsHV-1  $\mu$ Var genotypes.

The EU issued a Regulation relating to OsHV-1  $\mu$ Var in 2010 (EU Commission, 2010) which requires testing for detection/absence of OsHV-1  $\mu$ Var when increased mortality in *C. gigas* is reported. This is required as there are still great uncertainties regarding the emerging disease situation.

### Summary

- OsHV-1, OsHV-1Var and OsHV-1  $\mu$ Var have been associated with disease in young *C. gigas*.
- OsHV-1 and OsHV-1Var have been associated with disease in young *C. gigas* and *R. philippinarum*
- OsHV-1 has been associated with disease in young *C. gigas* and *R. discussatus*.
- Only limited comparative testing has been undertaken, primarily by French scientists. Reports in the literature either do not use the C2/C6 primer set, which enables discrimination between OsHV-1, OsHV-1Var and OsHV-1  $\mu$ Var, or very limited if any sequence analysis is undertaken.
- More research is required, targeting different regions of the OsHV-1 genome to enable detailed comparisons between the reference and variants strains.
- Infectivity trials are required to enable any virulence comparisons between the reference and variant OsHV-1 genotypes.
- There is one full OsHV-1 genome sequence and one C2/C6 OsHV-1 genome sequence in the public domain (GenBank) so comparisons of the Australian OSHV-1 sequences with exotic reference OsHV-1 and variants are very limited.

## References

Arzul et al (2001a) Evidence for interspecies transmission of oyster herpesvirus in marine bivalve. *J Gen Virol* 82: 865-870

Arzul et al (2001b) French Scallops: A New Host for Ostreid Herpesvirus-1. *Virology* 290: 342-349

Arzul et al (2002) Detection of oyster herpesvirus DNA and proteins in asymptomatic *Crassostrea gigas* adults. *Virus Res* 84: 151-160

Batista et al (2007) Detection of ostreid herpesvirus 1 DNA by PCR in bivalve mollusks: A critical review. *J Virol Methods* 139: 1-11

EU Commission (2010) COMMISSION REGULATION (EU) No 175/2010 of 2 March 2010 implementing Council Directive 2006/88/EC as regards measures to control increased mortality in oysters of the species *Crassostrea gigas* in connection with the detection of Ostreid herpesvirus 1  $\mu$ var (OsHV-1  $\mu$ var)

Friedman et al (2005) Herpes virus in juvenile Pacific oysters *Crassostrea gigas* from Tomales Bay, California, coincides with summer mortality events. *Dis Aquat Org* 63: 33-41

Moss et al (2007) Pathogens in *Crassostrea ariakensis* and other Asian oyster species: implications for non-native oyster introduction to Chesapeake Bay. *Dis Aquat Org* 77: 201-223

Segarra et al (2010) Detection and description of a particular *Ostreid herpesvirus* 1 genotype associated with massive mortality outbreaks of Pacific oysters, *Crassostrea gigas*, in France in 2008. *Virus Res* 153: 92-99

**Association between OsHV-1, OsHV-1Var and OsHV-1 $\mu$ Var and mollusc mortalities**

Date	Location	Species	Mortalities	Detection Method				Sequence Analysis	Reference
				TEM	PCR A	PCR B	PCR C		
1995-1999	France	<i>O. edulis</i> (n=3)	No	+	+	+	+	OsHV-1	Arzul et al (2001) Evidence for interspecies transmission of oyster herpesvirus in marine bivalve. J Gen Virol 82: 865-870
		<i>C. gigas</i> (n=3)	Yes	+	+	+	+	OSHV-1	
		<i>R. descussatus</i> (n=3)	Yes	+	+	+	+	OSHV-1	
		<i>C. gigas</i> (n=18)	Yes	+	+	+	-	*OsHV-1Var	
		<i>R. philippinarum</i> (n=3)	Yes	+	+	+	-	*OsHV-1Var	
Larval samples tested PCR A: A3/A4, PCR B: B1/B2, PCR C: C1/C6 *OsHV-1Var only detected in the 2 species in a single hatchery during one episode of mortality									

Date	Location	Species	Mortalities	Detection Method				Sequence Analysis	Reference
				TEM	PCR G	PCR B	PCR C		
2000	France	<i>P. maximus</i> (n=4)	Yes	Yes	+	+	+	OsHV-1Var	Arzul et al (2001) French Scallops: A New Host for Ostreid Herpesvirus-1. Virology 290: 342-349
Larval samples tested PCR G: Gp3/Gp4, PCR B: B3/B4, PCR C: C2/C4.									

Date	Location	Species	Mortalities	Detection Method				Sequence Analysis	Reference
				ISH	IHCT	PCR B	PCR C		
2000	France	<i>C. gigas</i> (n=30)	No	+	+	+	+	OSHV-1	Arzul et al (2002) Detection of oyster herpesvirus DNA and proteins in asymptomatic <i>Crassostrea gigas</i> adults. Virus Res 84: 151-160
Adult samples tested PCR B: B3/B2, PCR C: C2/C6									

Date	Location	Species	Mortalities	Detection Method				Sequence Analysis	Reference
				Histo	PCR A <sub>1</sub>	PCR A <sub>2</sub>	PCR C		
2002-2003	USA	<i>C. gigas</i>	Yes	+	N/A	+	-	*OsHV-1	Friedman et al (2005) Herpes virus in juvenile Pacific oysters <i>Crassostrea gigas</i> from Tomales Bay, California, coincides with summer mortality events. Dis Aquat Org 63: 33-41
Larval and juvenile samples tested Mortalities associated with elevated water temperatures PCR A <sub>1</sub> : A3/A4, PCR A <sub>2</sub> : A5/A6 (nested PCR), PCR C: C2/C6 *No PCR C positive material so unable to determine if it was the variant									

NOTES from Batista et al (2007) Detection of ostreid herpesvirus 1 DNA by PCR in bivalve mollusks: A critical review. J Virol Methods 139: 1-11

- Viral infections have been observed in adult bivalves but adults are apparently less sensitive to such infections compared to younger stages
- A variant of OsHV-1 (OsHV-1var) was also described in larvae of different bivalve species and OsHV-1 and OsHV-1var are considered representatives of a single viral species.
- The C region encodes parts of two proteins of unknown functions and is present twice in the genome (being located in the inverted repeats TR<sub>L</sub> and IR<sub>L</sub>).
- The differences observed in herpesvirus DNA detection suggested that the one-round PCR with the C2/C6 primer set was more useful for epidemiological surveys than the nested PCR using the A3/A4 and A5/A6 primer set.
- No amplification of OsHV-1var DNA with the C1/C4 and C1/C6 primer set, smaller amplicons produced with the C2/C4 and C2/C6 primer sets. Identical sized amplicons for both OsHV-1 and OsHV-1var using the A2/A4, B1/B2, B2/B4 and Gp3/Gp4 primer sets.
- C2/C6 allows differentiation of OsHV-1 and OsHV-1var but failed to amplify OsHV-1 detected in the USA.

Date	Location	Species	Mortalities	Detection Method				Sequence Analysis	Reference
					PCR A <sub>1</sub>	PCR A <sub>2</sub>			
2007	China Japan Korea	<i>C. ariakensis</i> <i>C. siakmea</i> <i>C. gigas</i> <i>C. hongkongensis</i>	No		N/A	+		*OsHV-1 (SNP differences in the A region)	Moss et al (2007) Pathogens in <i>Crassostrea ariakensis</i> and other Asian oyster species: implications for non-native oyster introduction to Chesapeake Bay. Dis Aquat Org 77: 201-223
PCR A <sub>1</sub> : A3/A4, PCR A <sub>2</sub> : A5/A6 (nested PCR) *3 polymorphic sites in the A region: 2 genetic strains in Japan, 1 in Korea and 2 in China. Suggest sequencing of additional gene regions									

Date	Location	Species	Mortalities	Detection Method			Sequence Analysis	Reference
				PCR A		PCR C		
1995-2007	France	<i>C. gigas</i> (32 isolates)	Yes	+		+	OsHV-1	Segarra et al (2010) Detection and description of a particular <i>Ostreid herpesvirus 1</i> genotype associated with massive mortality outbreaks of Pacific oysters, <i>Crassostrea gigas</i> , in France in 2008. Virus Res 153: 92-99
2008	France	<i>C. gigas</i>	Yes	+		+	17 OsHV-1 15* OsHV-1 $\mu$ Var	

PCR A: IA1/IA2 (new PCR primers), PCR C: C2/C6  
\* OsHV-1 $\mu$ Var different to OsHV-1Var

- In one batch, both OsHV-1 and OsHV-1 $\mu$ Var were detected. Sequence comparison between OsHV-1 and OsHV-1 $\mu$ Var but no sequence comparison with OsHV-1Var
- No relationship between geographical location and virus genotype
- Both OsHV-1 and OsHV-1 $\mu$ Var associated with mortality events in 2008
- Important information attributed to (data not shown) and (...., personal communication)
- More work is need to fully investigate the possible infectivity and virulence differences between the OsHV-1 and OsHV-1 $\mu$ Var genotypes
- Detection of the two genotypes in some samples collected in 2008 led suspect (sic) the presence of both genotypes in one individual. A study using the cloning technique has been carried out to investigate this aspect but has been inconclusive (data not shown).
- Our work revealed the emergence of a third genotype, OsHV-1 $\mu$ Var, associated with abnormal mortalities of *C. gigas* in France.

Date	Location	Species	Mortalities	Sequence Analysis	Reference
2008	France Ireland	<i>C. gigas</i>	Yes	OsHV-1 OsHV-1 $\mu$ Var	COMMISSION REGULATION (EU) No 175/2010 of 2 March 2010 implementing Council Directive 2006/88/EC as regards measures to control increased mortality in oysters of the species <i>Crassostrea gigas</i> in connection with the detection of <i>Ostreid herpesvirus 1</i> $\mu$ var (OsHV-1 $\mu$ var)
2009	Ireland United Kingdom	<i>C. gigas</i>	Yes	Suggestion OsHV-1 $\mu$ Var played a role	

- When increased mortality in *C. gigas* is reported, testing for detection/absence of OsHV-1 $\mu$ Var should be carried out
- The availability of accurate and timely information on the situation as regards the detection of OsHV-1  $\mu$ var in the Member States is a key element to ensure a proper control of the emerging disease situation. For that purpose, Member States should inform the Commission and the other Member States of the first confirmed presence of the OsHV-1  $\mu$ var virus on their territories in 2010 without undue delay.
- As there are, the measures provided for in this Regulation should apply until the end of December 2010.
- For the purposes of this Regulation, OsHV-1  $\mu$ var means a genotype of the virus *Ostreid herpesvirus-1* (OsHV-1) which is defined on the basis of partial sequence data exhibiting a systematic deletion of 12 base pairs in ORF 4 of the genome in comparison with OsHV-1 (GenBank # AY509253).
- The following primers must be used: CF and CR (These primers or descriptions thereof may be obtained from the Community Reference Laboratory for Mollusc Diseases (LGP-Ifremer, av de Mus de Loup, 17390 La Tremblade, France). The presence of OsHV-1  $\mu$ var in a sample is indicated by the presence of a band of the appropriate size (157 bp instead of 173 bp for OsHV-1) on a 2.5 % agarose gel with all negative controls negative and all positive controls positive.

### SUMMARIES OF KEY FINDINGS, KNOWLEDGE GAPS AND RESEARCH PRIORITIES

#### GROWERS

##### Key findings

- Growers agreed that the similarity between the virus genotypes found in Australia and New Zealand and those in Asia was a surprising result considering that no reports of mortalities associated with herpesvirus or even unexplained mortalities had been made from Asia. Given the importance of shipping routes between Australia and Asia it seems unusual that there have been no reports of suspected occurrence of the disease in Asia.
- The issue of water temperature limits is confusing. Research first suggested 18° C was the lower limit but with further research this estimate has changed to 16° C then 14° C. It was noted that this change in temperature limit could be misconceived as being as result of a further mutating by OsHV-1  $\mu$ Var. In discussion it was pointed out that this apparently variable temperature threshold is likely to be due to the interplay of a variety of other factors.
- Stock movements appear to be an important factor for several reasons. It was felt that over-handling of stock stresses the animals and leaves them more vulnerable to disease.
- The testing for OsHV-1  $\mu$ Var is confusing. Different tests are used at the different laboratories and this is confusing to producers and gives the impression that perhaps one may be more sensitive than another.

##### Knowledge Gaps

- At the moment little or nothing is known of any possible presence or distribution of OsHV-1  $\mu$ Var in Asia. As it is still an emerging disease and not an OIE listed disease countries are not compelled to report suspected cases.
- The possible risks other carrier species play in the life cycle of the disease is not well understood and could provide some important clues.
- Translocation of spat grown out at 18° C is a possible mechanism for making spat more hardy and reducing mortalities. More work could be done in looking at this as an option.
- The important question of whether vertical transmission occurs.

## Research priorities or actions needed

- More collaboration with Asian oyster industry is needed. Reports of mortalities, disease investigations and other possible evidence presence of OsHV-1  $\mu$ Var in Asia would be most helpful in determining spread, possible transmission mechanisms and strengthening our scientific understanding of the disease.
- It was suggested that avoiding any unnecessary movement and minimising handling in routine management practices should be recommended as a strategy to reduce oyster susceptibility to disease. Restrictions on stock movements during an investigation into mortalities were also seen as crucial in the mitigation of further transmission of the disease.
- Data collection of stock movements and management activities should be improved and would potentially be a powerful tool for surveillance and epidemiological investigations.
- Improvements in selective breeding for resistance also have the potential to be useful. France has seen a reduction in mortalities in those farmers who have used lines bred for resistance.
- Genetic research seems an important priority.
- Important for all concerned with the disease to reiterate wherever appropriate that the Oyster Herpesvirus and OsHV-1  $\mu$ Var pose no threat to human health and are not zoonotic. This will allay public misconceptions about any connection with human herpesvirus simplex and is an important message to make in order to protect market confidence in the product.
- Standardisation of testing in laboratories in both state and federal laboratories should be ensured. At the moment only the laboratories at EMAI and AAHL can test for the presence of the virus. This testing should be made available at state level laboratories in order to save time and perhaps money. Outbreak investigations should send samples to both state and national (AAHL) laboratories for urgent testing.
- Development of good management practices for routine data collection, immediate response, containment, control and surveillance. These need to be clear and well documented action plans which will ensure consistency across the industry. This is urgent with summer approaching.
- A clear understanding of government bodies and personnel who are responsible for various activities in the event of an outbreak or mortality report. For example, head agency, management groups in each state and any protocols they may have, national oyster industry POMS working group etc.
- The development of a framework to action the priorities from the workshop. Establishment of a committee or advisory group. In discussion, it was suggested that the existing FRDC AAHS may be able to take this role initially.

- The importance of continuing the dialog between researchers, regulators and industry on this issue. This may be the same group or a smaller group to meet on a regular basis (every year or two) and if the virus spreads being able to meet earlier.
- Recognising the importance of preventing the spread of the virus from NSW i.e. biosecurity management, translocation, education of industry and public (disposing of oysters into land fill etc.)
- That industry is represented on any working groups that are formed. Hatcheries will be crucial in any recovery from an outbreak.
- Selective breeding be recognised as one of the most important long term strategies. With Tasmania and SA isolated from NSW it is hoped that there is time to have 5 to 10 years of breeding under our belt before the disease spreads. This may be unrealistic but we have to be positive.
- Workshop findings need to be presented to industry, WOS4 in Tasmania in September a must.
- Linking with international research groups is important.
- All Pacific oyster farming states to implement a Pacific Oyster Health Surveillance program, Tasmania has one and has data from the past 15 years on oyster health.

## SCIENCE

### Key points

- Causes of mortality in the oyster
- Host-pathogen interaction
- Role of other pathogens, e.g. *Vibrio*, and other mollusc species, e.g. scallops
- Genetic background of Pacific oysters
- Biosecurity measures
- Hatchery methods for producing disease free spat
- Breeding for genetic resistance
- Water temperature (16°C) and overall temperature tolerance of the virus are important factors
- Cost balance of whether to leave oysters to die *in situ* or harvest
- Environmental impacts

### Research priorities

- Harmonize and validate diagnostics including a definition of mortalities
- Confirmation of the global distribution of the disease
- Pathogenicity studies

- Development of an experimental infection model
- Genetic analysis of the virus for insights into virulence and pathogenicity
- Better understanding of the genome and ORFs of significance
- Selective breeding for resistance
- Environmental risk factor analysis

## REGULATORS AND MANAGEMENT

### Actions recommended

- Integration and coordination of current activities and future initiatives is required
- Surveillance pre-summer 2011 in NSW, TAS and SA to improve capacity for early warning system
- Industry to work with states to ensure reporting mechanisms are in place for unexplained mortalities. Facilitating disease reporting and investigation – clarify disease reporting channels including when, what and how to report. Industry training in sample collection and dispatch.
- Rapid emergency response and quick turnaround of results from laboratories – roles, responsibilities and limitations
- Development of biosecurity guidelines – good practice for farmers particularly with translocation
- Industry to be better advised of testing protocols, time frames for results and costs
- DAFF OsHV-1  $\mu$ Var entry pathways project fully supported
- National survey which will detail the status of the disease in Australia should report results in August/September, 2011. It should inform national response and objective setting, future priorities and planning strategies.
- FRDC industry capacity building project (including Australian industry representatives for France and Ireland) fully supported
- Consideration of alternative species for production. Trialling *Ostrea angasi* (Australian flat oyster) as a third commercial oyster species in Australia. Trialling pipis as a potential species for aquaculture re-seeding. Possible change in producer business models to diversify stock and diminish risk.
- Examining resistant oyster lines
- National database for mortalities/events which will also inform growers and allow for benchmarking
- Testing production strategy to make sales prior to the water temperature reaching 15°C
- Examine the applicability of Performance of Veterinary Services (PVS) tool proposed by Geoff Grossel

- National listing of the disease is under consideration at the moment. Possible quarantine impacts and potential changes to other legislative powers.

#### Other points raised in final discussion

- AquaHealth.Net is a potentially useful information sharing tool.
- The Australian Centre of Excellence for Risk Analysis (ACERA) may be an important point of collaboration for analysis of risk factors.
- Collaboration and information sharing to be enhanced
  - Histology via ABIN
  - Phylogenetic (gene sequencing) studies
  - NSW and AAHL (will also undertake sequencing with approval from NSW CVO)
  - NZ (undertaking sequencing) and AAHL
  - Publishing of gene sequencing with GenBank
  - Other oyster producing countries
- Key recommendation: Use of the existing FRDC AAHS as an advisory and coordinating body for future activities and research. Needs to rejuvenate industry cooperation and ensure state jurisdictional contributions. International collaboration also required to widen its international engagement, i.e. informal links with IFREMER, OIE Reference Laboratory.
- Greater cooperation and also a more sensitive appreciation of different standpoints between oyster industry, shipping authorities, quarantine, science etc to enable further work on route of introduction: shipping, biofouling, ballast water, equipment imports, public waste disposal etc. in order to reduce the introduction of marine pests and diseases.
- Multifactorial approach needed when examining causal agents or risk factors. There is a complex web of causation, for example temperature is an important but not definitive switch for the disease. The difference in effects of temperature on virus and on host appears complex also.
- State laboratories should request standard positive controls from AAHL to ensure the consistency of tests (demand for standard positive controls need to be industry driven) – TAS has agreed to make this request.
- Cost sharing in Tasmania between Government and industry for ongoing surveillance and testing.
- Virus characterisation. Research required into phylogenetic relationships of all isolates globally.
- Reporting is hampered by the lack of a definition of increased mortality. A clear case definition for “increased mortality” including the life stage or production system affected and a mortality threshold is still not available.

## ANNEXE 5: SELECTED INTERNET LINKS

EURL for Mollusc Diseases, IFREMER. Tutorial on OsHV-1.

<http://wwz.ifremer.fr/crlmollusc/Main-activities/Tutorials/Herpes-virus-OsHV-1>

IFREMER OsHV-1 detection and quantification by real-time polymerase chain reaction.

[http://wwz.ifremer.fr/crlmollusc/content/download/42545/578238/file/OsHV-1%20RTPCR\\_1.pdf](http://wwz.ifremer.fr/crlmollusc/content/download/42545/578238/file/OsHV-1%20RTPCR_1.pdf)

Oyster mortalities in connection with OsHV-1. Commission Regulation (EU) No 175/2010 implementing Council Directive 2006/88/EC as regards measures to control increased mortality in oysters of the species *Crassostrea gigas* in connection with the detection of Ostreid herpesvirus 1  $\mu$ var (OsHV-1  $\mu$ var).

[http://www.megapesca.com/megashop/FH201103\\_i89/Oyster\\_Mortalities.htm](http://www.megapesca.com/megashop/FH201103_i89/Oyster_Mortalities.htm)

Guidance document on the establishment of surveillance programmes for ostreid herpesvirus 1  $\mu$ var (OsHV-1  $\mu$ var). European Commission, March, 2011

[http://ec.europa.eu/food/animal/liveanimals/aquaculture/guidance\\_document/OsHV-1%20surveillance\\_en.pdf](http://ec.europa.eu/food/animal/liveanimals/aquaculture/guidance_document/OsHV-1%20surveillance_en.pdf)

Dataquest: Inventory of data sources relevant for the identification of emerging diseases in the European aquaculture population. EFSA.

<http://www.efsa.europa.eu/en/supporting/pub/90e.htm>

Scientific Opinion on the increased mortality events in Pacific oysters, *Crassostrea gigas* 1. EFSA Panel on Animal Health and welfare (AHAW) 2, 3. European Food Safety Authority (EFSA), Parma, Italy.

<http://www.qualita.legapesca.it/documenti/parere%20EFSA%20su%20moria%20ostriche.pdf>

AFFSA. 2008. Avis de l'agence Francaise de securite sanitaire des aliments sur l'évaluation des risques zoo-sanitaires lies a l'exportation ou aux échanges intra-communautaires d'huitres dans un contexte de surmortalite d'huitres crues sur le littoral metropolitain. Affsa-saisine n. 2008-SA-0214.

Alvarez Vilaseñor R. 2006. Estudio taxonómico de bacterias del género *Vibrio* aisladas de ostión (*Crassostrea gigas*) de cultivo. MSc Thesis, CIAD Mazatlan, Mexico.

Arzul I, Garcia C, Joly JP. 2010. Report of the 2010 Annual Meeting of the National Reference Laboratories for Mollusc Diseases, Nantes 23-24 March 2010, 106 pp.

Arzul I, Nicolas JL, Davison AJ, Renault T. 2001a. French scallops: A new host for ostreid herpesvirus-1. *Viol.*, 290, 342-349.

Arzul I, Renault T, Lipart C. 2001b. Experimental herpes-like viral infections in marine bivalves: Demonstration of interspecies transmission. *Dis. Aquat. Org.*, 46, 1-6.

Arzul I, Renault T, Lipart C, Davison AJ. 2001c. Evidence for interspecies transmission of oyster herpesvirus in marine bivalves. *J. Gen. Virol.*, 82, 865-870.

Arzul I, Renault T, Thébault A, Gérard A. 2002. Detection of oyster herpesvirus DNA and proteins in asymptomatic *Crassostrea gigas* adults. *Virus Res.*, 84, 151-160.

Arzul I, Langlade A, Chollet B, Robert M, Ferrand S, Omnes E, Lerond S, Couraleau Y, Joly J-P, Francois C, Garcia C. Can the protozoan parasite *Bonamia ostreae* infect larvae of flat oysters *Ostrea edulis*? *Vet. Parasitol.* In Press. Publisher's official version: <http://dx.doi.org/10.1016/j.vetpar.2011.01.060>

Arzul I, Nicolas J-L, Davison AJ, Renault T. 2001. French scallops: A new host for ostreid herpesvirus-1. *Viol.*, 290, 342-349.

Arzul I, Renault T, Lipart C. 2001. Experimental herpes-like viral infections in marine bivalves: demonstration of interspecies transmission. *Dis. Aquat. Org.*, 46, 1-6.

Arzul I, Renault T, Lipart C, Davison A. 2001. Evidence for interspecies transmission of oyster herpesvirus in marine bivalves. *J. Gen. Virol.*, 82, 865-870.

Arzul I, Renault T, Thebault A, Gerard A. 2002. Detection of oyster herpesvirus DNA and proteins in asymptomatic *Crassostrea gigas* adults. *Virus Res.*, 84, 151-160.

- Azandegbe A. 2010. Etude de la structure des communautés bactériennes du sédiment et de l'écologie de *Vibrio aestuarianus* pathogène de l'huître creuse *Crassostrea gigas* dans deux sites ostréicoles. PhD Thesis, Université Européenne de Bretagne. <http://archimer.ifremer.fr/doc/00032/14277/>
- Barbosa-Solomieu V, Dégremont L, Vázquez-Juárez R, Ascencio-Valle F, Boudry P, Renault T. 2005. Ostreid Herpesvirus 1 (OsHV-1) detection among three successive generations of Pacific oysters (*Crassostrea gigas*). *Virus Res.*, 107, 47-56.
- Barbosa-Solomieu V, Miossec L, Vázquez-Juárez R, Ascencio-Valle F, Renault T. 2004. Diagnosis of Ostreid herpesvirus 1 in fixed paraffin-embedded archival samples using PCR and *in situ* hybridisation. *J. Virol. Meth.*, 119, 65-72.
- Batista F, Arzul I, Pepin J-F, Ruano F, Friedman CS, Boudry P, Renault T. 2007. Detection of ostreid herpesvirus 1 DNA by PCR in bivalve molluscs: A critical review. *J. Virol. Meth.*, 139, 1-11.
- Batista F, Taris N, Boudry P, Renault T. 2005. Detection of ostreid herpesvirus-1 (OsHV-1) by PCR using a rapid and simple method of DNA extraction from oyster larvae. *Dis. Aquat. Org.*, 64, 1-4.
- Beattie JH, Chew KK, Hershberger WK. 1980. Differential survival of selected strains of Pacific oysters (*Crassostrea gigas*) during Summer Mortality. *Proc. Nat. Shellfish Assoc.*, 70, 184-189.
- Beaz-Hidalgo R, Diéguez A, Cleenwerck I, Balboa S, Doce A, Vos P, Romalde J. *Vibrio celticus* sp. nov., a new *Vibrio* species belonging to the *Splendidus* clade with pathogenic potential for clams. *Systematic Appl. Microbiol.* (in press)
- Berthe F. 2004. Report about mollusc diseases. Mediterranean aquaculture diagnostic laboratories, 49, 33-48. <http://archimer.ifremer.fr/doc/00000/3300/>
- Berthe F. 2005. Diseases in mollusc hatcheries and their paradox in health management. *Diseases in Asian Aquaculture*. <http://archimer.ifremer.fr/doc/00000/3289/>
- Bodoy A, Garnier J, Razet D, Geairon P. 1990. Mass mortalities of oysters (*Crassostrea gigas*) during spring 1988 in the bay of Marennes-Oléron, related to environmental conditions: ICES CM 1990/K, 11, 1-23.
- Bouget J-F, Mazurie J. 2005. Croissance des huîtres creuses, *Crassostrea gigas*, en baie du mont Saint-Michel, avant la restructuration de 2004. <http://archimer.ifremer.fr/doc/00000/1637/>

Buchet V, Bluteau A. 1996. Etude d'un élevage intégré bars-huîtres.

<http://archimer.ifremer.fr/doc/00000/1917/>

Buestel D, Ropert M, Prou J, Gouilletquer P. 2009. History, status, and future of oyster culture in France. *J. Shellfish Res.*, 28, 813-820.

Burge C. 2010. Report of the 2010 Annual meeting of the National Reference Laboratories for Mollusc Diseases, Nantes, 23-24 March 2010. IFREMER, La Tremblade, France.

Burge CA, Griffin FJ, Friedman CS. 2006. Mortality and herpesvirus infections in the Pacific oyster *Crassostrea gigas* in Tomales Bay, California, USA. *Dis. Aquat. Org.*, 72, 31-43.

Burge C, Judah L, Conquest L, Griffin F, Cheney D, Suhrbier A, Vadopalas B, Olin PG, Renault T, Friedman CS. 2007. Summer seed mortality of the Pacific oyster, *Crassostrea gigas* Thunberg grown in Tomales Bay, California, USA: The influence of oyster stock, planting time, pathogens, and environmental stressors. *J. Shellfish Res.*, 26, 163-172.

Chang PH, Kuo ST, Lai SH, Yang HS, Ting YY, Hsu CL, Chen HC. 2005. Herpes-like virus infection causing mortality of cultured abalone *Haliotis diversicolor supertexta* in Taiwan. *Dis. Aquat. Org.*, 65, 23-27.

Chavez-Villaba J, Arreola-Lizárraga A, Burrola-Sánchez S, Hoyos-Chairez F. 2010. Growth, condition, and survival of the Pacific oyster *Crassostrea gigas* cultivated within and outside a subtropical lagoon. *Aquaculture*, 300, 128-136.

Chavez-Villaba J, Villelas-Avila R, Caceres-Martinez C, 2007. Reproduction, condition and mortality of the Pacific oyster *Crassostrea gigas* (Thunberg) in Sonora, Mexico. *Aquaculture Res.*, 38, 268-278.

Cheney D, Elston R, MacDonald B, Kinnan K, Suhrbier A. 2001. The roles of environmental stressors and culture methods on the summer mortality of the Pacific oyster *Crassostrea gigas*. *J. Shellfish Res.*, 20, 1195.

Cheney DP, MacDonald BF, Elston RA. 2000. Summer Mortality of Pacific oysters, *Crassostrea gigas* (Thunberg): Initial findings on multiple environmental stressors in Puget Sound, Washington, 1998. *J. Shellfish Res.*, 19, 353-359.

Cochennec-Laureau N, Baud J-P, Bedier E, Boudry P, Huvet A, Nicolas J-L, Pepin J-F, Petton B. 2010. Bilan des « Journées Surmortalité des huîtres creuses, *Crassostrea gigas* » du Programme P7 « Aquaculture Durable » des 8 et 9 décembre 2009. «

Journées Surmortalité des huîtres creuses du Programme P7 » 2009.

<http://archimer.ifremer.fr/doc/00000/7393/>

Collet B, Boudry P, Bougrier S, Heurtebise S, Phelipot P, Ledu C, Morand B, Heral M, Gerard A. 1997. Etudes des bases génétiques et de la variabilité des caractères physiologiques impliqués dans la croissance chez l'huître creuse *Crassostrea gigas*. Journées Conchylicoles Ifremer 1997. <http://archimer.ifremer.fr/doc/00000/3243/>

Comps M. 1988. Epizootic diseases of oysters associated with viral infections. American Fisheries Society Special Publication, 18, 23-37.

<http://archimer.ifremer.fr/doc/00000/5912/>

Comps M, Cochenec N. 1993. A herpes-like virus from the European oyster *Ostrea edulis*. L. J. Invert. Pathol., 62, 201-203.

Comps M, Herbaut Ch., Fougerouse A. 1999. Virus-like particles in pearl oyster *Pinctada margaritifera*. Bull. Eur. Assoc. Fish Pathol., 19, 85-88.

Corbeil S, Colling A, Williams LM, Wong FYK, Savin K, Warner S, Murdoch B, Cogan NOI, Sawbridge TI, Fegan M, Mohammad I, Sunarto A, Handler J, Pyecroft S, Douglas M, Chang PH, Crane MStJ. 2010. Development and validation of a TaqMan PCR assay for the Australian abalone herpes-like virus. Dis. Aquat. Org., 92, 1-10.

Costil K, Royer J, Ropert M, Soletchnik P, Mathieu M. 2005. Spatio-temporal variations in biological performances and Summer Mortality of the Pacific oyster *Crassostrea gigas* in Normandy (France). Helgoland Marine Research, 59, 286-300.

da Silva PM, Fuentes J, Villalba A. 2005. Growth, mortality and disease susceptibility of oyster *Ostrea edulis* families obtained from brood stocks of different geographical origins, through on-growing in the Ria de Arousa (Galicia, NW Spain). Mar. Biol., 147, 965-977.

da Silva PM, Renault T, Fuentes J, Villalba A. 2008. Herpesvirus infection in European flat oysters *Ostrea edulis* obtained from brood stocks of various geographic origins and grown in Galicia (NW Spain). Dis. Aquat. Org., 78, 181-188.

Dang C, Gonzalez P, Mesmer-Dudons N, Bonami J-R, Caill-Milly N, De Montaudouin X. 2009. Virus-like particles associated with brown muscle disease in Manila clam, *Ruditapes philippinarum*, in Arcachon Bay (France). J. Fish Dis., 32, 577-584.

Davison AJ, Eberle R, Ehlers B, Hayward GS, McGeoch DJ, Minson AC, Pellett PE, Roizman B, Studdert MJ, Thiry E. 2009. The order Herpesvirales. *Arch. Virol.*, 154, 171-177.

Davison AJ, Trus BL, Cheng NQ, Steven AC, Watson MS, Cunningham C, Le Deuff RM, Renault T. 2005. A novel class of herpesvirus with bivalve hosts. *J. Gen. Virol.*, 86, 41-53.

De Decker S. 2010. Approches multifactorielles pour l'étude d'interactions entre l'huître creuse *Crassostrea gigas* et deux *Vibrio* pathogènes, *V. splendidus* et *V. aestuarianus*: épidémiologie, variabilité de la sensibilité de l'hôte et pathogénèse. PhD Thesis, Université de La Rochelle. <http://archimer.ifremer.fr/doc/00016/12697/>

De Decker S, Normand J, Saulnier D, Pernet F, Castagnet S, Boudry P. 2011. Responses of diploid and triploid Pacific oysters *Crassostrea gigas* to *Vibrio* infection in relation to their reproductive status. *J. Invert. Pathol.*, 106, 179-191.

Dégremont L. 2003. Etude des bases génétiques de la mortalité estivale et des relations avec la croissance chez les juvéniles de l'huître creuse *Crassostrea gigas*. PhD Thesis, Université de Caen/Basse-Normandie. <http://archimer.ifremer.fr/doc/00000/202/>

Dégremont L, Bedier E, Soletchnik P, Ropert M, Huvet A, Moal J, Samain JF, Boudry P. 2005. Relative importance of family, site, and field placement timing on survival, growth, and yield of hatchery-produced Pacific oyster spat (*Crassostrea gigas*). *Aquaculture*, 249, 213-229.

Dégremont L, Boudry P, Ropert P, Samain J, Bédier E, Soletchnik P. 2010. Effects of age and environment on survival of summer mortality by two selected groups of the Pacific oyster *Crassostrea gigas*. *Aquaculture*, 299, 44-50.

Dégremont L, Boudry P, Soletchnik P, Bédier E, Ropert M, Huvet A, Moal J, Samain JF. 2003. Genetic basis of summer mortality in juvenile cupped oysters. *J. Shellfish Res.*, 22, 327-333.

Dégremont L, Soletchnik P, Boudry P. 2010. Summer mortality of selected juvenile Pacific oyster *Crassostrea gigas* under laboratory conditions and in comparison with field performance. *J. Shellfish Res.*, 29, 847-856.

Delaporte M, Soudant P, Lambert C, Jegaden M, Moal J, Pouvreau S, Dégremont L, Boudry P, Samain JF. 2007. Characterisation of physiological and immunological differences between Pacific oysters (*Crassostrea gigas*) genetically selected for high or low survival to summer mortalities and fed different rations under controlled conditions. *J. Exp. Mar. Biol. Ecol.*, 353, 45-57.

Deniau S. 2000. Essais de propagation in vitro de virus de type herpès infectant les mollusques bivalves marins et contribution à l'étude des interactions hôte-virus. PhD Thesis, Ecole Pratique des Hautes Etudes.

<http://archimer.ifremer.fr/doc/00025/13631/>

Didier Y. 2000. Apoptose et infections à virus de type herpès chez les bivalves marins: Détection de séquences codant pour des protéines inhibitrices de l'apoptose.

<http://archimer.ifremer.fr/doc/00000/1768/>

Dubuisson-Quellier S. 2001. Impacts des informations produites par l'IFREMER sur la qualité des eaux littorales dans la production et la commercialisation des coquillages. <http://archimer.ifremer.fr/doc/00000/1740/>

Dundon WG, Arzul I, Omnes E, Robert M, Magnabosco C, Zambon M, Gennari L, Toffan A, Terregino C, Capua I, Arcangeli G. 2011. Detection of Type 1 Ostreid Herpes variant (OsHV-1 mu var) with no associated mortality in French-origin Pacific cupped oyster *Crassostrea gigas* farmed in Italy. *Aquaculture*, 314, 49-52.

Duperthuy M. 2010. Effecteurs moléculaires de l'association *Crassostrea gigas*/*Vibrio splendidus*: Role de la porine OmpU dans les mécanismes de résistance et d'échappement à la réponse immunitaire de l'hôte. PhD Thesis, Université Montpellier 2. <http://archimer.ifremer.fr/doc/00028/13905/>

EFSA (European Food Safety Authority). 2008. Scientific Opinion of the Panel on Animal Health and Welfare on a request from the European Commission on aquatic animal species susceptible to diseases listed in the Council Directive 2006/88/EC. *The EFSA Journal*, 808, 1-144.

Elandaloussi L, Carrasco N, Andree K, Furones D, Roque A. 2009. Esdeveniments de mortalitat de l'ostró del Pacífic (*Crassostrea gigas*) en el delta de l'Ebre- Estudi de cas. Proceedings of the II Simposi d'aqüicultura de Catalunya. Sant Carles de la Rapita, Spain.

Elston RA, Hasegawa H, Humphrey KL, Polyak IK, Häse CC. 2008. Re-emergence of *Vibrio tubiashii* in bivalve shellfish aquaculture: Severity, environmental drivers, geographic extent and management. *Dis. Aquat. Org.*, 82, 119-123.

Epaud C. 2000. Recherche dans des échantillons d'eau de claires ostréicoles d'ADN génomique du virus de type herpès de l'huître.

<http://archimer.ifremer.fr/doc/00032/14342/>

Estes R, Friedman C, Elston R, Herwig R. 2004. Pathogenicity testing of shellfish hatchery bacterial isolates on Pacific oyster *Crassostrea gigas* larvae. *Dis. Aquat. Org.*, 58, 223-230.

FAO (Food and Agricultural Organization of the United Nations). 2008. The State of World Fisheries and Aquaculture 2008.

Available from: <http://www.fao.org/docrep/011/i0250e/i0250e00.htm>.

Farto R, Montes M, Perez MJ, Nieto TP, Larsen JL, Pedersen K. 1999.

Characterization by numerical taxonomy and ribotyping of *Vibrio splendidus* biovar I and *Vibrio scophthalmi* strains associated with turbot cultures. J. Appl. Microbiol., 86, 796-804.

Fleury E. 2009. Exploration fonctionnelle de gènes différentiellement exprimés entre les souches d'huîtres creuses *Crassostrea gigas* résistantes et sensibles à la mortalité estivale. PhD Thesis, Université de Rennes I.

<http://archimer.ifremer.fr/doc/00000/6462/>

Fleury E, Moal J, Boulo V, Daniel J-Y, Mazurais D, Henaut A, Corporeau C, Boudry P, Favrel P, Huvet A. 2010. Microarray-based identification of gonad transcripts differentially expressed between lines of Pacific oyster selected to be resistant or susceptible to summer mortality. Mar. Biotechnol., 12, 326-339.

Fleury P-G, Goyard E, Mazurie J, Claude S, Bouget JF, Langlade A, le Coguc Y. 2001. The assessing of Pacific oyster (*Crassostrea gigas*) rearing performances by the IFREMER/REMORA network: Method and first results (1993-98) in Brittany (France). Hydrobiologia, 465, 195-208.

Fleury P-G, Simonne C, Claude S, Palvadeau H, Guilpain P, D'Amico F, Le Gall P, Vercelli C, Pien S. 2005. Réseau Mollusques des Rendements Aquacoles (REMORA - huître creuse); résultats des stations NATIONALES. Année 2003.

<http://archimer.ifremer.fr/doc/00000/2412/>

Fouche D. 1997. Etat des connaissances sur la pathologie chez les mollusques bivalves. <http://archimer.ifremer.fr/doc/00000/1923/>

Friedman CS, Estes RM, Stokes NA, Burge CA, Hargove JS, Barber BJ, Elston RA, Burreson EM, Reece KS. 2005. Herpes virus in juvenile Pacific oysters *Crassostrea gigas* from Tomales Bay, California, coincides with Summer Mortality episodes. Dis. Aquat. Org., 63, 33-41.

Gagnaire B. 2005. Etude des effets de polluants sur les paramètres hématocytaires de l'huître creuse, *Crassostrea gigas* - Interactions entre environnement, mécanismes de défense et maladies infectieuses. PhD Thesis, Université de la Rochelle.

<http://archimer.ifremer.fr/doc/00000/730/>

Gagnaire B, Frouin H, Moreau K, Thomas GH, Renault T. 2006a. Effects of temperature and salinity on haemocyte activities of the Pacific oyster, *Crassostrea gigas* (Thunberg). *Fish Shellfish Immunol.*, 20, 536-547.

Gagnaire B, Soletchnik P, Madec P, Geairon P, Le Moine O, Renault T. 2006b. Diploid and triploid Pacific oysters, *Crassostrea gigas* (Thunberg), reared at two heights above sediment in Marennes-Oleron Basin, France: Difference in mortality, sexual maturation and hemocyte parameters. *Aquaculture*, 254, 606-616.

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*. *Microbial Ecol.*, 53, 187-196.

Gay M. 2004. Infection expérimentale chez *Crassostrea gigas*: étude de deux souches pathogènes apparentées à *Vibrio splendidus*. PhD Thesis, Université de La Rochelle. <http://archimer.ifremer.fr/doc/00000/328/>

Gay M, Berthe F, Leroux F. 2004. Screening of *Vibrio* isolates to develop an experimental infection model in the Pacific oyster *Crassostrea gigas*. *Dis. Aquat. Org.*, 59, 49-56.

Gay M, Lancelot G, Cholet B, Renault T, Cochenec C, Berthe FJ, Lambert C, Choquet G, Paillard C, Gouy M, Le Roux F, Gouletquer P. 2003. Characterisation of *Vibrio* isolated from Pacific oyster spat suffering from Summer Mortality outbreaks. *J. Shellfish Res.*, 22, 331.

Gay M, Renault T, Pons AM, Le Roux F. 2004. Two *Vibrio splendidus* related strains collaborate to kill *Crassostrea gigas*: taxonomy and host alterations. *Dis. Aquat. Org.*, 62, 65-74.

Gay M, Renault T, Le Roux F. 2003. Characterization of *Vibrio* isolated from *Crassostrea gigas* spat suffering summer mortality outbreaks (slides). 95. Ann. Meeting National Shellfisheries Assoc., New Orleans LA, USA, 13-17 April 2003. <http://archimer.ifremer.fr/doc/00000/3343/>

Gay M, Waechter M, Lambert C, Escoubas J-M, Cochenec N, Nicolas J-L, Berthe F, Le Roux F. 2001. Caractérisation de bactéries pathogènes, *Vibrio splendidus*, isolées de bivalves marins. Journées Conchylicoles Ifremer 2001. <http://archimer.ifremer.fr/doc/00000/3277/>

Gerard A. 1998. Avancées récentes sur la reproduction des huîtres. *La pisciculture Française*, 134, 71-76. <http://archimer.ifremer.fr/doc/00000/6535/>

- Gerard A, Naciri-Graven Y, Boudry P, Launey S, Heurtebise S, Ledu C, Phelipot P. 1995. Contrôle de la gamétogénèse des huîtres creuses et plates. Relations "reproduction" et "génétique". Groupe de travail sur la Reproduction des Mollusques. Bivalves Aquaculture Marine.  
<http://archimer.ifremer.fr/doc/00000/3210/>
- Gonzalez M. 2005. Etude de la réponse immunitaire chez l'huître *Crassostrea gigas*: Caractérisation et fonctions de protéines de reconnaissance aux LPS et d'effecteurs antimicrobiens. PhD Thesis, Université Montpellier II.  
<http://archimer.ifremer.fr/doc/00000/2098/>
- Goodman LB, Loregian A, Perkins GA, Nugent J, Buckles EL. 2007. A point mutation in a herpesvirus polymerase determines neuropathogenicity. PLoS Pathog 3(11): e160. doi:10.1371/journal.ppat.0030160
- Gouletquer P. 1998. Shellfish culture in France: present status and new approaches to optimise production. Shellfish Assoc. Great Britain, London.  
<http://archimer.ifremer.fr/doc/00000/3094/>
- Gouletquer P, Heral M, Rotschild B. 1994. Causes of decline of oyster production (*Crassostrea virginica*) in the Maryland portion of the Chesapeake Bay: A literature study. Haliotis, 23, 87-112. <http://archimer.ifremer.fr/doc/00000/3081/>
- Gouletquer P, Soletchnik P, Le Moine O, Razet D, Geairon P, Faury N, Taillade S. 1998. Summer Mortality of the Pacific cupped oyster *Crassostrea gigas* in the Bay of Marrennes-Oleron (France). ICES Mariculture Committee CM 1998/CC, 14-21.  
<http://archimer.ifremer.fr/doc/00000/3093/>
- Grizel H. 1987. Les maladies des mollusques: étiologie et progrès récents des recherches. Océanis, 13, 357-370. <http://archimer.ifremer.fr/doc/00000/3105/>
- Gubbins M. 2010. Recent mass mortality events affecting Pacific oysters in Europe. Powerpoint presentation. Fish health Inspectorate. Cefas, Weymouth UK.  
<http://www.shellfish.org.uk/files/32955Gubbins.pdf>
- Hedgecock D, Chow V, Waples RS. 1992. Effective population numbers of shellfish broodstocks estimated from temporal variance in allelic frequencies. Aquaculture, 108, 215-232.
- Hedgecock D, Gaffney PM, Gouletquer P, Guo X, Reece K, Warr G. 2005. The case for sequencing the Pacific oyster genome. J. Shellfish Res., 24, 429-441.

Heral M. 1993. Evolution of oyster aquaculture: Problems and perspectives. CIEM Conseil International pour l'Exploration de la mer.

<http://archimer.ifremer.fr/doc/00000/3067/>

Hine PM. 2002. Significant diseases of molluscs in the Asia-Pacific region. In: Diseases in Asian Aquaculture IV. Eds Lavilla-Pitogo CR and Cruz-Lacierda ER. Fish Health Section, Asian Fisheries Society, Manila, 187-196.

Hine PM, Thorne T. 1997. Replication of herpes like viruses in haemocytes of adult flat oysters *Ostrea angasi* - An ultrastructural study. Dis. Aquat. Org., 29, 189-196.

Hine PM, Wesney B, Besant P. 1998. Replication of a herpes-like virus in larvae of the flat oyster *Tiostrea chilensis* at ambient temperatures. Dis. Aquat. Org., 32, 161-171.

Hine PM, Wesney B, Hay BE. 1992. Herpesviruses associated with mortalities among hatchery-reared larval Pacific oysters *Crassostrea gigas*. Dis. Aquat. Org., 12, 135-142.

Huvet A, Degremont L, Labreuche Y, Daniel J-Y, Dubrunfaut T, Boudry P, Haure J, Bedier E, Ropert M, Herpin A, Cunningham C, Moal J, Samain J-F. 2005. The identification of genes from the oyster *Crassostrea gigas* that are differentially expressed between progeny exhibiting opposed susceptibility to summer mortality. International Marine Biotechnology Conference.

<http://archimer.ifremer.fr/doc/00000/3447/>

Huvet A, Herpin A, Degremont L, Labreuche Y, Samain J-F, Cunningham C. 2004. The identification of genes from the oyster *Crassostrea gigas* that are differentially expressed in progeny exhibiting opposed susceptibility to summer mortality. Gene, 343, 211-220.

Huvet A, Normand J, Fleury E, Quillien V, Fabioux C, Boudry P. 2010. Reproductive effort of Pacific oysters: A trait associated with susceptibility to summer mortality. Aquaculture, 304, 95-99.

IFREMER (Institut Français de Recherche pour l'Exploitation de la Mer). 2009. online. Bilan 2009 du réseau REPAMO. Réseau National de surveillance de la santé des mollusques marins des mollusques marins. Rapport IFREMER, DRV/RA/LPG. Ifremer La Tremblade, 45 pp.

[http://wwz.ifremer.fr/repamo/content/download/75792/522894/file/If\\_Rapport\\_REPAMO\\_2009.pdf](http://wwz.ifremer.fr/repamo/content/download/75792/522894/file/If_Rapport_REPAMO_2009.pdf)

Imai T, Numachi K, Oizumi J, Sato S. 1965. Studies on the mass mortality of the oyster in Matsushima Bay II. Search of the cause of the mass mortality and the possibility to prevent it by transplantation experiment. Bull. Tohoku Reg. Fish. Res. Lab. J., 25, 27-38.

Kan-no H, Sasaki M, Sakurai Y, Watanabe T, Suzuki K. 1965. Studies on the mass mortality of the oyster in Matsushima Bay I. General aspects of the mass mortality of the oyster in Matsushima Bay and its environmental conditions. Bull. Tohoku Reg. Fish. Res. Lab. J., 25, 1-26.

Koeck M, Farre M, Martinez E, Gajda-Schranz K, Ginebreda A, Navarro A, Alda M, Barcelo D. 2010. Integrated ecotoxicological and chemical approach for the assessment of pesticide pollution in the Ebro River delta (Spain). J. Hydrol. (Amsterdam), 383, 73-82.

Labreuche Y, Soudant P, Goncalves M, Lambert C, Nicolas J-L. 2006. Effects of extracellular products from the pathogenic *Vibrio aestuarianus* strain 01/32 on lethality and cellular immune responses of the oyster *Crassostrea gigas*. Dev. Comp. Immunol., 30, 367-379.

Lacoste A, Jalabert F, Malham S, Cueff A, Gélébart F, Cordevant C, Lange C, Poulet S. 2001. A *Vibrio splendidus* strain associated with Summer Mortality of juvenile oysters *Crassostrea gigas* in the Bay of Morlaix (North Brittany, France). Dis. Aquat. Org., 46, 139-145.

Lambert C, Nicolas JL, Cilia V, Corre S. 1998. *Vibrio pectenocida* sp. nov., a pathogen of scallop (*Pecten maximus*) larvae. Int. J. Syst. Bacteriol, 48, 481-487.

Langdon C, Ford E, Jacobson D, Blouin M. 2003. Yields of cultured Pacific oysters *Crassostrea gigas* Thunberg improved after one generation of selection. Aquaculture, 220, 227-244.

Lapegue S, Heurtebise S, Harrang E, Morga B, Flahauw E, Sauvage C, Boudry P. 2010. The use of SNPs in characterizing oyster genomes and their resistance to pathogens. Aquaculture Europe - The annual meeting of the European Aquaculture Society, Porto, Portugal October 5-8, 2010.

<http://archimer.ifremer.fr/doc/00015/12584/>

Le Deuff R-M, Lipart C, Chollet B, Haffner P, Delsert C, Cochenec N, Renault T. 1997. Etude du virus de type herpes observé chez les huîtres. Journées Conchylicoles Ifremer 1997. <http://archimer.ifremer.fr/doc/00000/3248/>

Le Deuff R-M, Nicolas JL, Renault T, Cochenec N. 1994. Experimental transmission of a herpes-like virus to axenic larvae of Pacific oyster, *Crassostrea gigas*. Bull. Eur. Assoc. Fish Pathol., 14, 69-72.

Le Deuff R-M, Renault T. 1999. Purification and partial genome characterization of a herpes-like virus infecting the Japanese oyster, *Crassostrea gigas*. J. Gen. Virol., 80, 1317-1322.

Le Deuff R-M, Renault T, Gerard A. 1996. Effects of temperature on herpes-like virus detection among hatchery-reared larval Pacific oyster *Crassostrea gigas*. Dis. Aquat. Org., 24, 149-157.

Le Roux F. 2004. Taxonomie et virulence de vibrions pathogènes d'huîtres creuses *Crassostrea gigas*. HDR. <http://archimer.ifremer.fr/doc/00000/3699/>

Le Roux F, Gay M, Lambert C, Waechter M, Poubalanne S, Chollet B, Nicolas J-L, Berthe F. 2002. Comparative analysis of *Vibrio splendidus*-related strains isolated during *Crassostrea gigas* mortality events. Aquatic Living Res., 15, 251-258.

Lemery N. 1997. Etude de la croissance d'une population d'huîtres creuses *Crassostrea gigas*. <http://archimer.ifremer.fr/doc/00033/14378/>

Li H. 2008. Comportements cellulaires et régulation génétique au cours des réactions d'immunité innée chez la moule *Mytilus galloprovincialis*. PhD Thesis, Université de Montpellier 2. <http://archimer.ifremer.fr/doc/00000/6241/>

Li H, Toubiana M, Monfort P, Roch P. 2009. Influence of temperature, salinity and *E. coli* tissue content on immune gene expression in mussel: Results from a 2005-2008 survey. Dev. Comp. Immunol., 33, 974-979.

Lipart C, Renault T. 2002. Herpes-like virus detection in *Crassostrea gigas* spat using DIG-labelled probes. J. Virol. Meth., 101, 1-10.

Lipp PR, Brown B, Liston J, Chew K. 1976. Recent findings on the summer diseases of Pacific oysters. Proc. Nat. Shellfish Assoc. in Garnier, 65, 9-10.

Lodato MI. 1997. Mortalité estivale de l'huître creuse, *Crassostrea gigas*, sur les bancs ostréicoles de Perquis et Ronce (Bassin de Marennes-Oléron): Etude des pratiques culturelles et des caractéristiques biologiques et spatiales des élevages. Thèse. Ecole Nationale Vétérinaire de Nantes, 127 pp.

Luna-Gonzalez A, Romero-Geraldo M de J, Campa-Córdova A, Orduña-Rojas J, Valles-Jimenez R, Ruiz-Verdugo CA. 2008. Seasonal variations in the immunological and physiological parameters of the Pacific oyster *Crassostrea gigas* cultured in the bahia de Macapule (Sinaloa, Mexico). Aquaculture Res., 39, 1488-1497.

Macián MC, Garay E, Gonzalez-Candelas F, Pujalte MJ, Aznar R. 2000. Ribotyping of *Vibrio* populations associated with cultured oysters (*Ostrea edulis*). Syst. Appl. Microbiol., 23, 409-417.

Malham SK, Cotter E, O'Keeffe S, Lynch S, Culloty SC, King JW, Latchford JW, Beaumont AR. 2009. Summer mortality of the Pacific oyster, *Crassostrea gigas*, in the

Irish Sea: The influence of temperature and nutrients on health and survival. *Aquaculture*, 287, 128-138.

Mann R, Burreson EM, Baker K. 1991. The decline of the Virginia oyster fishery in Chesapeake Bay: Considerations for introduction of a non-endemic species *Crassostrea gigas* (Thunberg, 1973). *J. Shellfish Res.*, 10, 379-388.

Martenot C, Oden E, Travaillé E, Malas JP, Houssin M. 2010. Comparison of two real-time PCR methods for detection of ostreid herpesvirus 1 in the Pacific oyster *Crassostrea gigas*. *J. Virol. Meth.* doi:10.1016/j.jviromet.2010.09.003

Martin A-G, Tige G, Hirata T, Kuntz G, Le Coguic Y. 1999. Bilan des travaux de la cellule zoosanitaire de La Trinité Sur Mer. Réseau de Pathologie des Mollusques. Secteurs Nord-Loire. Année 1998. <http://archimer.ifremer.fr/doc/00000/1920/>

Martin A-G, Tige G, Hirata T, Le Coguic Y, Chollet B, Robert M, Thebault A. 2000. Réseau de Pathologie des Mollusques - Secteurs Nord-Loire - Année 1999. Bilan des travaux de la cellule zoosanitaire de La Trinité-sur-Mer. <http://archimer.ifremer.fr/doc/00000/1921/>

Maurer D, Comps M, His E. 1986. Caractéristiques des mortalités estivales de l'huître *Crassostrea gigas* dans le bassin d'Arcachon, *Haliotis*, 15, 309-317.

Mazurie J, Fleury P-G, Claude S, Hirata T, Langlade A, Martin A-G, North B. 2000. Comparaison des performances d'élevage et de la vitalité de naissain d'huîtres creuses *Crassostrea Gigas*, en 3 sites du Morbihan (Rivière d'Auray et Baie de Quiberon), de mai 1999 à mars 2000. <http://archimer.ifremer.fr/doc/00025/13632/>

Meyers TR, Burton T, Evans W, Starkey N. 2009. Detection of viruses and virus-like particles in four species of wild and farmed bivalve molluscs in Alaska, USA, from 1987 to 2009. *Dis. Aquat. Org.*, 88, 1-12.

Minguez X. 1997. Suivi de croissance d'une génération d'huîtres creuses *Crassostrea gigas* dans le cadre du programme "Genephys 1996-2000". <http://archimer.ifremer.fr/doc/00033/14379/>

Minson AC, Davison A, Eberle R, Desrosiers RC, Fleckenstein B, McGeoch DJ, Pellett PE, Roizman B, Studdert MJ. 2000. Family Herpesviridae. In: *Virus Taxonomy: Seventh Report of the International Committee on Taxonomy of Viruses*. Eds van Regenmortel MHV, Fauquet CM, Bishop DHL, Carstens EB, Estes MK, Lemon SM, Maniloff J, Mayo MA, McGeoch DJ, Pringle CR and Wickner RB. Academic Press, San Diego, 203-225.

Miossec L, Allain G, Arzul I, Francois C, Garcia C, Cameron A. 2009a. First results of an epidemiological study on oyster (*Crassostrea gigas*) mortality events in France during summer 2008. ISVEE XII – Inter. Symp. Vet. Epidemiol. Economics.

<http://archimer.ifremer.fr/doc/00000/6772/>.

Miossec L, Allain G, Françoise C, Garcia C, Arzul I, Cameron A. 2009b. Epidemiological study on *Crassostrea gigas* mass mortality observed in 2008 in France. In: Report of the Annual Meeting and Seventh Combined Technical Workshop Meeting of the National Reference Laboratories for Mollusc Diseases, 16-19 March 2009. IFREMER, La Tremblade.

Miossec L, Le Deuff R-M, Gouilletquer P. 2009. Alien species alert: *Crassostrea gigas* (Pacific oyster). <http://archimer.ifremer.fr/doc/00000/6945/>

Moloney B J, Frances J, Lyall I, Hick P, Kirkland PD. 2011. Pacific Oyster Mortality Syndrome (POMS) in NSW: Outbreak and surveillance. Paper presented at the Aus. Coll. Vet. Sci. Science Week, Epidemiology Chapter, Gold Coast, Qld, 2011.

Montagnani C, Avarre J-C, De Lorgeril J, Quiquand M, Boulo V, Escoubas J-M. 2007. First evidence of the activation of Cg-timp, an immune response component of Pacific oysters, through a damage-associated molecular pattern pathway. Dev. Comp. Immunol., 31(1), 1-11.

Moreau K. 2005. Approche moléculaire des effets de polluants sur l'infection à virus OsHV-1 (Ostreid Herpesvirus 1) chez l'huître creuse, *Crassostrea gigas*.

<http://archimer.ifremer.fr/doc/00032/14339/>

Mori K. 1968. Changes in oxygen consumption and respiratory quotient in the tissue of oysters during the stages of sexual maturation and spawning. Tohoku J. Agric. Res., 19, 136-143.

Moss JA, Burreson EM, Cordes JF, Dungan CF, Brown GD, Wang A, Wu X, Reece KS. 2007. Pathogens in *Crassostrea ariakensis* and other Asian oyster species: implications for non-native oyster introduction to Chesapeake Bay. Dis. Aquat. Org., 77, 207-223.

Nealson KH, Wimpee B, Wimpee C. 1993. Identification of *Vibrio splendidus* as a member of the planktonic luminous bacteria from the Persian Gulf and Kuwait region with luxA probes. Appl. Environ. Microbiol., 59, 2684-2689.

Nell JA. 2002. Farming triploid oysters, *Aquaculture*, 210, 69-88.

Nicolas JL, Comps M, Cochennec N. 1992. Herpes-like virus infecting Pacific oyster larvae, *Crassostrea gigas*. Bull. Eur. Assoc. Fish Pathol., 12, 11-13.

OFIMER. 2008. Key figures for the fisheries and aquaculture sector in France. 36 pp. [www.ofimer.fr/ PDF/obsec/Chiffres\\_cles\\_2008.ang.pdf](http://www.ofimer.fr/PDF/obsec/Chiffres_cles_2008.ang.pdf).

Olicard C. 2002. Etude des mécanismes antiviraux développés par les huîtres lors d'infections à virus de type herpes. <http://archimer.ifremer.fr/doc/00000/1769/>

Olicard C, Didier Y, Marty C, Bourgougnon N, Renault T. 2005. *In vitro* research of anti-HSV-1 activity in different extracts from Pacific oysters *Crassostrea gigas*. Dis. Aquat. Org., 67, 141-147.

Olicard C, Renault T, Torhy C, Benmansour A, Bourgougnon N. 2005. Putative antiviral activity in hemolymph from adult Pacific oysters, *Crassostrea gigas*. Antiviral Res., 66, 147-152.

Pearce I, Handlinger JH, Hallegraef GM. 2005. Histopathology of the Pacific oyster (*Crassostrea gigas*) spat caused by the dinoflagellate *Prorocentrum rhathymum*. Harmful Algae, 4, 61-74.

Peeler EJ, Reece A, Thrush MA. 2010. Investigation of oyster herpes virus infection and mortality in the Republic of Ireland in 2009 - a questionnaire survey, CEFAS 2010 (unpublished report).

Pepin J-F, Riou A, Renault T. 2007. Rapid and sensitive detection of ostreid Herpesvirus 1 in oyster samples by real-time PCR (Poster). 13th EAFP International Conference on 'Diseases of Fish and Shellfish'. <http://archimer.ifremer.fr/doc/00000/3653/>

Pepin J-F, Riou A, Renault T. 2008. Rapid and sensitive detection of ostreid herpesvirus 1 in oyster samples by real-time PCR. J. Virol. Meth., 149, 269-276.

Pepin J-F, Sollic G, Vigneron V, Thebault A, Lamothe J, Renault T. 2008. Recherches d'ADN d'herpes virus infectant les mollusques dans les populations d'huîtres en milieu naturel et dans leur environnement. ASPS Association Santé Poissons Sauvages. <http://archimer.ifremer.fr/doc/00000/3716/>

Perdue JA, Beattie JH, Chew KK. 1981. Some relationships between gametogenic cycle and summer mortality phenomenon in the Pacific oyster (*Crassostrea gigas*) in Washington State. J. Shellfish Res., 1, 9-16.

Pernet F, Barret J, Le Gall P, Malet N, Pastoureaud A, Munaron D, De Lorgeril J, Bachere E, Vaquer A, Huvet A, Corporeau C, Normand J, Boudry P, Moal J, Quere C, Quilien V, Daniel J-Y, Pepin J-F, Saulnier D, Gonzalez J-L. 2010. Mortalité du naissain d'Huître creuse *Crassostrea gigas* dans l'étang de Thau en 2009. <http://archimer.ifremer.fr/doc/00002/11354/>

Quillet E, Boudry P, Lapegue S. 2007. Variabilité génétique de la réponse aux organismes pathogènes: Un outil pour améliorer la santé des mollusques et poissons d'élevage. INRA Productions Animales, 20, 239-252.

Open Access version: <http://archimer.ifremer.fr/doc/00000/2979/>

Ren W, Renault T, Cai Y, Wang C. 2010. Development of a loop-mediated isothermal amplification assay for rapid and sensitive detection of ostreid herpesvirus 1 DNA. J Virol. Meth., 170, 3036.

Renault T. 1998. Infection herpétiques chez les invertébrés: Détection de virus de type herpes chez les mollusques bivalves marins. Ann l'Institut Pasteur Virol., 2, 401-403. <http://archimer.ifremer.fr/doc/00000/2903/>

Renault T. 2006. Viruses of bivalve shellfish. Ann. l'Institut Pasteur Virol., 10, 35-41. <http://archimer.ifremer.fr/doc/00000/2925/>

Renault T. 2007. Tenter de maîtriser les maladies infectieuses chez les mollusques: Une clé pour une aquaculture durable. HDR. <http://archimer.ifremer.fr/doc/00000/3486/>

Renault T. 2008. Genomics and mollusc pathogens: trends and perspective. J. Vet. Clin. Sci., 1, 4-14. <http://archimer.ifremer.fr/doc/00000/4574/>

Renault T. 2008. Shellfish viruses. In: Encyclopedia of Virology (B.W.J. Mahy and M. H. V. Van Regenmortel, Editors), Oxford, 5, 560-567. Open Access version: <http://archimer.ifremer.fr/doc/00000/4932/>

Renault T, Allain G, Arzul I, Chollet B, Cobret L, De Decker S, Faury N, Ferrand S, Francois C, Garcia C, Haffner P, Joly J-P, Miossec L, Morga B, Nicolas J-L, Omnes E, Pepin J-F, Saulnier D, Schikorski D, Segarra A. 2009. Summer mortality outbreaks of French Pacific oysters *Crassostrea gigas* in 2008 and 2009. 14th EAAP International Conference, Diseases of Fish and Shellfish, 14th to 17th September 2009, Prague, Czech Republic. <http://archimer.ifremer.fr/doc/00014/12554/>

Renault T, Arzul I. 2001. Herpes-like virus infections in hatchery-reared bivalve larvae in Europe: specific viral DNA detection by PCR. J. Fish Dis., 24, 161-167.

Renault T, Arzul I, Lipart C. 2004. Development and use of an internal standard for oyster herpesvirus 1 detection by PCR. J. Virol. Meth., 121, 17-23.

Renault T, Cochenne N, Le Deuff R-M, Chollet B. 1994a. Herpes-like virus infecting Japanese oyster (*Crassostrea gigas*) spat. Bull. Eur. Assoc. Fish Pathol., 14, 64-66.

- Renault T, Faury N, Barbosa V, Moreau K, Haffner P, Pepin J-F. 2008. Antiviral immunity in the Pacific oyster, *Crassostrea gigas*: Last development and perspective. WAS - World Aquaculture Society. <http://archimer.ifremer.fr/doc/00000/4035/>
- Renault T, Le Deuff R-M, Chollet B, Cochenec N, Gerard A. 2000b. Concomitant herpes-like virus infections in hatchery-reared larvae and nursery-cultured spat *Crassostrea gigas* and *Ostrea edulis*. Dis. Aquat. Org., 42, 173-183.
- Renault T, Le Deuff R-M, Cochenec N, Chollet B, Maffart P. 1995. Herpes-like viruses associated with high mortality levels in larvae and spat of Pacific oysters, *Crassostrea gigas*: A comparative study, the thermal effects on virus detection in hatchery-reared larvae, reproduction of the disease in axenic larvae. Vet. Res., 26, 539-543.
- Renault T, Le Deuff R-M, Cochenec N, Maffart P. 1994b. Herpesviruses associated with mortalities among Pacific oyster, *Crassostrea gigas*, in France - Comparative study. Revue de Médecine Vétérinaire, 145, 735-742.  
<http://archimer.ifremer.fr/doc/00000/2898/>
- Renault T, Le Deuff R-M, Lipart C, Delsert C. 2000a. Development of a PCR procedure for the detection of a herpes-like virus infecting oysters in France. J. Virol. Meth., 88, 41-50.
- Renault T, Lipart C, Arzul I. 2001. A herpes-like virus infects a non-ostreid bivalve species: virus replication in *Ruditapes philippinarum* larvae. Dis. Aquat. Org., 45, 1-7.
- Renault T, Lipart C. 1998. Diagnosis of herpes-like virus infections in oysters using molecular techniques. Eur. Aquaculture Soc., Special Publication, 26, 235-236.
- Renault T, Novoa B. 2004. Viruses infecting bivalve molluscs. Aquat. Living Res., 17, 397-410.
- Renault T, Lipart C. 1998. Diagnosis of herpes-like virus infections in oysters using molecular techniques. Aquaculture and water: fish culture, shellfish culture and water usage. <http://archimer.ifremer.fr/doc/00000/2902/>
- Renault T, Lipart C, Arzul I. 2001. A herpes-like virus infects a non-ostreid bivalve species: Virus replication in *Ruditapes philippinarum* larvae. Dis. Aquat. Org., 45, 1-7.
- Renault T, Lipart C, Arzul I. 2001. A herpes-like virus infecting *Crassostrea gigas* and *Ruditapes philippinarum* larvae in France. J. Fish Dis., 24, 369-376.
- Renault T, Novoa B. 2004. Viruses infecting bivalve molluscs. Aquatic Living Res., 17, 397-409.

Riou A. 2006. Amélioration du protocole de PCR quantitative pour la détection de l'OsHV1 chez l'huître creuse, *Crassostrea gigas*, dans le cadre du projet TRIPLOFIMER et suivi d'individus lors du phénomène de mortalité estivale dans le cadre du projet AQUAFIRST. <http://archimer.ifremer.fr/doc/00033/14459/>

Roch P, Yang Y, Toubiana M, Aumelas A. 2008. NMR structure of mussel mytilin, and antiviral-antibacterial activities of derived synthetic peptides. *Dev. Comp. Immunol.*, 32, 227-238.

Roncarati A, Felici A, Dees A, Forlini L, Melotti P. 2010. Trials on Pacific oyster (*Crassostrea gigas* Thunberg) rearing in the middle Adriatic Sea by means of different trays. *Aquaculture International*, 18, 35-43.

Royer J, Robert M and Costill K. 2007. Spatio-temporal changes in mortality, growth and condition of the pacific oyster, *Crassostrea gigas*, in Normandy (France). *J. Shellfish Res.*, 26, 973-984.

Samain J-F, Degremont L, Soletchnik P, Haure J, Bedier E, Ropert M, Moal J, Huvet A, Bacca H, Van Wormhoudt A, Delaporte M, Costil K, Pouvreau S, Lambert C, Boulo V, Soudant P, Nicolas J-L, Leroux F, Renault T, Gagnaire B, Geret F, Boutet I, Burgeot T, Boudry P. 2007. Genetically based resistance to summer mortality in the Pacific oyster (*Crassostrea gigas*) and its relationship with physiological, immunological characteristics and infection processes. *Aquaculture*, 268, 227-243.

Samain J-F, McCombie H. 2008. Summer mortality of the Pacific oyster *Crassostrea gigas*. The Morest Project, Editions Quae, Versailles. 379 pp.

Saulnier D, De Decker S, Haffner P, Cobret L, Robert M, Garcia C. 2010. A large-scale epidemiological study to identify bacteria pathogenic to Pacific oyster *Crassostrea gigas* and correlation between virulence and metalloprotease-like activity. *Microbial Ecology*, 59, 787-798.

Sauvage C. 2008. Développement de marqueurs moléculaires liés à la résistance à la mortalité estivale chez l'huître creuse *Crassostrea gigas* - Approche QTL. PhD Thesis, Université de La Rochelle. <http://archimer.ifremer.fr/doc/00000/4544/>

Sauvage C, Boudry P, De Koning D-J, Haley CS, Heurtebise S, Lapegue S. 2010. QTL for resistance to summer mortality and OsHV-1 load in the Pacific oyster (*Crassostrea gigas*). *Animal Genetics*, 41, 390-399.

Sauvage C, Heurtebise S, De Koning D, Haley C, Boudry P, Lapegue S. 2008. Mapping QTL for resistance to summer mortality in the Pacific oyster *Crassostrea gigas*. XIV Plant and Animal Genome Conference. <http://archimer.ifremer.fr/doc/00000/3504/>

Sauvage C, Pepin J-F, Lapegue S, Boudry P, Renault T. 2009. Ostreid herpes virus 1 infection in families of the Pacific oyster, *Crassostrea gigas*, during a summer mortality outbreak: Differences in viral DNA detection and quantification using real-time PCR. *Virus Res.*, 142, 181-187.

Sawabe T, Kita-Tsukamoto K, Thompson FL. 2007. Inferring the evolutionary history of vibrios by means of multilocus sequence analysis. *J. Bacteriol.*, 189, 7932-7936.

Schikorski D, Faury N, Pepin J-F, Saulnier D, Tourbiez D, Renault T. 2011. Experimental ostreid herpesvirus 1 infection of the Pacific oyster *Crassostrea gigas*: Kinetics of virus DNA detection by q-PCR in seawater and in oyster samples. *Virus Res.*, 155, 28-34.

Schikorski D, Renault T, Saulnier D, Faury N, Moreau P, Pepin J-F. 2011. Experimental infection of Pacific oyster *Crassostrea gigas* spat by ostreid herpesvirus 1: Demonstration of oyster spat susceptibility. *Vet. Res.*, 42, 1-13.

Schmitt P. 2010. Diversité moléculaire des effecteurs antimicrobiens chez l'huître creuse *Crassostrea gigas*: Mise en évidence et rôle dans la réponse antimicrobienne. PhD Thesis, Université Montpellier 2.

<http://archimer.ifremer.fr/doc/00028/13906/>

Segarra A, Pepin J-F, Arzul I, Morga B, Faury N, Renault T. 2010. Detection and description of a particular Ostreid herpesvirus 1 genotype associated with massive mortality outbreaks of Pacific oysters, *Crassostrea gigas*, in France in 2008. *Virus Res.*, 153, 92-99.

Sheldon RW. 1968. The effect of high population density on the growth and mortality of oysters *Ostrea edulis*, *ICES*, 31, 352-363.

Sheridan AK. 1997. Genetic improvement of oyster production - A critique. *Aquaculture*, 153, 165-179.

Smolarz K, Renault T, Soletchnik P, Wolowicz M. 2005. Neoplasia detection in *Macoma balthica* from the Gulf of Gdansk: comparison of flow cytometry, histology and chromosome analysis. *Dis. Aquat. Org.*, 65, 187-195.

Sniesko SF. 1974. The effect of environmental stress on the outbreaks of infectious disease of fishes. *J. Fish Biol.*, 6, 197-208.

Sobecky PA, Mincer TJ, Chang MC, Toukdarian A, Helinski DR. 1998. Isolation of broad-host-range replicons from marine sediment bacteria. *Appl. Environ. Microbiol.*, 64, 2822-2830.

- Solé M, Porte C, Pastor D, Albaigés J. 1994. Long-term trends of polychlorinated biphenyls and organochlorinated pesticides in mussels from the Western Mediterranean coast. *Chemosphere*, 28, 897-903.
- Soletchnik P, Faury N, Gouletquer P. 2006. Seasonal changes in carbohydrate metabolism and its relationship with summer mortality of Pacific oyster *Crassostrea gigas* (Thunberg) in Marennes-Ole'ron bay (France). *Aquaculture*, 252, 328-338.
- Soletchnik P, Le Moine O, Faury N, Razet D, Geairon P and Gouletquer P. 1999. Summer mortality of the oyster in the Bay Marennes-Oleron: Spatial variability of environment and biology using a geographical information system (GIS). *Aquatic Living Res.*, 12, 131-143.
- Soletchnik P, Ropert M, Mazurie J, Fleury P-G, Le Coz F. 2007. Relationships between oyster mortality patterns and environmental data from monitoring databases along the coasts of France. *Aquaculture*, 271, 384-400.
- Solliec G, Vigneron V, Montanie H, Renault T. 2003. Recherche d'AND d'herpèsvirus infectant les bivalves dans des échantillons d'eau de claires ostréicoles. In: Les mollusques dans la recherche actuelle. <http://archimer.ifremer.fr/doc/00000/2856/>
- Spiga B. 2006. Treatment trials in *Crassostrea gigas* against *Polydora ciliata* infection. Thesis on "Marine Productions", Faculty of Biology, University of Sassari, Italy.
- Stachowski-Haberkorn S, Quiniou F, Nedelec M, Robert R, Limon G, la Broise D. 2008. *In situ* microcosms, a tool for assessment of pesticide impacts on oyster spat (*Crassostrea gigas*). *Ecotoxicology*, 17, 235-245.
- Sugumar G, Nakai T, Hirata Y, Matsubara D, Muroga K. 1998. *Vibrio splendidus* biovar II as causative agent of bacillary necrosis of Japanese oyster *Crassostrea gigas* larvae. *Dis. Aquat. Org.*, 33, 111-118.
- Tamate H, Numachi K, Mori K, Itikawa O, Imai T. 1965. Studies on the mass mortality of the oyster in Matsushima Bay - VI. Pathological studies. *Bull. Tohoku Reg. Fish. Res. Lab. J.*, 25, 89-104.
- Tan J, Lancaster M, Hyatt A, van Driel R, Wong F, Warner S. 2008. Purification of a herpes-like virus from abalone (*Haliotis* spp.) with ganglioneuritis and detection by transmission electron microscopy. *J. Virol. Meth.*, 149, 338-341.
- Thompson CC, Thompson FL, Vicente AC, Swings J. 2007. Phylogenetic analysis of vibrios and related species by means of atpA gene sequences. *Int. J. Syst. Evol. Microbiol.*, 57, 2480-2484.

Thompson FL, Gevers D, Thompson CC, Dawyndt P, Naser S, Hoste B, Munn CB, Swings J. 2005. Phylogeny and molecular identification of *Vibrios* on the basis of Multilocus Sequence Analysis. *Appl. Environ. Microbiol.*, 71, 5107–5115.

Thompson FL, Hoste B, Vandemeulebroecke K, Swings J. 2001. Genomic diversity amongst *Vibrio* isolates from different sources determined by fluorescent amplified fragment length polymorphism. *Syst. Appl. Microbiol.*, 24, 520–538.

Van Bannig. 1997. Summer Mortality in the Netherlands. In: IFREMER meeting 6, 9 October, 1997.

Varsamos S., Pepin J-F, Sauvage C, Renault T. 2007. Development of a new diagnostic tool (Mini-Array) for Ostreid Herpesvirus 1 (OsHV-1) detection in the Pacific oyster *Crassostrea gigas*. 13th EAFP International Conference on 'Diseases of Fish and Shellfish'. <http://archimer.ifremer.fr/doc/00000/3652/>

Vásquez-Yeomans R, Cáceres-Martínez J, Huerta AF. 2004. Herpes-like virus associated with eroded gills of the Pacific oyster *Crassostrea gigas* in Mexico. *J. Shellfish Res.*, 23, 417-419.

Vásquez-Yeomans R, García-Ortega M, Cáceres-Martínez J, 2010. Gill erosion and herpesvirus in *Crassostrea gigas* cultured in Baja California, Mexico. *Dis. Aquat. Org.*, 89, 137-144.

Vigneron V. 2002. Détection et étude de la stabilité de l'ADN de virus de type herpes infectant les huîtres dans des échantillons d'eau. <http://archimer.ifremer.fr/doc/00032/14350/>

Vigneron V, Sollic G, Montanie H and Renault T. 2004. Detection of Ostreid Herpesvirus 1 (OsHV-1) DNA in seawater by PCR: Influence of water parameters in bioassays. *Dis. Aquat. Org.*, 62, 35-44.

Waechter M, Le Roux F, Nicolas JL, Marissal E, Berthe F. 2002. Characterisation of *Crassostrea gigas* spat pathogenic bacteria. *Comptes Rendus Biologies*, 325, 231-238.

Webb SC, Fidler A and Renault T. 2007. Primers for PCR-based detection of ostreid herpes virus-1 (OsHV-1): Application in a survey of New Zealand molluscs. *Aquaculture*, 272, 126-139.

Yamada S, Matsumoto Y, Takashima Y and Otsuka H. 2005. Mutation hot spots in the canine herpesvirus thymidine kinase gene. *Virus Genes*, 31, 107-111.