Understanding and planning for the potential impacts of OsHV1 µvar on the Australian Pacific oyster industry (FRDC 2011/043)

French Study Tour Report

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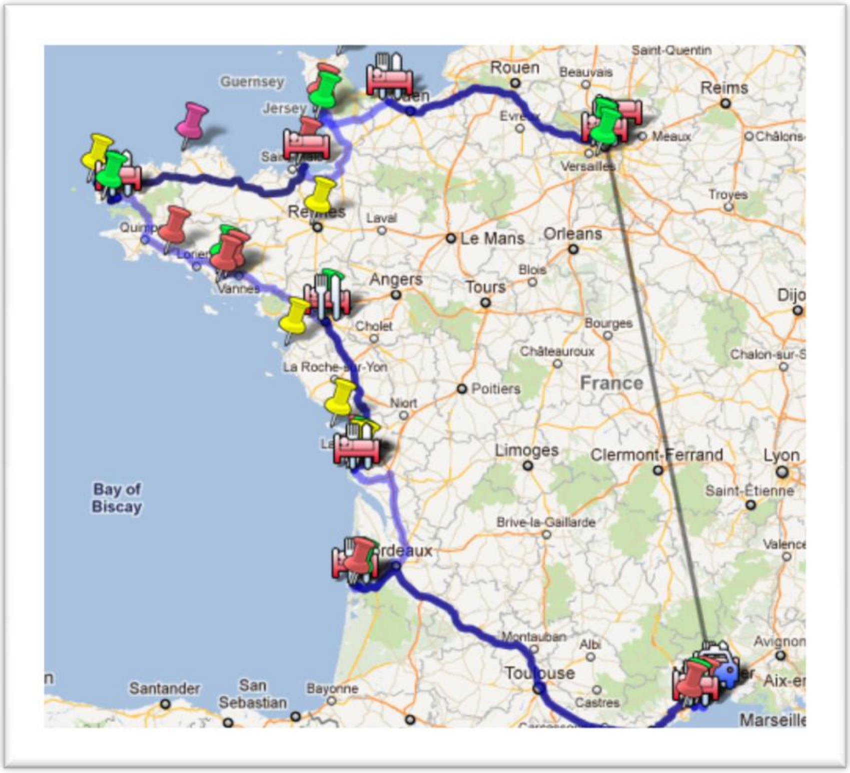

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oysters australia

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The project is being managed by Tom Lewis at RDS Partners. RDS Partners is a multi-disciplinary team dedicated to facilitating positive social, economic and environmental outcomes in rural and regional Australia. RDS specialises in projects within the agriculture, seafood and not-for profit sectors.

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Introduction

Following the late 2010 confirmation of Pacific oyster mortalities in New Zealand and NSW associated with the presence of the OsHV1 µvar virus, the Australian industry and FRDC supported[1] an industry study tour to France to gain first hand information of the effect on the French industry of the virus and the response to this threat by industry, researchers and regulators.

The study tour team comprised:

- Growers: Bruce Zippel, Rob Moxham, James Calvert;
- Epidemiological expertise, cultural attaché, translation and tour logistics: Angus Cameron, Cate Mackenzie (AusVet Animal Health Services, www.ausvet.com.au); and,
- Project manager: Tom Lewis.

Between 1 and 10 November 2011, the study team travelled from Paris to Normandy, around the French coast to the Mediterranean and back to Paris, meeting with growers, processors, industry representatives, researchers and government agencies.

A daily "blog" for this trip (www.oystertour.wordpress.com) was maintained to provide information in real time to interested parties and to enable them to provide feedback and ask questions during the tour.

The blog remains online as a resource to add detail to the contents of this report, which, in turn, provides a summary of the team's thinking at the end of the tour.

[1] James Calvert's participation was funded by Tas Prime Oysters. All others were supported through a combination of FRDC, Tas, SA and NSW oyster industry research council contributions.

Key recommendations

Research priorities

1. Increase selective breeding focus on developing virus resistant family lines that maintain the economic value already realised.
2. Establish a trial in the Georges River NSW to test the effect of growing height and oyster density on mortalities (possibly 3 heights, 3 densities, 3 replicates = 27 baskets).
3. Establish a series of trials in the Georges River (NSW) to test the effectiveness of other growing systems including adjustable longline systems and the floating basket system in use in the NSW oyster industry.
4. Adapt the French infectivity models as published by IFREMER in an Australian biosecure facility as the basis for direct research into different aspects of the virus.
5. Standardised protocol for PCR testing for the virus within Australia to provide confidence in result comparison between testing agencies.
6. Run a temperature “stress” trial to establish if increasing the culture temperature by about 1C per day to above 17C will elicit disease in sub-clinically infected oysters (if successful, this would be used as a fast and cheap test for the presence of virus in oysters).
7. Research the ability of other bivalve species to act as translocation and/or disease vectors.
8. Determine whether vertical transmission of the virus occurs.
9. Establish if virus has spread (e.g. north and south of Sydney Harbour).

Industry actions

1. Develop and implement plan for discussing tour findings with industry, researchers and regulators in SA, NSW and TAS.
2. Undertake an immediate risk assessment on likely vectors of transferring the virus within a state and between states.
3. Investigate the use of sentinel populations in high risk areas of potential viral infection. This may involve a mixture of cultivated and feral oyster populations.
4. Develop national capacity and capability to report and monitor non-harvest stock movements between states and within each state.
5. Develop a plan (or plans) for preparing an OsHv1 µvar-focussed, industry -owned and coordinated emergency response plan in each of SA, NSW and TAS. This plan (or plans) should include details of agreed:
    a. technical response options, including contingency planning and learning how to live with the disease and knowing what the options are for maintaining commercial production in an infected area

b. regulatory response options

c. financial options available at the individual farm level and at an industry level, including sources of assistance during the recovery phase

d. social and other sources of support for producers coping with this sort of problem

Summarised findings

This study tour provided information and contacts that should prove useful to the Australian industry. The study team did find, however, a certain lack of consistency in opinion about the effect that different factors have on virus-related mortalities. This could mean that:

- The effects are different in different locations / environments;
- There are other important factors influencing survival that we are not yet aware of;
- The factors being considered have little impact on survival, and observed differences are just random variation; and/or
- Desperate farmers are clutching at straws, hoping that different ideas may work, but with little support or proof (we have heard of a range of different ideas that sound rather improbable but which some farmers are keen to implement without any firm evidence that they may actually help).

Despite this there are some issues about which information has been more consistent and that the team feels merit careful consideration.

Hatchery/Nursery related issues

Virus transmission

We heard differing reports as to whether true vertical transmission (this means transfer of the virus from broodstock to larvae via infected eggs and/or sperm) occurs with this virus. The consensus is that it doesn’t, and that if larvae get infected, it is from virus particles in the water, probably shed by the broodstock. If this is true, then good technique should be able to produce virus-free larvae from infected broodstock, although this may need some level of further research.

Hatchery Management

There was some level of conflicting reports as to whether hatchery management techniques had some effect on reducing virus-related mortalities. It did seem to be important from what we could gather to use broodstock with previous exposure to the virus , but weren’t showing any clinical signs of being affected by it.

There was a general consensus that wild caught oysters fared better against the virus than did hatchery spat. It was also acknowledged that the quality of the spat from a hatchery definitely influenced mortality rates, and some hatcheries had a better reputation for having stock that was able to withstand the virus than other hatcheries.

We were also told that occasional batches of oysters that went through hatcheries survived quite well, but the reasons for this better survival were not known. We asked about the importance of not pushing oysters quickly through the hatcheries, and we received a mixed response. One response was that it was important not to push the growth of hatchery and nursery stock, yet others said it didn’t matter and that the quality of the broodstock was more important.

The feeling of our group was that perhaps it did matter to some extent, as the Australian experience suggests that the quickest growing oysters in a batch tended to more susceptible to husbandry problems (remembering that the fastest growers of a batch are usually culled by the hatchery in Australia).

In regards to the IFREMER hatcheries, we were told that some interesting research was going to be released in regards to hatchery management for the virus, but it was yet to be peer reviewed etc. Hopefully this will information will be available be before too long.

Growth rate

As discussed later (see Husbandry related issues) it seems that faster growing oysters, including larvae and spat, are more susceptible to mortalities. It could be important for hatcheries to manage larvae and spat growth rates to help manage this. Managing growth rates could also be an important component of on farm husbandry to manage the virus.

Timing

Larvae and spat can be produced to match industry understanding of the safest timing (e.g. size, age, water temperature).

Selection of spat

The French did not provide any evidence that there was any difference in mortality rates between diploid, triploid, wild-caught or hatchery-produced, although the French peak industry representative body was of the opinion that wild caught spat fared better than normal hatchery spat. The team feels it is unlikely that these variables alone have any significant influence on mortality. It is more likely that the genetics and husbandry of spat will play a larger role in determining susceptibility to the virus.

Husbandry related issues

Good husbandry practice is thought to be an effective means for limiting virus-related mortalities. This adds another strong incentive to keep your farm and stock in good order.

There is a limited range of husbandry techniques that can be considered in attempting to minimise the impacts of the virus.

Timing of stock input and movements

We heard regularly that the age and/or size of oysters, including spat, can have an influence on their mortality during times of increased viral activity. We also heard, and the French acknowledged, conflicting reports on this issue for oysters grown in different areas.

Once better understood in the Australian (or state, or bay, or culture system) context, this knowledge could provide useful insights to any management tools (e.g. spat can be transferred to farms at different times, so that they are bigger or smaller, older or younger, at times of peak risk (when water temperature is higher).

Growth rates

We heard regularly that rapidly growing oysters are more susceptible to viral-related mortality than slower growing ones. The exact mechanism for this has not been described with any consistency. It may be simply because rapidly dividing cells provide a better opportunity for the virus to replicate itself. Others point to physiological stress in rapidly growing oysters, and others suggest that shell strength plays a role (that is, it was felt that oysters grown in conditions that slightly inhibited growth and produced strong “solid” shells were more resilient to disease).

A number of strategies appear to have developed in response to the hypothesis of the importance of growth rate, involving speeding up or slowing down growth of different classes of oysters at strategic times.

Acting to decrease oyster growth rate, especially during times when the virus is active (e.g. when the water temperature is over 17°C), is thought to lead to decreased mortalities.

This has been achieved by growing oysters:

- at different heights in intertidal systems;
- closer to the surface in sub-tidal systems; and/or,
- at higher densities [and in this case, density should be considered at two SCALES: 1) at a local scale (number of oysters per bag/tray etc), and 2) at a larger scale (number of bags/trays per hectare in a growing area).

Transfers

It appears that stock transfer is one of the most effective ways of moving the virus from an infected area to a non-infected area. This means that the issue of when and how to limit stock transfers needs to be considered very carefully.

However, the French experience is that stock can be moved between different areas to influence growth rates or to avoid or delay exposure to heavy virus loads and warmer water temperatures.

Grading and handling

It appears that handling, even just washing, oysters during times of increased virus activity can lead to increased mortality. One grower on the Atlantic Coast told us that the virus seems to come in waves about a month apart during summer. This grower said he prepared his oysters for the disease by having them more “rumbled” (or other means of slowing the growth) about a month prior to the expected onset of the virus. Then, after the first monthly wave, he would handle oysters only if he had to, and would do everything to minimise shellfish stress, including immediate placement back into the ocean.

The timing for purchasing spat or ongown oysters seemed to influence survival depending on the strategy in place by the grower. Some will buy in the spring and then allow for moderate growth prior to preparing the oysters for summer. Others will buy in the autumn and then try and get greater growth to preparing the oysters for increased survival during the following summer. There did not seem to be a clear pattern suggesting which approach would be better.

Management of survivors

Oysters that have been previously exposed to the virus but have not died were seen to be treated in different ways by different growers:

- some consider that these oysters have been weakened by the virus and are more susceptible to dying with any later challenge;
- others think that they represent a population with some immunity; and,
- yet others think that they are likely to be carriers and therefore risk spreading the disease.

Temperature

One of the few things that people are pretty confident of is the effect of temperature on triggering the mortalities. If the virus is present, rising temperature triggers the onset of disease. The threshold at which mortalities start to appear varies a bit, which is what one would expect if a range of other factors are also involved (e.g. other sources of stress, other opportunistic pathogens such as *Vibrio* spp, age, resistance etc).

Most people we spoke to suggested that the threshold in most of France is around 17°C. IFREMER has also suggested that there is an upper temperature limit to the mortalities, around 24° C, but no similar comments have been made by producers and it is not certain what the practical importance of this would be in France. It may be relevant in Australia, however.

In some parts of France, farmers with multiple leases in different areas take advantage of this temperature effect. Some small areas have lower temperatures than surrounding areas and oysters may be transferred here to avoid the wave of mortalities that accompanies increasing water temperature. In Australia, there is a significant range of water temperatures between different oyster growing areas, but restrictions on interstate movements mean that the opportunities to exploit this type of approach will be limited to certain intra-state transfers.

Age/size

The early observations in France have been that young oysters are more susceptible. This still appears to be the case in most areas, to the point that farmers on the Atlantic coast seem to feel that adults are not at significant risk. They were surprised by the observations from Australia and New Zealand that older oysters also suffered very high mortality rates.

In contrast, in the Mediterranean (and possibly some other areas), adults appear to suffer heavy losses. Based on the hypothesis that genetic resistance is the main determinant of survival, some farmers prefer to have their spat heavily challenged and suffer high levels of mortality, so that the survivors have a lower chance of dying as adults. Losing adults after all the effort of growing them clearly has a very demoralising effect.

What is not clear is the interaction between age, size and growth rate. Definitions of adult and juvenile are generally based on size, but may describe oysters of very different ages, depending on the growing area. Significant differences in nutrient levels and growth rates, and therefore age/size relationships, are likely to be evident in Australia as well.

The group was informed that when mortalities started due to the virus, it was more likely to affect oysters across all sizes and age classes. In the Thau Lagoon, we were told that if oysters were brought in that hadn't been affected or exposed to the virus (some virus-free pockets exist in the Mediterranean apparently), those oysters die very quickly, regardless of size or age.

Viral load

Another relatively consistent message was the importance of viral load. It appears that oysters exposed to the virus may well continue to carry the virus, even if it is not detectable with PCR tests. It is not known if they excrete the virus in this subclinical state.

One suggestion (that has apparently been patented in the Mediterranean) is to grow mussels between the rows of oysters, based on the theory that they may act as a barrier to the movement of virus, or 'soak up' virus from the environment without being affected. There is little information available about the effectiveness of this approach. One IFREMER study showed that the mortality of oysters grown in a range of different environments (surrounded by mussels, other adults, or spat) were very similar, suggesting that it may not be effective.

On the other hand, the importance of viral load in the environment was demonstrated by an experiment in which spat that tested negative on PCR were grown in a previously unused area that was separated from existing (heavily contaminated) oyster growing areas by land and hydrological barriers, and suffered no mortalities despite having suitable water temperatures. This indicates that it is possible to avoid the disease by using a clean environment and spat that are either free or have infection which is undetectable by PCR.

Genetics

Genetics / Breeding for resistance is the area that is seen as the principle hope for the French oyster industry to combat this virus. There has been much effort spent so far in this area, and under Research Related issues (below), a selective breeding project is outlined that will require significant cash and in-kind resources.

The past lines that were originally bred for resistance for the classical OHSV-1 were released after being crossed with IFREMER tetraploids. It would appear that there were major problems getting enough of these oysters produced, and they failed under commercial conditions.

We were told that the next, genetically improved line of this breeding trial has performed well in trial conditions within the claires[2], and hopefully that line will be produced in commercial quantities for the upcoming summer (although it wasn't clear if there were any in hatcheries at the time of our visit).

Research related issues

Virus-related research in France was discussed by the group with IFREMER representatives at some length, with the following areas covered:

- Breeding for resistance

  Development of virus resistant family lines is being undertaken by both government and private hatchery operations.

[2] The "claires" are ponds dug in the clay and they are generally ancient salt pans reconverted in the19th century, after the decline of the production of salt. After their growth in the sea, oysters are placed in the "claires" where they acquire their tint and their taste (e.g. ref: http://bernezac.com/huitres_uk.htm).

The French have an ambitious aim of producing up to 2-3000 family lines over the next few years, with the aim of producing at least 100 virus-resistant lines as a basis for introducing a genetically diverse resistant population into the wild population (on which 80% of spat production relies). This project will be undertaken by IFREMER, but is likely to need to cooperation of private hatcheries to reach such a high number of family lines in the first season.

It is important to note that, in France, commercially bred lines can only be released as triploids. The French are very determined to protect the genetic diversity of their wild pacific oysters. Tetraploids of any sort, and in this case used for crossing with diploids to create triploids, can only be held by IFREMER.

IFREMER only release male tetraploids to commercial hatcheries and the shells of those oysters are micro chipped and must be returned to IFREMER after commercial use.

- Understanding potential vectors, including other species

We heard many and varied reports of other species that may carry and/or be affected by this virus. Needless to say this area needs much more work.

IFREMER is planning studies in their coming spring and summer to look into possible live and particulate vectors for this virus. It will be important for us to keep an eye on this research.

- Understanding the genetic relationship between the virus in different countries

As more work is done on the virus, it is becoming apparent that there is potentially greater genetic diversity between strains from different countries than previously thought.

Increased efforts on understanding the differences, and similarities, of different strains will provide greater understanding of its origin and path of spread around the world.

- Monitoring the virus

It is believed possible, even likely, that the virus may be difficult to detect, even with PCR analysis, when it isn’t active (particularly when from water below temperatures that coincide with mortalities). This was carefully noted by the group, hence the discussions about the usefulness of laboratory-based “stress tests” in which oysters are held in tanks and warmed to >17°C (by about 1°C per day) to stimulate the onset of the disease if the virus is present.

One other interesting point raised was the belief of one scientist that the virus does not spread easily in the water column, and may not spread from infected oysters to uninfected oysters over distances greater than about 1.5 kilometres.

Another area discussed within this topic was the possible use of pooling samples rather than testing individual oysters. It was felt by the IFREMER representatives that it was definitely possible to pool a sample of 10 – 12 oysters from a region and detect the virus if it is present and active. If the intent was simply to see whether the virus was present, then this process would be acceptable. If the virus is detected in a pooled sample, then individual oysters could then be tested to determine the location and level of infection.

- Infectivity Models.
  The group asked about infectivity models for laboratory transmission of the virus for the purposes of experimentation. It would appear that 2 models have been published, one based on direct injection of the virus into the adductor muscle, and one based on cohabitation with infected oysters.

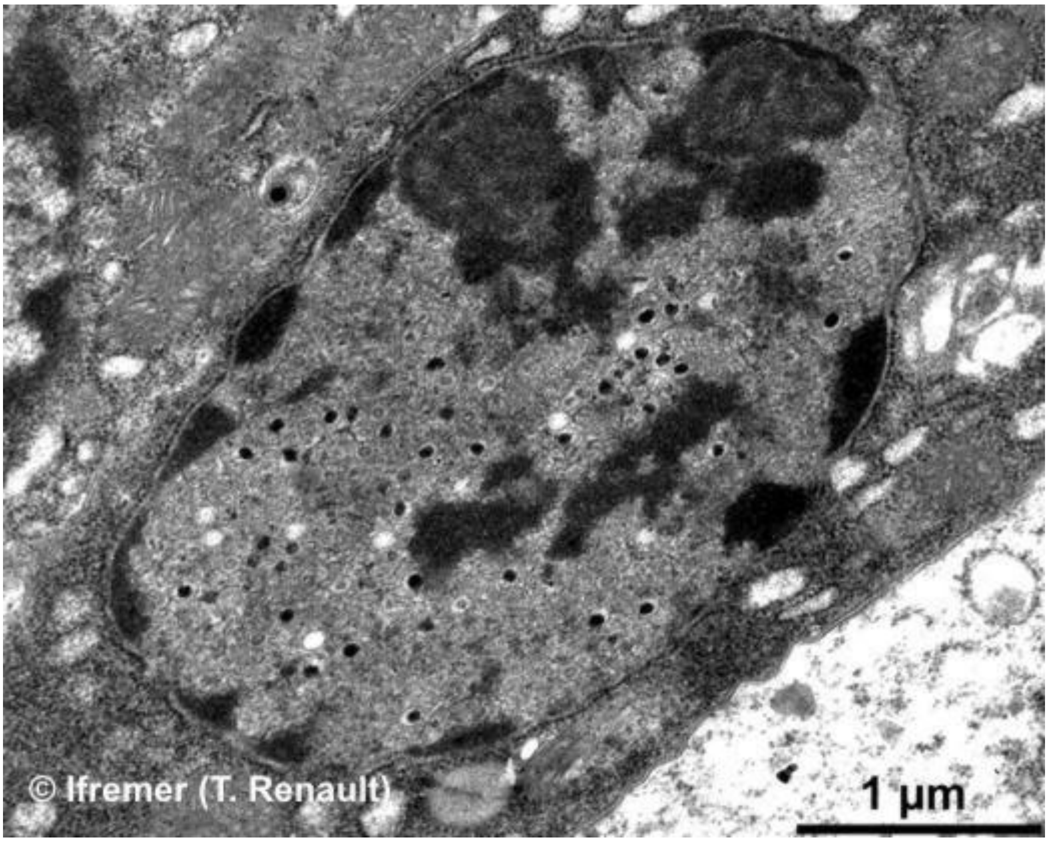
  It was felt that if there was any move for researchers in Australia to develop another model, then there would have to be a very clear reason to do so. In Australia, this would obviously have to be done in a biosecure facility.

- The role of *Vibrio* spp bacteria in oyster mortality.
  One IFREMER scientist was quite adamant that pathogenic *Vibrio* spp. bacteria had a key role to play in mortalities associated with the virus. He spoke of research that showed 1) a greater correlation between mortality and the presence of *Vibrio splendius* than with the presence of the virus, and 2) that when pathogenic *Vibrio* strains were removed in a laboratory experiment, the oysters did not die.

  The scientist also reported that that the mortalities in the experiment only occurred in the temperature range of 17 – 24 °C.

Based on our many conversations in France, the team considers that the following research topics should be included in Australian industry plans:

- Management options for decreasing mortality
- Cheaper, more practical tests for surveillance
- Development of effective clinical surveillance and transfer data capture systems
- Spatial network analysis
- Role of other species in spreading the disease
- Development of an experimental model
- Genetic analysis



OsHV-1 virus particles (black dots) in Pacific oyster tissue
(Transmission Electron Micrograph courtesy Dr T. Renault, IFREMER)