Future Oysters CRC-P: advanced understanding of POMS to guide farm management decisions in Tasmania

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Contents

List of Tables

List of Figures

1. Acknowledgments

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2. Abbreviations

ASI:	Australian Shellfish Industries
BT:	Biosecurity Tasmania POMS:Pacific Oyster Mortality Syndrome
DIIS	Department of Industry, Innovation and Science
EBV:	Estimated Breeding Value
FO CRC-P	Future Oysters Cooperative research Centre - Project
IMAS:	Institute for Marine and Antarctic Studies
OsHV-1:	Ostreid herpesvirus
qPCR	Quantitative polymerase chain reaction

3. Executive Summary

What the report is about.

The Tasmanian Pacific Oyster aquaculture industry was severely impacted by an outbreak of the disease Ostreid herpesvirus OsHV-1 µVar, known as Pacific Oyster Mortality Syndrome (POMS) in Australia, in January-February 2016. Massive oyster mortalities occurred on farms in four oyster growing areas in south-eastern Tasmania, and the two major hatcheries which supplied approximately 90% of oyster spat to SA and NSW were also in the infected area. This had a significant immediate impact on the supply of oysters to the market place, as well as a longer-term effect on the supply of Pacific oyster seed across Australia. In response to this devastating disease, the 'Future Oysters Cooperative Research Centre – Project' (FO CRC-P) was approved in August 2016 by the Department of Industry, Innovation and Sciences (DIIS), Australian Government to address the disease and production issues in the oyster industry. As part of this program, the Tasmanian Institute for Marine and Antarctic Studies (IMAS) was contracted to conduct research to advance the understanding of POMS disease and guide farm management systems that minimise the impact of POMS in Tasmania. This work compliments the research being conducted on selective breeding of oysters for POMS disease resistance, as well as other research undertaken as part of the FO CRC-P. Researchers at IMAS have worked closely with oyster farmers to be able to predict high risk periods and locations for POMS, to develop farming practices that reduce oyster mortalities from POMS disease and to document the effects of POMS on the Tasmanian oyster industry.

Background

POMS is a worldwide disease of Pacific Oysters that was first identified in Australia in the Georges River NSW in 2010, spread to the Hawksbury River in 2013, and then to four major oyster growing areas at Pitt Water, Pipe Clay Lagoon, Little Swanport and Blackman Bay in south-eastern Tasmania in January 2016, where 75-90% of oysters died on most farms. Various farm management techniques have been developed in other countries to minimise the impact of POMS, such as exposing large quantities of spat to the virus and ongrowing the survivors or determining the most cost-effective size and/or time of year to introduce spat to farm grow-out conditions. However, despite large research efforts overseas, there are still many unknowns about the OsHV-1 virus and POMS, including the reservoirs, carriers and hosts for this virus.

Aims

The objectives of our research have been to determine the high-risk periods for POMS infection and to develop a predictive framework so that the farmers can forecast danger periods for POMS. This includes developing a better understanding of where the virus exists in the environment and the factors that drive POMS disease outbreaks. We also aimed to work with the oyster industry to develop farm husbandry and handling protocols that maximise oyster production in POMS infected growing areas. Additionally, we surveyed the oyster farmers affected by POMS to get an overall view of the impact of POMS, especially socio-economic aspects.

Methodology

Our research was conducted on POMS infected Pacific Oyster farms in south-eastern Tasmania using commercially available oysters. Sentinel oysters were placed on farms approximately every fortnight to monitor survival rates during times when POMS outbreaks were likely to occur. Environmental data were collected using automatic, continuously monitoring data loggers recording temperature, salinity and other parameters every 10-30 minutes. Research trials investigating various farm management practices were developed in conjunction with oyster growers and were based around standard farming practices. They included replicate trials investigating the effects of handling, oyster density in culture containers, age and size of oysters

and chilling on mortalities due to POMS. The role of feral oysters as a potential reservoir for the POMS virus was also examined. The surveys of the effects of POMS on oyster growers were confidential and involved voluntary structured face to face interviews with oyster farmers individually. Human ethics approval was obtained from the University of Tasmania.

Key findings

Our research supports other studies that warm water temperature is a major driver of POMS outbreaks, with temperatures in south-eastern Tasmanian growing areas of 19 °C and above for around one week providing a high risk for a disease event to occur. The risk period for POMS disease outbreaks ranges from mid-November to late March. Other environmental factors likely to be important include water movements and density of infected oysters in a water body. Growing areas with extensive intertidal flats and poor water circulation, such as Pittwater, or with a high biomass of farmed and feral oysters in a relatively small area, such as Pipe Clay Lagoon, have shown to be more susceptible to POMS disease than the other farming areas. As feral oysters in Pipe Clay had a relatively high prevalence of OsHV-1, they may be contributing to the reservoir host of the virus.

Studies on farming practices conducted in close collaboration with oyster growers suggest that density of oysters in culture containers has limited effect on mortality rates, and that some handling is required during the POMS season to reduce biofouling and maintain stocking densities conducive to good growth and survival. Younger and smaller oysters are more susceptible to infection that larger and older juvenile and adult oysters. For oysters of the same age cohort, fast growers had higher mortalities than slow growers.

The surveys of oyster growers on the impacts of POMS on their farming operations has shown that mortalities from POMS have rapidly declined from an average of 67% of stock in 2016 to 9% in 2018/19. Changes to farming practices that have occurred during this time include a large increase in stock selectively bred for POMS disease resistance, reduced and more careful handling of oysters during the summer POMS season, selling a higher percentage of stock before the POMS high risk period, and purchasing spat when temperatures are declining.

Implications for relevant stakeholders

The impact of our research to develop a better understanding of the drivers of POMS disease and new farm management techniques to minimise POMS mortalities, along with major advancements in oyster selective breeding for POMS resistance, increased biosecurity measures and changes to farm management implemented by the oyster growers themselves, has led to a rapid turnaround in the Tasmanian oyster industry. It has changed from devastation and despair after the initial viral outbreak in 2016 to a positive outlook for the future in just over three years. Many farmers expect to be back to pre-POMS production levels by 2020 and have assessed their businesses as strong and more efficient than before POMS.

Recommendations

Although major progress has been made with selective breeding for POMS resistance and changed farm practices to minimise POMS mortalities, it is still early days for this disease and consequently its management in Tasmania. The selective breeding program needs to continue to ensure greater reliability of disease resistance. Additionally, it is important that oyster farmers regularly observe and keep records of oyster health, mortalities and environmental conditions on their farms, especially during extreme heat events, in case disease outbreaks occur in the future. There are also still many unknowns about the OsHV-1 virus, which have important implications for management of POMS disease, and further research in recommended to better understand the reservoirs, carriers and hosts for this virus. Interactions with bacteria and the role of oyster health and family line genetics in the likelihood and severity of POMS disease events also require further study.

Keywords

Pacific Oysters, *Crassostrea gigas*, Ostreid herpesvirus OsHV-1, oyster farm management, Pacific Oyster Mortality Syndrome POMS

4. Introduction

The Ostreid herpesvirus µVar (OsHV-1) was first identified in France in 2008 and has caused widespread and large-scale mortalities of Pacific Oysters (*Crassostrea gigas*) in several regions around the world. It spread to the Georges River in New South Wales (NSW), Australia, and to New Zealand in 2010 with rapid onset of mass mortality of oysters. Known as Pacific Oyster Mortality Syndrome (POMS) in Australia, it was subsequently diagnosed in Pacific Oysters in the Hawkesbury River in 2013, and then in south-eastern Tasmania in January 2016. Although considerable research effort has been expended in France, New Zealand and NSW in recent years on the epidemiology of this virus, there are still many unknowns, including how it disperses, especially at the local scale, and whether a combination of environmental variables are involved in triggering disease outbreaks.

In Tasmania in January 2016 massive mortality of Pacific Oysters was observed on farms in several major oyster growing areas, including upper and lower Pitt Water, Pipe Clay Lagoon, Blackman Bay and Little Swanport. It was also detected in oysters in the Derwent River, the major port for the capital city of Hobart. In nearby regions such as Bruny Island and Great Swanport the virus was detected at very low levels in several oysters and a disease outbreak did not occur. The only subsequent outbreak of POMS in Tasmania was at Gardners Bay in the D'Entrecasteaux Channel in 2018.

Prior to the OsHV-1 disease outbreak, Tasmanian oyster farms produced around four million dozen Pacific Oysters per year with an estimated farm gate value of $26 million, and Tasmanian hatcheries were supplying approximately 90% of the Pacific Oyster spat grown on farms in Australia (Davis 2016). As the disease occurred in the growing areas where the two main hatcheries were located, this has had a major effect on the supply of Pacific Oyster spat across Australia. Significantly, the South Australian Government in consultation with the SA Oyster Industry banned the importation of Pacific Oyster spat from Tasmania, impacting many South Australian oyster growers because they could not get oyster seed for their farms.

Selective breeding for disease resistance is widely accepted as the most likely means of reducing the impact of the OsHV-1 virus; however, farm management practices are also considered to be important. Developing a high level of disease resistance takes several generations of oysters and years to be highly effective. In the meantime, Pacific Oyster farmers needed to develop farming methods that maximised survival of oysters in POMS infected areas. Because the POMS disease event in Tasmania was unexpected, limited data were available on environmental conditions during the disease outbreak to support the development of an early warning system for farmers. Our project proposed to collect environmental data, both in real time and for post event analysis, to determine the period of infection and associated environmental conditions. It also aimed to better understand why differences in mortality rates occurred between POMS-infected Tasmanian oyster growing areas in Tasmania.

Various farm management techniques have been developed in other countries to minimise the impact of POMS, such as exposing large quantities of spat to the virus and ongrowing the survivors or determining the most cost-effective size and/or time of year to introduce spat to farm grow-out conditions. Our research aimed to support Tasmanian farmers to modify their current farming practices to enable them to operate successfully in POMS infected areas, especially during the next few years while selective breeding for POMS resistance was being developed.

The proposed research was planned to complement the development of genetically selected POMS resistant oysters, and to provide added assurance to oyster farmers that Pacific Oyster aquaculture would continue to be a commercially viable industry in Australia.

5. Objectives

1. To determine i) the periodicity of infection of OsHV-1 µVar virus in Tasmania, ii) advance the understanding of the drivers of POMS disease outbreaks, and iii) develop a predictive framework that allows the Tasmanian Pacific Oyster industry to forecast danger periods for POMS.

2. To develop farm husbandry and handling protocols to maximise Pacific Oyster production in POMS infected growing areas by investigating oyster survival in relation to: i) subtidal versus intertidal culture, ii) high water flow areas compared with low flow, iii) reduced handling, iv) size and timing of spat onto growout farms, and v) stocking density.

3. 2016/17 To enhance commercial production of Pacific Oysters in a POMS infected area through analysis of past farm production and management records, and a contemporary study of farm production systems and oyster survival.

Objective 3 was a PhD project to be managed by The Yield. However, after staff changes at The Yield, followed by a discontinuation of The Yield's operations on oyster farms in Tasmania and a lack of response from suitable PhD students, this PhD project was not undertaken. Industry representatives at the annual meeting of the Steering Committee for this project on 22 June 2017 expressed a strong interest in research on the effects of chilling on POMS mortalities. A change to Objective 3 was approved by the Steering Committee to:

3a 2017/18 Effects of chilling on the occurrence and severity of mortalities due to POMS.

The research conducted over the summer of 2017/18 found no effect of chilling on Pacific Oyster mortalities, so Objective 3 was again changed at the annual meeting of the Steering Committee for this project on 28 August 2018, to:

3b 2018/19 Investigating the source of the OsHV-1virus, including surveying and testing feral Pacific Oysters.

To obtain a more comprehensive account of the response by individual farmers and the industry as a whole to POMS, we conducted a survey of Pacific Oyster farmers, which helped inform Objectives 1 - 3. In this final report we are adding an additional objective:

4. To survey Pacific Oyster farmers for changes in farm management practices since the first POMS outbreak in 2016.

6. Method

Objective 1: Periodicity, drivers and prediction of OsHV-1 outbreaks

Methods used for Objective 1 were initially based on the research being conducted in Prof. Richard Whittington's laboratory at the School of Veterinary Science, The University of Sydney, described in Whittington et al. (submitted). Training was provided to Christine Crawford and Sarah Ugalde at this laboratory on the collection of Pacific Oyster meats in the Hawkesbury River and q-PCR analysis for OsHV-1 µVar.

Periodicity of Infection

Periodicity of Infection, 2016/17 OsHV-1 Season

Sentinel Pacific Oyster spat (collected on 2240 µm mesh, and Estimated Breeding Value (EBV) = 40%) donated by Shellfish Culture Pty. Ltd. were maintained in an aquarium at the Institute for Marine and Antarctic Studies (IMAS), Taroona, with daily algal feeding and a controlled water quality system to ensure no contamination with the OsHV-1 virus. Every fortnight over the warmer months of the year (November to April) approximately 250 of these oyster spat were deployed in 1.2 mm plastic mesh socks at each of the four growing areas that had been impacted by POMS in the initial outbreak in January-February 2016 (*Figure 1*); Pipe Clay Lagoon, Pitt Water including Island Inlet, Blackman Bay, and Little Swanport. Each sock was zip tied to the bottom of a housing unit that consisted of duplicate 6 mm mesh oyster tubes (SEAPA basket), linked together and attached to floatation, to reduce surface rumbling while ensuring the oysters were continually submerged just below the water surface. Two duplicate housing units were placed at four sites within each growing area approximately 20 – 50 m apart from each other (*Figure 2*). The sites were selected based on farmer recommendations to ensure locations were representative of the area and past patterns of OsHV-1 distribution, could be easily accessed, were in low-traffic areas, and the housing units would be continually submerged (*Figure 3*).

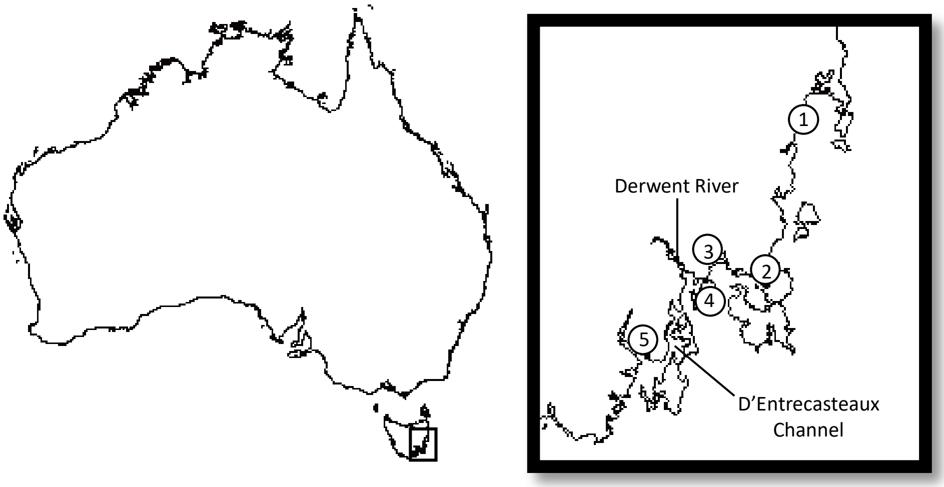

Figure 1. Map of oyster growing areas in Tasmania that have experienced mass mortality of Pacific Oysters due to the POMS virus. 1 = Little Swanport, 2 = Blackman Bay, 3 = Pitt Water (including Island Inlet), 4 = Pipe Clay and 5 = Gardners Bay. 1 - 4 were infected in 2016, whereas 5 first recorded POMS in 2018.

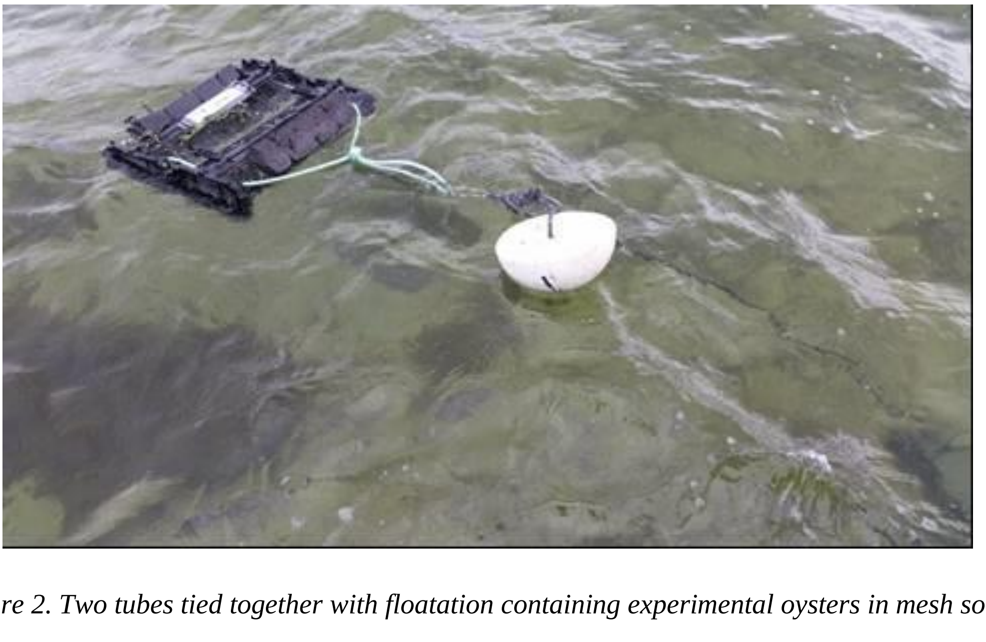

Figure 2. Two tubes tied together with floatation containing experimental oysters in mesh socks.

Timing of deployment was based on results from NSW and France where OsHV-1 viral outbreaks only occurred when water temperatures increased over summer (Paul-Pont et al. 2014, Pernet et al. 2014, Petton et al. 2013). The first batch of oysters were deployed in early November 2016 when water temperatures were around 16 °C and new batches of oysters were deployed approximately every fortnight until late March 2017. Temperature loggers (UA-001-08 Hobo Pendant) were zip tied to the bottom of one tube at each site and were recording every 30 minutes.

After the oyster spat had been in place for approximately two weeks, socks were opened in the field, and two subsamples were taken each of approximately 50 spat; one for live:dead counts (average spat counted: 59 +/- 20), and the other for later OsHV-1 detection using qPCR analysis. A fresh batch of 250 spat in a sock was added in to the tube at this time.

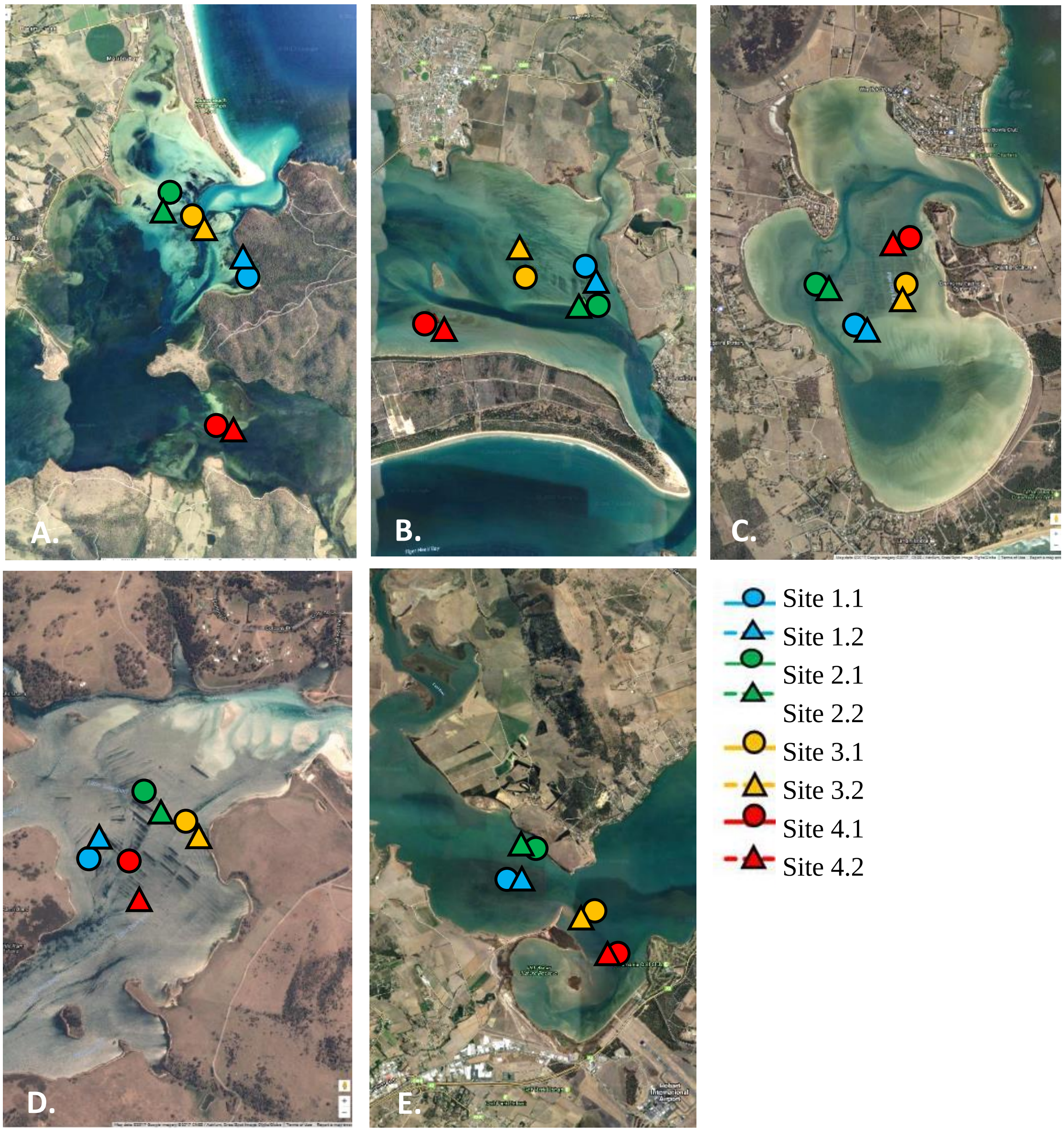

Figure 3. Location of duplicate tubes containing sentinel Pacific Oyster spat in the four infected growing areas, with Pitt Water divided into two areas; (A) Blackman Bay, (B) Iron Inlet (lower Pitt Water), (C) Pipe Clay, (D) Little Swanport, and (E) Upper Pitt Water.

The spat retrieved every fortnight were taken back to the IMAS laboratory for immediate processing. All spat were counted as either live or dead by submerging them in freshwater, removing the floating shells (dead) and, if required, by observing them using a Zeiss dissecting microscope. The hinge of each spat was compressed to help determine between live and dead. Spat that were either dead or dying (degradation of flesh, weak adductor) were counted as dead.

For OsHV-1 detection using qPCR analysis, approximately 50 spat were crushed using a sterile and disposable implement and preserved in 90% ethanol for later analysis of OsHV-1 DNA using qPCR at the School of Veterinary Science, The University of Sydney.

Infection with OsHV-1 was defined by qPCR results of <35 copies/mg, and indeterminate if OsHV-1 DNA was detected by qPCR results of $\geq$35 copies/mg. A POMS disease outbreak from farmer observations was defined by mortalities > approximately 15% during summer with water temperatures >18 °C and where there were no other obvious causes of mortality, such as low salinities, extreme air temperatures or excessive biofouling. On many occasions farmer observed disease outbreaks were verified by positive qPCR results for OsHV-1 DNA. Subclinical infections were defined as positive qPCR results but mortality <15%.

Periodicity of Infection, 2017/18 OsHV-1 Season

Due to some discrepancies in results between mortalities observed in sentinel spat, qPCR results for OsHV-1 virus, and farmer observations of mortalities, methods were modified from 2016/17 as follows:

The two farming areas which had recorded the highest mortalities in 2016/17, at Pitt Water and Pipe Clay, were selected as the only two growing areas in which to deploy spat. The same four sites at each location as the previous year were used based on advice from farmers and previous observed patterns of POMS distribution and severity (*Figure 4*). However, instead of deploying all spat in floating containers as in the previous year, half the spat were deployed in tubes on racks next to commercially produced oysters, with the other half in the same floatation housing as used last year (*Figure 5*). This was done because of concerns that the spat in the floatation housing were not experiencing the same environmental conditions as the farmed oysters on racks. Temperature loggers recording every 30 minutes (UA-001-08 HOBO pendant) were tied to tubes at each site.

The spat were 2240s with EBV 80%, donated by Shellfish Culture Pty Ltd. and housed in the Pipe Clay hatchery for the duration of the project, until each fortnightly deployment. Hatchery conditions were standard but with reduced food, i.e. enough to maintain spat health while minimalizing growth in order to avoid large changes in spat size. Before each deployment of spat on the farms, approximately 200 spat were sampled in the hatchery for later background mortality counts and qPCR analysis, as required. Approximately 100 spat were placed in 1.6 mm mesh socks on the leases for approximately two weeks.

The spat were also processed slightly differently for qPCR analysis compared to the previous year and in accordance with the requirements from the Tasmanian Government Animal Health Labs. The whole meats from three to six randomly collected spat in each sock were removed and preserved in 95 % ethanol for qPCR analysis. All equipment used was sterilised between each sample. qPCR testing was performed at the Animal Health Laboratory, Mt Pleasant, at the end of the POMS season.

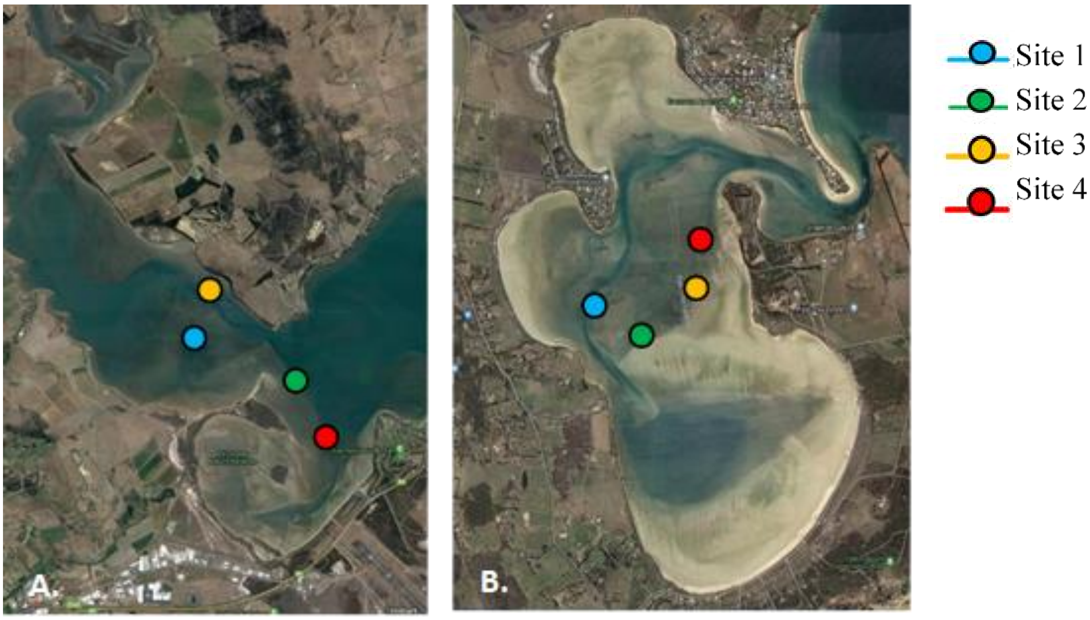

Figure 4: Location of single tubes containing sentinel Pacific Oyster spat in the two infected growing areas; (A) Upper Pitt Water, and (B) Pipe Clay.

Figure 5: Tubes on racks containing experimental Pacific Oysters.

Periodicity of Infection, 2018/19 OsHV-1 Season

The sentinel spat trials were not continued in 2018/19. There were inconsistencies in mortality among the sentinel spat used in the trial described above, in farmer observation of their own stock and results from qPCR testing for OsHV-1 DNA performed by Biosecurity Tasmania (BT). Furthermore, the fortnightly sampling method was relatively time consuming for both researchers and farmers, and costs for analysis for OsHV-1 using qPCR were relatively high. For the 2018/19 summer we documented mortalities observed by several farmers on leases at Pitt Water and Pipe Clay.

OsHV-1 Predictive Framework

Temperature data

The temperature data were collected as part of the Periodicity of Infection Project (Hobo Pendant Temperature Data Loggers, UA-001-08, logging every 30 mins), in addition to temperature, salinity and tidal height that were also available from The Yield Seabird sensors at Pitt Water and Pipe Clay Lagoon (logging every 10 mins).

Daily Pacific Oyster mortality data

Daily Pacific Oyster mortality data were recorded by farmers in Pitt Water and Pipe Clay and were classified as 0 for no signs of disease, 1 for some sign of disease such as weak and ‘dozey’ oysters with low mortality, and 2 for higher than expected mortality, that is >15%.

Development of a statistical model to predict POMS

A model was developed using water temperatures and daily Pacific Oyster mortality data provided by the farmers at Pitt Water and Pipe Clay Lagoon. Water temperatures were provided by The Yield and the Hobo temperature loggers for time periods 1/11/2016 – 31/5 2017 and 1/11/2107 – 31/5/2018. Prior to building the model, the temperature data were averaged both spatially and temporally to provide daily time series.

A generalized linear model (GLM) was constructed to measure the predictability of Pacific Oyster mortality based on surface temperature data. The GLM was used because it is the most accessible and explicable approach to determining the statistical connection between binary (oyster mortality) and numeric (temperature) variables (Nelder and Wedderburn, 1972). Different from traditional linear regression assuming response variables and errors following strict normal distribution, GLM allows the existence of exponential families, such as binomial distribution in this case. The general expression of GLM with binomial distribution is:

$$link\left(\frac{p}{1-p}\right) \sim V1 + V2 + \cdots$$

where p is the fitted probability for the existence of true (1) case in response variable, Vn indicates regressors and link() indicates a particular link function to adjust the probability distribution of response variable into normal distribution. Laaksonen (2006) identified GLMs commonly applied to link functions which are used for binominal distribution: (1) logit; (2) prob and (3) cloglog. Compared to the other two link functions, the logit link function provides better interpretability of fitted coefficients because of its lesser complexity. Based on this, the logit link function was selected for the construction of this model.

Objective 2: Farm Husbandry and Handling Protocols

Effects of Handling and Stocking Density

Many POMS-affected oyster farmers believed that handling during the POMS season stressed the oysters and made them more susceptible to the OsHV-1 virus. As a consequence, many did not handle their oysters for several months over summer. This project was designed in conjunction with industry to examine the effects of handling on survival and condition of oysters.

Pacific Oysters were donated from Barilla Bay Oysters, and the experiment was designed with industry input. Two groups of 30 mm oysters with 80 % EBV from the same spawning batch THO 16D (spawning: 14/11/2016) were used for the experiment: unchallenged oysters grown at Dunalley and pre-exposed oysters grown at Pitt Water. Unchallenged oysters (UC) were deployed at Pitt Water, Pipe Clay, and Blackman Bay, and pre-exposed (PX) oysters were at Pitt Water only due to low availability of these oysters.

Oysters were deployed in October until late February to early March (see Table 3.1). They were deployed before the expected POMS season to allow them to acclimatise to the environmental conditions.

Oysters were held in typical oyster growing tubes on intertidal longlines at two densities: High (200 oysters per tube) and Low (100 oysters per tube), on an active oyster lease using standard farm management regimes. The oysters were exposed to three handling regimes: no handling (‘No Handling)’, gentle hand sorting on the vessel (‘Hand Sorting’), and rougher onshore mechanical or hand grading (‘Mechanical Grading’). No handling oysters were not touched for the duration of the project. Hand sorting minimised handling stress to the oysters by gently sorting them in water in buckets on the boat immediately after retrieval from the racks. Mechanical grading oysters were subjected to rougher treatment by taking them ashore overnight, and either sorted with a mechanical grader or roughly hand sorting. These treatments approximated the standard handling procedures used by farmers before POMS occurred. Pipe Clay and Blackman Bay had four replicates of each treatment, while Pitt Water had four to seven replicates depending on oyster availability.

At the commencement of the project, during both types of monthly handling and on completion (*Table 1*), the following measurements were taken:

- Mortality: The number of live and dead oysters were counted.
- Growth: Photographs were taken (top shell facing down) of at least a dozen oysters from each tube for later image analysis of shell length, width, and area.
- Biofouling: Estimated biofouling of the outside of the tube, expressed as % cover.
- Predation: All predators and competitors in the tubes were identified and counted.

Table 1: Deployment and handling dates for unchallenged Pacific Oysters at three growing area locations in South-eastern Tasmania.

	Deployment and Handling Dates			
	Deployment	December	January	February / March
Blackman Bay	5/10/2017	13/12/2017	18/1/2018	28/2/2018
Pitt Water	19/10/2017	18/12/2017	22/1/2018	7/3/2018
Pipe Clay Lagoon	24/10/2018	14/12/2017	30/1/2018	5/3/2018

Effects of Age and Size

Several oyster farmers were keen to investigate whether there was a most cost-effective size or age at which to purchase spat. For example, a number of farmers opted to buy large quantities of the smallest spat (2240 µm in length) as they were least expensive to purchase, and to expose them to POMS so that spat susceptible to POMS died, and time and effort (and cost) was expended in farming only the more resistant surviving oysters. However, there was potentially an optimal age/size at which the combination of purchase costs and mortality rates resulted in overall greatest profitability.

Pacific Oysters were donated by Shellfish Culture and Barilla Bay Oysters. Four batches of oysters of the same genetic family lines were used; 16A, 16D, 16G, and 16I, across 4 ages (14, 11, 7.5, and 5.5 months) and 5 sizes (30, 8, 6, 5, 4 mm; *Table 2*). This project focused on smaller oysters because they are more susceptible to POMS. The 30 mm size was opportunistically available. Oyster size refers to sieve mesh size that oysters are retained on when graded.

Table 2: Descriptive statistics at beginning of experiment.

Hatchery Batch #	Age (months)	Size (mm)	Replicates	Background Mortality (%)	Shell Area (cm)	Shell Length (cm)	Width (cm)	Density (g/tube)	Tube Size (cm)
16A	14	6	4	29	2.09 ± 0.78	1.80 ± 0.30	1.43 ± 0.32	450	3
16A	14	30	4	2	3.48 ± 1.00	2.40 ± 0.38	1.81 ± 0.27	1000	6
16D	11	4	4	7	0.52 ± 0.13	0.87 ± 0.15	0.68 ± 0.14	400	3
16D	11	6	4	8	1.10 ± 0.11	1.12 ± 0.21	0.98 ± 0.10	450	3
16D	11	8	4	3	1.24 ± 0.43	1.45 ± 0.30	1.07 ± 0.17	600	3
16G	7.5	4	3	7	0.51 ± 0.12	0.92 ± 0.13	0.70 ± 0.09	400	3
16G	7.5	5	4	1	0.68 ± 0.23	1.20 ± 0.19	0.82 ± 0.21	400	3
16G	7.5	6	4	2	0.82 ± 0.15	1.25 ± 0.18	0.95 ± 0.12	450	3
16G	7.5	8	4	0	1.27 ± 0.24	1.55 ± 0.16	1.04 ± 0.12	600	3
16I	5.5	4	4	2	0.58 ± 0.15	0.86 ± 0.15	0.73 ± 0.11	400	3
16I	5.5	5	3	3	0.70 ± 0.21	1.09 ± 0.18	0.81 ± 0.13	400	3

Oysters were deployed on the 27 November 2017 until 22 March 2018 at Pipe Clay. Based on farmer observations and results from the Periodicity of Infection Project, it is likely that the oysters experienced two distinct outbreaks. Four replicate tubes for each age-size treatment were deployed on the oyster lease according to normal farm management practices. This included marginally lower than normal densities to account for minimal handling over the summer season. To keep tube biofouling and densities low, tubes were handled once onshore by hand on the 16th February 2018, and oysters were subsequently split into two or three tubes where oysters had grown and densities (biomass)in the tubes had become too high.

The following measurements were made at the beginning and end of the experiment:

- Mortality: The number of live and dead oysters in 3 randomly chosen groups of approximately 100 oysters were counted.
- Growth: Photographs were taken (top shell facing down) of at least a dozen oysters from each tube for later image analysis of shell length, width, and area.
- Weight: Total oyster wet weight (g) in each tube was taken in November and March only.
- Biofouling: Estimated biofouling of the outside of the tube, expressed as % cover.
- Predation: All predators and competitors in the tubes were identified and counted.

Objective 3: Chilling and OsHV-1 Source

Effects of Chilling

Over the summer of 2016/17 several oyster farmers found that placing 50+ mm Pacific Oysters in a chiller for 1-3 days at <5 °C after harvesting increased oyster survival rate. The oysters were placed in the chiller when POMS appeared imminent and were then graded, with larger oysters sent to market. Smaller Pacific Oysters approx. (40-50mm) were returned to the water and were observed to have lower mortality rates, compared with similar oysters retained on the farm. Farmers requested more information on effects of chilling – e.g. test viral load on consecutive days in the chiller and after returning to the farm.

Oysters were deployed at Pitt Water on 29 October 2017 until 11 January 2018. Two groups of 80 % EBV oysters from the same spawning batch (THO 16D spawning 14/11/2016) were used for the experiment; 20 – 30 mm unchallenged oysters previously grown at Dunalley (exposure treatment: ‘Unchallenged’) and 30 – 40 mm pre-exposed oysters previously grown at Pitt Water (exposure treatment: ‘Pre-Exposed’). Oysters were sorted before being housed in tubes on racks at a density of 100 oysters per tube.

The oysters were subjected to treatments ranging in timing of chilling and duration of chilling:

- Just prior to the first POMS outbreak, chilling for either one day (pre-POMS 1d) or three days (pre-POMS 3d).
- During the first POMS outbreak (during POMS 3d).
- Weekly chilling treatments over the POMS season (Weekly 3d).
- No chilling at all and oyster left untouched on the farm (On farm 0d).

Weekly chilling treatments involved bringing in the oysters from the farm for chilling for two to three days every week, depending on farm schedules, and then returning them to the water on the farm.

Source of the Virus (Feral Oyster Survey)

To obtain more information on the source of the OsHV-1 virus and the potential role of feral Pacific Oysters as a host for the virus, we surveyed and monitored both farmed and feral oysters in Pipe Clay. Pipe Clay contains 11 oyster leases and several dense feral Pacific Oyster reefs, as well as clumps of feral oyster scattered around the lagoon (*Figure 6*).

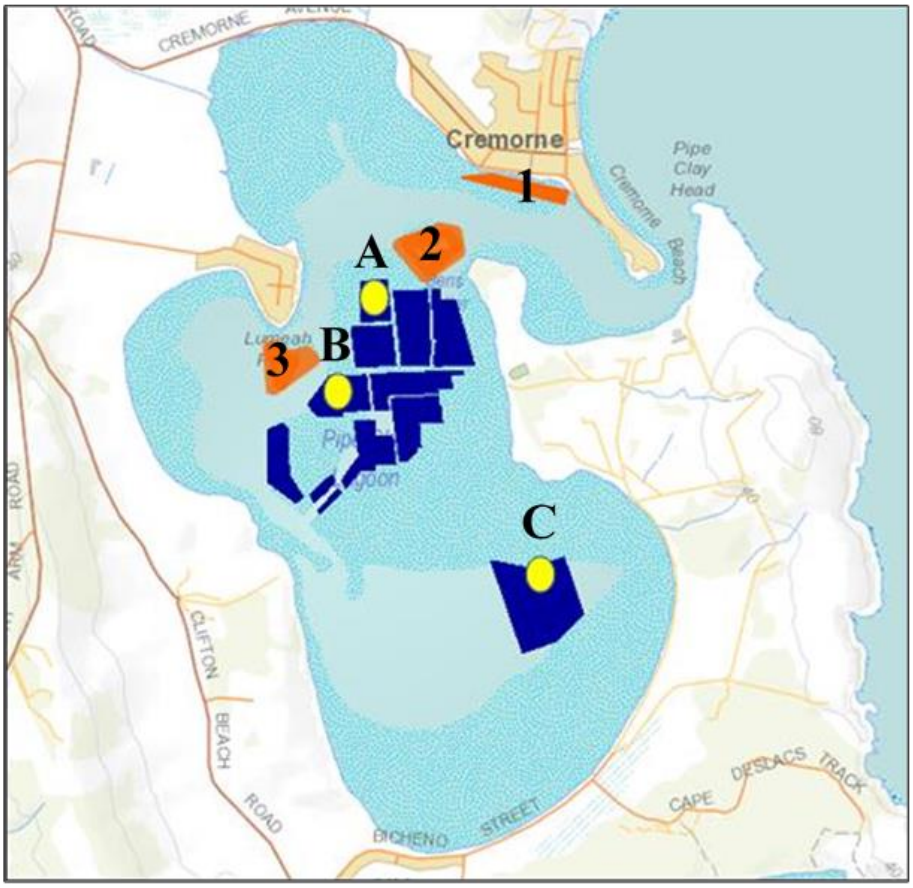

Figure 6: Location of feral and farm sampling sites in Pipe Clay. Oyster leases are shown in dark blue, feral Pacific Oyster reefs (1-3) in orange and farmed oyster sample sites (A-C) in yellow. Blue shading shows the shallowest areas of the lagoon which are exposed during low-tides.

We monitored three feral Pacific Oyster reefs: two were clumped and dense vertical reefs on fine sand near farmed stock and one was scattered among cobbles and boulders that form an artificial rock wall towards the entrance of the lagoon (*Figure 7*). Hatchery reared commercial diploid stock were distributed to the three farmed sites (length 50 - 65 mm) and held in four replicate baskets on racks at Site C and tubes on lines at Sites A and B at the commercial stocking density used by that farm (n = 100).

Figure 7: Photographs of the three feral oyster sites sampled.

The size, structure and density of the three feral Pacific Oyster populations were surveyed using 0.25 m^2 quadrats (site 1 n = 29; site 2 n = 35; site 3 n = 28) during low tide. The percentage of substrate cover and the number of live and 'new' or 'old' dead oysters was recorded for each quadrat. The size of oysters was categorised by length as small (<40 mm), medium (41 – 60 mm), large (61 – 81 mm) or extra-large (>81 mm) as a percentage of the total number of oysters within each quadrat.

OsHV-1 Prevalence

Both farmed and feral Pacific Oysters were collected in November (n = 160) and December (n = 35) 2018, and in January (n = 34), February (n = 37), April (n = 37) and June (n = 160) 2019 within a sampling period of three days. 160 oysters were sampled on the first and last sampling events as this number was calculated as being required to represent the population with a 2% prevalence of OSHv1.

Feral oysters were predominately medium to large in length. A small sample of gill/mantle from each oyster was preserved in 95% ethanol and sent to the Australian Animal Health Laboratory (AAHL) for detection of OsHV-1 using qPCR analysis.

Several blue mussels, *Mytilus edulis*, were observed to express severe shell gape and were slow to respond to stimuli during summer at Pipe Clay Lagoon, with some mortality. Samples of *M. edulis* were collected opportunistically from Pipe Clay and tested for OsHV-1 using qPCR. All mussel samples returned negative test results, except for one that returned an indeterminant result. A further 28 *M. edulis* were collected from Pipe Clay and transported to the IMAS aquaculture PC2 facilities at Taroona. These animals were divided among four recirculating tanks (n = 7). After one week the temperature of two tanks was gradually increased to a maximum temperature of 3 °C at a rate of 1.5 °C each day to stimulate viral activity and replicate upper extreme temperatures experienced during low tide at Pipe Clay. The other two tanks were held at ambient temperature (18.69 ± 0.51 °C), similar to that experienced at Pipe Clay Lagoon over summer. After 15 days, tissue was collected from each mussel and sent to AAHL for detection of OsHV-1 using qPCR analysis. All mussel samples returned negative test results, except for three that were indeterminant.

Objective 4: Farmer Surveys

Farmer Survey 1 on the effects of POMS on Pacific oyster farmers 2016/2017

This study aimed to improve our knowledge of the effects of Pacific Oyster Mortality Syndrome (POMS) on oyster growers in south-east Tasmania by recording the views, data and observations of farmers during the summer season 2016/17. The survey information was expected to contribute to the evolution of farm management and husbandry techniques to reduce the impact of POMS and identify the industry's research priorities and information gaps.

Human ethics approval was attained through a Minimal Risk Application to the Tasmanian Social Sciences Human Research Ethics Committee, University of Tasmania (ethics reference number: H0016495). Participation in the survey was voluntary and confidential, and the survey conducted for each lease was issued a unique identifier code to comply with ethical requirements.

Survey data were collected for each lease by conducting structured, face-to-face interviews with oyster farmers from bays infected with POMS (Pitt Water, Pipe Clay, Blackman Bay, and Little Swanport).

Farmer Survey 2: A Survey of Changes to Oyster Farming in Tasmania since the OsHV-1 Outbreak in January 2016

This survey of all oyster farmers in Tasmania, including those not directly impacted by OsHV-1 disease outbreaks, followed similar procedures to the first survey, with voluntary structured face-to-face interviews and an extension of the human ethics approval from the University of Tasmania. However, this survey was conducted primarily at a company level, rather than at a lease level, as the aim of the survey was to document changes in oyster farming that have occurred over four summers since the first POMS outbreak. This will provide the oyster industry with an overall view of how their industry has adapted and act as a benchmark for future developments in the industry.

7. Results

Objective 1: Periodicity of Infection

Periodicity of Infection, 2016/17 OsHV-1 Season

Temperature Regimes

Temperatures provided by The Yield at Pitt Water, Pipe Clay Lagoon and Little Swanport over the 2016/17 summer showed that water temperatures were more extreme at Pitt Water than at the other sites, with higher peaks over much of summer, and then declining much more quickly in March (*Figure 8*). Pipe Clay Lagoon and Blackman Bay had relatively similar temperature regimes, except for the occasional higher peak in Pipe Clay Lagoon early in summer and at Blackman Bay later in the season.

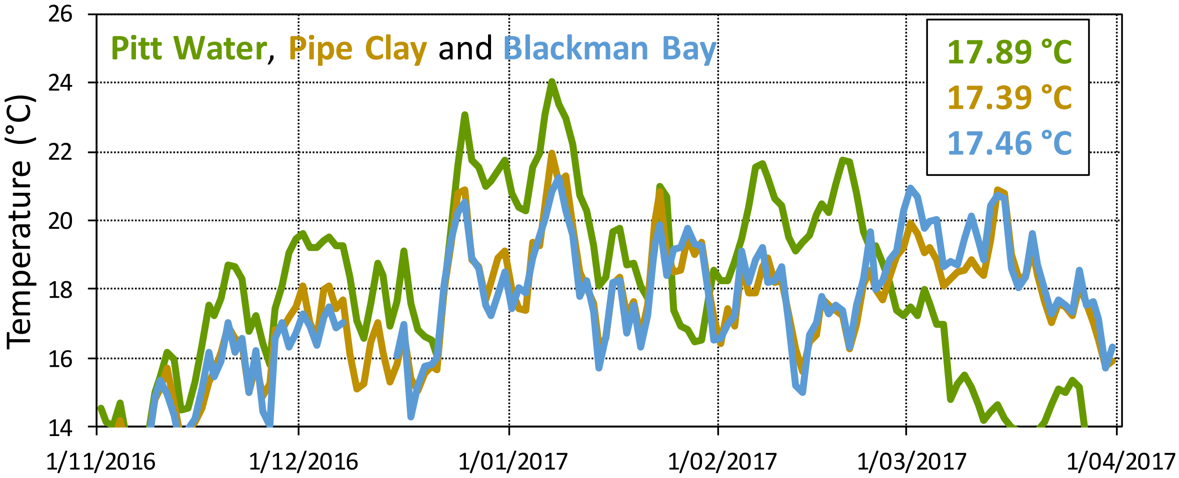

Figure 8: Average daily water temperatures at Pitt Water, Pipe Clay Lagoon and Blackman Bay provided by The Yield sensors at farms at each location.

Periodicity of Infection

Mortalities in sentinel Pacific Oyster spat showed considerable variation spatially and temporarily across each growing area (*Figure 9A-E*). Spat from one site at each location in the infected growing areas were analysed by qPCR for OsHV-1, and positive OsHV-1 results are shown in *Figure 8*. These figures also show the times when significant mortalities (at least 15% of an oyster cohort) were observed by at least two oyster farmers on their leases in each growing area. They also show the dates when oysters sampled by BT had positive OsHV-1 results. All oyster farmers are required as part of their licence agreements to report major mortalities to BT, and for the first report of mortalities in a growing area in each season, BT samples approximately 30 oysters across a lease to test for OsHV-1. Thus the results from BT are generally only from one lease in each growing area and for the first outbreak of the season.

At Blackman Bay, sentinel spat had an indeterminant OsHV-1 infection in both early December and January at two sites and a positive qPCR in mid-January only at site 4, furthermost in the Bay (*Figure 9A*). BT also recorded positive OsHV-1 at site 4, but earlier in

January in 20-30 mm stock. Farmer observations were disease outbreaks, minor in late November, substantial in early January, and a second outbreak in late January – early February.

Island Inlet located at the mouth of Pitt Water estuary (*Figure 9B*) only showed positive OsHV-1 in the sentinel spat in early January at site 4. However, both BT and farmers recorded positive OsHV-1 and disease outbreaks in early December across the growing area. Another outbreak was recorded by several farmers in mid January.

At Pipe Clay (*Figure 9C*) sentinel spat showed positive for OsHV-1 at three sites in early January. Similarly, BT and farmers recorded positive OsHV-1 and major mortalities across the growing area at this time. Three farmers also recorded mortalities in mid-March.

All results for OsHV-1 in sentinel spat were negative at Little Swanport (*Figure 9D*). However, all farmers reported significant mortalities in early January and again in mid March. BT also recorded positive OsHV-1 in 20-40 mm spat at Little Swanport on 11 January 2017.

In Upper Pitt Water (*Figure 9E*) sentinel spat showed positive for OsHV-1 at three sites in early January, similar to Pipe Clay. However, farmers reported a significant disease outbreak in early December which was confirmed by BT as positive OsHV-1. Farmers also reported a minor outbreak in mid-January.

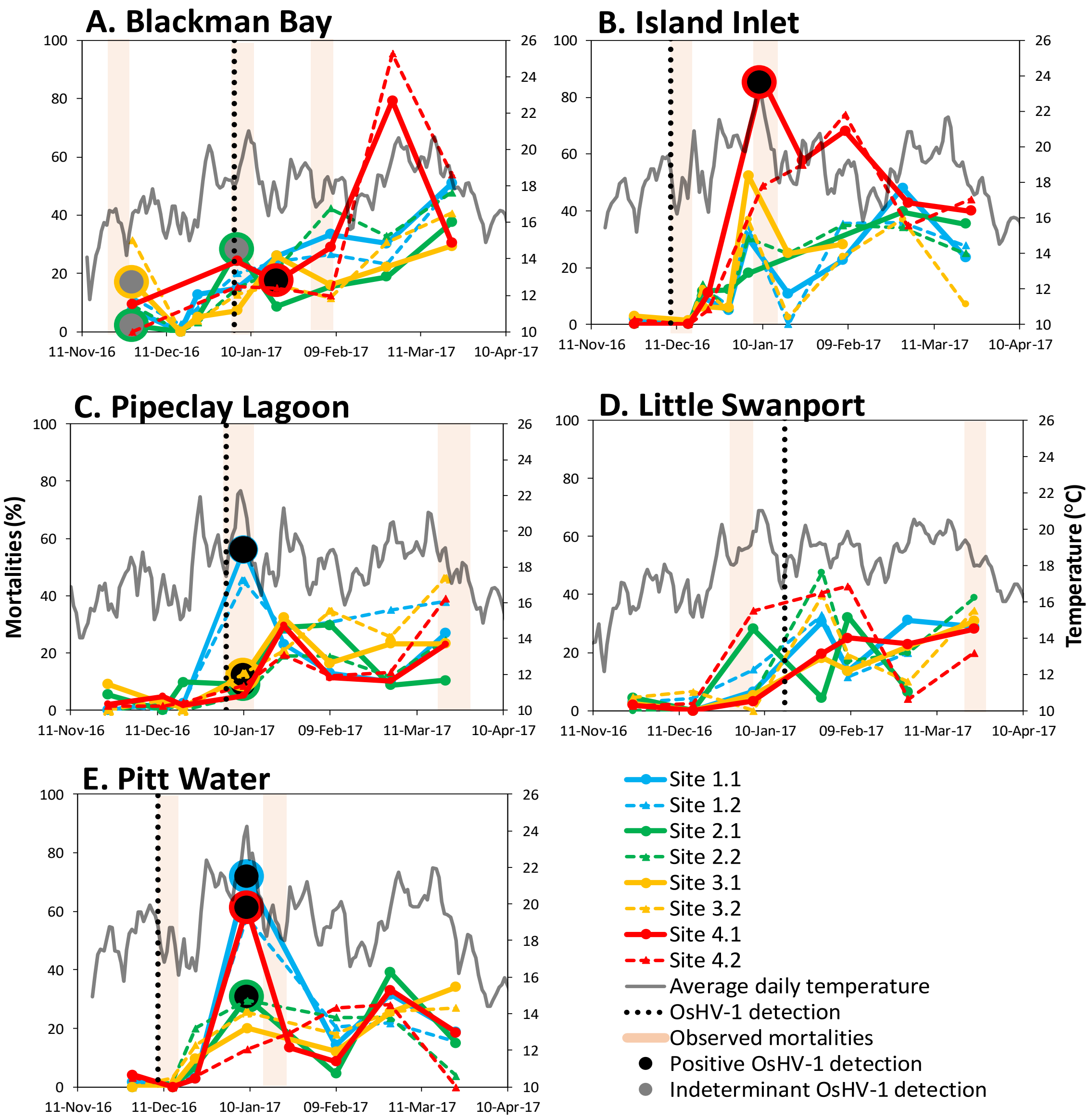

Figure 9: Percentage mortality of sentinel Pacific Oyster spat over time at four replicate sites in five growing areas. The dashed line shows when oysters were first tested positive for OsHV-1 by Biosecurity Tasmania, the pink line when oyster growers reported mass mortalities and the large black dots when sentinel spat were positive for OsHV-1. Average daily temperatures are shown by the black line.

Periodicity of Infection, 2017/18 OsHV-1 Season

Temperature Regimes

Pitt Water again exhibited higher temperatures than at Pipe Clay Lagoon, on average by at least 1 °C for both racks (Pitt Water average = 19.8 °C; Pipe Clay average = 18.7 °C) and floats (Pitt Water average = 20.1°C; Pipelcay avearage = 19.0 °C). However, temperature patterns between racks and floating packs were similar at both Pitt Water and Pipe Clay (*Figure 10*). The average rack temperatures across all sites were slighly lower than floating tempeartures in both Pitt Water (racks average = 19.8 °C; floats average = 20.1 °C) and Pipe Clay (racks avearge = 18.7 °C; floats average = 19.0 °C) across the whole POMS season.

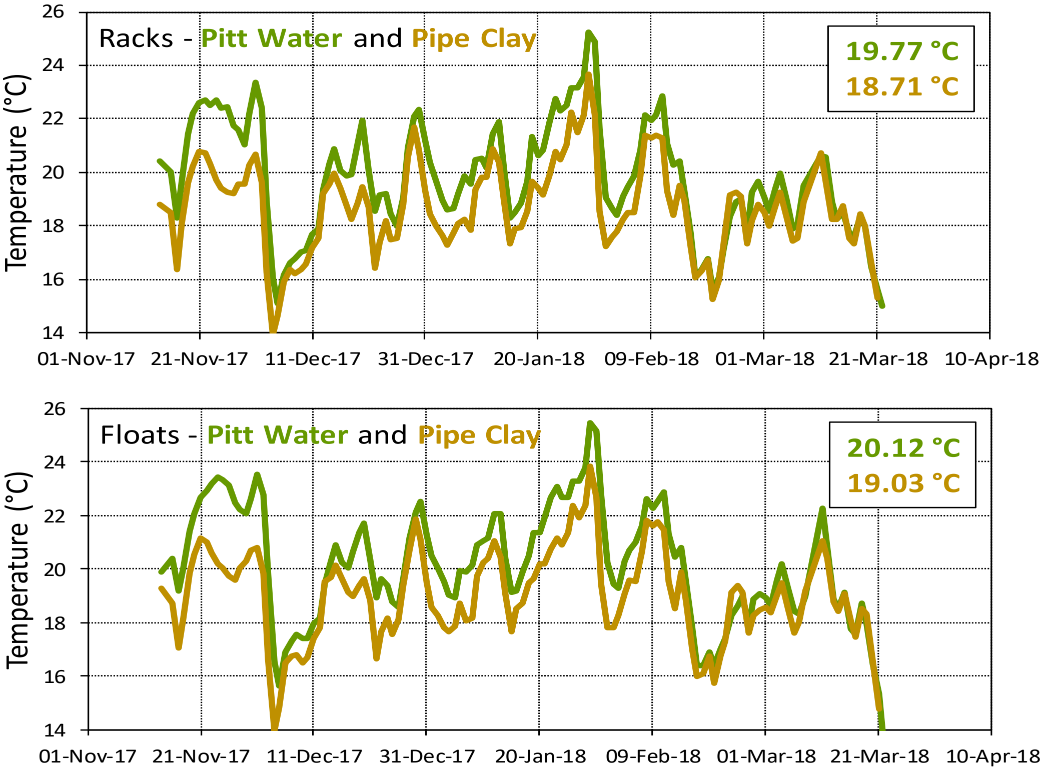

Figure 10: Comparison of water temperature (daily averages) on racks and in floating tubes between Pitt Water and Pipe Clay Lagoon.

Periodicity of Infection

Prior to deployment, the background mortality of Pacific Oyster spat in the hatchery was low (6.48 % ± 0.03 %). 8 'fortnightly' sampling periods from 13 November 2017 to 22 March 2018 ranged from 13 to 25 days, depending on industry schedules.

Of the total 128 samples, 19 had mortality greater than 10 %, 29 had mortality 5-10 %, and 96 were < 5 %. Very high mortality (>80%) was recorded only in floating sentinel spat in early and mid-February at site 1, and lower but significant moralities (>20%) were recorded at site 3, also in the upper reaches of the farming area, in mid-December and early February (*Figure 11A*). However, these floating spat did not show positive for OsHV-1 by qPCR analysis across the summer (*Figure 11A*). At Pitt Water mortalities were consistently low in sentinel spat attached to racks, although positive qPCR results were recorded in late November and mid-late March (*Figure 11B*). Farmer observations detected a major mortality event in late November after exceptionally high temperatures for that time of year, and this was confirmed by qPCR conducted by BT, with relatively high concentrations of the virus (*Figure 11A-B*). Minor mortality events were observed by several farmers in early and late January after increases in water temperature (*Figure 11A-B*).

In contrast, at Pipe Clay very high mortalities were recorded in sentinel spat at all sites in both floating and rack oysters at the first sampling on 24 November, and positive OsHV-1 was reported by BT (*Figure 11C-D*). In early February mortalities were also very high at site 1 in floating spat and at site 2 in spat attached to racks, and lower at site 2 in floating spat and site 3 in spat attached to racks (*Figure 11C-D*). Positive qPCR results were only obtained from these sentinel spat at Pipe Clay on racks in mid-January, but high concentrations of the virus were recorded by BT in late November (*Figure 11C-D*).

Of the 139 samples tested for disease presence with qPCR, only three returned positive results; all in Pitt Water in spat on racks, and with high Ct values (over 30) indicating only low levels of the virus. It is also noted that mortalities of spat on the racks were very low for the entire sampling period, but positive qPCR results were also obtained in late March. However, the farmers did not report significant mortalities at this time.

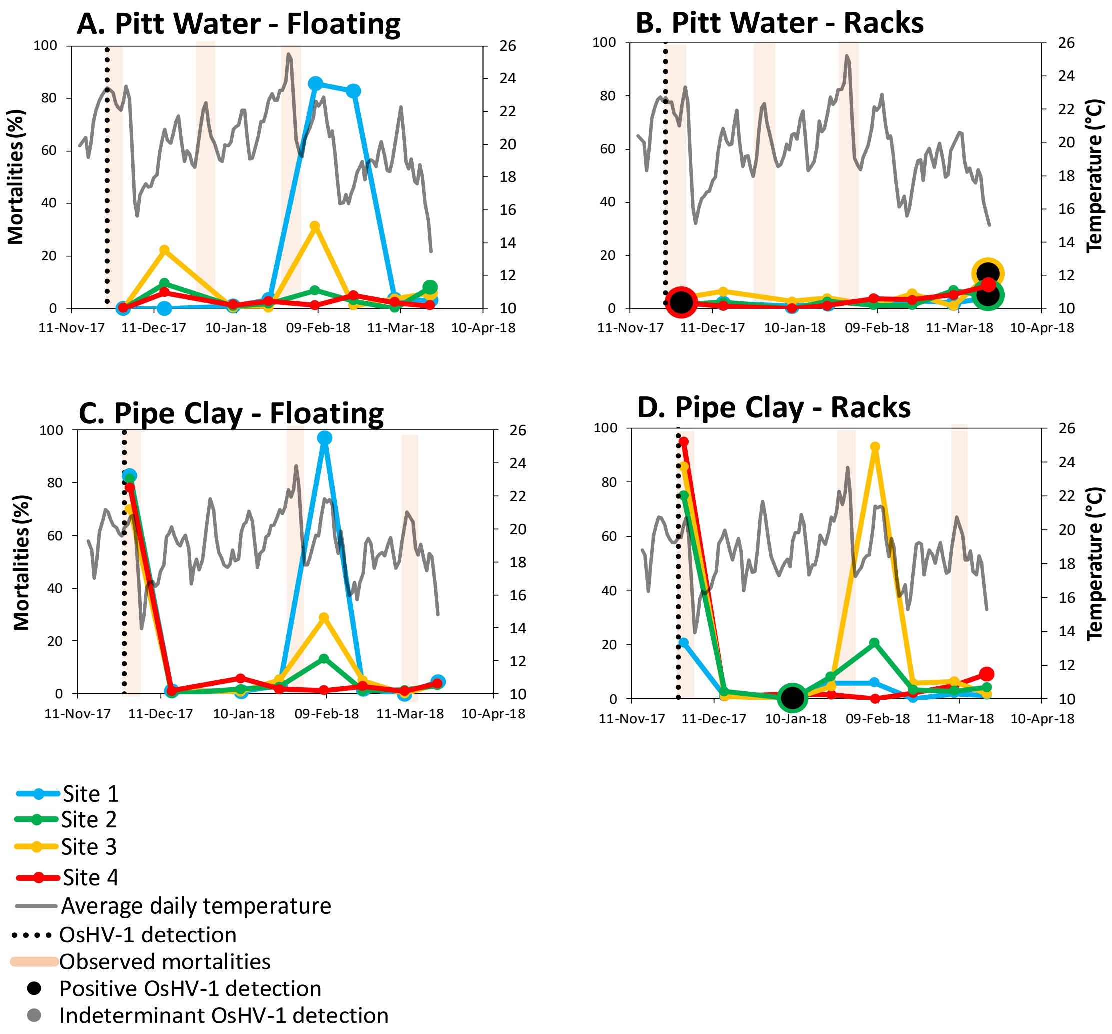

Figure 11: Percentage mortality of Pacific Oyster spat over time at Pitt Water and Pipe Clay in floating tubes and tubes attached to racks.

Periodicity of Infection, 2018/19 OsHV-1 Season

Although mortalities were much lower in 2018/19, Pitt Water experienced two mortality outbreaks that were observed by farmers, in mid-December and mid-late January (*Figure 12A*). In Pipe Clay, two mortality events were recorded in mid-December and mid-January (*Figure 12B*).

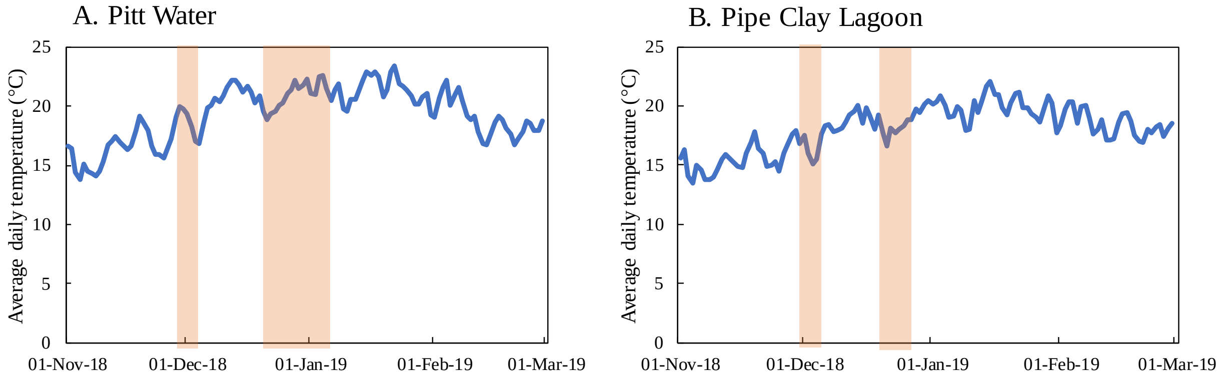

Figure 12: Farmer observations of timing of above average Pacific Oyster mortalities at Pitt Water and Pipe Clay (pink lines) over 2018/19 POMS season. Average daily temperatures are shown by the blue line.

OsHV-1 Predictive Framework

The model was described by:

$$ln\left(\frac{p}{1-p}\right) \sim b0 + b1\, ln(Temp) + b2loc + b3\, ln(Temp) : loc$$

where ln() is the default format for logit link function, ln(Temp) indicates the logarithmic transformation of daily surface temperature, loc is a binary variable indicating the location for each data set at Pipe Clay (PC) or Pitt Water (PW) and ln(Temp):loc is the interaction (product) between ln(Temp) and loc. b0-3 are fitted coefficients for each model, separately representing default constants, fingerprints of logarithmic transformation of daily surface temperature, influence of different locations, and the performance of temperature on oyster mortality in different locations. If we consider PC as 0 and PW as 1, the fitted model equation would have two different types: for Pipe Clay (PC)

$$ln\left(\frac{p}{1-p}\right) = b0 + b1ln(Temp)$$

And for Pitt Water (PW)

$$ln\left(\frac{p}{1-p}\right) = (b0 + b2) + (b1 + b3)ln(Temp)$$

Since this model is built from two datasets (Sensors, Floats), we obtained four equations to model the predictability of oyster mortality based on surface water temperature. All four equations could be expressed in the format of

$$ln\left(\frac{p}{1-p}\right) = a + bln(Temp)$$

The fitted coefficients a and b in all four equations are summarized in following table.

a	**PC**	**PW**
Sensor	-37.724	-28.857
Float	-37.645	-16.532

b	**PC**	**PW**
Sensor	13.1693	9.92644
Float	13.0532	5.68156

The fitted coefficients (a and b) reveal some characteristics of temperature's influence on Pacific Oyster mortality, including that temperature increases influence higher oyster mortality, and this is more significant in Pitt Water than in Pipe Clay (shown by higher b in PC). Temperature influences on oyster mortality were similar in Sensor and Float data (shown by similar b).

We used the model to calculate the probability of mortality occurring across the annual average temperature range of Pipe Clay Lagoon and Upper Pitt Water (Figure X). Pipe Clay had a slightly higher probability of mortality than Upper Pitt Water at a given temperature across the expected range of summer temperatures.

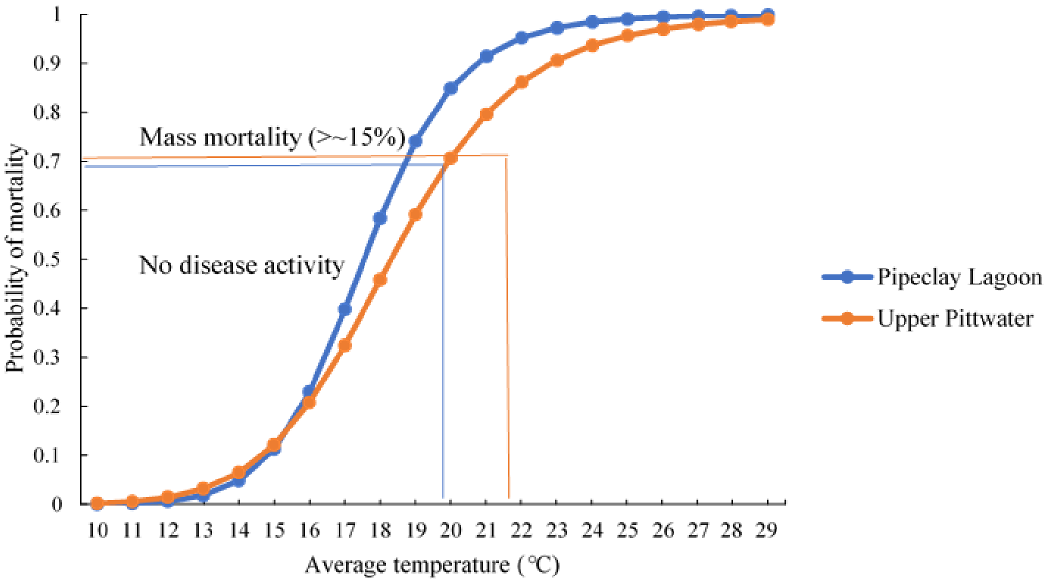

Figure 13: Probability of mortality calculated using the model developed from results of mortality due to POMS and temperature regimes in Pitt Water and Pipe Clay Lagoon in 2016- 2018.

The average temperatures at which farmers observed high Pacific Oyster mortalities at Pitt Water and Pipe Clay Lagoon in the 2018/19 ‘POMS season’ were then plotted in Figure 13. High mortality was observed in Pipe Clay at 19.4 °C, and at 21.4 °C for Upper Pitt Water and probability of mortality was just below and just above 0.8, respectively. The model fits well with the farmer observations of mortalities in 2018/19.

Objective 2: Farm Husbandry and Handling Protocols

Effects of Handling and Stocking Density

Mortality rates were highest up to mid-December at both Pipe Clay and Pitt Water following exceptionally highwater temperatures over that time. Mortality rates were then lower in subsequent sampling days (*Figure 14*).

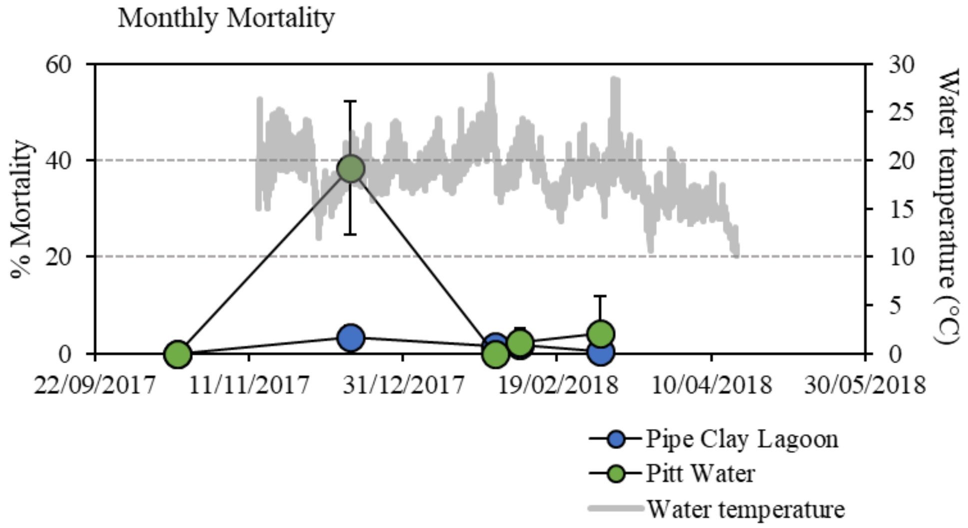

Figure 14: Average percentage cumulative Pacific Oyster mortality over the sampling period. Water temperature data collected by the Yield sensor deployed in Pitt Water 1/11/2017 to 31/5/2018.

Two-ways ANOVAs were performed using IBM SPSS 24 to explore whether the final percentage cumulative mortality of oysters at Pipe Clay and Pitt Water, separately was influenced by handling and stocking density. Mortalities between sites were not statistically compared due to the different environmental conditions at each site. Statistical analysis was also not applied to Blackman Bay because mortalities were minimal (<5 % in all tubes, *Figure 15A*) and is not discussed in depth, although the presence of OsHV-1 was confirmed by BT using qPCR analysis from taken on 4/1/2018.

At Pipe Clay, final mortalities of unchallenged oysters were only significantly affected by density with higher mortality recorded in high density treatments when compared to low density (p = 0.015; *Figure 15B*). At Pitt Water, final mortalities were not significantly affected by either handling or density treatments, and the interaction term was also not significant (*Figure 15C*). These results suggest that high density oysters can be more susceptible to mortality, although the survivability and impact of OsHV-1 on oysters is site-specific.

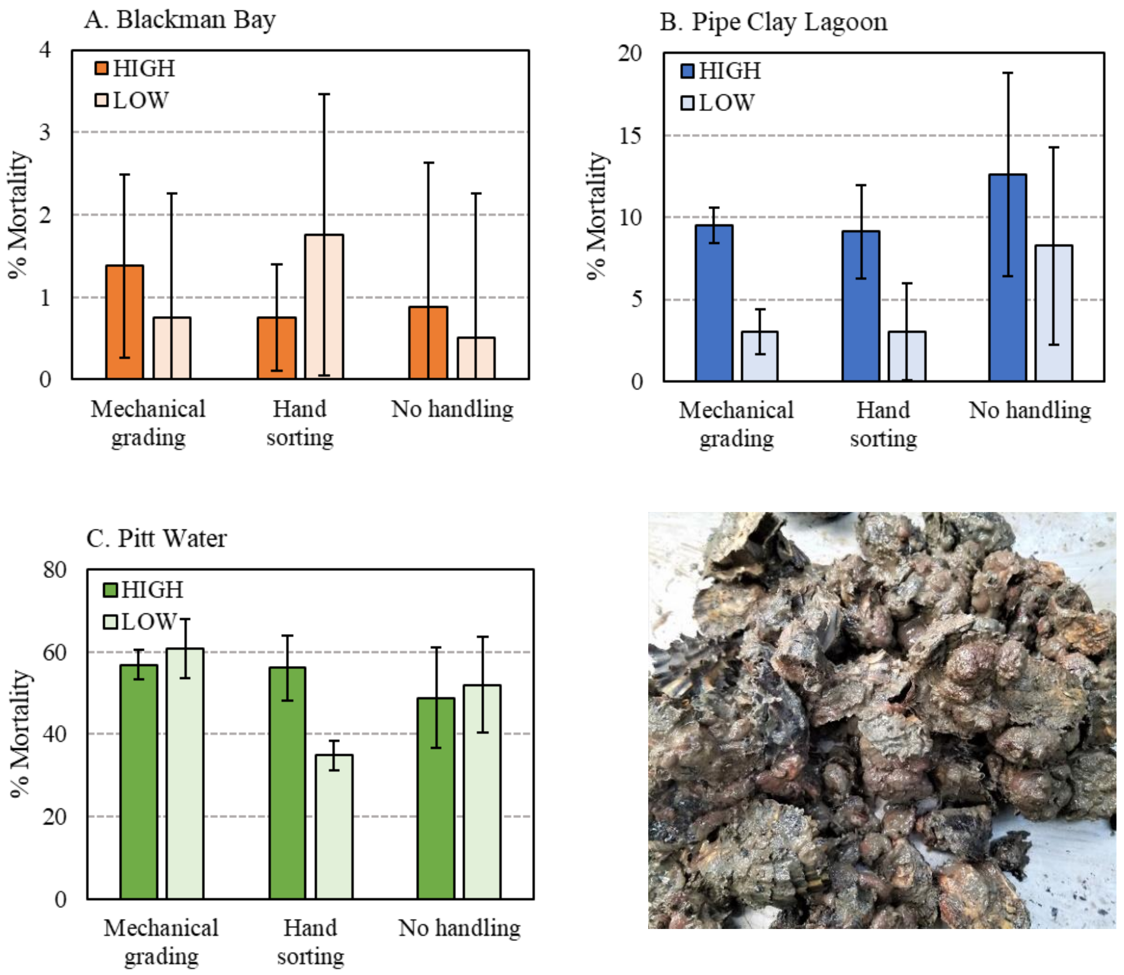

Figure 15: Average Pacific Oyster percentage mortality over 4.5 month trial period between handling treatments [error bars = SD] at Blackman Bay (A), Pipe Clay (B), and Pitt Water (C), and cunjevoi covering oysters observed in Pitt Water no handling treatment at the end of the trial (D.)

A three-way ANOVA using IBM SPSS 24 explored whether differences in POMS exposure (unchallenged vs pre-exposed) at Pitt Water affected final percentage mortality, along with factors of handling treatment and oyster density (*Figure 16*). Unchallenged oysters had a significantly higher mortality than pre-exposed ($p = 0.044$). In addition, there was also a significant interaction between handling and POMS exposure. A simple effects analysis revealed only one significant effect between mechanical grading and POMS exposure ($p = 0.002$) indicating that pre-exposed oysters were able to withstand harsher grading than unchallenged oysters.

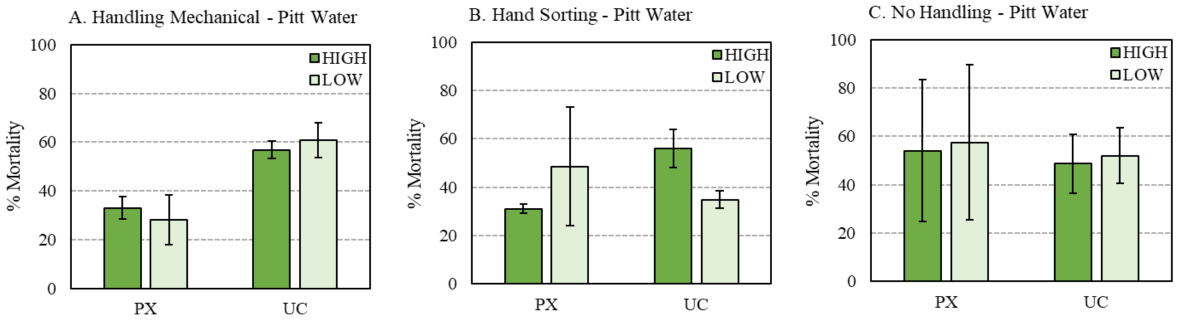

Figure 16: Mortality of pre-exposed (PX) and unchallenged Pacific Oysters (UC) under different handling treatments: A - Mechanical Handling, B - Hand Sorting and C – No Handling and Oyster Density – High and Low.

Although biofouling recorded at Pipe Clay was negligible, Pitt Water experienced heavy biofouling, predominately cunjevoi (*Pyura* sp.), within and around some tubes (*Table 3*). A three-way ANOVA assessed whether handling, density, and POMS contact (challenged vs pre-exposed) influenced the biofouling (expressed as percentage cover). Biofouling was significantly affected by handling ($p = 0.002$) and POMS exposure ($p = 0.000$; Table 3), but not density. These results suggest that if oysters are not handled for long periods of time then biofouling increases, especially in pre-exposed oysters.

Table 3: Covering of biofouling under different handling and density treatments: Hand Sorting, Mechanical Grading, and No Handling, and High and Low Oyster Density for Unchallenged (UC) and Pre-Exposed (PX) oysters.

Location	**Treatment**	**Density**	**Split**	**Biofouling (% cover of tube)**		**Cunjivoi (% cover of oysters)**	
				UC	**PX**	**UC**	**PX**
Blackman Bay	Hand	High	.	0	.	0	.
		Low	.	0	.	0	.
	Mechanical	High	.	0	.	0	.
		Low	.	0	.	0	.
	No Handling	High	.	0	.	0	.
		Low	.	0	.	0	.
Pipeclay	Hand	High	Feb	0	.	0	.
		Low	Feb	0	.	0	.
	Mechanical	High	Feb	0	.	0	.
		Low	Feb	0	.	0	.
	No Handling	High	.	0	.	0	.
		Low	.	0	.	0	.
Pittwater	Hand	High	.	5	4	1	0
		Low	.	3	0	2	0
	Mechanical	High	.	1	4	0	0
		Low	.	6	1	0	0
	No Handling	High	.	13	38	5	58
		Low	.	9	21	13	8

Farmers also scored the oysters out of 20 for condition factors such as fat coverage, meat:shell ratio, shape, defects, and shell and abductor strength (*Figure 17*). Low density oysters not only had lower mortality, but also better condition. No handling oysters with high mortality had less favourable condition, especially for shell and abductor strength. The example below shows oysters from Pipe Clay across all treatments and scored out of 20 by farmers.

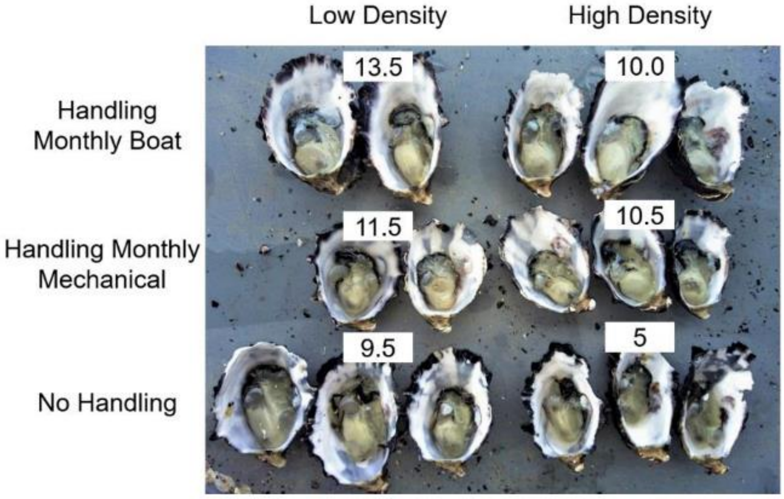

Figure 17: Scoring of condition of Pacific Oysters at Pipe Clay under different handling and density regimes.

Effects of Age and Size

Two-way ANOVAs were performed using IBM SPSS 24 to explore differences in the dependent variables of final Pacific Oyster mortality, shell area, and weight at Pipe Clay with the dependent variables of oyster age and size class (length mm). Oyster mortalities (expressed as a final percentage) were found to be significantly affected by age ($p = 0.005$) and size class ($p = 0.015$), although no significant interaction was observed (*Figure 18A*).

Similarly, shell area (cm^2) and final oyster weight (expressed as g per 12 oysters) were significantly affected by oyster age ($p = 0.001$ for both variables) and size class ($p = 0.00$ for both variables), and a significant interaction for age and size was only detected for oyster shell area ($p = 0.002$; *Figure 18B,C*). Generally, oyster mortality increased with size of spat in each age group, indicating that fast growing oysters (i.e. ‘front runners’) are more susceptible than slower growing oysters. Mortality also decreased with age, except for the youngest age group at 5.5 months. No notable biofouling or predation was recorded on the oysters or in the tubes.

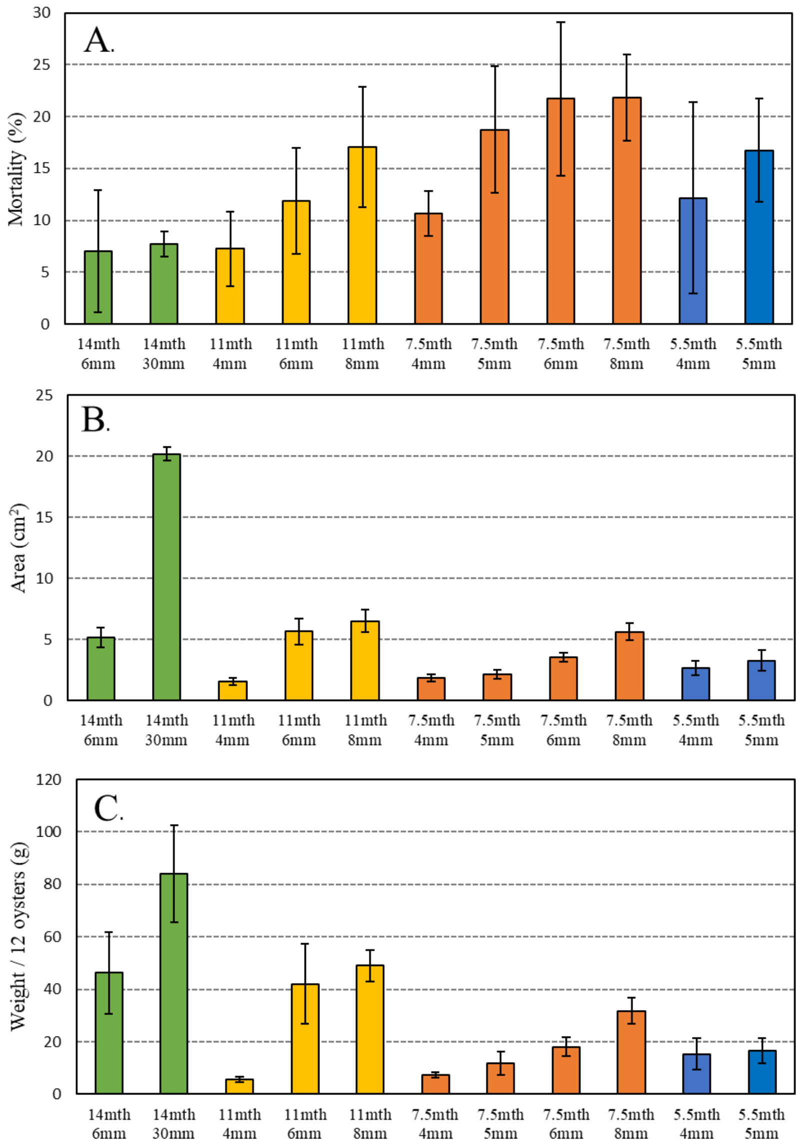

Figure 18: Mortality (A), shell area (B), and Pacific Oyster weight (C) of different age and size classes over summer 2017/18.

The cost per thousand surviving oysters, based on approximate commercial spat purchase prices shows the cost increases quickly with size compared with relatively small difference in mortality rates between the size/age groups (*Table 4*). These results suggest that it is more cost effective to purchase smaller rather than larger oysters.

Table 4: Cost per thousand surviving Pacific Oysters at different sizes/ages.

Hatchery Batch #	Age (months)	Size (mm)	Survival (%)	Approx Cost (1000 Oysters)	Approx Cost (1000 Surviving Oysters)
16A	14	6	93.0	$45.00	$48.39
16A	14	30	92.3	$230.00	$249.22
16D	11	4	92.7	$27.00	$29.12
16D	11	6	88.1	$45.00	$51.05
16D	11	8	83.0	$61.00	$73.53
16G	7.5	4	89.3	$27.00	$30.23
16G	7.5	5	81.3	$31.00	$38.15
16G	7.5	6	78.3	$45.00	$57.48
16G	7.5	8	78.1	$61.00	$78.06
16I	5.5	4	87.8	$27.00	$30.74
16I	5.5	5	83.3	$31.00	$37.24

Objective 3: Chilling and OsHV-1 Source

Effects of Chilling

The results from the chilling experiment with juvenile oysters indicates that chilling had no effect on the survival of Pacific Oysters. Instead, they emphasise the difference in mortality rates between OsHV-1 unchallenged and pre-exposed oysters, with unchallenged having significantly higher mortality than pre-exposed oysters.

Two-way ANOVAs were performed using IBM SPSS 24 to explore differences in final Pacific Oyster mortality (expressed as a percentage) and weight gain (kg per 100 oysters) at Pitt Water with factors of oyster POMS exposure (pre-exposed and unchallenged) and chilling treatments. Oyster mortality was found to be significantly affected by Pre-exposure/Unchallenged ($p = 0.000$), with no other significant factors detected (*Figure 19A*).

Oyster weight was significantly affected by Pre-exposure/Unchallenged ($p = 0.000$) and chilling treatment ($p = 0.000$; *Figure 19B*). There was no significant interaction detected. This demonstrates that the pre-exposed oysters not only had an improved survival when compared with unchallenged oysters, but also had improved growth.

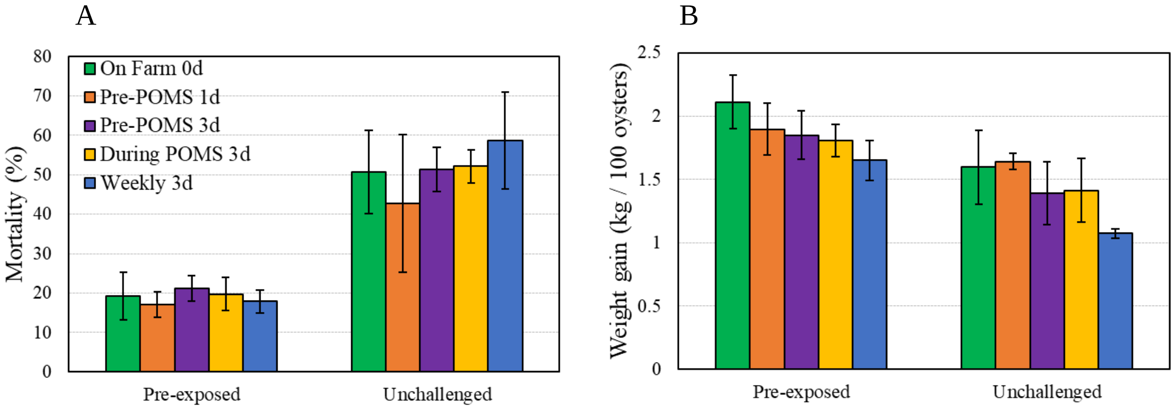

Figure 19: A. Percentage mortality of pre-exposed and unchallenged and pre-exposed Pacific Oysters across chilling treatments. B. Weight gain in chilled oysters.

Source of the Virus (Feral Pacific Oyster Survey)

The survey of feral Pacific Oyster reefs in Pipe Clay showed that the two main reefs close to oyster farms had high densities of live oysters (average 152 oysters m^{-2}), and an average of 39 dead oysters m^{-2} (*Figure 20*). Over 80% of these oysters were extremely large (> 80 mm), with a small percentage < 60 mm (*Figure 21*). Site 1 oysters that were clustered along an artificial seawall differed with a lower density of 37 live oysters m^{-2} (32 dead oysters m^{-2}), and a higher proportion of smaller oysters with 39 % of oysters < 60 mm and 12 % of oysters > 80 mm (*Figure 21*).

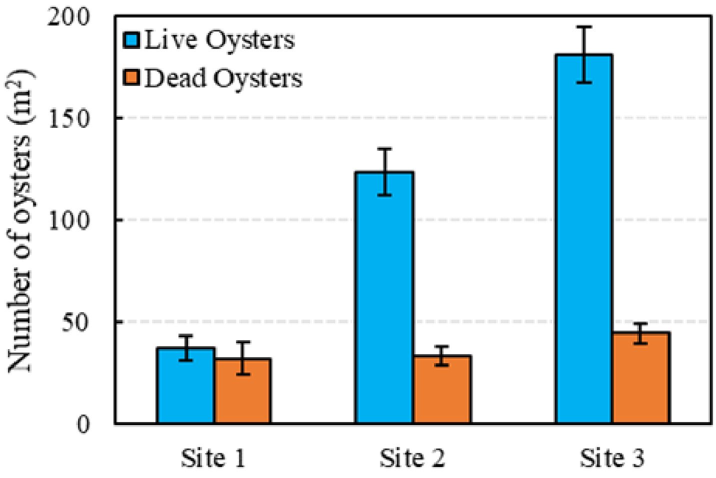

Figure 20: Average number of live and dead Pacific Oysters (m^{-2}) across the three sampled feral Pacific Oyster reefs at Pipe Clay Lagoon.

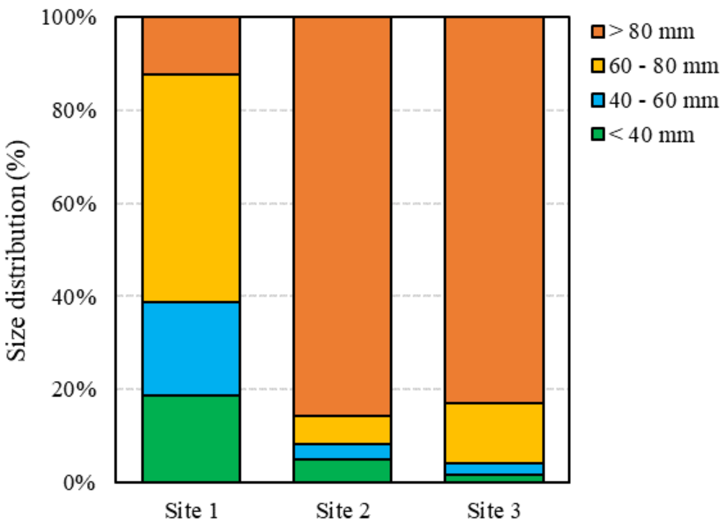

Figure 21: Average size distribution of Pacific Oysters expressed as a percentage of the total abundance across the three sampled feral Pacific Oyster reefs at Pipe Clay Lagoon.

A higher percentage of feral Pacific Oysters had positive and indeterminate qPCR results than farmed oysters across the 2018/19 summer, peaking at 25 % in January for feral oysters, and 8% for farmed oysters in February (*Figure 22*). Over the course of the trial, feral oysters at Site 3 (6 % of oyster tested positive for OsHV-1) had less than half the number of positive samples at feral oysters at Site 1 (16 % tested positive for OsHV-1) and Site 2 (14 % tested positive for OsHV-1), whereas all farmed sites had similar numbers of positive OsHV-1 samples.

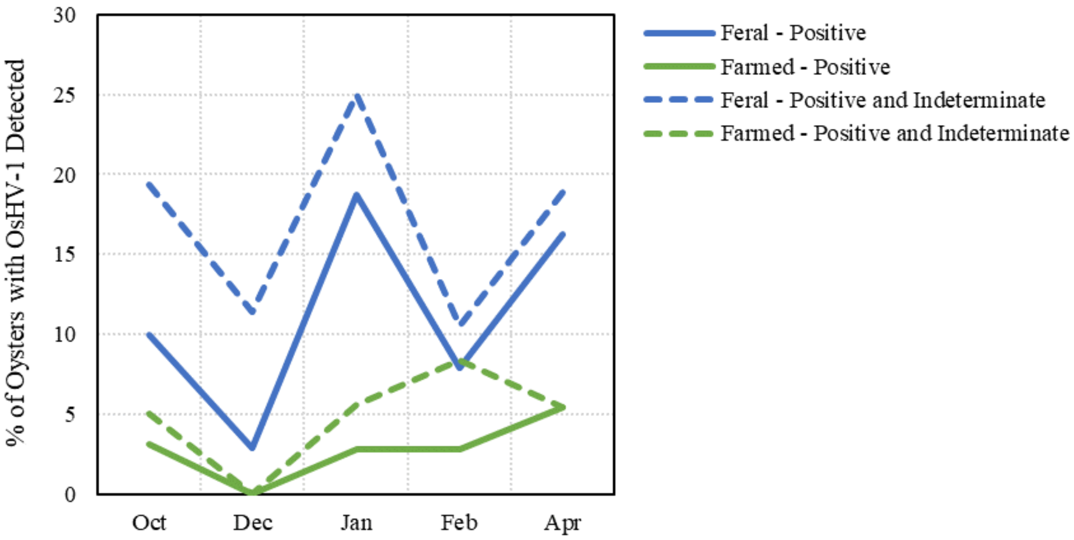

Figure 22: Percentage of the total number of farmed and feral Pacific Oysters that returned positive or indeterminant qPCR test results for OsHV-1. Pipe Clay Lagoon, Tasmania 2018-2019.

Objective 4: Farmer Surveys

Farmer Survey 1 on the effects of POMS on Pacific Oyster farmers 2016/2017

The following manuscript has been published:

Ugalde SC, Preston J, Ogier E, Crawford C (2018). Analysis of farm management strategies following herpesvirus (OsHV-1) disease outbreaks in Pacific Oysters in Tasmania, Australia. Aquaculture 495, 179 – 186.

Abstract:

The microvariant genotype of Ostreid herpesvirus 1 (OsHV-1 µVar) has severely disrupted oyster production in Europe, New Zealand, and Australia by causing repeated and seasonal outbreaks of mass mortality in Pacific Oysters (*Magallana gigas*). The virus was first detected in Tasmania, Australia, in January 2016, and mortalities of up to 87% were reported (de Kantzow et al. 2017). This study surveyed 95% of Tasmanian oyster farmers in OsHV-1 infected growing areas one year following initial detection, and recorded mortalities and associated farm management strategies in the 2016/2017 season, compared with the initial outbreak and before OsHV-1 occurrence. The survey was comprised of 37 open- and closed-ended questions, with data collected on background information, mortalities, environmental, genetic, and husbandry information. Perceived business viability was overall strong (75%), with changes to farm management occurring on 88% of leases in response to the virus. Commercial oyster farming businesses ranked handling regimes and stocking densities as the most important husbandry factors for influencing mortalities. Water temperature was ranked as the most important environmental factor, with 60% of businesses considering mean water temperature of 18 – < 20 °C sufficient to activate disease. Mortalities for oyster size classes across multiple years are also reported. This survey has provided an expedient and cost-effective method to obtain information on the impact of a highly virulent disease and associated environmental conditions across an industry. These results will inform future management strategies and associated research.

For publication, see Appendix 1

For a full list of survey questions, see Appendix 2

Farmer Survey 2: A Survey of Changes to Oyster Farming in Tasmania since the OsHV-1 Outbreak in January 2016- (Appendix 3)

The survey was conducted in April - July 2019 with 17 companies participating. This is lower than the number of businesses that completed the survey in 2017 because of company mergers. The majority of farmers answered all questions.

Section 1: POMS Mortality and Oyster Production

Overall, farmers have experienced a reduction in Pacific Oyster mortality from an average of 67% in 2016 to 9% in 2018/2019 (*Table 5*).

Table 5: Pacific Oyster mortality recorded by farmers.

% Mortality	**2016**	**2016/17**	**2017/18**	**2018/19**
Average	67	37	23	9
Minimum	15	10	1	0
Maximum	95	75	56	30

A majority (80%) of the of businesses surveyed experienced lower production in 2018-19 than pre-POMS. Production levels were reduced on average by 37% (minimum 14%, maximum 65%). Two businesses reported increased production and one business stated their production was the same as pre-POMS levels. Of the twelve businesses that reported lower production, 10 are aiming to get production back to pre-POMS levels; most by 2020. These businesses reported that since POMS they have not been able to purchase the required quantity of spat, and they are only now back to being able to purchase the number of stock at the larger size that they prefer.

Section 2: Oyster Farm Operations

All farmers changed their farm management in response to POMS (*Figure 23*). Most farmers reported a reduction in Pacific Oyster handling during the POMS season, an increase in percentage of stock selectively bred for POMS disease resistance, sold a higher percentage of stock before POMS season, and generally farmed lower densities of stock, largely because there was not the stock available for purchase (*Figure* 24).

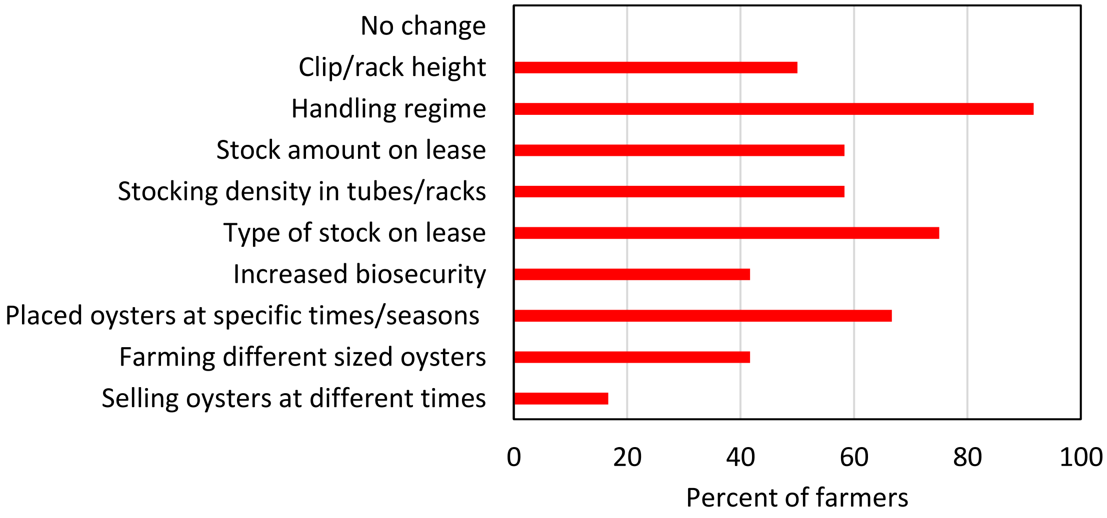

Figure 23: How did you vary your farm management in response to POMS?

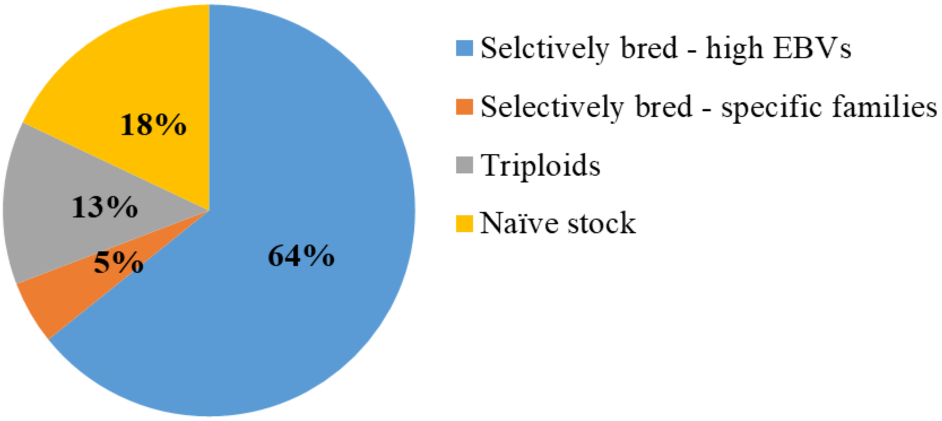

Figure 24: What percentage of each type of stock do you farm?

Most farmers (73%) said they bought Pacific Oyster spat at different times during 2018/19 compared to pre-POMS, including not buying spat during ‘risky’ summer months. After POMS hit many farmers bought smaller spat than pre-POMS because larger stock was not available, but several businesses reported that they are now returning to normal stocking patterns, i.e. purchasing larger spat and rearing at similar stocking levels to pre-POMS.

Farmers sold ‘matures’ at different sizes or times compared to pre-POMS (64%). Some farmers mentioned that their businesses have now recovered from the initial effects of the POMS outbreak and have returned to a business as usual scenario. Half of farmers also said they continue to move stock between farms or growing areas similar to pre-POMS.

Regarding employment levels, 33% of businesses responded that their level of employment was less and 33% that their employment was greater than pre-POMS. Although these percentages indicate some recovery in the level of employment post POMS, it is difficult to attribute these numbers to actual employment recovery as the industry has experienced consolidation through business acquisitions and market restructure.

Companies noted that they have not been able to produce the same quantity of oysters as pre-POMS so they have exported less stock, and consolidated their Australian markets. Some have increased the number of oysters sold through direct retail sales. The increase in price was welcomed by farmers as it counteracted lost income from reduced production. Many farmers commented that they are uncertain about retaining the increased price as production from South Australia rises over the next couple of years (*Figure 25*). 77% of farmers stated they do not sell to different markets. Businesses that have sold to different markets have stated they now sell more ‘matures’ to domestic markets because of the limited stock available.

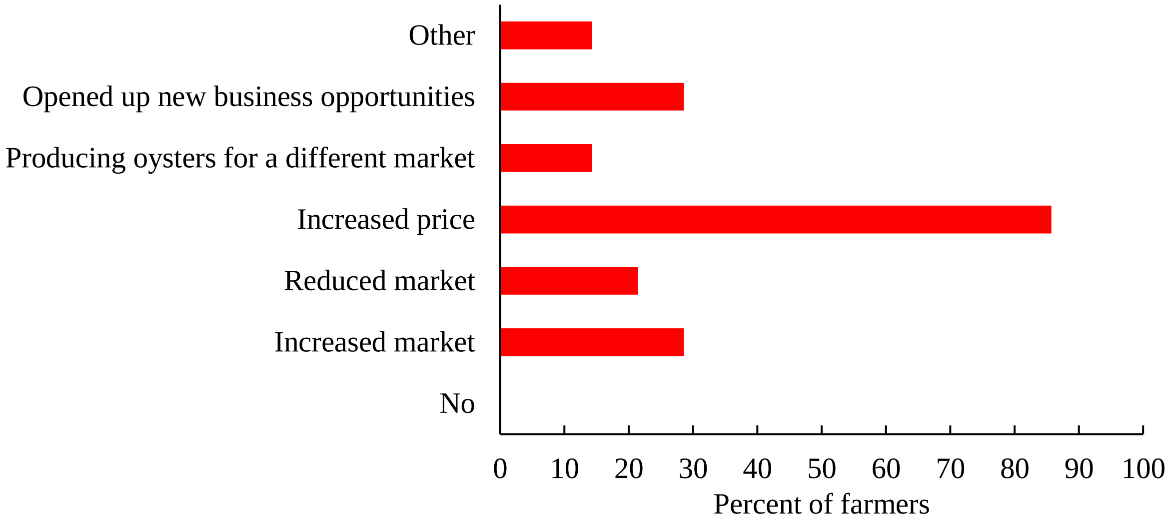

Figure 25: Has POMS affected your marketing of Pacific Oysters?

Section 3: Environmental Information

Water temperature was clearly considered by farmers to be the major driver of POMS outbreaks, followed by air temperature and hydrology, tides and water movements (*Figure* 26).

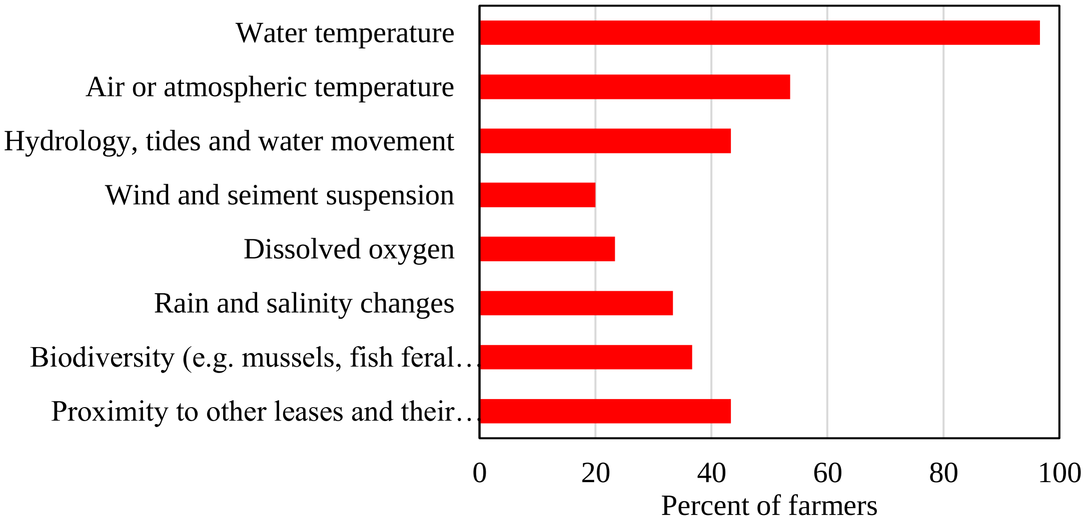

Figure 26: In general, which environmental factors listed below do you think are important to POMS outbreaks on your leases?

On average, companies considered a temperature of 19 (± 0.1) ° C for a duration of 9 (± 1.9) days was required for a POMS outbreak. One business considered this temperature to be as low as 12 °C and another business as high as 23 °C for a single day. In addition to watching water temperature, 80% of businesses also consider weather and/or tidal patterns are important during the POMS season.

67% of farmers said they did not observe any pattern in Pacific Oyster mortality across their lease of growing area. Of the remaining that reported noticing patterns, several companies noted that mortality was concentrated in spat and small juveniles, and one company reported that mortalities commenced at the back of the bay in the warmer shallower water, and progressively moved out across the bay.

77% of farmers thought that the biomass of shellfish influences POMS outbreaks. Farmer comments included that high biomass led to greater infection of naïve stock, or conversely, led to removal of particulate matter. In relation to feral Pacific Oysters, 71% of farmers stated there were large populations of feral in their growing area in 2019, and of these 81% were unsure if the feral populations were affected by POMS, and 19% reported no POMS in ferals. Some farmers responded that the feral populations did have POMS in previous years, but they have not been checked recently.

Section 4: Farm Management

40% of business operators rated the overall effect of POMs as being a major negative experience, compared with 33% and 27% rating it as being a minor negative and major positive experience, respectively. Farmers that rated the overall effect of POMS as major positive also stated the impact was initially devastating, emotionally and financially, and would have initially rated the impact as a major negative. However, at the end of this fourth summer of POMS, 86% of businesses rated the viability of their oyster operation as strong and 14% rated their viability as medium.

This generally positive response to business viability is partly due to improved farm efficiently; 92% of those surveyed stated their business was now more efficient. Many farmers commented that due to severe financial pressure, they had to become better organised, with more efficient time management to reduce costs of operation. Several farmers also noted that this increased efficiency has been a positive outcome from the POMS disease infestation. Increased efficiencies have occurred through lower stock numbers and less handling allowing for a reorganisation of staff time and effort.

In addition, many businesses have changed their business structure (54% of farmers), including the sale of mature stock, more POMS resistant stock, merger, expansion and purchase of businesses, more seasonal than permanent staff, retail investment and more emphasis on sales to the tourism trade. However, most businesses (73%) are not considering diversification. The remainder were considering farming other shellfish and/or selling to new markets.

Almost half of farmers (44%) do not expect major POMS related mortalities to occur in the future, whilst 38% are unsure and 18% anticipate higher mortalities. Comments included that another couple of years of selective Pacific Oyster breeding is required before they are confident that major mortalities will not occur.

Surveyed farmers were also asked what areas of research, if any, would benefit their operation in the future. The two major areas of research were harmful algal blooms, including depuration, and the continuation of selective breeding research. Although research in farm management influencing OsHV-1-associated mortalities and impacts was not generally mentioned, 79% of farmers rated this research as having had a high impact. Reasons given included greater communication of results through newsletters and workshops, and personal interactions between researchers and oyster growers. Three farmers rated the research impact as medium, stating the relevance of the work is now not as high as when POMS first occurred, and that some work was inconclusive, such as the period of infection study.

Farmer Survey 2: 2018/19, Uninfected Growing Areas (Appendix 4)

Five oyster growers from POMS-free growing areas around Smithton, north west Tasmania and one from St Helens, east Tasmania were surveyed in May-June 2019.

All farmers have changed the type of stock on their farms with a significant increase in the number of disease resistant oysters (*Figure 27*). Many have been purchasing stock at a smaller size of 2 mm as that is what has been available from hatcheries. Farmers commented that they have handled stock more gently, reduced their time out of water, graded less often, managed biofouling and conditioning by managing growing heights, and have placed greater attention on temperature management, particularly in the warmer months of January to April.

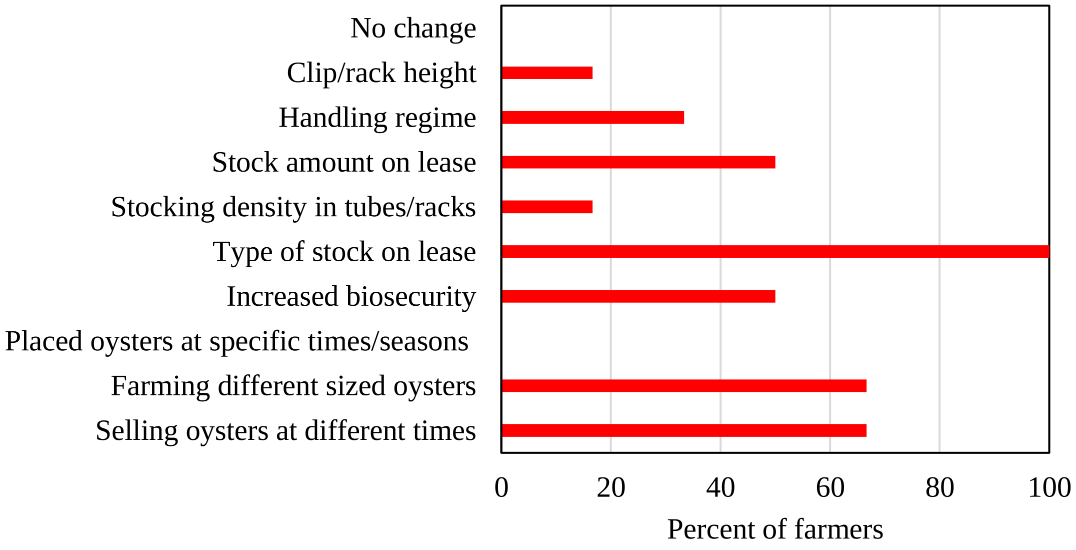

Figure 27: How have you changed your farm management practices from pre-POMS?

All farmers surveyed have moved away from naïve Pacific Oyster stock and 88% are farming selectively bred oysters and the remaining 12% farming triploids. All companies have been buying spat at different times of the year and at a smaller size compared to pre-POMS due to supply issues. Further, three farmers have been selling ‘matures’ at different time/sizes. One farmer did not respond as they do not sell ‘matures’ and another company said their mature sales have not changed and continue to be governed by food safety quality, river flows and rainfall.

In contrast to the responses from business in infected areas, four businesses in uninfected areas were very concerned and two business were concerned about the impact of POMS on their farm in the future. However, the majority of farmers rated the viability of their business as being strong. All six business have increased their biosecurity. These include all stock movements being traced and recorded, and stock only being sourced from bio-secure hatcheries and disease-free regions of Tasmania. Furthermore, 2/3 of the business are considering diversification to farm alternate species and increasing retail opportunities. All businesses would like to see continued selective breeding and further research on species diversification. Three businesses would like to see more research on harmful algal blooms and biotoxins. One business would like to see additional POMS farm management research.

8. Discussion

Mortality events resulting from an OsHV-1 disease outbreak in Tasmania in January-February 2016 have had a major impact on the Pacific Oyster aquaculture industry across southern Australia. Four growing areas, representing 60% of the Tasmanian oyster production initially experienced massive mortalities, including the two major hatcheries that supplied approximately 90% of Pacific Oyster spat to SA and NSW. Our research related to improved farm management practices to reduce OsHV-1-associated mortality and overall impact has worked in conjunction with research to selectively breed oysters for POMS disease resistance. The overall result is a major turnaround of the Pacific Oyster aquaculture industry in Tasmania from disaster and despair to a very positive outlook for the future in less than four years.

Objective 1: Periodicity of Infection

In common with all previous studies on OsHV-1, warm water temperatures are clearly required for disease outbreaks to occur. Our research with oyster growers in Tasmania shows that average water temperatures of 19 °C for over a week indicate a risk of mortalities occurring, and above 20 °C the risk of mortality from POMS is very high. Some farmers reported that average temperatures were important, whereas others maintained that minimum daily temperatures above around 18 °C were required. Oyster growers also reported mortalities as late as mid-end of March when water temperatures were starting to drop to 17-18 °C. These temperatures for OsHV-1 disease events are similar to those recorded for POMS outbreaks in NSW where mortalities commenced when the mean water temperature rose above approximately 20 °C (Whittington et al. in press). However, as discussed by Whittington et al. (in press), these temperatures are some 4-5 °C warmer than the temperatures required to stimulate disease outbreaks in European countries, but reasons for these differences are unknown.

Taking into account these records of mortalities from the sentinel oysters and farmer observations of mortalities, the period of risk for POMS disease outbreaks in south-eastern Tasmania ranges from mid-November to late-March, with the highest risk commencing in mid-December in most years. However, in November 2017 when an exceptionally warm water temperature event occurred due to the more southerly extension of the East Australian current of eastern Tasmania, the high-risk period commenced earlier than other years, with oyster farmers at Pitt Water and Pipe Clay reporting mass mortalities in mid-late November. This seasonal period of POMS susceptibility from mid-November to late March in Tasmania is slightly less than that noted by Whittington et al. (in press) in NSW where mortalities were mostly widespread and frequent between December and April, although they could occur as early as late October or as late as May. This is to be expected as water temperatures are warmer for a greater period of the year in NSW than in Tasmania.

Relating temperature data to POMS mortalities in 2016/7 and 2017/18 at Pitt Water and Pipe Clay Lagoon, a model was developed which calculated the risk of mortality occurring across the annual average range of temperatures. Pitt Water had a slightly higher probability of mortality at a given temperature than Pipe Clay Lagoon, which is in accordance with the higher average daily temperatures regularly recorded at this site than at Pipe Clay. This model can be used to predict the likelihood of a disease outbreak for a given temperature at each of these growing areas.

In our sentinel oyster spat the mortalities we observed were not always consistent with the prevalence of the OsHV-1 virus from qPCR analysis of these spat, or with qPCR analysis of oysters taken from the same growing area by BT. For example, oysters sampled by BT in 2016/17 showed that OsHV-1 was present weeks earlier than shown by the qPCR results from sentinel spat at Blackman Bay, Upper Pittwater and Island Inlet. The results from Biosecurity Tas. also showed that the virus was present at Little Swanport, whereas we did not have any positive records from this site. In 2016/17 we kept the spat continually immersed in floating cylinders so that the oysters could continually filter and be exposed to higher viral loads. However, because of discrepancies in results between farmer observed mortalities and mortalities in sentinel spat, the oyster growers were concerned that the spat in floating containers were not representative of their farmed spat on racks. Consequently, in 2017/18 we held sentinel spat in cylinders both floating and attached to racks next to commercially grown oysters. Again, OsHV-1 results from the racks were not always consistent with results from BT and farmer observations. For example, in November 2017 sentinel spat on racks at Pipe Clay Lagoon did not test positive for OsHV-1, even though we counted high mortality at all sites, BT's samples were positive at this time and high mortalities were recorded by farmers across the growing area. Conversely, at Pittwater our results from one site and those from BT were positive for OsHV-1in late November and farmers recorded mass mortality, but we had very low mortality in our sentinel spat.

Although we do not have positive identification of OsHV-1 presence for all farmer observations of mortality, we have a high level of confidence that the farmers were able to identify mass mortalities due to OsHV-1 from other causes. They reported 'dozy' oysters which struggled to remain shut when disturbed just before mass mortality occurred, followed by rapid death and a distinctive smell of the dead oysters on the farms. These conditions had not been observed prior to the initial detection of POMS in Tasmania.

Additionally, in 2017/18 our samples were analysed for OsHV-1 concentration by the same laboratory as BT, at the Tasmanian Government Animal Health Laboratories and methods of preparation of samples were similar, so analytical methods are unlikely to have affected the results.

The inconsistencies in results between sentinel spat, farmer observations and BT samples possibly occurred because our sentinel oysters were small (2240s) and they were taken directly from a sheltered hatchery environment to farm sites where they were exposed to winds, tides and currents. The stress of handling and tougher environmental conditions may have impacted on spat behaviour, such as filtration rates. Also, our sentinel spat had been selectively bred for resistance to OsHV-1, whereas some of the farmer observations were for naïve spat and triploids with minimal selective breeding. As we sampled approximately every two weeks, we may have missed major mortality events, leaving only dead shells or live oysters which avoided/survived the virus. The results also show that the prevalence rate for the virus was low, and decreased from 2016/17 to 2018/19, requiring large sample numbers for accurate assessment. Because of the cost of analysis at registered laboratories, we were restricted in the number of samples that could be analysed.

Whittington et al. (in press) conducted more intensive sampling with greater replication of qPCR testing in two NSW estuaries and concluded use of sentinel oysters for surveillance for OsHV-1 was only effective at an estuary wide scale and required an intensive sampling program. Exposure to OsHV-1 at small scale (within farms, meters) and medium scale (between sites within an estuary, kilometres) was affected by clustering, making systematic monitoring unreliable. This clustering of oysters infected with OsHV-1 was considered by

Whittington et al. (in press) to be consistent with OsHV-1 being a water borne infection with indirect transmission via particles in the water column, and consequently subjected to local hydrodynamic patterns and biological influences. However, as farming ceased in the POMS infected estuaries in NSW after the OsHV-1 outbreaks in 2010 and 2013, Whittington et al. (in press) did not have farmer records or observations of mortality events to compare with results from their sentinel oysters.

The first mass mortality event each year occurred at Pittwater, a shallow estuary with large intertidal areas that rapidly increases in temperature, especially in upper Pittwater where there is less flushing and high evaporation compared to other estuaries because of natural and artificial constraints to water movement. Mortalities were also higher in this estuary and in Pipe Clay Lagoon, a small marine embayment with a larger proportion of the area occupied by Pacific oyster aquaculture, than at Blackman Bay and Little Swanport. Our results suggest that the most susceptible growing areas to POMS are where water temperatures rise quickly on shallow sand/mud flats and in upper reaches of estuaries where exchange with cooler oceanic waters is reduced. The density of oysters in the water body is also likely to be important. Differences in timing and severity of disease outbreaks have been observed between other Pacific oyster growing areas, for example between Georges River and Hawkesbury River in NSW, but without any apparent reasons (Whittington et al. in press), and between oyster growing estuaries in New Zealand (Vince Syddall, pers. comm. 2017).

Although warmer summer water temperatures are clearly required for disease outbreaks, there is not a direct relationship between temperature and mortality, and mortality events have been observed in mid-March when temperatures have dropped to 17-18 °C, which suggests that factors additional to OsHV-1 are involved. This is consistent with studies elsewhere that have indicated that mass mortality events are more complex than just OsHV-1 being the causative agent. The influence of bacterial communities is increasingly being considered to be important (Petton et al. 2015, Dégremont et al. 2019, de Lorgeil et al. 2018, King et al. 2019). For example, King et al. (2019) found that the microbes of oyster families susceptible to OsHV-1 were significantly different to those of disease-resistant families. The microbiome of oysters infected with OsHV-1 µvar is being investigated in the Future Oysters CRC by the University of Technology Sydney and we are co-partnering with this research by providing OsHV-1 infected oysters from Tasmania to better understand the causes of mortality in Tasmania.

Within each growing area, oyster mortalities clearly differed between sites on many sampling occasions, as shown in *Figure 28*, and especially when large mortalities occurred. For example, in 2016/17 mortality levels were often very different between sites that were only 10-20 m apart. Many Tasmanian oyster farmers also reported no obvious spatial patterns in mortalities across their farms. High variability in the occurrence of OsHV-1 at low spatial scale, both within an oyster farm (10 m scale) and across a growing area (km scale), has been widely reported (Pernet et al. 2018, Whittington et al. 2018, in press). Water movements around a lease are likely to be very important in determining where the virus will be effective, however, these water patterns are affected by many factors, including wind, current, tides and oyster farming infrastructure

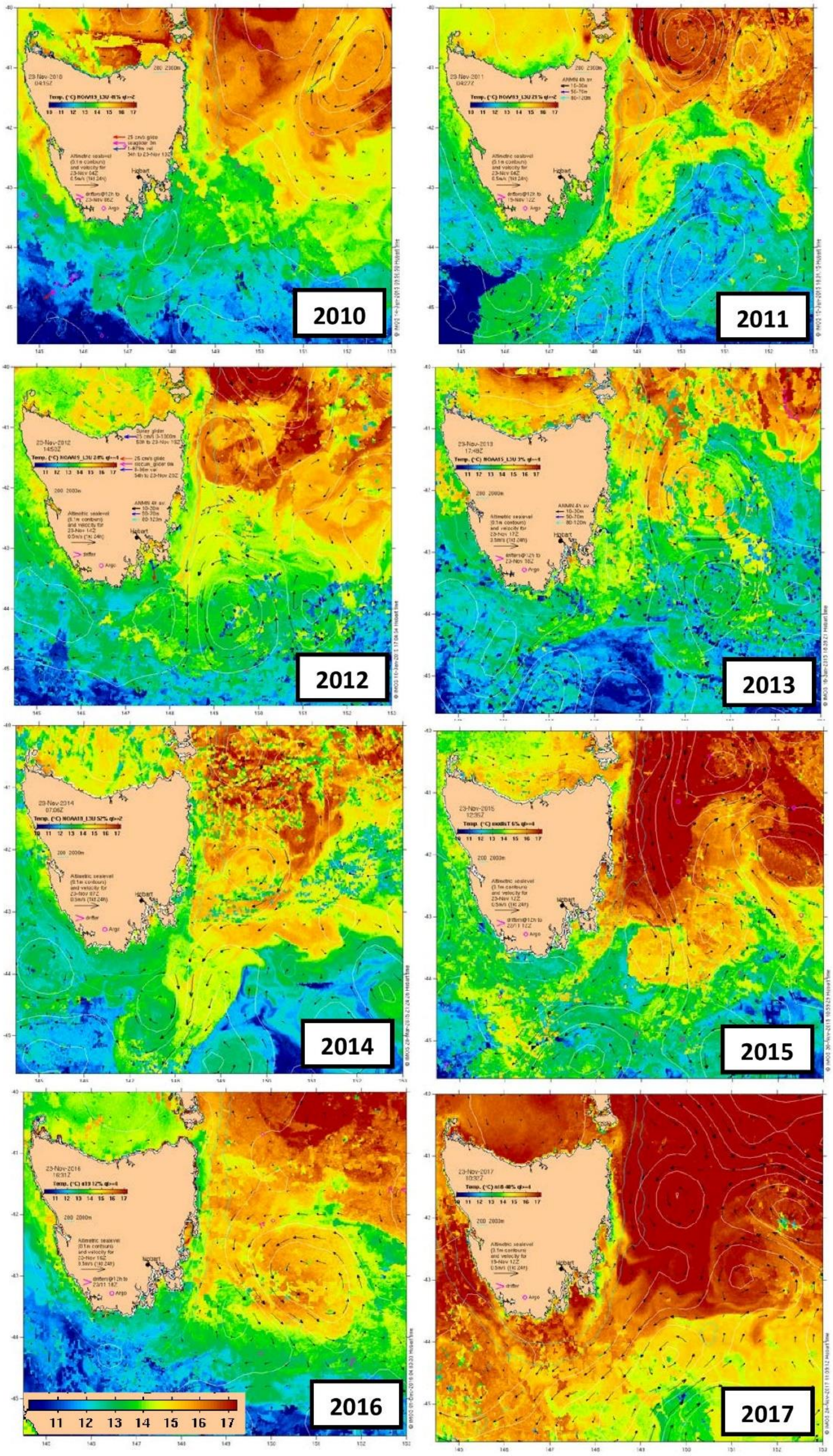

Figure 28: Sea surface temperature adjacent to Tasmania for 23rd November 2010 to 2017. Data sourced from the Integrated Marine Observing System data portal (http://oceancurrent.imos.org.au/)

Objective 2: Farm Husbandry and Handling Protocols

After the first POMS outbreak in southern Tasmania, many impacted farmers stopped handling their oysters, that is grading and cleaning the tubes, once the temperature rose to around 18 °C until temperatures started to decline in March. They believed that handling the oysters, especially mechanical grading and leaving them onshore overnight, stressed the oysters and made them more vulnerable to disease. This was supported by a study of risk factors for mortality during the first POMS outbreak in Tasmania by de Kantzow et al. (2017), which concluded that handling oysters in the week prior to the disease outbreak resulted in higher mortality and should be avoided during high risk periods. However, this practice often resulted in slower growth, poor condition of market sized oysters and the cumulative effect on mortality over the summer period was unknown. Our research showed that at Pitt Water and Pipe Clay Lagoon the high mortalities were not significantly different between hard mechanical grading and no handling. At Pitt Water many of the no handling oysters and tubes were smothered by biofouling, especially the tunicate ‘cunjevoi’. Most oyster farmers in the POMS infected areas resumed handling in the 2018/19 POMS season, but at a lower frequency that before POMS. Many farmers also commented that they treat the oysters more carefully, they avoid keeping them out of water for long periods and avoid handling them during hot windy weather (Farmer surveys 2019).

The relationships between oyster age and size and OsHV-1 mortality have been investigated in several studies in Europe and Australia, with mixed results and a recommendation for further studies on the effects of age and size and associated factors, such as family lines and oyster physiological state (Azéma et al. 2017, Hick et al. 2018, Rodgers et at., 2018).

Our trial with oyster juveniles of the same genetics but graded into different sizes by on-farm screening for several age groups showed that oyster age and size independently affected mortalities, with mortality increasing with size for oysters of the same age, that is, the faster growers (front runners) had higher mortality. However, in NSW larger oysters had significantly lower cumulative mortality than smaller ones within two age groups (spat and adults; Hick et al. 2018.) Similarly, Azéma et al. (2017) found that smaller oysters had a higher susceptibility to OsHV-1 than larger oysters when deployed at 15 months of age on farms in north-west France. They posit that OsHV-1 may actively use the host’s cellular mechanism for replication, and thus the smaller oysters which had twice the growth rate of the larger oysters during the first year of the experiment more quickly reached the viral load required for mortality to occur. This could also be the reason for our front runners having higher mortality if OsHV-1 is using the fast growing oysters’ cellular system to replicate itself.

As the commercial price for oysters in Tasmania is primarily based on screen mesh size that the oysters are retained on, that is length rather than age, and as the price increases quickly with size, but changes in mortality are relatively slower between the size groups, this suggest that it is more cost effective to purchase smaller rather than larger oysters. This supports the farm management strategy adopted by several farmers to challenge oysters at a small size to remove the individuals most susceptible to POMS, and only expend time and resources on culturing oysters that have higher chance of surviving future POMS exposure.

We also detected that older oysters were generally less susceptible to OsHV-1 than younger ones, for oysters of the same size. Hick et al. (2018) manipulated the growth of oysters using clip heights to change immersion times to produce oysters of the same age but different sizes and found that the cumulative mortality was higher in both the small and large size classes of oyster spat than in adults. Thus, age of the oysters was important for survival, and size alone did not provide protection from mortalities due to OsHV-1.

Although our original objective included investigating differences in survival between Pacific Oysters grown intertidally to subtidal culture, we were not able to do this because no large subtidal farming operations were impacted by POMS. Interestingly, oyster meat samples collected from a subtidal farm in Great Oyster Bay in December 2015 as part of the Tasmanian Shellfish Market Access Program were subsequently shown to hold a low level of the virus, but mortalities did not occur, and retesting could not confirm the result as a true positive. The origin of the virus in this area has never been confirmed, but tracing by BT suggested it may have been transferred in oyster movements from infected areas (Kevin Ellard, per. comm. July 2019).

Similarly, a direct comparison of the effects of POMS in high water flow areas compared with low flow was not conducted due to a lack of suitable sites. However, as discussed above, growing areas with relatively high current flow, such as Blackman Bay with a current of up to 8 knots in the channel and full water exchange on every tidal cycle (de Kantzow et al. 2016) have had an increasingly low level of mortality compared with Pittwater where water flow is restricted, especially in the upper reaches of the estuary. However, reasons for the higher overall mortality in Blackman Bay than Pitt Water in the initial POMS outbreak, observed by de Kantzow et al. (2017) are unknown. Oyster growers in Pipe Clay Lagoon have also observed that mortalities start first at the upper section of the bay where it is shallower, water is warmer in summer, and flow rate is less.

Oyster density, and thus host availability and concentration of the virus in the water has also been found to be a factor affecting POMS disease episodes (Pernet et al. 2018, Petton et al. 2015). The odds of disease mortality were found by Petton et al. (2015) to increase with increasing biomass of neighbouring infected oysters and markedly decrease with water renewal, under controlled conditions, which they related to the dilution and concentration effects of viral particles. Oysters cemented to ropes *in situ* at low densities had much lower mortalities than oysters in baskets at approximately six times the stocking density, which was attributed to increased flushing rate (Pernet et al. 2012). Several studies have suggested that OsHV-1 disease outbreaks are more likely in inshore lagoonal or estuarine areas where water movements are generally slower than open coastal waters such as the Mediterranean Sea (Pernet et al. 2018, Petton et al. 2015, Rodgers et al. 2018). The high density of both farmed and feral oysters in the water body at Pipe Clay Lagoon may be contributing to the higher disease risk in this growing area.

Objective 3: Chilling and OsHV-1 Source

We investigated the effect of chilling during a POMS outbreak on Pacific Oyster mortalities because of interest in this method from several oyster growers. Although chilling spat for various times did not increase survival during a POMS disease event, several oyster growers still consider that chilling market sized oysters at the first signs of a POMS outbreak is cost-effective because of higher survival in the chiller than on the farm. The chilling process presumably slows oyster metabolism and viral development. Most of the oysters in the chiller have been observed to survive for several weeks and can be progressively marketed over this time. Pernet et al. (2015) also found that low water temperature treatments, albeit only as low as 10 and 13 °C, were not a viable option for reducing oyster seed mortalities. They concluded that OsHV-1 persists in oysters at low temperatures as high levels of mortality occurred in the cold-acclimated seed when the temperature was suddenly raised to 21 °C.

The pattern of occurrence of the OsHV-1 virus in south-eastern Tasmania strongly suggests that this virus is transported across large distances (10-1000's of km) by live oysters, most likely as biofouling on ships or possibly by the movement of edible oysters. OsHV-1 suddenly appeared in southern Tasmania in January 2016, presumably coming from POMS infected areas in NSW, but bypassed oyster growing estuaries in southern NSW and north-eastern Tasmania. Dead and dying oysters were first observed in Tasmania on an oyster farm in Iron Inlet (lower Pitt Water) which is adjacent to the River Derwent. Subsequent testing of oysters from the Derwent in early February 2016 showed that the virus was also present there. The oyster grower who reported the first mass oyster mortality had distributed juvenile oysters from his farm to grow-out areas at Pipe Clay Lagoon, Blackman Bay and Little Swanport only a short time, hours to days, prior to the major mortality event and these areas all became infected with POMS, and are still the only major growing areas impacted by this virus in Tasmania. After the first POMS disease outbreak was identified in Tasmania, BT immediately imposed a ban on the movement of oysters from infected areas and have continued to manage oyster movements around the State to avoid disease transmission. As noted by Whittington et al. (2018), all occurrences of POMS in Australia have occurred in close proximity to the major ports of Sydney, Hobart and Adelaide, suggesting that biofouling is the likely means of transferal. This is further supported by OsHV-1 virus being identified in oyster biofouling on a commercial ship in Port Adelaide, South Australia in 2018 (Whittington et al. 2018).

It was initially predicted that POMS would rapidly spread around south-eastern Tasmania, especially to the D'Entrecasteaux Channel which converges with the River Derwent at the estuary mouth (Figure 1) and has an almost contiguous, and in many areas very dense, population of feral Pacific Oysters in the intertidal zone. There are around 20 oyster leases in this growing area. However, this spread has not occurred over the four summers since POMS was first detected. The only additional growing area that was confirmed as OsHV-1 positive was a small isolated farm at Gardners Bay, Port Cygnet in the Channel and the most likely means of transfer of the virus was either from live oysters as it is a popular mooring site for yachts or transfer on oyster farming equipment from an infected site.

The survey of feral Pacific Oysters in Pipe Clay Lagoon showed that these beds contained a high density of live extra-large oysters (>81 mm), many of which presumably survived the first major POMS outbreak in 2016. Recruitment has been low, but it is unknown whether this is due to POMS mortality or environmental conditions that have not been conducive to oyster spawning and spat settlement. As OsHV-1 virus was detected in a relatively high percentage of feral oysters over the summer months, this suggests that these oysters could be a host and reservoir for OsHV-1, especially as they have survived the virus and can live for up to 30 years. Thus, populations of feral Pacific oysters may need to be managed to reduce the risk of POMS. Fewer farmed oysters were infected with the virus, presumably because the farmed population is younger and at lower density. However, Evans et al. (2017) considered that Pacific Oysters are not a likely reservoir host for the OsHV-1virus because prevalence of the virus and viral loads were consistently low in stock that had been pre-exposed to the virus.

OsHV-1 DNA has also been detected in oysters during the colder winter months in southern Tasmania, albeit at low prevalence and low concentration, suggesting that subclinical infections occur throughout the year. Whittington et al. (in press) also observed subclinical infections in NSW from early October to late June, and several studies in Europe have reported low prevalence and low concentrations of the virus in farmed Pacific Oysters in cooler water temperatures over winter (Pernet et al. 2015, 2018, Petton et al. 2015). However, hosts for the virus, carriers and reservoirs where it is maintained during the colder winter months has not been fully elucidated (Pernet et al. 2016). The OsHV-1 virus has been found in other bivalves and invertebrates in NSW estuaries (Evans et al. 2017), and a review by Rodgers et al. (2018) reported a wide range of bivalve host species. Infection of OsHV-1 µVar was recently reported in the widely distributed European shore crab, *Carcinus maenas* (Bookelaar et al. 2018), and laboratory trials showed the virus could be transmitted from crabs to naïve oysters. Bookelaar et al. (2018) suggest that the virus can sustain itself in the ecosystem outside the host species for periods of time.

Objective 4: Farmer Surveys

Although the surveys of oyster farmers in Tasmania were not originally planned as part of the project, they have provided valuable information on farmer observations and opinions across the industry, and on the socio-economic impact of POMS.

Observations and records on environmental factors impacting on POMS outbreaks provided by the oyster growers in the surveys generally agreed with our research results and informed additional research. The surveys also provided information on new management practices that were implemented by the farmers to reduce the impact of POMS, including buying selectively bred stock for disease resistance, selling as many oysters as possible before the temperature increase over summer, rearing juveniles in POMS free areas during the warmer summer months, not purchasing spat over the summer months, and not handling oysters during the POMS season.

Farmer observations of average percentage dead oysters each year decreased from 70-90% in 2016 to 5-20% in 2018/19. During this time the number of oyster growers farming stock selectively bred for disease resistance increased and the Estimated Breeding Value (EBV) also increased from 40% to 80%, that is the predicted survival for one year old spat increased from 40-80%. Whittington et al. (in press) also observed declining mortalities and viral load over their study period from 2012-13 to 2016-17 in NSW, and they discuss the three most likely reasons for this as i) natural attenuation of the virus, ii) a reduction in the available dose of OsHV-1, and iii) increased resistance in sentinel spat over time. For the POMS disease outbreaks in Tasmania, it is unlikely that the natural attenuation would occur as quickly as over three years, and as shown by our study of hosts for the virus in Pipe Clay Lagoon, there were numerous farmed and feral Pacific Oysters available which were positive for OsHV-1, so a reduction in available dose is unlikely. The sentinel spat used in our study were bred each year for increasing disease resistance, and although the EBVs, which increased each year were estimates for survival of juveniles at 12 months of age, it is expected that the spat used in this study were increasing in resistance each year. Consequently, the spat used in our study are highly likely to have increased resistance to those used by Whittington et al. (in press), especially as they used mainly triploids which had less selective breeding for resistance than diploids (Matt Cunningham, pers. comm. 2019).

Important results from the surveys related to socio-economic aspects. These included 75% of oyster companies in 2017 rating their businesses as strongly viable, even though the industry still appeared the be severely impacted by POMS. This increased to 86% in 2019 and most farmers rated their operations in 2019 as more efficient than pre-POMS. Many farmers found it difficult to rate the effect of POMS on their farming business because initially it was a major negative both financially and emotionally, but by 2019 was generally considered to have a positive impact because of greater efficiencies that were enforced on farmers and also because of increased prices in the market place.

Many of the farmers surveyed in 2019 thought that they would be back to pre-POMS production levels by 2020, which is a relatively short period of time for recovery from a major disease outbreak. This was significantly helped by the Australian Pacific Oyster growers having a selective breeding program already in place for improved production before POMS occurred, and then rapidly adjusting the program to selectively breed oysters for POMS resistance (see http://www.asioysters.com.au/). There is a relatively high level of optimism for the future of the oyster industry as shown by only 18% of companies surveyed in 2019 anticipating that high mortalities will continue, although many commented that another couple of years of selective breeding is required before they will be strongly confident about stabilised production outputs from their farms.

Communication and Extension

Although implicit in our research project, communication and extension with the oyster growers impacted by POMS and with the main State government body responsible for managing the disease, BT, has been a major component of our project, and we believe contributed to its success. As we originally did not have biosecure facilities at IMAS Taroona, most of our research was conducted on-farm. We regularly worked alongside growers who supported our research by providing sites to conduct trials and boat transport to these sites. The periodicity of infection project, in particular, involved fortnightly sampling at a range of farms across the infected growing areas. These regular farm visits provided the opportunity to update farmers on our research and to hear from them about their on-farm observations, issues and changes to their farming operations.

We also regularly produced POMS Update newsletters, 12 in total, in conjunction with BT, which were emailed to oyster growers and Government managers in Tasmania. These newsletters contained information on our research and biosecurity aspects, as well as many other issues relevant to POMS disease, such as progress with the selective breeding project, developments in hatcheries etc. Particularly in the early stages of the POMS outbreak when there was much confusion and a severely impacted and stressed industry, it was important to provide factual information and regularly update the oyster growers on developments such as research progress, Government support available and biosecurity surveillance programs and movement permits. To our surprise, there was broad interest in our newsletter from oyster growers, researchers and managers across southern Australia, and our newsletter was also distributed to oyster growers in NSW and South Australia. Our final circulation list extended to hundreds of people across Australia and to researchers and industry in New Zealand. As the oyster industry has recovered from the initial devastation of the POMS outbreak, and as Oysters Tasmania and the ASI selective breeding program have developed their own newsletters, the need for our newsletter has declined and our ongoing research results will be provided in the Oysters Tasmania newsletter and on the IMAS website.

We convened a forum in mid-2017 specifically for oyster growers in Tasmania who were heavily impacted by POMS to provide an update on research activities and Government support and management of the industry, as well as an opportunity for farmers to interact. This forum was attended by over 80 Tasmanian oyster growers. Final presentations were also given at the NSW, TAS, and SA Oyster Conferences in August 2019, along with other projects within the FO CRC-P. Additionally, the surveys of oyster farmers on the impacts of POMS on their farming operations involved almost every oyster farmer in Tasmania, and significantly strengthened the communication between researchers and oyster growers, especially those with relatively small operations and in isolated areas. This was verified in our final POMS survey in autumn-winter 2019 when an overwhelming majority of farmers rated our research as having a high impact. Reasons given included greater communication of results through newsletters and workshops, and personal interactions between researchers and oyster growers.

Future Developments

As mentioned above, there remain a number of knowledge gaps about OsHV-1 which could be highly relevant to future management of the disease. Of importance is that the mechanisms for transmission of the virus, where its main reservoirs are located, and concentrations required for disease outbreaks are not fully understood. If these could be more clearly identified, then containment of the virus would be a more viable option. The interaction of the microbiome of oysters with OsHV-1 is increasingly being considered as a significant factor in contributing to mass mortality events. Oyster physiology as well as family line genetics are also thought to play a role in disease events, but are not well understood.

Research to date points to shallow estuaries and bays, where oyster aquaculture is concentrated, regularly having favourable conditions for disease transmission and mortality. These areas are most at risk of OsHV-1 disease events, but this risk is minimised in open waters with good water circulation (Pernet et al. 2018, Rodgers et al. 2018). As technology advances for offshore aquaculture, this may be the area for development of shellfish farming in the future. The results also indicate that any expansion of oyster farming should include spatial planning which takes into account the hydrodynamics of the area and the biomass of oysters in the water body from an epidemiological as well as carrying capacity perspective (Petton et al. 2015).

Data for the next two to five years are going to be vital in determining whether the optimism of the Tasmanian oyster growers that their industry is back to normal has been justified, particularly if another extreme heat event occurs. It will be important to continue recording the mortalities and environmental conditions that affect this industry each year so that the knowledge base of diseases and associated factors continues to expand.

9. Conclusion

Surveys of Pacific Oyster growers who suffered mass oyster mortalities on their farms in south-eastern Tasmania have shown that the industry has recovered well from the devastation and despair after the initial viral outbreak in 2016 to confident and progressive in just over three years. Many farmers expect to be back to pre-POMS production levels by 2020 and assessed their businesses as strong and more efficient than pre-POMS. This turnaround in the industry has occurred through Future Oysters CRC-P research, including the development of new farm management techniques to minimise POMS mortalities and the selective breeding program for OsHV-1 disease resistance, in combination with changes to farming operations implemented by the oyster growers.

Our research supports other studies on the OsHV-1 virus that warm water temperature is a major driver of disease outbreaks, with temperatures in south-eastern Tasmanian growing areas of 19 °C and above for around one week providing a high risk for a disease event to occur. The risk period for POMS disease outbreaks ranges from mid-November to late March. Other environmental factors likely to be important include hydrodynamics and biomass of infected oysters in the water body. Growing areas with extensive intertidal flats and poor water circulation, such as Pittwater, or with a high biomass of farmed and feral oysters in a relatively small inlet, such as Pipe Clay Lagoon, have shown to be more susceptible to POMS disease and mass mortalities than the other farming areas. As feral Pacific Oysters in Pipe Clay had a relatively high prevalence of OsHV-1, they may be contributing to the reservoir host of the virus.

Studies on farming practices conducted in close collaboration with oyster growers suggest that density of oysters in culture containers has limited effect on mortality rates, and that some handling is required during the POMS season to reduce biofouling and maintain stocking densities conducive to good growth and survival. Younger and smaller oysters are more susceptible to infection that larger and older juvenile and adult oysters. For oysters of the same age cohort, fast growers had higher mortalities than slow growers.

It will be important to continue surveillance of POMS in Tasmania into the 2020’s to assess the ongoing success of the selective breeding program and to increase the knowledge base of the disease and associated oyster physiology and environmental factors.

10. Implications

This research project has been conducted in close collaboration with the oyster growers impacted by POMS and with BT, the Tasmanian Government agency responsible for disease management in primary industries. As such, our research was directly applicable to industry and was conducted collaboratively with government managers. The impact of our research, along with major advancements in selective breeding of oysters for resistance to the OsHV-1 virus, increased biosecurity measures and changes to farm management implemented by the oyster growers themselves, has resulted in a rapid return to almost pre-POMS production levels and a more efficient industry. This has occurred more quickly than POMS disease outbreaks elsewhere, such as NSW and New Zealand.

Outcomes from our research include increased knowledge of the OsHV-1 virus which supports changes to farm operations to minimise mortalities. This includes a better understanding of the high-risk period for disease outbreaks – when temperatures reach around 20 °C, the effects of handling on mortality and hosts for the virus. Our surveys of oyster growers on the impacts of POMS has provided an industry-wide view of the effects of the virus and how industry has adapted and moved forward.

A final question on our survey of oyster farming companies in May-July 2019 was:
‘***Impact of our research on your farming operations’.***

79% of farmers rated the Future Oysters CRC-P research reported here as having a high impact. Reasons given included greater communication of results through newsletters and workshops, and personal interactions between researchers and oyster growers. Three farmers rated the research impact as medium, stating the relevance of the work is now not as high as when POMS first occurred, and that some work was inconclusive, such as the period of infection study.

11. Recommendations

Further Developments

There are still many unknowns about the OsHV-1 virus, in particular where the reservoir for this virus resides in the environment, and how it is dispersed. These factors have important implications for management of this disease and further research in recommended to better understand the reservoirs, carriers and hosts for this virus. Additionally, the results imply that although warm water temperatures are a major driver of OsHV-1 outbreaks, there are other triggers involved which we do not clearly understand. These include OsHV-1 density dependent factors and the viral concentrations required to trigger disease events, as well as interactions with microbial communities. The role of oyster physiology and genetics, including family lines, in the likelihood and severity of POMS disease events is also poorly understood.

Although major progress has been made with selective breeding for POMS resistance and changed farm practices to minimise POMS mortalities, it is still early days for this disease and consequently its management in Tasmania. The selective breeding program needs to continue to develop further to ensure greater disease resistance. Additionally, it is important that oyster farmers regularly observe and keep records of oyster health, mortalities and environmental conditions on their farms, especially during extreme heat events, in case disease outbreaks occur in the future.

12. Extension and Adoption

Communication about our project with the end user - oyster growers and managers, has been extensive throughout the project. As most of our research was conducted on-farm, we regularly worked alongside growers on their farms, who supported our research by providing sites to conduct trials and boat transport to these sites. We also regularly produced POMS Update newsletters, 12 in total, in conjunction with BT, which were emailed to oyster growers and Government managers in Tasmania. These newsletters contained information on our research and biosecurity aspects, as well as many other issues relevant to POMS disease, such as progress with the selective breeding project, developments in hatcheries etc. As a consequence, there was broad interest in our newsletter from oyster growers, researchers and managers in NSW and South Australia, and our final circulation list extended to hundreds of people across Australia.

We also held a forum in mid-2017 specifically for oyster growers in Tasmania who were heavily impacted by POMS to provide an update on research activities and Government support and management of the industry, as well as an opportunity for farmers to interact. This forum was attended by over 80 Tasmanian oyster growers.

A final project debriefing to industry, including a summary of results from our research was presented at the annual oyster industry conferences in New South Wales, Tasmania and South Australia in August 2019.

Ongoing information on our research will be provided to industry through the recently developed newsletter “The Filter” by Oysters Tasmania, and through presentations at industry events such as Shellfish Futures and other oyster grower association meetings.

Our research reports, newsletters and presentations to industry are available on the IMAS website at https://www.imas.utas.edu.au/research/fisheries-and-aquaculture/publications-and-resources under ‘OYSTERS’.

Project coverage

This project attracted Australia-wide media attention:

- IMAS research explores big chill theory to battle Pacific oyster mortality syndrome in Tasmania. The Mercury, November 22, 2017.
- Oyster research nets national award and solutions for growers. The Examiner, March 19, 2018.
- ABC Country Hour March 2017: POMS in Tasmania
- ABC Country Hour October 2018: Effect of POMS in Tasmania on the South Australian oyster industry.
- ABC Radio Hobart, January 24, 2018. What can Robo Oysters do to save their fellows from POMS?
- ABC Landline February 2017: Oyster Industry Update and the Response to the Pacific Oyster Mortality Syndrome.

Parts of this project were presented at national conferences:

1. CRC-P: Advanced understanding of POMS to guide farm management decisions in Tasmania (#: FN30643). Report for industry and steering committee, July 2018.
2. Tasmanian Oyster Industry Annual Conference (Shellfish Futures), *Advanced understanding of POMS to guide farm management decisions in Tasmania,* Hobart, 2018
3. POMS Forum, *Oyster farming post POMS the new reality – introduction to research,* Hobart, 2018 [C Crawford].
4. POMS Forum, *Survey of POMS Survey and Information Related to Farm Management*, Hobart, 2018 [J. Preston]
5. POMS Forum, *Window of Infection*, Hobart, 2018 [S. Ugalde]
6. Australian Marine Science Association Conference, *Major Impacts of POMS Disease on Pacific Oyster Farming in Australia*, Perth, 2019 [C. Crawford]
7. Oysters Australia Research and Development Day, *Advanced Understanding of POMS to Guide Farm management Decisions in Tasmania: Latest results on effects of farm management practices on oyster survival in POMS affected areas,* Sydney, 2018 [S. Ugalde]
8. South Australian Oyster Industry Annual Conference, *Advanced understanding of POMS to guide farm management decisions in Tasmania*, Streaky Bay, 2019 [Crawford]
9. New South Wales Oyster Industry Annual Conference, *Advanced understanding of POMS to guide farm management decisions in Tasmania*, Wallis Lake, 2019 [Crawford]
10. Tasmanian Oyster Industry Annual Conference (Shellfish Futures), *Advanced understanding of POMS to guide farm management decisions in Tasmania*, Orford, 2019 [Crawford]

Intellectual Property

This project generated no intellectual property that requires protection; all outcomes have been disseminated to the Australian oyster industry.

Some environmental data were collected and provided by The Yield. The data has been used in this report with the permission of The Yield and relevant oyster growers.

Commercial in confidence material emerged in the farmer surveys.

Researchers and Project Staff

The following are research and staff associated with this project:

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- Dr Jeff Ross, Senior Research Fellow (Institute for Marine and Antarctic Studies)
- Dr John Wright, Research Assistant (Institute for Marine and Antarctic Studies)
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Whittington, R.J., Liu, O., Hick, P., Dhand, N. and Rubio, A. (in press). Long-term temporal and spatial patterns of *Ostreid herpesvirus 1* (OsHV-1) infection and mortality in sentinel Pacific oyster spat (*Crassostrea gigas*) inform farm management. *Aquaculture.*

Whittington, R.J., Paul-Pont, I., Evans, O., Hick, P. and Dhand, N.K., 2018. Counting the dead to determine the source and transmission of the marine herpesvirus OsHV-1 in *Crassostrea gigas*. *Veterinary research*, 49(1), 34.

14. Appendix

Appendix 1: Project Materials Developed

Industry communications:

POMS Update, issue 1 to 12. Available online http://www.imas.utas.edu.au/research/fisheries-and-aquaculture/publications-and-resources

A Survey of the Effects of Pacific Oyster Mortality Syndrome (POMS) on Pacific Oyster Farms in Tasmania, 2016 – 2017. Sarah Ugalde, Christine Crawford. Available online http://www.imas.utas.edu.au/__data/assets/pdf_file/0015/1007313/POMS_Survey_Industry-Report21July.pdf

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FRDC FISH Magazine 25(2) 2017. Resistance warranted. The Pacific Oyster industry's determination to fight back after the incursion of POMS has received a funding boost.

FRDC FISH Magazine 27(3) 2019. POMS: where is the Pacific oyster industry now?

POMS Update, issue 1 to 12. Available online http://www.imas.utas.edu.au/research/fisheries-and-aquaculture/publications-and-resources

Peer reviewed publications and presentations

Ugalde SC, Preston J, Ogier E, Crawford C (2018). Analysis of farm management strategies following herpesvirus (OsHV-1) disease outbreaks in Pacific Oysters in Tasmania, Australia. Aquaculture 495, 179 – 186.

Oysters in hot water. YouTube, May 4, 2018 (https://www.youtube.com/watch?v=KiuiZT4RykA). First place winner of the Pitch It Clever Video Competition (Vice Chancellors' Award), Universities Australia 2018.

Oysters in hot water. YouTube, May 4, 2018 (https://www.youtube.com/watch?v=KiuiZT4RykA). First place winner of the Australian Society for Fish Biology Video Competition for Science Communication 2019.

Appendix 2: Ugalde et al. (2018)

For a copy of full published paper:
www.researchgate.net/publication/325341664_Analysis_of_farm_management_strategies_following_herpesvirus_OsHV-1_disease_outbreaks_in_Pacific_oysters_in_Tasmania_Australia

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Review

Analysis of farm management strategies following herpesvirus (OsHV-1) disease outbreaks in Pacific oysters in Tasmania, Australia

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ABSTRACT

The microvariant genotype of *Ostreid herpesvirus 1* (OsHV-1 μVar) has severely disrupted oyster production in Europe, New Zealand, and Australia by causing repeated and seasonal outbreaks of mass mortality in Pacific oysters (*Magallana gigas*). The virus was first detected in Tasmania, Australia, in January 2016, and mortalities of up to 87% were reported (de Kantzow et al., 2017). This study surveyed 95% of Tasmanian oyster farmers in OsHV-1 infected growing areas one year following initial detection, and recorded mortalities and associated farm management strategies in the 2016/2017 season, compared with the initial outbreak and before OsHV-1 occurrence. The survey was comprised of 37 open- and closed-ended questions, with data collected on background information, mortalities, environmental, genetic, and husbandry information. Perceived business viability was overall strong (75%), with changes to farm management occurring on 88% of leases in response to the virus. Commercial oyster farming businesses ranked handling regimes and stocking densities as the most important husbandry factors for influencing mortalities. Water temperature was ranked as the most important environmental factor, with 60% of businesses considering mean water temperature of 18– < 20 °C sufficient to activate disease. Mortalities for oyster size classes across multiple years are also reported. This survey has provided an expedient and cost-effective method to obtain information on the impact of a highly virulent disease and associated environmental conditions across an industry. These results will inform future management strategies and associated research.

1. Introduction

Ostreid herpesvirus-1 microvariant (OsHV-1 μVar, hereafter 'OsHV-1'), also referred to as Pacific Oyster Mortality Syndrome (POMS) in Australia, is a highly contagious and lethal virus to Pacific oysters (*Magallana gigas, previously known as Crassostrea gigas*) (Salvi et al., 2014). First detection occurred in France 2008, and the virus is now seasonally active during warmer months throughout several countries in Europe, New Zealand, and Australia (Friedman et al., 2005; Renault and Novoa, 2004; Segarra et al., 2010; Jenkins et al., 2013; Keeling et al., 2014). The high mortality rate and seasonal reoccurrence of OsHV-1 in oyster growing regions causes significant economic and production loss, and considerable effort is being invested into establishing best farm management strategies to reduce OsHV-1-associated mortalities and overall impact, in conjunction with selective breeding programs for disease resistance.

OsHV-1 was first detected in Tasmania, Australia, in January 2016 and rapidly spread to four major growing areas (Pipeclay Lagoon, Little Swanport, Blackman Bay and Pitt Water). Mortalities in all infected growing areas of up to 87% on commercial *M. gigas* leases were reported (de Kantzow et al., 2017). At the time of detection 100 oyster leaseholders were active in Tasmania producing 3029 tons, almost entirely *M. gigas*, with an estimated value in 2014–15 of $23 million (Australian Bureau of Agriculture and Resources Economics and Sciences; Mobsby and Koduak, 2017). Almost one third of active leases were affected by OsHV-1 which is the only known disease affecting *M. gigas* production in Tasmania. This joins with marine biotoxins produced by harmful algal blooms as the major challenges now facing the industry.

Farmer environmental observations during potential OsHV-1 seasons could contribute to an understanding of complex lease and growing area dynamics, and this information could be utilised to develop predictive tools and improved farm management. Seasonal OsHV-1 outbreaks occur during warmer months, and historically, water temperature has been the primary predictive tool. Water temperature thresholds for disease activation varies. Studies in France report temperatures between 16 and 20 °C, which are considered to be the risk threshold for disease activation (Petton et al., 2013; Pernet et al., 2014;

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Dégremont, 2013). In Australia, mortalities have been observed between 21 and 27 °C (Paul-Pont et al., 2014), with an estimated increased risk between 18 and 26 °C (de Kantzow et al., 2016). However, water temperature patterns are complex, and are characterised by large temperature swings driven by sharp peaks and troughs. These are influenced by broad- and fine-scale hydrology (e.g. tides and currents) and atmospheric/climatic drivers (e.g. atmospheric heat, rainfall and wind), making predictions based on temperature particularly challenging. In addition, OsHV-1 dynamics could also be influenced by the surrounding biodiversity, including natural populations and over-catch on farming infrastructure by a variety of bivalves (Pernet et al., 2014). Lease-specific observations in conjunction with growing area data provided by oyster farmers who have a consistent daily presence at these farms will likely assist in the development of environmental predictive tools and identification of risk thresholds.

Farm management strategies, along with genetic breeding for increased resistance (Dégremont et al., 2016), are considered to be crucial to reduce OsHV-1-associated mortalities (de Kantzow et al., 2017; Paul-Pont et al., 2013a). Various management strategies to mitigate the effect of OsHV-1 outbreaks have been investigated in other regions infected with the virus, including handling regimes (Peeler et al., 2012), lease infrastructure (Pernet et al., 2012), oyster age and size (Dégremont, 2013; Paul-Pont et al., 2014), and stock growing height in the water column (Paul-Pont et al., 2013a; Whittington et al., 2015a; Azéma et al., 2017). Individually, as well as combinations of, these management strategies impose varying levels of physiological stress, altering the vulnerability of oysters to disease. However, best management practices have been difficult to elucidate, particularly due to high seasonal and spatial variability, inconsistencies and contradictions in observations, difficulties in detecting and quantifying disease, differences in farm management strategies and infrastructure, and limited data sources (Pernet et al., 2016). By collecting information through a well-designed survey, some of these difficulties can be strategically minimalised by utilising the first-hand experiences of farmers in a structured and systematic approach.

In this study, we surveyed oyster farmers in OsHV-1 infected areas in Tasmania to increase knowledge of OsHV-1 disease events, in particular (i) drivers of outbreaks to support the development of a predictive framework that forecasts risk of OsHV-1 disease activation, and (ii) farm management practices that reduce OsHV-1 mortalities and overall impact.

2. Methods

2.1. Farm survey

Human ethics was attained through a Minimal Risk Application from the Tasmanian Social Sciences Human Research Ethics Committee, University of Tasmania. All leaseholders in OsHV-1 infected bays in south-eastern Tasmania (Fig. 1) were invited to participate in the survey conducted in May 2017, approximately one year following initial detection and after the second summer of disease events. Leaseholders were initially contacted through industry newsletters and communications, and directly through phone and email. Participation in the survey was voluntary and confidential, and each farmer and lease was issued a unique identifier code to comply with ethical requirements. Subsequently, 30 leases across 21 commercial businesses from all four infected bays participated, representing 95% of eligible respondents. As part of standard monitoring procedures, initial mortalities observed by leaseholders were tested by Biosecurity Tasmania to confirm the presence of OsHV-1 by use of qPCR analysis (data not shown).

2.2. Survey questions

Survey questions were developed based on similar surveys conducted elsewhere (Carlier et al., 2013; Castinel et al., 2015; Peeler et al., 2012) and discussions with various industry representatives. The survey consisted of 37 open- and closed-ended questions in 5 sections; background information, lease mortalities, and environmental, genetic, and husbandry information. Surveys were completed on-farm during visitation by one or two researchers, with the exception of one survey that was completed over the phone. Survey respondents were small-scale company owners or managers of larger companies. In addition to the structured questions, respondents were also encouraged to provide information on specific observations, trials, and future research direction. Researchers conducting the interview scribed responses to all questions, and undertook a scribe standardisation process to reduce biases.

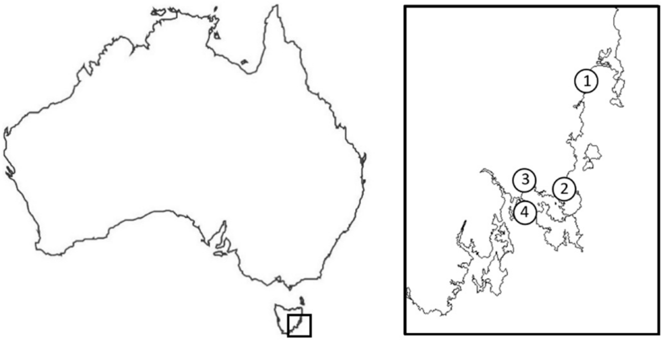

Fig. 1. OsHV-1 infected areas in Tasmania – Little Swanport (1; −42.340497, 147.937958), Blackman Bay (2; −42.854509, 147.831351), Pitt Water (3; −42.80806, 147.494742), and Pipeclay Lagoon (4; −42.969895, 147.524052).

2.3. Data analysis

Data was investigated on one of two levels; lease-level and business-level. Lease-level data looked at differences between all leases, regardless of some business owning or managing multiple leases, where specific business decisions did not skew results (e.g. '*How much stock do you have on this lease of each type and size class?*'). Business-level data looked at differences from a company perspective (e.g. '*What temperature regimes to do you consider to be required for an outbreak?*') (see supplementary material). Both levels were compared between growing areas. Statistics were performed in SPSS (IBM, Statistics 24) using ANOVAs where sample size met assumptions.

One open-ended question relating to future research priorities required semi-quantitative content analysis using descriptive statistics, in which responses were categorised into topic codes and expressed as a percentage of total suggested topic priorities at a company-level.

3. Results

3.1. Characteristics of lease sites and farm operations

The mean developed lease size was 7.8 ± 1.1 (standard error, SE) ha, range: 0.3 to 20.0 ha (Table 1). Most leases employed intertidal racks (20 in total), with 15 leases employing both intertidal racks and injection moulded plastic baskets attached to adjustable lines (SEPA-type) baskets (Fig. 2). Two leases involved shallow sub-tidal farming.

The type and size of oysters deployed on leases as of 1 November 2016 (pre-season) are summarised in Table 2. These included a combination of genetically selected (i.e. Australian Seafood Industries Pty Ltd. family lines), pre-exposed (i.e. oysters exposed to OsHV-1 in the previous year), and naïve stock (i.e. oysters not exposed to OsHV-1 previously).

Records of oyster farm operations were updated daily for 72.2% of leases, compared with 25.0% and 2.7% updating weekly or 'other' (e.g. only when farm is managed), respectively. These records were mostly managed through detailed white board notes (42.6%), notebooks

Table 1
Business and lease descriptive statistics - number of businesses, total number of operational leases, average area of lease in hectares, and farming methods used (intertidal racks and SEPA-type baskets; subtidal not shown) across 4 growing areas.

				Farm method used on lease	
Growing area	No. businesses	No. leases	Average area of developed lease (ha)	Intertidal racks	SEPA-type baskets
Pipeclay lagoon	5	10	4.45	10	7
Little swanport	3	5	8.16	3	5
Blackman bay	7	8	7.34	2	5
Pitt water	6	7	12.68	5	7

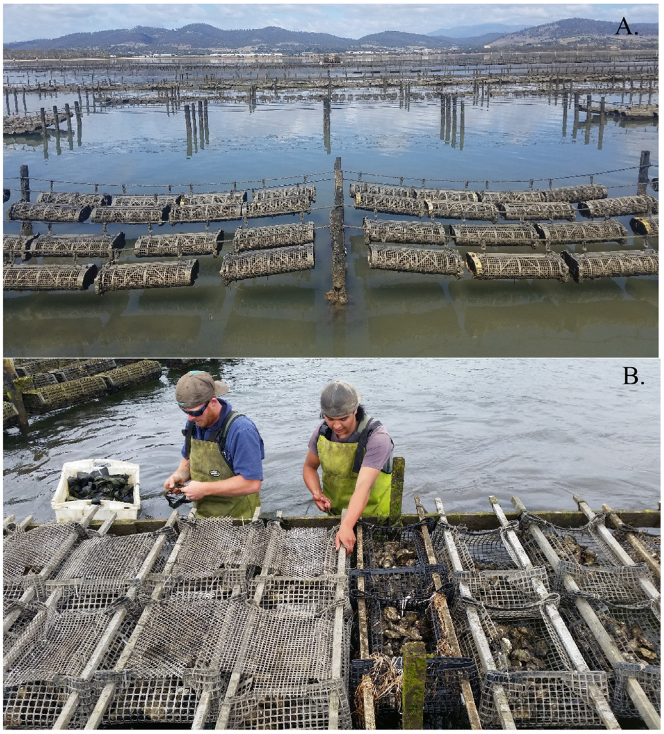

Fig. 2. Farming method (A) SEPA-type baskets and (B) intertidal racks with open baskets.

Table 2
Total amount of diploid and triploid oysters across leases and size classes on 1 November 2016.

	Small spat (< 4 mm)	Large spat (4–10 mm)	Juvenile (10–50 mm)	Market (> 50 mm)	Total
Diploids	20,100,000	8,321,000	31,179,000	16,359,500	75,959,500
Triploids	0	1,275,996	2,249,500	956,000	4,481,496
Total	20,100,000	9,596,996	33,428,500	17,315,500	80,440,996

(25.9%), or specific oyster management software (e.g. 'Shellfish Data Management', 16.7%). The remainder (3.7%) kept limited written records or used excel spreadsheets (16.7%). Records for 66.7% of leases used a combination of more than one recording-keeping method.

3.2. Mortality information

Mean lease percentage mortalities significantly varied between years for all oyster sizes; small spat ($p = 0.006$), large spat ($p = 0.021$), juvenile ($p = 0.022$), and market ($p = 0.001$; Fig. 3A). Pre-OsHV-1 small spat had significantly lower mortalities than both 2015/16 ($p = 0.007$) and 2016/17 ($p = 0.021$) seasons, whereas pre-OsHV-1 large spat had significantly lower mortalities than 2015/16 season only ($p = 0.007$). Pre-OsHV-1 juvenile and market sized oysters were significantly different to 2015/16 season ($p = 0.007$ and 0.001, respectively), with only market size varying between 2015/16 and 2016/17 seasons ($p = 0.020$).

Percentage mortalities differed between years and sites at Pipeclay Lagoon, Pitt Water, and Blackman Bay growing areas (Fig. 3B,C and D) because at the time of the first OsHV-1outbreak in 2015/16 most spat < 4 mm length were grown in the relatively calmer waters at Pipeclay. In 2016/17 these small spat were trialled in Pitt Water but suffered major mortalities, whereas in Pipeclay in this year mortalities were highest in the 4–10 mm spat size group.

More than one OsHV-1 event was observed on 61% of leases, compared with 17.9% for just one event. The remainder were unsure. Within growing areas, 40% of Pipeclay Lagoon leases exhibited more than one event, and at Blackman Bay 50.0% of leases had more than one OsHV-1 event and the other half were unsure. One OsHV-1 event was observed on 85.7% of leases at Pitt Water, and 80.0% of leases at Little Swanport. Mortality estimates could be given on 41.7% of leases exhibiting more than one OsHV-1 event, with the remainder either unsure/not checked between events (37.5% of leases) or mortalities not able to be estimated (e.g. minimal mortalities, lease de-stocked, 20.8% of leases), and of these, 72.7% had higher mortalities in the first event than the following event(s).

3.3. Genetic information: ploidy

Five leases from three locations stocked diploids (with some selective breeding) and triploids (all naïve) that were reported to be of similar age and size. On these leases, all triploids had higher mortalities (mean: 80.0%) than diploids (mean: 43.0%). Percentage mortality for unchallenged naïve stock was higher than pre-exposed stock for spat (large and small spat combined), juvenile and market oysters (Table 3). Similarly, percentage mortality for unchallenged genetically selected stock (estimated breeding value, EBV, ∼40%) was higher than pre-exposed genetically selected stock for spat, juvenile, and market oysters (Table 3). Unchallenged spat with an EBV 80% (no larger stock available) were across 5 leases only, and showed lower mortality than unchallenged spat of EBV 40%.

3.4. Spawning, age, and size

A full spawning was only observed on 28.6% of leases, and a partial spawn on 35.7%. No spawning was observed on 25.0% of leases, and 10.7% were unsure if a spawning had occurred. Several respondents commented on a connection between spawning and increased mortalities.

48.0% of businesses considered that both oyster size and age were equally important, whereas 40.0% considered that age was more important than size in surviving OsHV-1 disease. Front-runners (i.e. the quickest growing oysters in a batch) had a higher mortality than the rest of the stock in 50.0% of leases. From these leases observing higher mortalities in stock front-runners, 14.7% of their stock were considered to be front-runners and they had an estimated 46.9% mortality.

3.5. Environmental information

All businesses ranked water temperature as the most important factor in influencing mortalities, followed by hydrology/water movement and proximity to other leases (Fig. 4). 90.5% of businesses monitored water temperatures using live-streaming fixed sensors provided by Government for monitoring shellfish food quality-related parameters (Seabird Electronics, SBE 38), and 52.4% used their own sensors/thermometers.

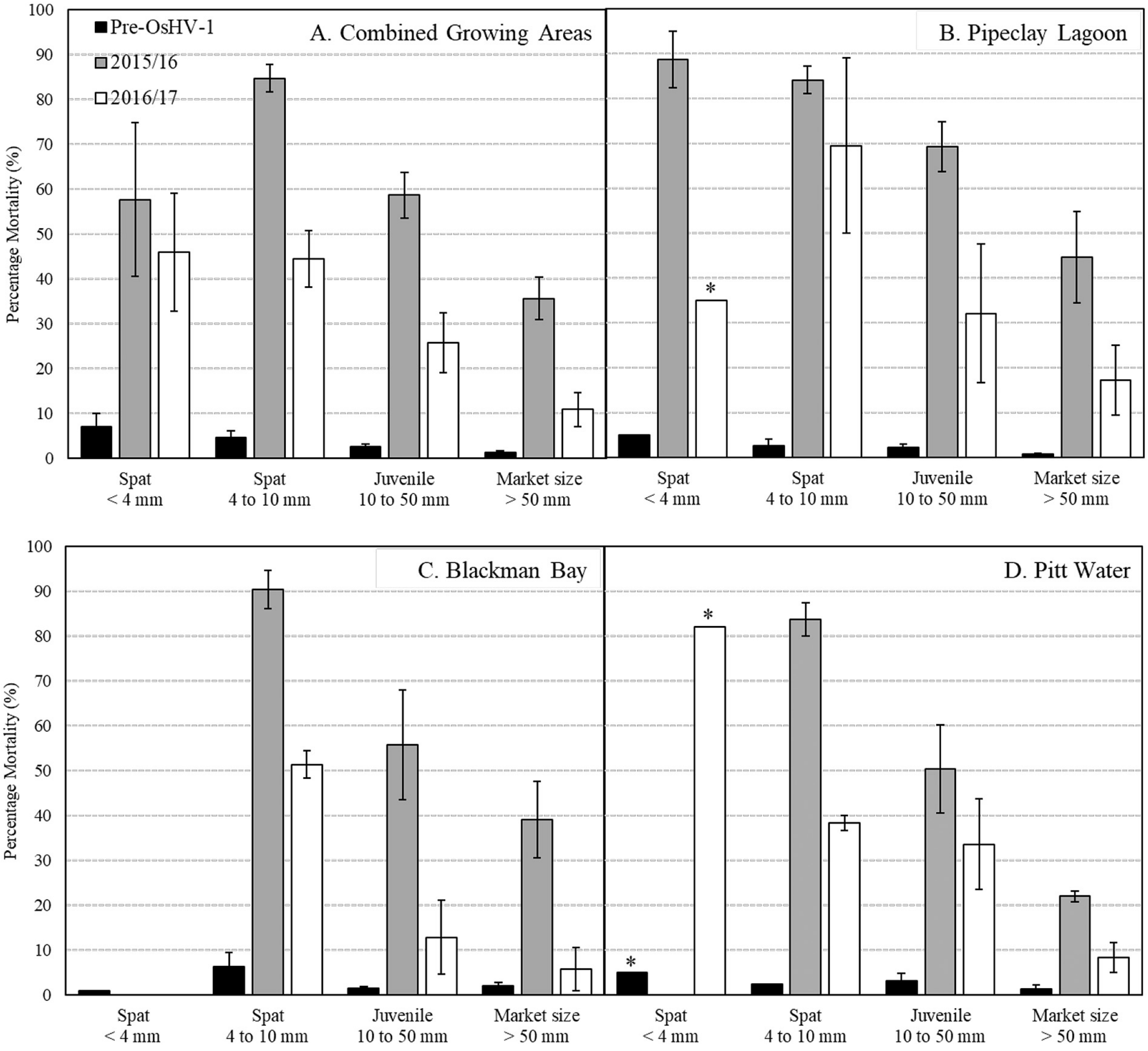

Fig. 3. Mean percentage mortality ± SE among leases for oyster size classes across multiple years for all growing areas combined (A), Pipeclay Lagoon (B), Blackman Bay (C), and Pitt Water (D). Little Swanport is not shown due to the small number of leases operating in the area. Percentage mortalities with n = 1 is denoted with *.

60% of businesses considered mean water temperatures of 18 to < 20 °C will activate disease, whereas 25% considered temperatures < 18 °C and 10% considered 20 to 22 °C are required. These temperatures need to be maintained for 4 to 5 days (25% of businesses), 7 days (30% of businesses), or > 2 weeks (25% of businesses). Water temperature spikes (maximum) and troughs (minimum) were also identified as playing an important role in disease activation (68.2% of businesses), and of these, 55.6% considered spikes more important than troughs. Of the leases that exhibited more than one disease activation, 35.0% had an observed difference in temperature regimes leading up the activation (e.g. a steady increase in mean temperature for the first activation, compared with high temperature spikes and troughs for the second activation, 50% had no observed difference).

OsHV-1 affected one area of a lease more than another area for 37.9% of leases, compared with 48.3% having no observable differences across the lease. Water flow was not observed to be involved in the transfer or severity of disease for 72.4% of leases, although both high and low flow were suggested by different farmers to be associated with the disease.

Table 3
Mean percentage morality (± SE, [range]) for 2016/17 season across all leases and size classes for genetics and previous exposure to OsHV-1.

Genetics and exposure	Spat (0–10 mm)	Juvenile (10–50 mm)	Market (> 50 mm)
Naïve, UC	75 ± 6.0% [46–85%]	28 ± 16% [0–75%]	33 ± 14% [6–90%]
Naïve, PE	50.0% [one lease]**	21 ± 6.6% [0–60%]	10 ± 2.7% [0–20%]
40% EBV, UC	63 ± 8.5% [50–79%]**	28 ± 17% [0–60%]	16 ± 14% [2–30%]**
40% EBV, PE	34 ± 1.9% [30–40%]	13 ± 3.8% [5–30%]**	2.0 ± 0.3% [1.5–2.5%]**
80% EBV, UC	40 ± 17% [10–82%]		

UC = unchallenged, PE = pre-exposed. EBV = approximate estimated breeding value. Mortalities marked with ** are from 3 or less leases.

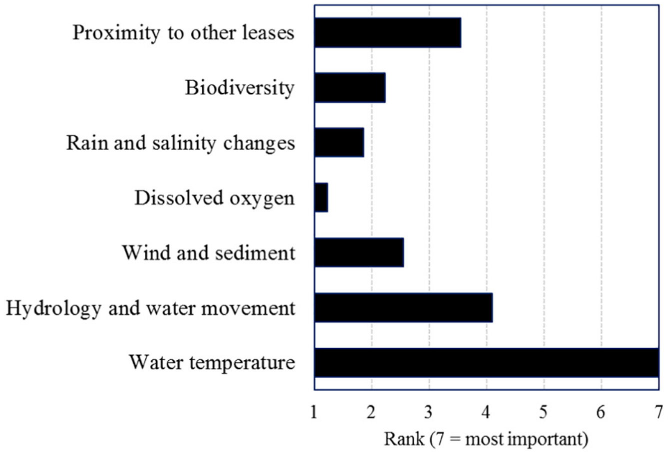

Fig. 4. Ranked environmental factors most important for influencing mortalities as reported by commercial businesses (7 = most important, 1 = least important).

3.6. Husbandry information

Businesses ranked husbandry factors as shown in Fig. 5, with handling regimes and stocking densities considered the most important factors influencing mortality. The reported changed farm management strategies, as captured by the survey, supports this ranking. Farm management on 88% of leases changed in response to OsHV-1, with only 8.0% opting for no change (Fig. 6). In addition, mostly the same management strategies would be applied on 80% of leases in the next OsHV-1 season (Table 4).

Stock was handled on 65.2% of leases 1–2 weeks prior to observed mortalities. Of these, 26.7% continued to handle once mortalities were observed. Rack or clip height was varied in response to OsHV-1 on 46.7% of leases (Fig. 6), and 25.8% of these observed higher mortality when oysters were held low in the intertidal zone (Fig. 7). Stocking density was varied on 75.0% of leases in response to OsHV-1 (Fig. 6), with a mean reduction of 35% compared with pre-OsHV-1 years, primarily because of low stock availability.

3.7. Business viability and research areas

As a gauge for perceived business viability, 75.0% of businesses rated their operation after the 2016–17 season as strongly viable, compared with 20.5% and 4.5% rating average and uncertain viability, respectively.

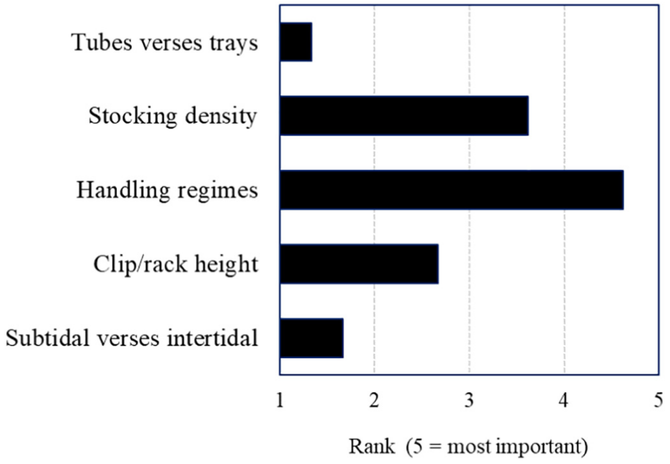

Fig. 5. Ranked husbandry factors most important for influencing mortalities as reported by commercial businesses (5 = most important, 1 = least important).

Businesses identified areas of research that would be beneficial to future operations (Table 5). Only data relating to farm management has been supplied, with harmful algal blooms/biotoxins and genetic breeding research areas excluded. Continuous data collection and predictive modelling was the highest priority for industry, followed by virus dynamics and oyster physiology.

4. Discussion

This survey strategically facilitated the collation and reporting of large amounts of diverse and quantifiable information with minimal resource investment, and importantly, can be repeated annually to record industry's changing farm management strategies and business perceptions. Face-to-face interviews were performed rather than surveys conducted by telephone or email to encourage participation and provision of accurate, detailed responses to open- and closed-ended questions. The survey captures the perspective of the whole industry, as well as information on lease-specific dynamics from those who spend the most time in the farm environment. The information can be used to contribute to the identification of disease outbreak drivers and the evolution of predictive tools and effective farm management strategies for reducing OsHV-1-associated mortalities and overall impact.

Pre-OsHV-1 in Tasmania, the broad success of applied farm management strategies is evident by the low background mortality (average 4% across all size classes). Following first OsHV-1 detection in the 2015/16 season, the virus rapidly spread to four major growing areas and resulted in an overall mean mortality of 59% across all size classes. In the following 2016/17 season, overall mortality reduced to 32% across all size classes, and no additional areas were infected. The reduction in mortalities may be due to almost all businesses taking action to modify their farm management strategies (92% of leases), including modifying handling regimes and increased amount of stock with genetic resistance. In addition, the extreme mortalities experienced during the initial detection 2015/16 season resulted in a reduced amount of overall stock available, and therefore, reduced stocking densities both across a lease and within stock housing (e.g. amount of stock per tubes or baskets). This reduced stocking density may alleviate physiological stress by allowing increased access to available food and water flow and reduced handling requirements. Many farmers commented that their oysters had had exceptionally good growth and condition. 80% of leases opted to continue with these changed farm management strategies in future OsHV-1 seasons, with minor modifications to facilitate the turnover of larger amounts of stock.

High spatial variability and patchiness in mortalities was observed on some leases (38% of leases), although growers had not observed any relationship with water flow and hydrology (72% did not notice a difference). OsHV-1 may have the capacity to attach to particles in seawater (Whittington et al., 2015c), the distribution of which can be influenced by physical disturbances such as lease location, orientation and infrastructure (Forrest et al., 2009), or farm management strategies such stock handling to manage biofouling and over-catch. Mortality patchiness may also be influenced by environmental characteristics, such as water chemistry and quality (e.g. temperature, salinity, quantity of organic matter, nutrients), phytoplankton and other microbiological communities (e.g. feed availability and nutrition) (Berthelin et al., 2000; Peeler et al., 2012). Although this survey could not attribute or eliminate any key drivers, it does suggest that mortality patchiness could be influenced, in part, by specific lease dynamics and farm management strategies (Paul-Pont et al., 2013a; Garcia et al., 2011).

In this survey, diploid oysters had lower mortality (43%) compared with triploids (80%). Although triploids can have faster growth and condition is not interrupted by spawning cycles (Nell, 2002; Normand et al., 2009), the advantages of diploid genetically-bred OsHV-1 resistance is lacking. The reduced diploid mortalities suggest some success in selective breeding for OsHV-1 resistance. Ploidy and stock genetics are increasingly becoming a crucial farm management decision

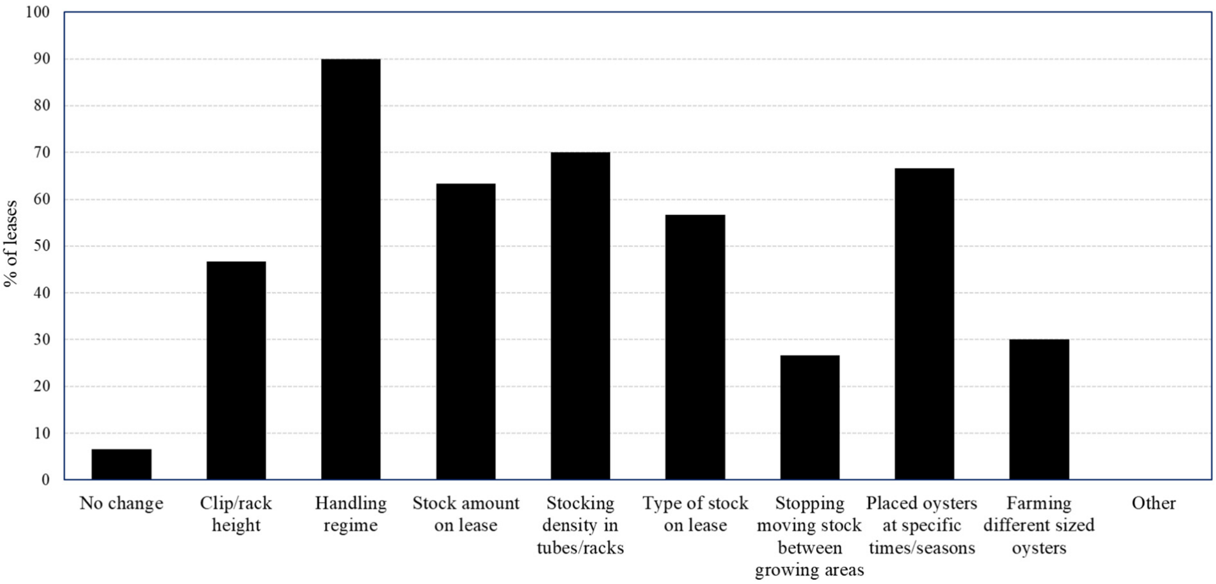

Fig. 6. Lease management changes in response to OsHV-1 in Tasmania, summer 2016/2017.

Table 4
Total number of responses (and percentages): 'Are you likely to use the same farm management strategy next OsHV-1 season?'.sssss

	# Responses	Percentage
Farm management strategies will be the same	5	16.7%
Farm management strategies will mostly be the same	24	80.0%
Farm management strategies will mostly change	0	.
Unsure if the farm management strategies will be the same	1	3.3%

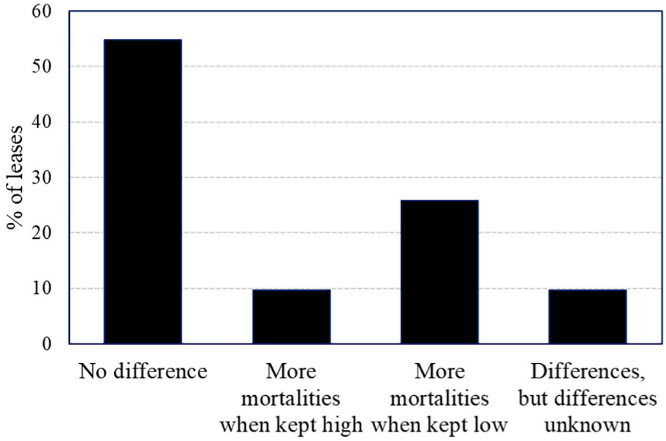

Fig. 7. Mortality observations in relation to clip or rack height.

Table 5
Research areas of interest as determined by Tasmanian oyster businesses. Results are expressed as a percentage (%) of all responses listed.

Research area	Response (%)
Continued data collection between multiple seasons and predictive OsHV-1 modelling	24
Virus dynamics and oyster physiology, including spat exposure to OsHV-1	20
Temperature moderation and manipulation, including chilling	16
Handling regimes	8
Infrastructure, including sub-tidal farming	8
Over catch and feral oysters	8
Stocking densities	4
Bay-specific differences	4
Oyster size in relation to disease susceptibility	4
Hydrology and water movement	4

that can have a major influence on oyster survival. In the current survey, diploid oysters on most leases did not experience a full spawn. The physiological changes and metabolic disturbances associated with spawning and reproductive effort can induce physiological stress and increase susceptibility to mortality (Huvet et al., 2010; Li et al., 2009; Wendling and Wegner, 2013), and this highlights an area of further research in which oyster condition, reproductive effort, and overall oyster physiological status may be utilised as indicators of disease dynamics, susceptibility to infection, and associated mortality.

In the current survey, water temperature was clearly perceived as the most important factor for influencing mortalities, and almost half of businesses used more than one method for monitoring temperature changes. Although it is well understood that water temperature plays a vital role in activating OsHV-1 disease, the complexities of in situ temperature regimes makes it difficult to give specific details. In Europe, 16 °C is considered the temperature threshold to activating disease, and events have been reported to occur between 16 and 24 °C (Oden et al., 2011; Pernet et al., 2012; Petton et al., 2013). In NSW, Australia, the temperature threshold appears to be higher at 22 to 25 °C, although it is not well defined (Jenkins et al., 2013; Paul-Pont et al., 2013b). Experimental infection and mortality has been reported to occur ≥18 °C (de Kantzow et al., 2016). In Tasmania, the majority of farmers consider 18–20 °C to be the temperature threshold, although there were varied opinions as to how long this needs to be maintained in order to activate disease. On a small temporal scale, temperature peaks (disease activation) and troughs (disease deactivation) within a tidal cycle may play an important role. The complexity is further demonstrated in the current study by half the leases that experienced more than one disease event, unlikely to be caused by stock movement, noted an observable difference in temperature regimes leading up to the events, such as a steady increase in mean temperature for the first event, compared with high temperature spikes and troughs for the second. With continued collation and analysis of temperature data, it is anticipated that temperature thresholds for OsHV-1 activation will be refined, and farmers will be able to develop additional indicators of disease outbreak.

In Australia, detectable amounts of virus has been found in natural populations of feral *M. gigas* and *S. glomerata* (Whittington et al., 2015b), as well as in mussels (*Mytilus* spp., *Trichomya hirsute*), cockles (*Anadara trapezia*), whelks (*Batillaria australis* and *Pyrazus ebeninus*), and barnacles (*Balanus* spp.) (Evans et al., 2017). In the current survey, half of the leases were reported to have large populations of nearby feral oysters, and of these, half were known to be affected by mortalities. There is a lack of information on the influence of surrounding biodiversity on virus dynamics, both in terms of quantity and richness of species. For example, mussels may reduce infection pressure on susceptible oysters, as shown by the reduced mortality of sentinel oysters deployed on *M. galloprovincialis* farms compared to empty or stocked oyster leases (Pernet et al., 2014). The management of surrounding biodiversity may be a useful management strategy in reducing disease risk and warrants further investigation.

Farm management strategies play a significant role in the spatial and temporal dynamics of oyster mortality (Pernet et al., 2012), and handling is clearly perceived as the most important practise for reducing OsHV-1-associated mortalities. Although, manual handling of spat has been associated with higher levels of mortality than mechanical handling (Peeler et al., 2012), gentle manual handling is considered to be less intrusive by Tasmanian farmers. In the current study, oyster size was considered important with smaller oysters much more susceptible than larger oysters, and this has been consistently observed in other studies (Burge et al., 2006; Peeler et al., 2012; Paul-Pont et al., 2013a; Pernet et al., 2014; de Kantzow et al., 2017; Azéma et al., 2017). From a farm management perspective, it is important to consider growth rates and to differentiate between oyster size and age. In the current study, half of the farmers observed faster growing oysters of the same age (i.e. ‘front-runners’), particularly at a small size, tended to exhibit a higher mortality (15% of stock categorised as front-runners with 47% mortality). In a French study, smaller oysters, that experienced higher mortality than larger oysters, had a higher daily specific growth rate, suggesting that OsHV-1 might actively use the host's cellular mechanisms to replicate, indicating that the risk factor or OsHV-1-associated mortality is increased in fast growing oysters (Azéma et al., 2017). The daily growth rate of oysters regularly decreases with age, and is consistent with a decrease of susceptibility to OsHV-1 from larvae or spat to adults (Azéma et al., 2016; Dégremont et al., 2016; Whittington et al., 2015a). However, this observation is not always supported by other studies that report no correlation between mortality and growth rate (Burge et al., 2006). Farmers can actively vary farm management practices to regulate growth rate (e.g. time held out of the water which limits food availability), and this may be a useful management tool to reduce disease risk. In the current study, farmer responses to mortalities associated with stocking density and rack height were not clear or consistent, despite being previously identified as a useful management tool (Peeler et al., 2012; Paul-Pont et al., 2013a). These inconsistencies may not be surprising, and could be due to oyster genetics (Azéma et al., 2017) or specific husbandry or environmental information not captured in this survey.

The majority of Tasmanian businesses perceive their future oyster farm operation as ‘strongly viable’ (79% of businesses). This level of confidence has not necessarily been obvious in other regions following initial OsHV-1 detection. Carlier et al. (2013) interviewed oyster farmers in France following initial detection, and reported the vast majority of farmers were concerned and recognised applied husbandry as contributing to mortalities, but only one third of farmers changed their practices to limit OsHV-1 disease spread and associated impacts on production. OsHV-1 disease in Spain has been identified as the causal agent for dramatic declines in *M. gigas* production from ~800 to 138 metric tons per year between 2006 and 2011 (Carrasco et al., 2017). In NSW, Australia, following initial OsHV-1 detection in November 2010, the total production of pacific oyster reduced by 48% within three years (NSW Department of Primary Industries, 2015). Tasmania's strong industry confidence may be due to the rapid response and support offered by industry representatives and government, the amount of information freely available from other regions already managing this disease, the already well-developed selective breeding program and commercial availability of genetically-selected OsHV-1 resistant family lines, and/or the specific temperature and environmental conditions experienced at the time. The main factors affecting perceived business viability are likely to change in future seasons, depending on environmental conditions, and the success of selectively bred family lines and modified farm management strategies.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aquaculture.2018.05.019.

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Appendix 2: Ugalde et al., (2018), Survey Questions

Participation in the survey was voluntary and confidential.

Section 1: Background Information

1. How many hectares is your lease?
2. How many developed hectares?
3. Which farming methods do you use on this lease?
4. How much stock do you have on this lease of each type and size class?
5. How do you keep records of your oyster farm operation?
6. How often do you update these records?
7. How do you rate the viability of your oyster operation after this second summer of POMS?

Section 2: POMS Mortality information

8. What average mortality have you experienced over summer when POMS was not present in Tasmania?
9. What average mortality have you experienced over summer when POMS was present in Tasmania for seasons 2015/16 and 2016/17?
10. What differences in mortalities have you experienced on this lease in relation to selective breeding for spat, juvenile, and market oysters?
11. Have you experienced several POMS events this summer?
12. If yes, details of when and overall mortality percentage on each occasion:

Section 3: Environmental information

13 In general, environmental factors do you think are most important for reducing mortalities on this lease?

14 How do you keep an eye on your water temperature?

15 What temperature regimes do you consider to be required for a POMS outbreak?

16 Do you think temperature spikes or troughs are a contributing factor in triggering POMS outbreaks?

17 If you experienced several POMS events, did the temperature regimes differ between POMS events?

18 Did POMS affect some parts of your lease more than other parts?

19 Did you observe any evidence for water movements being involved in the transfer or severity of POMS disease?

20 Are there large populations of feral oysters anywhere near you lease?

Section 4: Genetic Information

21 What was the difference in mortalities between triploids and diploids?

22 If you have triploids and diploids, were there differences between them (e.g. size, age, where on the lease)?

23 Did the oysters on your lease spawn this summer?

24 Do you believe oyster size or age from spawning is more important in surviving a POMS outbreak?

25 Have you noticed any difference in mortality in front-runners in your stock?

Section 5: Husbandry Information

26 In general, which husbandry factors do you think are most important for mortalities?

27 How did you vary your farm management in response to POMS?

28 Are you likely to use the same farm management strategy next POMS season?

29 Once POMS mortalities had been observed in your growing area, did you stop handling?

30 Did you observe any differences in mortalities between the same stock at different heights in the water column?

31 Did you observe higher mortalities in the same stock when they were held at different stocking densities?

35. Finally, can you identify any areas of research that you believe would be beneficial to your operation in the future (not compulsion to answer)?

Appendix 3: 2018/19, Survey Questions (Infected)

Section 1: Mortality information in 2018/19

1. What average mortality have you experienced this summer?
2. How did this mortality occur?
3. Have you changed any of your farming methods since last year?

Section 2: Environmental information

4. In general, which environmental factors listed below do you think are important to POMS outbreaks on your leases?
5. What temperature regimes do you consider to be required for a POMS outbreak?
6. Do you look at weather or tidal patterns before handling stock during the POMS season?
7. Have you observed any patterns in mortality across your leases, or your growing areas?
8. Are there large populations of feral oysters in your growing area?
9. Do you believe that the biomass of shellfish (farmed, feral oysters and other filter feeders) has an influence on POMS outbreaks?

Section 3: Farm Operations

10. Have you changed your farm management practices from pre-POMS?
11. Do you buy spat at different times and/or sizes compared to pre-POMS?
12. Do you sell matures at different times and/or sizes compared to pre-POMS?
13. What percentage of each type of stock do you farm?
14. Do you move oysters between farms differently to pre-POMS?
    Within growing areas? Between growing areas?
15. Are your oysters sold to a different market place now compared to pre-POMS?

Section 4: Production and Farm Management

16. What is your estimated overall mortality in each summer season since the first outbreak in 2016?
17. What is your production compared with Pre-POMS level?
    If lower, are you aiming to get your production back to Pre-POMS level?
18. Is your employment back to Pre-POMS level?
    If lower, are you aiming to get employment back to Pre-POMS level?

19. Do you consider that your farming operation is more efficient than pre-POMS? Any specific reasons why?
20. How would you rate the overall effect of POMS in south-eastern Tasmania on your farming business?
21. How do you rate the viability of your oyster operation after this fourth summer of POMS?
22. Has the business structure of your farming operations changed since POMS?
23. Has POMS affected your marketing of oysters?
24. Do you anticipate major mortalities (>30%) from POMS in the future?
25. Are you considering diversifying in the future, e.g. other species, products, markets?

Section 5: Future Research?

26. Are there any areas of research related to oyster farming that you believe would be beneficial to your operation in the future?

Section 6: Impact of our research

27. As part of the reporting for the CRC-P funding, we are required to provide information on how relevant and useful our research has been to Pacific oyster farming in Tasmania in POMS infected areas. How would you rate the impact of our research on your farming operation?

Appendix 4: 2018/19, Survey Questions (Uninfected)

Section 1: Background information

1. Your developed lease is ______________ha
2. You undeveloped lease area is _______________ha
3. Which farming methods do you use on this lease:

Section 2: Farming operations

4. How would you rate the overall effect of POMS in south-eastern Tasmania on your farming business?
5. Has your view on POMS and its effect on your operation changed significantly since the first outbreak in 2016?
6. Has POMS affected your marketing of oysters?
7. Are you concerned that POMS could occur on your farm in the future?
8. In general, which environmental factors are important in relation to POMS outbreaks?
9. In general, which husbandry factors are important in contributing to POMS mortalities?
10. Do you monitor your water temperature?
11. Are there large populations of feral oysters anywhere near your lease?
12. If POMS was to occur on your lease, are you confident that you can make the best farm management decisions to reduce POMS mortalities and overall impact?
13. What precautions, if any, have you taken to reduce your risk of major POMS mortality?
14. Have you increased biosecurity on your farm (e.g. movement of people and gear onto your farm)?
15. How do you rate the viability of your oyster operation after this third summer of POMS?
16. Finally, can you identify any areas of research related to POMS that you believe would be beneficial to your operation in the future (not compulsive to answer)?