

South Australian Pacific Oyster Selective Breeding Program

Building POMS resistance to reduce risk for the South Australian oyster industry

Penny Miller-Ezzy, Mark Gluis, Kathryn Wiltshire, Marty Deveney and Xiaoxu Li

June 2024

FRDC Project No. 2019-039



© 2024 Fisheries Research and Development Corporation and South Australian Research and Development Institute (Aquatic and Livestock Sciences). All rights reserved.

All rights reserved.

ISBN: 978-1-876007-53-9

South Australian Pacific Oyster Selective Breeding Program. Building POMS resistance to reduce risk for the South Australian oyster industry.

FRDC Project No. 2019-039

2024

Ownership of Intellectual property rights

Unless otherwise noted, copyright (and any other intellectual property rights, if any) in this publication is owned by the Fisheries Research and Development Corporation and The South Australian Research and Development Institute (Aquatic and Livestock Sciences).

This publication (and any information sourced from it) should be attributed to Miller-Ezzy, P., Gluis, M., Wiltshire, K., Deveney, M., and Li, X. The South Australian Research and Development Institute (Aquatic and Livestock Sciences), 2024, *South Australian Pacific Oyster Selective Breeding Program. Building POMS resistance to reduce risk for the South Australian oyster industry*, Adelaide, June. CC BY 3.0.

Creative Commons licence

All material in this publication is licensed under a Creative Commons Attribution 3.0 Australia Licence, save for content supplied by third parties, logos and the Commonwealth Coat of Arms.



Creative Commons Attribution 3.0 Australia Licence is a standard form licence agreement that allows you to copy, distribute, transmit and adapt this publication provided you attribute the work. A summary of the licence terms is available from https://creativecommons.org/licenses/by/3.0/au/. The full licence terms are available from https://creativecommons.org/licenses/by/3.0/au/.

Inquiries regarding the licence and any use of this document should be sent to: <u>frdc@frdc.com.au</u> **Disclaimer**

The authors warrant that they have taken all reasonable care in producing this report. The report has been through the SARDI internal review process, and has been formally approved for release by the Research Director, Aquatic and Livestock Sciences. Although all reasonable efforts have been made to ensure quality, SARDI does not warrant that the information in this report is free from errors or omissions. SARDI and its employees do not warrant or make any representation regarding the use, or results of the use, of the information contained herein as regards to its correctness, accuracy, reliability and currency or otherwise. SARDI and its employees expressly disclaim all liability or responsibility to any person using the information or advice. Use of the information and data contained in this report is at the user's sole risk. If users rely on the information they are responsible for ensuring by independent verification its accuracy, currency or completeness. The SARDI Report Series is an Administrative Report Series which has not been reviewed outside the department and is not considered peer-reviewed literature. Material presented in these Administrative Reports may later be published in formal peer-reviewed scientific literature.

The authors do not warrant that the information in this document is free from errors or omissions. The authors do not accept any form of liability, be it contractual, tortious, or otherwise, for the contents of this document or for any consequences arising from its use or any reliance placed upon it. The information, opinions and advice contained in this document may not relate, or be relevant, to a reader's particular circumstances. Opinions expressed by the authors are the individual opinions expressed by those persons and are not necessarily those of the publisher, research provider or the FRDC.

The Fisheries Research and Development Corporation plans, invests in and manages fisheries research and development throughout Australia. It is a statutory authority within the portfolio of the federal Minister for Agriculture, Fisheries and Forestry, jointly funded by the Australian Government and the fishing industry.

Researcher Contact Details		FRDC Contact Details	
Name:	Xiaoxu Li	Address:	25 Geils Court
Address:	2 Hamra Avenue, West Beach, SA, 5024		Deakin ACT 2600
Phone:	08 8207 5464	Phone:	02 6122 2100
Fax:	08 8207 5415	Email:	frdc@frdc.com.au
Email:	pirsa.sardiaquatics@sa.gov.au	Web:	www.frdc.com.au

In submitting this report, the researcher has agreed to FRDC publishing this material in its edited form.

Contents

Contents	iii
Acknowledgments	iv
Executive Summary	v
Introduction	1
Objectives	2
Method	
Results, discussion, and conclusion	9
Implications	12
Recommendations	13
Extension and Adoption	14
References	15
Appendices	17

Figures

Figure 1	Semi-continuous micro-algae system at SARDI West Beach, for feeding Pacific Oyster	2
	broodstock, larvae and spat	3
Figure 2	Strip spawning of Pacific Oyster gametes at SARDI, West Beach.	4
Figure 3	Pacific Oyster gametes were assessed for quality (A) and washed to remove excess	
	tissue (B)	4
Figure 4	Pacific Oyster families were fertilised in 10 litre buckets according to the mating	
	matrix provided by ASI	4
Figure 5	Pacific Oyster larvae rearing tanks at SARDI, West Beach	5
Figure 6	An example of Pacific Oyster larvae being washed/graded at SARDI, West Beach	5
Figure 7	An example of Pacific Oyster larvae being induced for metamorphosis with	
	epinephrine at SARDI, West Beach	5
Figure 8	Spat upwelling system at SARDI, West Beach	6
Figure 9	An example of Pacific Oyster spat grading at SARDI, West Beach	6
Figure 10	Spat of a selectively bred Pacific Oyster families ready for deployment to the field	7
Figure 11	Kaplan-Meier survival curve showing SARDI 2019-year class and unselected line	
	survival when challenged with OsHV-1	10
Figure 12	Weibull model survival probability estimates following exposure to OsHV-1 for the	
	SARDI 2019-year class families and unselected lines	11

Acknowledgments

Funding for this project was provided by the South Australian Research and Development Institute (SARDI), South Australian Oyster Growers Association (SAOGA), Australian Seafood Industries (ASI), Flinders Ports and the Fisheries Research and Development Corporation (FRDC) on behalf of the Australian Government.

The authors would like to acknowledge the following for their involvement throughout the project:

Members of the project steering committee (2019 – 2023) including Dr Mike Steer (SARDI; Committee Chair), Mr Wayne Hutchinson (FRDC), Dr Adam Main (PIRSA), Dr Shane Roberts (PIRSA Mr Randal Bonner (Flinders Ports), Mr Gary Zippel (SAOGA), and Mr Matt Cunningham (ASI).

Mr Jack Wolverson (SARDI) provided technical assistance. ASI staff assisted in transport of stock.

This report benefited from constructive review by Mr Wayne Hutchinson, Dr Sarah Catalano, Dr Jessica Buss, Dr Sasi Nayar and Dr Mike Steer.

Executive Summary

The Australian Seafood Industries (ASI) South Australian (SA) Pacific Oyster Selective Breeding Program was established in 2016 and federally funded through the Future Oysters CRC-P project (Stage 1) until 2019. A continuation of the program was needed to reach 90% Pacific Oyster Mortality Syndrome (POMS) disease resistance for greater than or equal to (\geq) one year old Pacific Oysters (*Magallana gigas*, previously *Crassostrea gigas*). The initial objective of this project was to reach the target level of POMS resistance in three years to protect the SA Pacific Oyster aquaculture industry from losses when an outbreak of POMS occurs in an oyster growing region. Later the Project Steering Committee decided to incorporate the top ten POMS resistant 2020-year class families selected and imported by ASI from Tasmania into the SA breeding program, and at the same time approve the Pacific Oyster spat survival (POSS) program in SA (through FRDC project 2020-064 South Australian Pacific Oyster mortality trials). The top POMS resistant 2020-year class families in Tasmania were produced from parents with estimated breeding value (EBV) of > 90% resistance to POMS.

A total of 221 new selectively bred families were produced at the South Australian Research and Development Institute (SARDI), West Beach, between 2019 and 2023, meeting project objectives. The 2019-year class was challenge tested in the Port River, SA for POMS resistance, after which the imported Tasmanian broodstock were incorporated into the program and the objective changed to improve both POMS resistance and POSS in SA. Details of the POSS field challenge tests and results will be available in the final report of FRDC project 2020-064. In the 2022 families, the last year class produced at SARDI, the average EBV of the top five POMS resistant families was 100% and the average EBV of the top five POSS families was 90%.

The provision of selectively bred Pacific Oysters to the SA aquaculture sector will help to protect against losses when an outbreak of POMS occurs in an oyster growing region and will increase the survival of newly stocked spat, thus increasing profitability and helping to protect and grow an important SA industry. The successful collaboration between SARDI, ASI, and all other parties involved in through the Project Steering Committee resulted in this project not only meeting its deliverables, i.e., establishing the required number of families and target level of POMS resistance, but also being extended by a further year (the 2022-year class) and allowing for the continuation of the SA Pacific Oyster breeding node into the future. The demand for selectively bred POMS-resistant and POSS ASI spat from SA oyster growers has increased substantially over the course of this project.

Keywords

Selective breeding, Pacific Oyster, Magallana gigas, South Australia, OsHV-1, POMS

Introduction

Pacific Oyster Mortality Syndrome (POMS), a disease caused by ostreid herpesvirus type one microvariants (OsHV-1), can cause high and rapid mortality (e.g., up to 100% mortality within days of initial detection) of Pacific Oysters (*Magallana gigas*, previously *Crassostrea gigas*). POMS has been the cause of mass mortality outbreaks globally resulting in significant economic loss (Friedman et al., 2005; Peeler et al., 2012; Roque et al., 2012; Hwang et al., 2013; Keeling et al., 2014; Mortensen et al., 2016; Abbadi et al., 2018; Burioli et al., 2018; Burge et al., 2021). POMS was first detected in Australia in New South Wales (NSW) in November 2010 (Jenkins et al., 2013), and was detected in south-eastern Tasmania in February 2016 (De Kantzow et al., 2017). The Tasmanian outbreak resulted in the Department of Primary Industries and Regions South Australia (PIRSA), in consultation with the South Australian Oyster Growers Association (SAOGA), temporarily preventing the import of live oysters into South Australia (SA). The ban included the selectively bred POMS resistance family oysters that Australian Seafood Industries (ASI) was developing. ASI is owned by peak oyster aquaculture industries and, prior to the Tasmanian outbreak, would distribute selectively bred families to oyster growers in Tasmania, SA, and NSW for performance evaluation. POMS was detected in feral Pacific oysters in the Port River, SA, in February 2018, but to date, has not been detected in any Pacific Oyster production areas in the state.

Establishing readiness for a POMS outbreak in the oyster farming regions is one of the most important priorities for the SA oyster industry. In 2020-21, the SA oyster industry had an on-farm value of \$44 million and employed approximately 583 people directly (BDO-EconSearch, 2022). Farming is regionally based (i.e., Denial Bay, Smoky Bay, Streaky Bay, Coffin Bay, Louth Bay, Franklin Harbour, Nepean Bay, and American River) and therefore very important to the rural economy of western and central regional SA. Outbreaks of POMS are temperature dependent. In Europe water needs to be above 16 °C for a disease outbreak to occur (Pernet et al., 2012; Renault et al., 2014), whereas outbreaks in NSW occur at 19-24 °C (Paul-Pont et al., 2013; Paul-Pont et al., 2014). Other shellfish species act as carriers of POMS and increase transmission and persistence of the disease (Arzul et al., 2001; Evans et al., 2017; O'Reilly et al., 2018). Dependent on life cycle and geographical location, POMS resistance has a reported heritability rate of 0.12 - 0.63 (Dégremont et al., 2015; Azéma et al., 2017; Camara et al., 2017; Gutierrez et al., 2018), hence it can be selected through a family-based selective breeding program. Having highly POMS resistant Pacific Oysters stocked on SA leases prior to any outbreak will help to protect this important industry from significant losses and financial impacts.

To address the inaccessibility of POMS resistant oysters to the SA oyster industry, the South Australian Research and Development Institute (SARDI) collaborated with ASI and SAOGA to initiate the SA Pacific Oyster Selective Breeding Program as part of the federal government funded Future Oysters CRC-P project (Stage 1). The breeding program was funded from 2016 to 2019 and developed 160 selected families. Estimated breeding values (EBV) were calculated for each family to assess their resistance to POMS. Where the ASI Tasmanian Selective Breeding Program reached the target of greater than (>) 90% POMS resistance in the top performing families prior to 2020, the SA Selective Breeding Program lagged. This lag was due to initially only having access to families a generation behind those available in Tasmania, a lack of robust and reliable challenge tests, and lower accuracy of EBV calculations. Field tests could only be completed in SA after the 2018 POMS outbreak in the Port River, and virus loads were low in consecutive years due to mild weather and biosecurity strategies to knock down feral oyster populations. EBV calculations lacked accuracy because only data from distant relatives (higher rank of cousins) could be used for calculating family EBV in SA, whereas information from close relatives (parents and brothers and sisters) were available for these calculations in Tasmania. This project was established to achieve a resistance level of over 90% for \geq one year old oysters within the SA Pacific Oyster Selective Breeding Program over a further three years.

In 2020, it was agreed by the Project Steering Committee (Appendix 1) to alter the second project objective to improve both POMS resistance and Pacific Oyster spat survival (POSS) in SA as a new project objective; the third objective in this report. This change arose from the SA Pacific Oyster aquaculture industry experiencing substantial mortality of deployed spat, up to 100% in some cases, over a prolonged period. SA spat mortality was variable between batches, locations, and seasons. It was, therefore, critical for the breeding program to understand how spat mortality correlated with genetics. Degremont et al. (2005) observed a significant

family × environment interaction on Pacific Oyster spat survival in France, with the largest amount of survival variation observed between families. It was also agreed to import selectively bred Tasmanian Pacific Oysters to incorporate with the SA stocks to increase resistance levels within the SA program. Selected Tasmanian stocks were the top performing families where > 90% POMS resistance had already been reached. These stocks were imported using the *Protocol for importation of hatchery reared Pacific Oyster* (Crassostrea gigas) *spat from jurisdictions where Pacific Oyster Mortality Syndrome (POMS), caused by Ostreid Herpesvirus 1 microvariant, has been detected* developed by PIRSA and SAOGA. Given the ability to import highly resistant Tasmanian stocks into the biosecure SARDI facilities and strong support from industry to focus on the improvement in both POMS resistance and POSS in SA, the project was extended by a year to incorporate both traits into the SA selective breeding program and the selective bred broodstock for commercial hatchery productions.

Objectives

- 1. Develop selectively bred families with 90% POMS disease resistance for ≥ one year old Pacific Oysters.
- 2. Support the SA industry by provision of high POMS resistant broodstock for commercial spat production.

A new objective was added during this project:

3. Support the SA industry by supplying broodstock with high POMS resistance and high POSS rate for commercial spat production.

Methods

Selection of broodstock

The mating design of this project was provided by ASI to initially maximise POMS resistance (2019/20 families) and later to incorporate the most POMS resistant Tasmanian families into the SA Breeding Program and maximise both POMS resistance and POSS. EBVs of parental families were calculated by ASI using pedigree data, inbreeding rates, and the results of challenge tests (see FRDC project 2020-064 South Australian Pacific Oyster mortality trials). A mating matrix was then provided by ASI to SARDI prior to each spawning.

Family production

All families within the ASI SA Pacific Oyster selective breeding program were spawned and cultured in the shellfish hatchery at SARDI, West Beach, in compliance with the site biosecurity plan.

Four species of microalgae were cultured semi-continuously (*Tisochrysis lutea, Pavlova lutheri* and *Chaetoceros muelleri*) (Figure 1) or statically (*Chaetoceros calcitrans*) using pasteurised seawater and Walne medium (Lavens and Sorgeloos, 1996) and fed to broodstock, larvae and spat at a ratio of 1:1 flagellates and diatoms



Figure 1 Semi-continuous micro-algae system at the South Australian Research and Development Institute, West Beach, for feeding Pacific Oyster (*Magallana gigas*) broodstock, larvae and spat.

Incoming seawater was UV treated and filtered to 1 μ m for broodstock and spat and ultra-filtered to 0.01 μ m for larvae. Broodstock were received from the field 6-10 weeks prior to planned spawning. Broodstock were held in quarantine within the constant environment room (CER) at SARDI, West Beach, and fed at the microalgal concentration adjusted according to their gonad development. Temperature was gradually increased from 14 °C to 22 °C over the conditioning time.

Spawning was completed on a single day for each spawning run of up to 42 families. When mature, broodstock were strip spawned using a scalpel blade to loosen eggs and sperm from the gonad tissue (Figure 2). Gametes of each broodstock were washed into individual plastic containers, rinsed through a 70 μ m screen to remove excess tissue, and assessed under the microscope for quality (e.g., egg shape, sperm mobility etc., Figure 3). Fertilisation of single pair crosses was performed in 10 litre buckets according to the mating matrix provided by ASI at an oocyte to sperm ratio of ~1:10 (Figure 4).



Figure 2 Strip spawning of Pacific Oyster (*Magallana gigas*) gametes at the South Australian Research and Development Institute, West Beach.



Figure 3 Pacific Oyster (Magallana gigas) gametes were assessed for quality (A) and washed to remove excess tissue (B).



Figure 4 Pacific Oyster (*Magallana gigas*) families were fertilised in 10 litre buckets according to the mating matrix provided by Australian Seafood Industries.

Larvae were reared in 200 L static conical tanks, one family per tank, up to 42 tanks per spawning run (Figure 5). Water temperature was 24 °C and oxygen was supplied via an airline to the bottom of the tank. Each tank was drained and cleaned daily, with the larvae washed, graded (using 40 μ m - 250 μ m screens), and returned to the same tank (Figure 6). Larvae were fed rations of microalgae across the day as required (based on size

and quantity of spat in each family). When the larvae were competent (> 236 μ m screen size, eyed, and foot present), metamorphosis was induced using epinephrine at 0.03 g / L for one hour, every day for a maximum of three times per family (Figure 7) following <u>APVMA Minor Use Permit PER80085</u>.



Figure 5 Pacific Oyster (*Magallana gigas*) larvae rearing tanks at the South Australian Research and Development Institute, West Beach.



Figure 6 An example of Pacific Oyster (Magallana gigas) larvae being washed/graded.



Figure 7 An example of Pacific Oyster (Magallana gigas) larvae being induced for metamorphosis with epinephrine

Spat were transferred to an upwelling system, cleaned daily, and fed according to the total biomass in each raceway (Figure 8). In general, spat were graded four times over the spat rearing period (approximately two

months) with 0.5 mm, 1 mm, 2 mm, and 3 mm screens respectively (Figure 9). When sufficient numbers (the aim was for a minimum of 5000) of spat between 2 - 3 mm screen size were available, spat were deployed to Smokey Bay, Ceduna, SA by ASI in 1.5 mm mesh size socks (Figure 10). Prior to deployment, sample spat were sent to Gribbles Veterinary (Glenside, SA) for pathology assessment and to confirm POMS free status.



Figure 8 Pacific Oyster (Magallana gigas) spat upwelling system at the South Australian Research and Development Institute, West Beach.



Figure 9 An example of Pacific Oyster (Magallana gigas) spat grading.



Figure 10 Spat of selectively bred Pacific Oyster (Magallana gigas) families ready for deployment to the field.

POMS challenge test of the 2019-year class

When this project was developed, field-based challenge tests within the Port River were being trialled. The original plan was to use this method to test POMS resistance of the selected families. However, low field mortality limited data inference and this challenge test method was abandoned in favour of the development of a lab-based challenge test. A lab-based challenge test has been commonly used to select for POMS resistance in European Pacific Oyster breeding programs (Degremont et al., 2015; Gutierrez et al., 2020).

The lab-based challenge test approach was developed in 2019 at the South Australian Aquatic Biosecurity Centre (SAABC), Roseworthy. The 2019-year class animals from families 206, 221, 235, 256 and 262 were included in the first formal trial and compared with three unselected commercial lines of Pacific Oyster spat. Approximately 250 animals from each family and unselected line were acclimated in one of nine 750L tanks of aerated seawater (35 ‰) at 17 °C at SAABC. 30 unselected donor Pacific Oysters of approximately 50 mm shell length were acclimated in a tenth 750 L tank. Oysters were fed daily either 15 L of a mixture of live algae (*Chaetoceros muelleri, Tisochrysis lutea,* and *Pavlova lutheri*) in approximately equal proportions to a combined cell count of ~8.5x10⁶ cells mL⁻¹ or equivalent algal paste (Reed Mariculture Shellfish Diet 1800[®]). Water was 100% exchanged three times weekly. Oysters were acclimated for 14 days. A viral extract using tissue from a Pacific Oyster from the 2018 Port River outbreak which returned a cycle threshold (CT) of ~18 when tested for OsHV-1 using the OIE Martenot qPCR assay (Martenot et al., 2010). The extract was prepared by homogenising 5 g of tissue with 2 mL of phosphate buffered saline (PBS). The viral extract was filtered through a 0.22 μ m filter, frozen in liquid nitrogen and stored at -80°C until use. A comparable viral extract of material from the initial NSW OsHV-1 outbreak provided by Dr Peter Kirkland (Elizabeth Macarthur Agricultural Institute, NSW DPI) was used as a positive control.

For the experiment, seven 750 L tanks were filled with seawater (35 g L⁻¹) at 17 °C at SAABC. Each tank was assigned 30 individual oysters from each family and unselected line, and a basket for donor oysters. Unselected donor Pacific Oysters of approximately 50 mm shell length were chosen randomly, relaxed using 50 g.L⁻¹ magnesium chloride in seawater (as permitted by <u>APVMA PER7250</u>), and injected with 0.1 mL of thawed Port River viral extract of 5×10^4 OsHV-1 DNA copies per 100 µL quantified by qPCR following Oden et al. (2011)ⁱ. Three of these donor oysters were added to 5 of the experimental tanks. Three donor oysters were injected with NSW OsHV-1 extract and placed in one 750L tank as a positive control and three donor oysters were injected with filtered Pacific Oyster homogenate confirmed by qPCR to be OsHV-1 free as a negative control and placed in one tank as a negative control. The temperature was raised over 2 days to 21-22 °C. Feeding was continued after exposure. The oysters were observed twice daily and assessed for morbidity and mortality for 21 days. Oysters were left in the system until dead and then were removed during water changes.

To visualize the results, Kaplan-Meier survival curves were generated using the *survival* package (Therneau and Grambsch, 2000; Therneau, 2015) and plotted using *ggplot* (Wickham, 2016) in *R* (R Core Team, 2021). A Weibull survival model was run to determine the effect of treatment (infected or control) and family on survival. This model was run in a Bayesian framework using integrated nested Laplace approximations (Rue et al., 2009) via the *R-INLA* package (Martins et al., 2013; Lindgren and Rue, 2015; Rue et al., 2017) with Treatment and Family as fixed effect and Tank as a random effect (= frailty in the context of survival analysis). Models including and excluding the Treatment x Family interaction were compared using the Watanabe-Akaike information criterion (WAIC) to determine whether the treatment response varied between families. Differences between families within the infected treatment were assessed using post-hoc tests implemented with the *inla.make.lincombs* function following Gomez-Rubio (2020). Family responses were considered significantly different where the 95% credible interval (CI) of the difference between coefficients did not include zero.

POSS challenge tests

Over 2019/2020, the first POSS field trial was undertaken using 2019-year class families produced in SA. SARDI prepared three replicate samples of 2 mm and 3 mm spat from each family (55 families) in 1.5 mm mesh size socks and delivered these to ASI who were responsible for deployment to the field, on farm maintenance and data collection. The same methods were used to test both SA 2020-year class (80 families)

and Tasmanian imported top 10 POMS resistant 2020-year class families in 2020/2021. In 2021/2022, the spat quality of the 2021-year class (67 families) was poor (see results, discussion, and conclusion for details) and the POSS trials were delayed until the spat were 7 mm and 12 mm in size to ensure only live, healthy spat were part of the trial. In 2022/23, spat quality returned to previous levels and replicates from the 2022-year class (41 families) were deployed at 2 mm and 3 mm screen size as described above. The full design and results of the POSS challenge tests will be detailed in the final report of FRDC project 2020-064 South Australian Pacific Oyster mortality trials.

Results, discussion, and conclusion

Family Production

The primary output for this project was the establishment of 60 full sibling (full-sib) Pacific Oyster families in 2019/20, 2020/21 and 2021/22, respectively. This was later extended to include the establishment of at least 30 full-sib Pacific Oyster families in 2022/23, which was achieved. Over the 2019/20 season, 55 families (2019-year class) were established and were sent to industry for grow-out and POSS field trials. SARDI procured extra larval rearing tanks and made up the shortfall (5 families) in the next season where 80 families (2020-year class) were produced.

Over the 2021/22 Pacific Oyster spawning season, SARDI produced 67 families (2021-year class), with >10,000 2-3mm spat per family, which were sent to ASI for grow out and POSS assessment. In comparison with the stock produced over the 2020/21 season, higher spat mortality and slower growth were observed within the 2021-year class after metamorphosis. After delivery to ASI and subsequent in field grading by ASI, a total of 59 families survived, of which 50 families had enough numbers to use in the breeding program. A key factor likely contributing to the poor spat performance at SARDI, and subsequent field survival, was poor water quality from the dredging at the West Beach boat ramp (concentrated at approximately -34.96, 138.50) adjacent to where the SARDI marine water intake pipes are located. Note, poor hatchery production of finfish was also experienced at West Beach over the dredging period. The pathology report on the spat supported the poor water quality hypothesis as the spat had dilation of the digestive gland lumens with extensive epithelial attenuation which can be caused by several environmental irritants, including copper toxicity, toxic algae, and decreased salinity. Samples of incoming water and storage tank sediment were collected on February 10th, 2022, and sent to the Australian Water Quality Centre (Adelaide) for testing, however no conclusive causative agent for the mortality could be identified. After the dredging activities at West Beach were finalised, an additional experiment to determine if charcoal filtration improved larvae/spat survival commenced in February 2022 using the same Pacific Oyster hatchery protocol that was used in the previous two seasons of family production and the broodstock for 2021 family production. The results were similar to those over the 2020/21 season, with >10,000 healthy 3mm spat produced in both treatments (N =3) four weeks after metamorphosis. After February 2022 it was also observed that there were no production issues with finfish fingerlings.

In the 2022/23 season, spat performance improved to a similar quality as the 2020/21 season, and 41 families (2022-year class) were established. During the project period, a total 237 Pacific Oyster families were produced and sent to industry for field POSS trials, of which 221 families had enough numbers to use in the breeding program, thus reaching the target number of families for the project.

The average POMS and POSS EBVs of the top five 2022-year class families produced at SARDI were 100% and 90%, respectively, whereas the averages of across the year classes were ~80% and ~70%, respectively.

Challenge tests

Initially, this project required the establishment of a reliable POMS challenge test system in SA for the provision of broodstock for commercial production of POMS resistant spat in SA. This changed during the

2021/22 season to focus on POSS in SA given that high POMS resistance was met through the importation of Tasmanian broodstock with > 90% resistance.

Field testing for POMS in SA was considered unreliable given low virus levels present in the Port River. In the POMS lab-based challenge test of the 2019-year class, the donor oysters (injected with OsHV-1) all died on day 2 or 3. Mortality commenced in the exposure oysters on day 4, and the oysters in the unselected lines had all died by day 12 (Figure 11). Some oysters survived 21 days. One control oyster died but tested negative for OsHV-1. There were no differences between the positive control and the Port River challenge response. Inclusion of the Treatment x Family interaction term in the survival model led to a decrease in WAIC of 2, providing minor support for inclusion of this term. The post-hoc analyses showed that mortality risk was greater for all unselected families than selected families, specifically that mortality risk for US1 > US2 > US3 > 256 > 206 = 221 = 235 > 262 (Figure 12). Although mortality did not occur as quickly in selected families, most experienced 100% mortality in the 21-day exposure. Family 262 had the highest survival with over 23% of oysters surviving to the end of the 21-day exposure.



Figure 11 Kaplan-Meier survival curve showing the South Australian Research and Development Institute 2019-year class (families 206, 221, 235, 256, 262) and unselected line (US1, US2, US3) survival when challenged with ostreid herpesvirus type one microvariants.



Figure 12 Weibull model survival probability estimates following exposure to ostreid herpesvirus type one microvariants for the South Australian Research and Development Institute 2019-year class (families 206, 221, 235, 256, 262) and unselected lines (US1, US2, US3).

The SARDI 2019-year class families survived OsHV-1 challenge significantly better than unselected lines, and, importantly, they took longer to die. Improvement in survival should be facilitated by incorporation into the breeding program of Tasmanian OsHV-1 resistant families (ten top families from the ASI 2020-year class) imported into South Australia in March 2021 which were quarantined and released to industry in April 2021. Further POMS challenge tests were not completed given the change in objective of this project.

Further tests will be required to establish the correlation in family POMS resistance ranks between the results from SAABC laboratory and direct field challenge tests. Disease resistance can vary between sites for families of selected oysters (Proestou et al., 2016) and assessing survival of SA-reared POMS resistant families should be an urgent priority following detection of OsHV-1 in any South Australian oyster growing region.

The results of the POSS challenge tests will be available in the final report of FRDC project 2020-064 South Australian Pacific Oyster mortality trials. Results of the POSS trials were used to select the broodstock for each generation of selective breeding.

Conclusions

This project met its objectives by establishing 221 new selectively bred Pacific Oyster families from 2019 to 2023. Incorporating the imported OsHV-1 resistant families of Tasmanian broodstock into the SA breeding program allowed the focus of this project to change from POMS resistance to improving POSS in SA while protecting the SA industry from losses when a POMS outbreak occurs in an oyster growing region. A successful partnership between SARDI, ASI and all other project partners through the Project Steering Committee was developed through this project allowing the establishment and continuation of the SA Pacific Oyster selective breeding node into 2023/24 and beyond.

Implications

This project has provided the SA oyster aquaculture industry with 221 new selectively bred Pacific Oyster families that achieved the target level of POMS resistance and substantially increased POSS in SA. The implications of these new families are the protection of the SA aquaculture industry from losses when a POMS outbreak occurs in an oyster growing region and the provision of spat with greater survival in SA waters. The strong relationship between SARDI, ASI and other project partners through the Project Steering Committee formed over the duration of this project implies greater collaboration in the future and a continuation of the SA Pacific Oyster selective breeding node.

In addition, a lab-based ostreid type 1 herpes virus (OsHV-1) challenge test has been established at SAABC, which could provide a unique opportunity to improve the POMS resistance at any post-metamorphosis stage or any time of the year when needed, although validation is needed to understand the correlation in family rank between laboratory and field test outcomes.

Recommendations

It is recommended that the SA Pacific Oyster selective breeding node continue beyond this project to maintain and further improve the SA EBV index which includes POMS resistance, POSS and other commercially important traits in for the SA oyster industry.

Extension and Adoption

Information derived from this project has been extended to key stakeholders including interested industry members, government departments and educational institutions.

The results of this project will also be available to and communicated to related state agencies (e.g., PIRSA Fisheries and Aquaculture), interested South Australian and Tasmanian oyster farmers and the public through this final report. Project updates have been provided at the SA POMS Working Group meetings over the project period.

Presentations of the most year class breeding results were given by Mr. Bryce Porker (ASI Regional Coordinator) at the annual meetings of SA Oyster Growers Association (SAOGA).

Project updates have also been provided in the ASI and SAOGA newsletters throughout this project.

The gains in POSS and POMS survival EBV's, and a high focus on breeding robust families with good commercial traits and appearance have resulted in the considerable increase in the demand for ASI spat in the SA market. Feedback received by ASI from all three SA based hatcheries is the preference and plan to produce more ASI thoroughbred this coming season than in the past. Recent SA mortality trial results have clearly identified that some ASI families have superior genetics when compared to others and survive considerably better in the event of environmentally driven SA mortality. Growers have heard this message from ASI and are very interested in buying ASI spat with high SA survival EBV's and this has been expressed to hatchery operators.

References

- Abbadi, M., Zamperin, G., Gastaldelli, M., Pascoli, F., Rosani, U., Milani, A., Schivo, A., Rossetti, E., Turolla,
 E., Gennari, L., Toffan, A., Arcangeli, G., Venier, P. (2018) Identification of a newly described OsHV-1 mu var from the North Adriatic Sea (Italy). *Journal of General Virology*, **99**(5), 693-703.
- Arzul, I., Nicolas, J.L., Davison, A.J., Renault, T. (2001) French scallops: A new host for ostreid herpesvirus-1. *Virology*, **290**(2), 342-349.
- Azéma, P., Lamy, J.B., Boudry, P., Renault, T., Travers, M.A., Dégremont, L. (2017) Genetic parameters of resistance to Vibrio aestuarianus, and OsHV-1 infections in the Pacific oyster, *Crassostrea gigas*, at three different life stages. *Genetics Selection Evolution*, **49**(1), 23.
- BDO-EconSearch (2022) The Economic Contribution of Aquaculture in the South Australian State and Regional Economies 2020/21. *A Report to PIRSA Fisheries and Aquaculture*, 1-63.
- Burge, C.A., Friedman, C.S., Kachmar, M.L., Humphrey, K.L., Moore, J.D., Elston, R.A. (2021) The first detection of a novel OsHV-1 microvariant in San Diego, California, USA. *Journal of Invertebrate Pathology*, **184**.
- Burioli, E.A.V., Varello, K., Lavazza, A., Bozzetta, E., Prearo, M., Houssin, M. (2018) A novel divergent group of Ostreid herpesvirus 1 mu Var variants associated with a mortality event in Pacific oyster spat in Normandy (France) in 2016. *Journal of Fish Diseases*, **41**(11), 1759-1769.
- Camara, M.D., Yen, S., Kaspar, H.F., Kesarcodi-Watson, A., King, N., Jeffs, A.G., Tremblay, L.A. (2017) Assessment of heat shock and laboratory virus challenges to selectively breed for ostreid herpesvirus 1 (OsHV-1) resistance in the Pacific oyster, *Crassostrea gigas*. Aquaculture, **469**, 50-58.
- De Kantzow, M.C., Hick, P.M., Dhand, N.K., Whittington, R.J. (2017) Risk factors for mortality during the first occurrence of Pacific Oyster Mortality Syndrome due to Ostreid herpesvirus – 1 in Tasmania, 2016. *Aquaculture*, **468**, 328-336.
- Degremont, L., Bedier, E., Soletchnik, P., Ropert, M., Huvet, A., Moal, J., Samain, J.F., Boudry, P. (2005) Relative importance of family, site, and field placement timing on survival, growth, and yield of hatchery-produced Pacific oyster spat (*Crassostrea gigas*). *Aquaculture*, **249**(1-4), 213-229.
- Dégremont, L., Lamy, J.B., Pépin, J.F., Travers, M.A., Renault, T. (2015) New insight for the genetic evaluation of resistance to ostreid herpesvirus infection, a worldwide disease, in *Crassostrea gigas*. *Plos One*, **10**(6), e0127917.
- Evans, O., Paul-Pont, I., Whittington, R.J. (2017) Detection of ostreid herpesvirus 1 microvariant DNA in aquatic invertebrate species, sediment and other samples collected from the Georges River estuary, New South Wales, Australia. *Diseases of Aquatic Organisms*, **122**(3), 247-255.
- Friedman, C.S., Estes, R.M., Stokes, N.A., Burge, C.A., Hargove, J.S., Barber, B.J., Elston, R.A., Burreson, E.M., Reece, K.S. (2005) Herpes virus in juvenile Pacific oysters *Crassostrea gigas* from Tomales Bay, California, coincides with summer mortality episodes. *Diseases of Aquatic Organisms*, 63(1), 33-41.
- Gomez-Rubio, V. (2020). Bayesian inference with INLA. Chapman and Hall/CRC, Boca Raton: 330 pp.
 Gutierrez, A.P., Bean, T.P., Hooper, C., Stenton, C.A., Sanders, M.B., Paley, R.K., Rastas, P., Bryrom, M., Matika, O., Houston, R.D. (2018) A Genome-Wide Association Study for Host Resistance to Ostreid Herpesvirus in Pacific Oysters (*Crassostrea gigas*). *G3 (Bethesda)*, **8**(4), 1273-1280.
- Hwang, J.Y., Park, J.J., Yu, H.J., Hur, Y.B., Arzul, I., Couraleau, Y., Park, M.A. (2013) Ostreid herpesvirus 1 infection in farmed Pacific oyster larvae *Crassostrea gigas* (Thunberg) in Korea. *Journal of Fish Diseases*, **36**(11), 969-972.
- Jenkins, C., Hick, P., Gabor, M., Spiers, Z., Fell, S.A., Gu, X.N., Read, A., Go, J., Dove, M., O'Connor, W., Kirkland, P.D., Frances, J. (2013) Identification and characterisation of an ostreid herpesvirus-1 microvariant (OsHV-1 mu-var) in *Crassostrea gigas* (Pacific oysters) in Australia. *Diseases of Aquatic Organisms*, **105**(2), 109-126.
- Keeling, S.E., Brosnahan, C.L., Williams, R., Gias, E., Hannah, M., Bueno, R., McDonald, W.L., Johnston, C. (2014) New Zealand juvenile oyster mortality associated with ostreid herpesvirus 1-an opportunistic longitudinal study. *Diseases of Aquatic Organisms*, **109**(3), 231-239.
- Lavens, P., Sorgeloos, P. (1996) Manual on the production and use of live food for aquaculture. *FAO Fisheries Technical Paper 361*, Food and Agriculture Organization of the United Nations, Rome, Italy.

- Lindgren, F., Rue, H. (2015) Bayesian spatial modelling with R-INLA. *Journal of Statistical Software*, **63**(19), 1-25.
- Martenot, C., Oden, E., Travaillé, E., Malas, J.P., Houssin, M. (2010) Comparison of two real-time PCR methods for detection of ostreid herpesvirus 1 in the Pacific oyster *Crassostrea gigas*. *Journal of Virological Methods*, **170**(1-2), 86-89.
- Martins, T.G., Simpson, D., Lindgren, F., Rue, H. (2013) Bayesian computing with INLA: new features. *Computational Statistics & Data Analysis*, **67**, 68-83.
- Mortensen, S., Strand, A., Bodvin, T., Alfjorden, A., Skar, C.K., Jelmert, A., Aspan, A., Saelemyr, L., Naustvoll, L.J., Albretsen, J. (2016) Summer mortalities and detection of ostreid herpesvirus microvariant in Pacific oyster *Crassostrea gigas* in Sweden and Norway. *Diseases of Aquatic Organisms*, **117**(3), 171-176.
- O'Reilly, A.J., Laide, C., Maloy, A., Hutton, S., Bookelaar, B., O'Sullivan, K., Lynch, S.A., Culloty, S.C. (2018) The role of the mussel Mytilus spp. in the transmission of ostreid herpesvirus-1 microVar. *Parasitology*, **145**(8), 1095-1104.
- Oden, E., Martenot, C., Berthaux, M., Travaillé, E., Malas, J.P., Houssin, M. (2011) Quantification of ostreid herpesvirus 1 (OsHV-1) in *Crassostrea gigas* by real-time PCR: Determination of a viral load threshold to prevent summer mortalities. *Aquaculture*, **317**(1), 27-31.
- Paul-Pont, I., Dhand, N.K., Whittington, R.J. (2013) Spatial distribution of mortality in Pacific oysters Crassostrea gigas: reflection on mechanisms of OsHV-1 transmission. Diseases of Aquatic Organisms, **105**(2), 127-138.
- Paul-Pont, I., Evans, O., Dhand, N.K., Rubio, A., Coad, P., Whittington, R. (2014) Descriptive epidemiology of mass mortality due to Ostreid herpesvirus-1 (OsHV-1) in commercially farmed Pacific oysters (*Crassostrea gigas*) in the Hawkesbury River estuary, Australia. *Aquaculture*, **422**, 146-159.
- Peeler, E.J., Reese, R.A., Cheslett, D.L., Geoghegan, F., Power, A., Thrush, M.A. (2012) Investigation of mortality in Pacific oysters associated with Ostreid herpesvirus-1 mu Var in the Republic of Ireland in 2009. *Preventive Veterinary Medicine*, **105**(1-2), 136-143.
- Pernet, F., Barret, J., Le Gall, P., Corporeau, C., Dégremont, L., Lagarde, F., Pépin, J.-F., Keck, N. (2012) Mass mortalities of Pacific oysters *Crassostrea gigas* reflect infectious diseases and vary with farming practices in the Mediterranean Thau lagoon, France. *Aquaculture Environment Interactions*, **2**(3), 215-237.
- R Core Team (2021) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.
- Renault, T., Bouquet, A.L., Maurice, J.-T., Lupo, C., Blachier, P. (2014) Ostreid herpesvirus 1 infection among Pacific oyster (*Crassostrea gigas*) spat: relevance of water temperature to virus replication and circulation prior to the onset of mortality. *Applied and Environmental Microbiology*, **80**(17), 5419-5426.
- Roque, A., Carrasco, N., Andree, K.B., Lacuesta, B., Elandaloussi, L., Gairin, I., Rodgers, C.J., Furones, M.D.
 (2012) First report of OsHV-1 microvar in Pacific oyster (*Crassostrea gigas*) cultured in Spain.
 Aquaculture, **324**, 303-306.
- Rue, H., Martino, S., Chopin, N. (2009) Approximate Bayesian inference for latent Gaussian models by using integrated nested Laplace approximations. *Journal of the Royal Statistical Society: Series B* (*Statistical Methodology*), **71**(2), 319-392.
- Rue, H., Rieble, A., Sørbye, S.H., Illian, a.B., Simpson, D.P., Lindgren, F.K. (2017) Bayesian Computing with INLA: A Review. Annual Reviews of Statistics and Its Applications, **4**, 395-421.
- Therneau, T. (2015) A Package for Survival Analysis in S. version 2.38 <u>https://CRAN.R-project.org/package=survival</u>.
- Therneau, T.M., Grambsch, P.M. (2000) *Modeling Survival Data: Extending the Cox Model*. Springer, New York.
- Wickham, H. (2016) ggplot2: elegant graphics for data analysis. Springer.

Appendices

Appendix 1

Project Steering Committee (2019 - 2023)

The Project Steering Committee was established with the following members:

Dr Michael Steer (SARDI; Committee Chair)

Mr Wayne Hutchinson (FRDC)

Dr Adam Main (PIRSA F&A)

Dr Shane Roberts (PIRSA F&A)

Mr Randal Bonner (Flinders Port)

Mr Gary Zippel (SA Oyster Growers Association)

Mr Matt Cunningham (Australian Seafood Industries)

Prof. Xiaoxu Li (SARDI; Project PI)