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Business Cooperative Research Centres Program

Future Oysters CRC-P: Final Report to the Department of Industry, Innovation & Science

(CRC-P50609 – CRC-P Round 1)





SM Clarke and M Cunningham (editors)

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Snapshot Summary

Introduction

The Future Oysters CRC-P project (CRC-P 2016-553805; Future Oysters) was funded by the Australian Government's Business Cooperative Research Centres (CRC) Program, which is managed by the Department of Industry, Innovation and Science (DIIS). The budget as documented in the agreement comprised a cash contribution by the Commonwealth through DIIS of $3,300,000, a cash contribution totalling $2,011,040 from the participants, and a total staff and non-staff in-kind contribution from participants of $6,292,189, which together add to a total project value of $8,303,229.

The Future Oysters CRC-P project was developed to undertake the research and development (R&D) needed to build and evolve the Australian oyster aquaculture industry, due to the severe impacts of disease on oyster production in Tasmania (TAS), South Australia (SA) and New South Wales (NSW) at the time of its initiation. The trigger for the project was the sudden spread of the viral disease ostreid herpesvirus-1 microvariant (OsHV-1), more commonly described as Pacific Oyster Mortality Syndrome (POMS), from NSW to TAS in February 2016. POMS rapidly affected 60% of the TAS production ($26 million per annum), with losses of approximately 100 jobs and $10 million of production at that time. POMS had already devastated Pacific Oyster farms in NSW in Botany Bay and Port Jackson (since 2010) and the Hawksbury River (since 2013). There was also growing potential for POMS to inflict further major losses on Australia's total oyster production ($104 million) given that 60% of this value was from Pacific Oyster production if it spread to SA, where farmers were already suffering from an 80% drop in spat supply because of biosecurity restrictions imposed on hatcheries supplying stock from TAS. Even without POMS, periodic unexplained mortalities, termed South Australian Mortality Syndrome (SAMS) were causing losses of Pacific Oysters in SA, and Sydney Rock Oyster farmers were also continuing to face substantial losses due to Queensland Unknown (QX) disease and Winter Mortality disease (WM).

This project's research and development (R&D) portfolio, developed in response to the substantial disease threats to the Australian oyster industry, with advice from Food Innovation Australia Ltd (FIAL), focused on accelerating the breeding of disease resistant oysters, improving the understanding of oyster diseases to improve farm management, increasing productivity and profitability, and diversifying risks to allow the industry to recover; and in future to grow domestic and international markets.

Governance

The Future Oysters CRC-P Management Committee and its "Terms of Reference' were established at the start of the project to provide effective administration. Membership comprised the key project participants: ASI the recipient of the project grant from DIIS; FRDC; Oysters Australia; representatives from the NSW, SA and TAS oyster industries; representatives of the key research participant in NSW, SA and TAS; a Chairperson and an Executive Officer, the latter appointed by the Committee. Twice yearly Management Committee meetings were held with agendas, briefing papers and minutes produced for each.

Quarterly progress reports were prepared for DIIS by the Future Oysters CRC-P Executive Officer who reviewed and collated contributions from Principal Investigators of each research project. The compiled report was approved and submitted to DIIS by the General Manager, Australian Seafood Industry (ASI). Copies of these reports were also provided to the Future Oysters CRC-P Management Committee and Oysters Australia for review and to the NSW, SA and TAS oyster industry peak bodies, as requested, for their information.

Financial information, both cash and in-kind, was sought from each partner as part of three monthly reporting to DIIS. The Executive Officer maintained a 'Summary Excel Spreadsheet' to collate the ongoing information, which provided the Future Oysters CRC-P Management Committee with the necessary data to manage the financials of the project. The Financial Officer, ASI also maintained a comprehensive record of project income and expenditure and organised the independent financial assessment at its completion.

Research

Research projects undertaken by the Future Oysters CRC-P were developed as applications submitted to the FRDC that evaluated and approved these in consultation with the Future Oysters CRC-P Management Committee. FRDC subsequently managed assessment and approval of project milestones and made payments to research agencies with funds recovered from ASI twice pa. Research was undertaken under three research themes, ‘Better Oysters’, ‘Healthy Oysters’ and ‘More Oysters’, with the following seven FRDC research related projects distributed within these.

Better oysters

2016-801 Enhancing Pacific oyster breeding to optimise national benefits

2016-802 Accelerated Sydney rock oyster breeding research

2016-803 New technologies to improve Sydney Rock Oyster breeding and production

Healthy oysters

2016-804 Advanced understanding of POMS to guide farm management decisions in Tasmania

2016-805 Polymicrobial involvement in OsHV-1 outbreaks (and other diseases)

2016-806 Advanced aquatic disease surveillance for known and undefined oyster pathogens

More oysters

2016-807 Species diversification to provide alternatives for commercial production

Two other FRDC projects addressed the ‘Management’ and ‘Communication and Extension’ of The Future Oysters CRC-P.

2016-800 Management and extension

2017-233 Communication and adoption

The overall focus of the Future Oysters CRC-P was on addressing Australian edible oyster disease related mortalities that were being experienced in NSW, SA and TAS, causing major impacts on regional economies and employment by negatively impacting on production and profitability.

The ‘Better Oysters’ theme targeted enhancing the selective breeding of Pacific Oyster to address POMS; accelerating existing Sydney Rock Oyster breeding research; and developing new technologies to improve Sydney Rock Oyster breeding and production.

The ‘Healthy Oysters’ theme focused on better understanding the nature of the key diseases of concern to the Australian edible oyster aquaculture industry, such as POMS, WM and SAMS, and how they can best be managed. The commissioned R&D projects targeted understanding the relationship between disease initiation and intensity and the Australian edible oyster growing environment and farm management practices. This was done so as to better guide farm management decisions and included determining the nature of the microbial community associated with aquacultured Australian edible oysters, establishing improved aquatic disease surveillance procedures for key known as well as unknown oyster pathogens, and developing more cost effective and rapid diagnostic techniques.

The ‘More Oysters’ theme focused on the interest of some Australian edible oyster aquaculture industry members to diversify the oyster species they farm so as to reduce the business risk of all their stock being negatively impacted by a single disease as well as to better utilise the diversity of habitat their existing leases often include. The commissioned R&D project targeted improving networking between Australian edible oyster aquaculture industry members interested in farming native Flat Oysters and/or Western/Sydney Rock Oysters and on-lease grow-out trials with select industry members in SA and TAS, so as to evaluate the growth and survival of native Flat Oysters in different farm environments.

Research Project Outcomes & Impacts

The key outcomes and impacts from each research theme for the Australian Sydney Rock Oyster and Pacific Oyster Industries were:

Sydney Rock Oyster Industry

Specific outcomes achieved from the ‘Better Oyster’ theme included: an acceleration in selective breeding by doubling the number of families produced, fertilisation success increased by 18%, improved storage techniques for gametes, and three million triploid oysters provided to industry. Collectively, the knowledge gained and the improvements to breeding achieved have been synthesized into a new plan for research that when validated is estimated to reduce the annual cost of breeding by 33%, that is from $350,000 to $250,000. This is expected to accelerate full adoption of the breeding program by industry and save $100,000 per annum on an ongoing basis. Overall, hatcheries are now estimated to be providing up to 20% of the stock used by industry, which equates to a farm gate value for hatchery stock of approximately $10 million per annum. The proportion of this stock derived from selective breeding is increasing and is expected to form the majority of hatchery seed supply in forthcoming seasons.

Specific outcomes achieved from the ‘Healthy Oysters’ theme included: an improved understanding of Sydney Rock Oyster WM disease based on a factually supported case definition to better understand the occurrence of WM, first time estimates of the heritability of WM resistance, optimisation of progeny tests for performance assessment and procedures for greater sampling efficiency; identification of the potential to address QX disease of Sydney Rock Oysters by selective breeding through molecular technologies, the use of seven identified gene expression markers and the use of a one-year breeding cycle that effectively doubles the rate of genetic progress for developing QX resistance; and an understanding of the potential to use microbial communities associated with oysters to enhance disease detection.

Pacific Oyster Industry

Specific outcomes achieved from the ‘Better Oysters’ theme included: establishment of an improved and biosecure breeding facility, improved rates of genetic improvement in the TAS population to fast track POMS resistance (about 10% per annum), improvement in-laboratory oyster POMS challenges, accelerated maturation, development of a SNP identification tool, and production of POMS resistant family lines and facilitation of an ongoing selective breeding program in SA.

Specific outcomes achieved from the ‘Health Oysters’ theme included: an improved understanding of when and where POMS is of higher risk through the development of a predictive model; improved POMS surveillance methodologies decreasing costs and enabling greater confidence in translocation testing and declarations of areas free from POMS; the establishment of a rapid, low cost, flow cytometry based POMS testing procedure, which has resulted in decreased surveillance costs, improved confidence about translocations and a faster response to address future incursions of POMS; and a greater understanding of SAMS.

Specific outcomes achieved from the ‘More Oysters’ theme targeted at diversifying the oyster species available to the Pacific Oyster industry included: the identification of optimal on-farm Flat Oyster farming techniques; enhanced communication between those interested in farming Flat Oysters; and the establishment of a translocation protocol to enable Western Rock Oyster translocation from WA to SA if the industry wishes to explore this opportunity. While species diversification continues to be of interest, industry members have different views as to the optimal farming and marketing approach, with further industry growth likely to be slow and niche orientated as alternative approaches are further evaluated.

Combined Industry Impact

An impact assessment of the Future Oysters CRC-P based on a range of assumptions and costing only some of the benefits, conservatively estimated that the about $8.3 million in total invested produced in present value terms an estimated total benefit of $126.6 million, net present value of $118.3 million, benefit-cost ratio of 15.3:1, internal rate of return of 187% and a modified internal rate of return of 38%. It was also estimated that if a POMS mortality event did occur across the Pacific Oyster growing areas in SA, the Future Oysters CRC-P would conservatively reduce its impact on the GVP in this state in the year it occurred by about $15.1 million.

Collaboration (including Education and Training)

As a result of the Future Oysters CRC-P, strong relationships were built between researchers from the research organisations involved and the industry participants and their organisations. About ten industry oyster farming companies directly supported components of the research undertaken on-farm and in extending it to the Australian oyster industry, the project outcomes were disseminated to greater than 300 'Small and Medium Enterprises' (SME), primarily oyster farmers.

The seven work experience, graduate and postgraduate students involved in the Future Oysters CRC-P benefited greatly from participating in aspects of the applied research and from opportunity for close interaction with industry, both on farm and at state based industry events.

Communications and Extension

Participants in the Future Oysters CRC-P provided information to populate the Australian oyster industries 'Oysters Australia Web Site' with project descriptions, updates and presentations on project progress and outcomes given. Researchers also attended annual oyster industry events in NSW, SA and TAS, with the three final 2019 events having a total of about 500 people attend.

To date, 17 scientific papers and reports have been published or are in press, in-review or at a late stage of preparation (with more expected to be produced over the 12-18 months following the project) and 16 posters and/or papers given at national and international scientific conferences. Over 130 industry communications have also taken place, these largely comprising presentations at conferences and workshop, newsletters and short articles, and video clips on Oyster Australia's web site. Some 20 media releases have also occurred.

Commercialisation and IP

The Future Oysters CRC-P research and development was structured so as be available to the Australian oyster industry through the direct transfer of research outcomes to the many industry participants within the project. This included the products (e.g. oyster broodstock, oyster family lines and triploid oyster spat) and services produced (e.g. improved surveillance procedures, improved hatchery biosecurity protocols and a rapid POMS diagnostic methodology using flow cytometry). As such, the Future Oysters CRC-P as a specific entity did not produce any products, services or IP that were marketed.

The Future Oysters CRC-P project data resides with Australian Seafood Industry (ASI) and the research organisation of the Principal Investigator of each FRDC project that comprised the Future Oysters CRC-P, except in a few instances where a small component of research was subcontracted to a university, whereby this data resides with the relevant university.

Benefits to Producers

At the completion of the Future Oysters CRC-P, the project impact assessment identified economically quantifiable benefits to the Australian edible oyster aquaculture industry in the areas of increased productivity and profitability, which flowed from:

- greater Pacific Oyster survival as a result of enhanced POMS resistance achieved through the ASI breeding programs in South Australia and Tasmania;

- improvements to Pacific Oyster and Sydney Rock Oyster survival as a result of enhanced breeding program methodologies, ; farm management and oyster production procedures; biosecurity and surveillance protocols; and a better understanding of the influence of the environment and microbiome on oyster health; and

- improvements to Flat Oyster growth as a result of improved farm management procedures.

The unquantified project benefits were identified as increased efficiency and capacity of future Australian edible oyster R&D and enhanced community well-being, which flowed from:

- strengthened Australian edible oyster aquaculture R&D networks across states, regions and industry participants and greater knowledge of edible oyster R&D associated with managing oyster diseases and health, including methodologies; and

- flow-on effects of a more productive, profitable and sustainable Australian edible oyster aquaculture industry on the social 'fabric' of Australian regional population centres and the environment where improved marine biodiversity and water quality are in part, respectively, a result of increased infrastructure that oyster farming adds to the marine environment and the filtering effect of the oysters as they feed.

In summary, the Future Oysters CRC-P has facilitated advancement of the Australian Oyster Industry resulting in:

- Tasmania: A re-energised and more efficient industry, with production approaching pre-POMS levels. A survey of TAS oyster farmers undertaken in May-July 2019 as part of FRDC Project No. 2016-804 (Crawford and Ugalde 2019), which included the question 'Impact of our research on your farming operations', identified that 79% of farmers rated the Future Oysters CRC-P research as having a high impact.

- New South Wales: An industry that is demonstrating new enthusiasm, with considerable improvements occurring in relation to hatcheries and on-farm technologies and production increasing.

- South Australia: A much better informed and prepared industry able to detect and address the associated issues if / when it is directly impacted on-farm by POMS.

- Other Parts of Australia: Other than in NSW, SA and TAS, the oyster industry is in an early development phase. WA in particular is presently seeking to develop a novel sub-tropical-tropical oyster industry, and in doing so is accessing and adapting information from the exiting Australian oyster industry to facilitate its growth and reduce risks. WA and NT oyster industry representatives were well represented at the NSW 2019 Oyster Industry Conference, in part, to capture the Future Oyster CRC-P outcomes presented at this.

Keywords

Pacific Oyster, *Crassostra gigas*, Sydney Rock Oyster, *Saccostrea glomerata*, Native Oyster, *Ostrea angasi*, Pacific Oyster Mortality Syndrome, Winter Mortality, South Australian Mortality Syndrome, aquaculture, disease, health, selective breeding, microbiome, farm management, growth, mortality.

1 General Introduction

1.1 Background

The Future Oysters CRC-P project was developed to undertake the research and development (R&D) needed to build and evolve the Australian oyster aquaculture industry, due to the severe impacts of disease on oyster production in New South Wales (NSW), South Australia (SA) and Tasmania (TAS) at the time of its initiation. The trigger for the project was the sudden spread of the viral disease ostreid herpesvirus-1 microvariant (OsHV-1), more commonly described as Pacific Oyster Mortality Syndrome (POMS), from NSW to TAS in February 2016. This rapidly affected 60% of the State's production ($26 million per annum), with losses of approximately 100 jobs and $10 million of production. At this time POMS had already devastated Pacific Oyster farms in NSW in Botany Bay and Port Jackson (since 2010) and the Hawksbury River (since 2013). There was also growing potential for POMS to inflict further major losses on Australia's total oyster production ($104 million), given that 60% of this value was from Pacific Oyster production, if it spread to South Australia, which was already suffering from an 80% drop in spat supply because of biosecurity restrictions imposed on hatcheries supplying stock from TAS. Even without POMS, periodic unexplained mortalities were causing losses of Pacific Oysters in South Australia (termed South Australian Mortality Syndrome - SAMS), and Sydney Rock Oyster farmers in parts of NSW were also continuing to face substantial losses due to Queensland Unknown (QX) disease and Winter Mortality disease (WM).

The R&D portfolio developed in response to the substantial disease threats to the Australian oyster industry, with advice from Food Innovation Australia Ltd (FIAL), were focused on accelerating the breeding of disease resistant oysters, improving disease management, increasing productivity and profitability, and diversifying risks with the production of new oyster species to allow the industry to grow and supply domestic markets and a growing global consumer demand for seafood.

1.2 Themes, Projects and Objectives

The Future Oysters CRC-P project, as approved by the Australian Government Department of Industry, Innovation and Science (DIIS), was structured to encompass three themes, which were supported through nine R&D projects managed through the Australian Government, Fisheries Research and Development Corporation (FRDC).

1.2.1 Theme 1: Better Oysters

Objective: Advance genetic selection and progeny testing to accelerate availability of disease resistant Pacific and Sydney Rock Oysters.

Three research project were developed to address Theme 1:

FRDC Project No. 2016-801: Enhancing Pacific Oyster breeding to optimise national benefits

Objectives:

- Design and implement a selective breeding strategy for Australian Seafood Industry (ASI) that meets the immediate and medium term (five year) needs of the national Pacific Oyster industry.
- Identify biosecurity constraints to the movement of ASI stock and develop a strategy to permit optimal flow of benefits across the national industry.
- Review, document and communicate protocols and procedures for the use of OsHV-1 exposed broodstock by hatcheries and the transfer of resulting progeny compliant with State regulations.

- Redefine the protocols for the laboratory family spat challenger model to improve the predictability of field survival, with the goal of a 70% correlation between the laboratory and field tests and to extend the application of the challenge model to include challenges to larvae.
- Develop a system, supported by general purpose algorithms that will allow ASI to routinely benchmark the estimated breeding values of ASI POMS resistant families against commercial performance of hatchery stock of known pedigree after exposure to OsHV-1 at different life stages.
- Document and implement a strategy to allow use, within the breeding program, of males and female broodstock at one year.
- Develop and verify a single-nucleotide polymorphism (SNP) based genetic test that can discriminate ASI oysters from non-ASI oysters and to identify oysters to family and implement a plan for this test to be commercially available to stakeholders.

FRDC Project No. 2016-802: Accelerating Sydney Rock Oyster (SRO) breeding research

Objectives:

- By 2019, to have doubled the number of family lines currently planned for the SoCo breeding program
- To reduce the generation time for QX and winter mortality disease resistance to one year.
- To have confirmed the value of "stress markers" in selective breeding of Sydney Rock Oysters.

FRDC Project No. 2016-803: New technologies to improve Sydney Rock Oyster breeding and production

Objectives:

- 20% of industry with access to triploid Sydney Rock Oysters.
- Reduce complete hatchery operation costs by 15% through a reduction in time for oyster conditioning.
- Increase Sydney Rock Oyster breeding program reliability.

1.2.2 Theme 2: Healthy Oysters

Objectives:

- Employ novel methods to assess oyster health and manage diseases.
- Identify environmental factors implicated in oyster diseases.

The objectives of Theme 2 were addressed with the following three research projects:

FRDC Project No. 2016-804: Future oysters CRC-P: Advanced understanding of POMS to guide farm management decisions in Tasmania

Objectives:

- To determine:
    - the periodicity of infection of OsHV-1 virus in TAS;
    - advance the understanding of the drivers of POMS disease outbreaks; and
    - develop a predictive framework that allows the TAS oyster industry to forecast danger periods for POMS.
- To develop farm husbandry and handling protocols to maximise oyster production in POMS infected growing areas by investigating oyster survival in relation to:
    - subtidal versus intertidal culture;
    - high water flow areas compared with low flow;
    - reducing handling;
    - size and timing of spat onto grow-out farms,;and
    - stocking density.
- 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.

FRDC Project No. 2016-805: Polymicrobial involvement in OsHV-1 outbreaks (and other diseases)

Objectives:

- Define microbial communities associated with oysters and identify threats.
- Link changes in environmental conditions to changing microbial communities.
- Better understand the association between microbial communities and disease.

FRDC Project No. 2016-806: Advanced aquatic disease surveillance for known and undefined oyster pathogens

Objectives:

- Winter Mortality disease (WM): causative agent investigation, case definition, management strategies, improved husbandry and validate WM resistance assessments for Sydney Rock Oysters.
- South Australian Mortality Syndrome (SAMS): causative agent investigation, improved understanding of causes, case definition, improved diagnostic technologies and improved husbandry to maximise survival for Pacific Oysters.
- Pacific Oyster Mortality Syndrome (POMS): improved surveillance for early detection, to manage spread and understand transmission, novel detection and enumeration method based on flow cytometry.

1.2.3 Theme 3: More Oysters

Objectives:

- Assess commercial potential of alternative species to diversify production.
- Develop and assess new technologies to increase production and profitability.

The objectives of Theme 3 were addressed with the following research project:

FRDC Project No. 2016-807: Species diversification to provide alternatives for commercial production

Objectives:

- Native Flat Oysters:
    - develop native Flat Oyster on-farm growing methods that maximise survival and growth in SA and TAS;
    - compare the performance between Pacific Oysters and native Flat Oysters in SA; and
    - establish a native Flat Oyster farmers network to share new techniques and knowledge.
- Western Rock Oysters:
    - develop translocation protocols for the safe translocation of Western Rock Oysters to SA; and
    - trial Western Rock Oysters in the field in SA to assess the performance and viability of a potential industry if agreed by industry and regulators.

1.2.4 Project Management and Communication

In addition to the three research themes, management and communication functions of the Future Oysters CRC-P were addressed with the following two projects:

FRDC Project No. 2016-800: Future oysters CRC-P: Management and extension

Objectives:

- Establish the Future Oysters CRC-P and its governance structures.
- Manage the Future Oysters CRC-P budget.
- Develop CRC-P Communication, extension and education and training plans.
- Monitoring and evaluation progress of Future Oysters CRC-P research project and approving milestone payments.
- Maintain cohesion and strategies direction of the Future Oysters CRC-P.
- Coordinate and deliver on reporting requirements to DIIS.
- Ensure that Future Oysters CRC-P delivers on its DIIS milestones as per the Funding Agreement.

FRDC Project No. 2017-233: Future oysters CRC-P: Commercialisation and adoption

Objectives:

- Effectively communicate findings and activities of all Future CRC-P R&D projects to industry, other researchers and stakeholders.
- Conduct specific extension activities that provide opportunities for growers to be exposed to findings of Future Oysters CRC-P R&D to maximise adoption and impact.
- Develop and maintain communication media and methods that effectively deliver Future Oysters CRC_P findings in a manner that is most effective for industry.
- Ensure attendance of CRC-P members at annual oyster industry conferences in each state to most effectively present findings and meet with and seek feedback from growers.
- Engagement of honours students with CRC-P research and development of their capacity to work effectively with industry.

1.3 Methods

Research projects undertaken by the Future Oysters CRC-P were developed as applications submitted to the FRDC that evaluated and approved these in consultation with the Future Oysters CRC-P Management Committee. FRDC subsequently managed assessment and approval of project milestones and made progress payments to research agencies with funds recovered from ASI (the CRC-P industry applicant) twice pa.

The methods used in the Future Oysters CRC-P projects varied greatly. Some projects involved a combination of hatchery, field and laboratory R&D (e.g. 2016-801 - Future oysters CRC-P: Enhancing Pacific Oyster breeding to optimise national benefits and 2016-802 - Future oysters CRC-P: Accelerating Sydney Rock Oyster (SRO) breeding research), whereas others were predominately laboratory orientated (e.g. 2016-803 - Future oysters CRC-P: New technologies to improve Sydney Rock Oyster breeding and production; 2016-805 - Polymicrobial involvement in OsHV-1 outbreaks (and other diseases); and 2016-806 - Advanced aquatic disease surveillance for known and undefined oyster pathogens) or field work (e.g. 2016-804 - Advanced understanding of POMS to guide farm management decisions in Tasmania and 2016-807 - Species diversification to provide alternatives for commercial production). All projects involved industry engagement, either with collaborating oyster farmers in the field, where research was both on and off lease and/or in various industry forums where the results of R&D were disseminated and discussed. Industry forums were facilitated by 2016-800 - Future Oysters CRC-P: Management and extension and 2017-233 - Future oysters CRC-P: Commercialisation and adoption).

2 Governance and Management

2.1 Background

The Future Oysters CRC-P involved the recipient, ASI and 14 project partners, the Fisheries Research and Development Corporation (FRDC); the Commonwealth Scientific and Industrial Research Organisation (CSIRO); the Department of Primary Industries and Regions South Australia (PIRSA) through the South Australian Research and Development Institute (SARDI); Flinders University; Macquarie University; the Department of Industry Skills and Regional Development through the New South Wales Department of Primary Industries (NSW DPI); Oysters Australia; the Select Oyster Company; The Yield Technology Solutions; the University of Adelaide; the University of Newcastle; the University of Sunshine Coast (USC); the University of Tasmania (UTAS) though the Institute of Marine and Atmospheric Science (IMAS); and the University of Technology Sydney (UTS). To effectively manage the activities of such a large group, key early objectives were to establish an appropriate governance structure and terms of reference so to ensure sound management of the project, including its R&D. milestones, budgets and communications.

2.2 Governance

Project agreements for the Future Oysters CRC-P existed at a number of levels. The overarching agreement was between the DIIS and the ASI, the recipient. ASI then established an agreement with the FRDC to administer a portfolio of research and development projects to deliver the required outcomes and outputs of their agreement with DIIS. To achieve this, FRDC established project agreements with each of the key participating research organisations. Where these participating organisations sought the delivery of services from other research organisations, they in turn established sub-agreements to deliver components of individual projects.

The Future Oysters CRC-P project was managed by the Future Oysters Management Committee, with the support of an Executive Officer who liaised regularly with the Principal Investigators of the projects. Most projects also involved a number of Co-Investigators and other research and technical staff and some included students. The Principal Investigators had primary responsibility for planning and managing research activities within their project to delivery upon agreed project objectives and outputs.

2.2.1 Future Oysters Management Committee

The composition of the Future Oyster Management Committee, which changed over time, comprised:

- a senior representative of ASI, the recipient of the grant for the Future Oysters CRC-P project: Mr Matt Cunningham, General Manager;
- a representative of FRDC: Jo-Anne Ruscoe then Mr Wayne Hutchinson, Research Portfolio Manager;
- a representative of Oysters Australia Pty Ltd the national oyster industry body: Ms Sue Grau, Executive Officer, Oysters Australia (and Oysters Tasmania);
- an industry representative from each participating state (NSW, SA and TAS): Mr Ian Duthie, Managing Director, SeaPerfect Pty Ltd, TAS; Mr Tony Troup, Owner/Operator, Camden Haven Oysters Pty Ltd, NSW and sometimes proxy Ms Caroline Henry, Owner/Operator, Wonboyn Oysters Pty Ltd, NSW; Ms Trudy McGowan and then Mr Judd Evans, Executive Officer, South Australian Oyster Growers Association (SAOGA) and South Australia Oyster Research Council (SAORC);

- a senior representative of each participating key state (NSW, SA and TAS) research organisation: Dr Wayne O'Connor, Senior Principal Research Scientist, NSW DPI; Prof. Gavin Begg, Research Director, SARDI; and Prof. Chris Carter, Deputy Executive Director and Academic Director, IMAS and sometime proxy Dr Christine Crawford, Senior Research Fellow, IMAS;
- the Chairperson appointed by the Committee: Prof.. Graham Mair, Senior Lecturer, Flinders University, followed by Mr Matt Cunningham, General Manager, ASI; and
- the Executive Officer appointed by the Committee: initially Mr Wayne Hutchinson, Oysters Australia and finally Mr Steven Clarke, Science Initiatives and Support Manager, SARDI.

2.2.2 Principal Investigators

The relevant Principal Investigator was the primary manager of each FRDC project, having responsibility for the project's activities and for the participating Co-Investigators, other research and technical staff, and in some instances students. The Principal Investigators of the nine associated FRDC projects that comprised the Future Oysters CRC-P were:

- Mr Matt Cunningham, General Manager, ASI (FRDC Project No: 2016-800, 2016-801 and 2017-233);
- Dr Michael Dove, Senior Research Scientist, Fisheries Research, NSW DPI (FRDC Project No: 2016-802 and 2916-803);
- Dr Christine Crawford, Senior Research Fellow, IMAS/UTAS (FRDC Project No: 2016-804);
- Prof. Justin Seymour, Ocean Microbiology Group Leader, Integrated Marine Observing System (IMOS) and NSW Node Leader, Climate Change Cluster, UTS DPI (FRDC Project No: 2016-805);
- Dr Marty Deveney, Marine Pests Subprogram Leader, Marine Ecosystem Program, SARDI (FRDC Project No: 2016-806); and
- Prof. Xiaoxu Li, Aquaculture Program Leader, SARDI (FRDC Project No: 2016-807).

When requested, Principal Investigators attended Future Oyster Management Committee meetings to provide more detailed face-to-face information.

2.3 Reporting

2.3.1 Future Oysters Management Committee

The Future Oysters Management Committee was established at the start of the project and a 'Terms of Reference' developed, which included that the Committee would meet at least twice yearly and that minutes of the meetings would be produced, both which occurred over the duration of the project.

The at least twice yearly meetings were arranged to best align with the Future Oysters CRC project schedule and with the availability of the members of the Committee. Typically they were held face-to-face and were of half to one-day in duration, with an occasional member dialling-in if unable to attend in person.

A comprehensive agenda with associated papers, including relevant three and six monthly reports and for the final year of the project, a 'Communication Database' and 'Student Database', were provided for each meeting, along with invited Principal Investigators, so that Committee members were kept fully informed and contributed to any project or budget changes/decisions required.

2.3.2 Quarterly Reporting to DIIS

Quarterly progress reports were provided by the Principal Investigators to the Executive Officer who collated them and forwarded them to Mr Matt Cunningham, AIS to review and sign and, typically to return to the Executive Officer to forward to DIIS. As required by the CRC-P Program Reporting Requirements these quarterly reports included:

- an update of progress against the Project and each relevant research theme Milestone;
- financial contribution and expense details;
- identification of any other matters that may affect compliance with the Funding Agreement; and
- the Program Data Questionnaire with each report that coincided with the end of the financial year.

For the final year of the project the 'Communication Database' and 'Student Database' were also provided along with each quarterly report.

All quarterly reports were completed, although a number were submitted to DIIS slightly late because of delays in receiving relevant technical or financial information from the 14 CRC-P participants. It was the Executive Officer's responsibility to follow-up information that was outstanding, review what was provided, and address any amendments sought by the Executive Officer, AIS, the Future Oysters Management Committee and/or DIIS.

2.3.3 Six Monthly Reporting to FRDC

Technical progress reports were provided each six months by the Principal Investigators to FRDC for each FRDC project. The collation, review and dissemination process was as for quarterly reporting to DIIS, but in this instance focused on the progress, financials and milestones of the FRDC projects.

All six monthly FRDC reporting was completed, with some amendments made to aspects of projects at various times, with these approved by the Future Oysters CRC-P Management Committee.

2.3.4 Final Reporting to FRDC

A comprehensive final technical report was completed for each of the seven FRDC R&D projects by their respective Principal Investigator in association with their Co-Investigators. FRDC agreed that the final reporting relating to the two projects: "Management and Extension" and "Communication and Adoption" would be adequately addressed by covering these topics in the final DIIS report.

Each FRDC report was completed in line with the FRDC template created for this purpose and reviewed by the Future Oysters CRC-P Executive Officer and / or FRDC representative, as well as by the Principal Investigator's institution through their formal 'Publication Review Process'.

2.3.5 Final Reporting to DIIS

This report, the final report for DIIS, addressed the requirements of the guidelines provided for this purpose and was reviewed by FRDC and DIIS. In general, the information contained was collated from the FRDC final technical reports but presented in less detail, particularly that relating to the research and development undertaken.

2.3.6 Financial Management and Budget Provisions

As highlighted above financial reporting was included with quarterly reports to DIIS and six monthly reports to FRDC, this covering both cash and in-kind contributions by all participants. In addition to providing the data available at the time of reporting, the Executive Officer also maintained a 'Working Excel Spreadsheet' that incorporated all the data for each participant, as well as summarising it for all. This spreadsheet provided the capacity to capture any late data submitted or any subsequent amendments once an error was detected and addressed in association with the relevant participant, so as to provide detailed reporting for the purposes of the Future Oysters CRC-P Management Committee. ASI's Financial Manager maintained a database of the cash income received from the Commonwealth Government and project participants, as well as the funds they distributed to participants as per the FRDC project agreements for the costs they incurred.

2.3.7 Management of Intellectual Property (IP)

During the Future Oysters CRC-P the Principal Investigator of each FRDC project maintained an IP register, as did the Executive Officer. However, little of relevance was recorded as the Future Oysters CRC-P research and development was structured so as be available to the Australian oyster industry through the direct transfer of research outcomes to the many industry participants within the project. This included the products (e.g. oyster broodstock, oyster family lines and triploid oyster spat) and services produced (e.g. improved surveillance procedures, improved hatchery biosecurity protocols and a rapid POMS diagnostic methodology using flow cytometry). As such, the Future Oysters CRC-P as a specific entity did not produce any products or services for market or retain any IP.

The Future Oysters CRC-P project data resides with ASI and the research organisation of the Principal Investigator of each FRDC project that comprised the Future Oysters CRC-P, except in a few instances where a small component of research was subcontracted to a university, whereby this data resides with the relevant university.

2.4 Assets

The Future Oysters CRC-P did not purchase any significant assets and as such none needed to be dispersed at the end of the project.

2.5 Independent Audit Statement

The ASI as the grant recipient had overall responsibility for the management of the finances of the Future Oysters CRC-P, but facilitated this by establishing and involving the Future Oysters CRC-P Management Committee. At the end of the project the AIS arranged an independent audit of the Future Oysters CRC-P as required by DIIS.

The independent audit is still in progress but is expected to confirm that the Future Oysters CRC received and expended the budget as documented in the agreement, which comprising a cash contribution by the Commonwealth of $3.3 million, a cash contribution of about $2.011 million from the participants, and a staff and non-staff in-kind contribution from participants of about $6.292 million; a total of about $8.303 million.

The independent audit document will be completed separately to this report.

3 R&D Project Results

3.1 Introduction

The Executive Summary for each of the seven FRDC R&D reports are reproduced here under the relevant theme "Better Oysters", "Healthy Oysters" or "More Oysters"; each full report can be accessed from the reference provided if further information is sought. FRDC agreed to forgo the two final reports relating to "Management and Extension" (2016-800) and "Communications and Adoption" (2017-233) based on the relevant information relating to these being incorporated in this report.

3.2 Theme 1: Better Oysters

3.2.1 FRDC Project No. 2016-801: Future oysters CRC-P: Enhancing Pacific Oyster breeding to optimise national benefits (Principal Investigator, Mr Matt Cunningham, ASI).

Executive Summary:

This report details the research undertaken as part of the FRDC project 2016-801 - Enhancing Pacific Oyster Breeding to Optimise National Benefits. This was undertaken as part of the Future Oysters Cooperative Research Centre Project (CRC-P 2016-553805; Future Oysters), conducted as part of the Australian Government's Cooperative Research Centres Program. The project was led by ASI with collaborating researchers from CSIRO, IMAS/UTASSARDI, Flinders University and the NSW DPI. Project activities occurred across the three major oyster producing states of Australia, NSW, SA and Tasmania TAS.

The research was conducted as a direct consequence of the 2016 TAS POMS outbreak that decimated parts of this State's Pacific Oyster (*Crassostrea gigas*) industry and caused numerous flow-on effects throughout the entire Australian industry. The project was aimed to allow continuation and improvement of the work that had been undertaken prior to the 2016 outbreak, which was not only a major disruptor to the industry but also the breeding program. New techniques needed to be established to allow continued breeding in TAS in the new POMS paradigm and operations were required to be established in SA due to the biosecurity restrictions brought about by the TAS outbreak. Aspects of the project also looked to increase the rate of genetic gains for POMS resistance by developing additional supporting technologies.

The project was conducted across multiple areas that reflected the objectives of the project. Researchers worked collaboratively to conduct research across breeding strategy development, capacity building in SA, genetic improvement, laboratory and field challenges, accelerated maturation and developing an identification tool.

The results have allowed the ASI selective breeding program to most importantly improve genetic resistance to POMS and allowed these results to be available to all Pacific Oyster growing regions. Specifically, the results of this project have allowed:

- continued breeding in TAS by development of a biosecure breeding facility at the IMAS aquaculture facility. Family production has continued at the same level prior to POMS and enabled ASI to breed from Pacific Oysters which have been expose to the virus. The facility now has an approved biosecurity plan which has been externally audited and signed off by the Tasmanian Chief Veterinary Officer (CVO);

- production of 160 families in SA to enable POMS resistant broodstock to be deployed to the SA oyster industry. A project has been developed which allows further breeding in South Australia to deliver breeding outcomes for the medium term;

- improved rates of genetic improvement in the TAS population to fast track POMS resistance. The program has now developed and implemented spat POMS challenge trials that have increased gains;
- improvement in laboratory challenges which have increased correlations to field survival and a laboratory challenge at IMAS, which has been an important precursor to future work on genomics;
- accelerated maturation that has allowed increased usage of one year old animals in the ASI breeding program, which have been shown to offer increased rates of genetic gains in POMS resistance; and
- development of an SNP identification tool which can differentiate between ASI and non ASI stock and different ASI families.

The results have had a massive impact on the Australian Pacific Oyster industry. In TAS there has been a significant recovery of the industry since the 2016 outbreak and this can be substantially attributed to the POMS resistant broodstock developed as part of this project. The recovery is almost complete and has resulted in the industry recovering to full stocking and employment levels within three years of the outbreak. This is internationally unprecedented, with other international industries taking much longer to recover. The growers in TAS are returning to profitability and now have a very positive outlook for the future. In SA the production and commercialization of ASI family lines will see that industry partially insulated from the effects of POMS if / when it reaches the growing regions. Whilst POMS resistance is currently lower in farm stock in SA than TAS, the stocking of these oysters on farms can avoid the crippling losses experienced in TAS during the 2016 outbreak. The knowledge of this "insurance policy" has allowed the industry in SA to invest in their businesses with greater confidence.

The other aspects of this project have allowed the breeding program to increase the rate of genetic gains and these have had direct impacts on Australian Pacific Oyster growers.

Reference: Cunningham, M., Kube, P., Trotter, A., Li, X., Kirkland, P., Robson, N., Smith, G., and Carter, C. (2020). Future Oysters CRC-P: Enhancing Pacific Oyster breeding to optimise national benefits. Final Fisheries Research and Development Corporation report, FRDC Project No. 2016-801, 87 pp. ISBN 978-0-646-81759-0

3.2.2 FRDC Project No. 2016-802 Accelerated Sydney Rock Oyster (SRO) Breeding Research (Principal Investigator, Dr Michael Dove, NSW DPI).

Executive Summary:

This project focussed on increasing genetic resistance of SOCo breeding program Sydney Rock Oyster (*Saccostrea glomerata*) families to QX disease and WM disease. NSW DPI has worked collaboratively with SOCo to develop a Sydney Rock Oyster family-based breeding program (BP) to replace the mass selection program used to develop fast growth and disease resistance since 1991. Family-based breeding has a number of distinct advantages over mass selection including; increased genetic gains, ability to select for disease resistance under biosecure conditions, improved selection methods for multiple traits, better estimates of genetic gains and trends as well as control over inbreeding. Annual family breeding runs commenced in 2014 to establish the SOCo breeding program. An FRDC project (No. 2015-230) provided genetic expertise to establish and refine breeding methodology for a family-based breeding program. The next step was greater understanding of the genetic parameters for QX and WM disease and how these related to other traits under selection, growth and meat condition. Genetic progress could be achieved by increasing the numbers of families available for selection, improved understanding of the genetic architecture of traits and reducing the length of breeding cycles for disease resistance. NSW DPI, SOCo, genetic specialists at CSIRO and oyster researchers at Macquarie University developed a multidisciplinary research program to deliver genetic progress for the SOCo breeding program.

The aims of this project were to:

- double the number of families produced for the SOCo breeding program from 80 to 160;
- halve the generation time for selection to QX and winter mortality resistance;
- determine the heritability of the trait of winter mortality resistance in Sydney Rock Oysters; and
- determine whether marker-assisted selection using reverse transcription can be incorporated into the SOCo breeding program.

Annual family production was approximately 40 when this project started and was increased to nearly 80 as a consequence of this project. Families in the 2014 and 2015 year classes continue to rank highly and are available for breeding runs taking the total number of families available to industry to more than 250.

Commercial hatchery production of Sydney Rock Oysters is more difficult compared to other species such as Pacific and Eastern Oysters. Further complexities are encountered when producing Sydney Rock Oyster families due to fertilisation deficiency in particular crosses, low rates of success when fertilisation occurs and poor levels of larval development in the period immediately after fertilisation. The increase in annual family production in this project was achieved through numerous stepwise improvements to protocols, facilities, husbandry and staff training. Whilst significant advances have been made, this area remains a high priority for further research. Improvements to guarantee better fertilisation success rates and larval yield in the early stages of a breeding run will further boost the number of Sydney Rock Oyster families possible in a single breeding run to a number well beyond 80. The ability to efficiently produce more families can provide increased genetic gains and program reliability in the future.

A one-year breeding cycle was successfully introduced for QX resistance, effectively doubling the rate of genetic progress for this trait. This required the use of quantitative genetics to understand the genetic architecture of traits, and knowledge of the reproductive and QX disease cycles to determine a design that considered the logistics of all breeding program operations, including those for the other primary traits of growth and meat condition. This understanding allowed the production of Sydney Rock Oyster families to be advanced so that spat from SOCo families could be challenged to QX during the season following production allowing data to be collected and breeding decisions to be made within 12 months.

During this project, WM disease expression through field exposures was low and inconsistent across multiple year classes and sites. This reduced the level of discrimination between family survival. WM resistance in Sydney Rock Oysters has low to moderate heritability, and thus would potentially respond to genetic selection. However, the rate of gain is likely to be lower for this trait than for traits such as QX resistance and growth rate. No correlations were found between WM resistance and other primary traits under selection (QX resistance, meat condition and growth). The best estimates were obtained from the Quibray Bay site using survival data measured in December from one year-old oysters. It is recommended that the SOCo breeding program continue WM field exposure trials of one year-old oysters at Quibray to obtain further data if resources permit. A decision about including WM resistance as part of the SOCo breeding objective is required. This decision will need to be made in consultation with industry and with consideration of the impact of how this will influence gains in other traits. Whilst that decision has not been made, the knowledge generated from this project has enabled this process to be done.

This project developed a method to sample oyster tissue for genetic analysis that does not affect oyster reproduction or survival. Seven gene expression markers were identified that could be incorporated into the breeding program once further validation steps are completed. Quantitative polymerase chain reaction (qPCR) is a cost-effective method for screening large numbers of oysters and can provide results in sufficient time to make breeding decisions. Marker-assisted selection has the potential to increase genetic gains for QX resistance by enabling within family selection, and this project has identified candidate markers. Further development is required to determine if these genetic markers can identify susceptible individuals and to incorporate marker-assisted selection into the current logistics and schedule of the breeding program.

Major changes have been made to breeding logistics and are now used in the SOCo breeding program to increase genetic gains for QX disease resistance. The SOCo breeding program is operational using targeted multi-trait selection to increase the rate of genetic gain. Breeding goals have been set by industry based upon the need for SOCo to have a commercially viable product that provides clear benefits to oyster growers. The breeding goals are for SOCo stock to demonstrate 70% survival through a QX disease outbreak, 30% growth advantage compared to wild oysters, and no difference in condition compared to wild oysters. Estimates have been made on the timelines to achieve these goals, using data generated from this project and elsewhere, and these goals will be met in one to five years, depending on the trait in question. The commercial release of oysters with 70% QX disease resistance is expected in March 2020, 30% growth advantage over wild oysters is in March 2021 and oysters with no change in condition is in March 2024. These time frames are conservative estimates and can be reduced by using a more aggressive selection strategy if required. This project changed the scale, logistics and schedule of the SOCo BP to reduce overall operating costs yet increase the genetic gains for QX disease resistance and now offers industry a viable risk mitigation strategy against disease impacts.

Reference: Dove, M., Kube, P., Lind, C. Cumbo, V., Raftos, D. and O'Connor, W. (2019). Accelerated Sydney Rock Oyster (SRO) Breeding Research. Final Fisheries Research and Development Corporation report, FRDC Project No. 2016-802, 33 pp. ISBN 978-1-76058-356-9

3.2.3 FRDC Project No. 2016-803: New technologies to improve Sydney Rock Oyster breeding and production (Principal Investigator, Dr Michael Dove, NSW DPI).

Executive Summary:

Hatchery production of Sydney Rock Oysters (*Saccostrea glomerata*) is a costly and high risk activity for the breeding program and industry exacerbated by factors such as: reliance on hatchery conditioning, low fertilisation success using strip-spawned gametes, extended larval rearing period compared to Pacific Oysters (*Crassostrea gigas*), and variable settlement rates. This project, one of a number that comprised the Future Oysters Cooperative Research Centre project (Future Oysters CRC-P), was developed through discussions with the Sydney Rock Oyster industry hatchery sector and was designed to target specific hatchery production challenges.

Four fundamental components were identified for research and development:

- production of tetraploid Sydney Rock Oysters for triploid hatchery production;
- decreasing the time frame and increasing the reliability of hatchery conditioning;
- producing a spawn inducing factor(s)/pheromone to trigger natural release of gametes; and
- physiological process that occurs following oocyte release to extend the duration of viability through the use of benchtop storage media.

Each component listed above is designed to either decrease hatchery operation costs, increase production reliability or increase the market for hatchery produced seed. The highest priority for further development of the Sydney Rock Oyster Breeding Program (BP) is increasing the success rate of single pair mated crosses. Research for production of tetraploid Sydney Rock Oysters, producing a spawn inducing factor and investigating oocyte viability will produce valuable information that can be directly applied to increase the success rate of single pair mated crosses.

Tetraploid inductions were performed by Southern Cross Shellfish (SCS) and the NSW DPI. Tetraploid inductions were not successful in producing a batch of tetraploid Sydney Rock Oyster that could be made available for commercial hatchery production of triploids using tetraploid male and triploid female crosses. Eleven attempts were made and the major challenges encountered was low egg numbers, asynchronous embryonic development in strip-spawned oocytes after fertilisation, poor development and poor larval survival. A very small number of spat were successfully settled from two trials, however, no tetraploids were found in these batches when oysters had reached a size where tissue could be taken to determine ploidy level.

Although the original milestones related to the tetraploid research were not achieved, there have been some significant outcomes from this Future Oysters CRC-P project with respect to production of triploid Sydney Rock Oysters and making these available to industry. Successful chemical triploid batches produced for the CRC-P tetraploid work have resulted in distribution of triploids from QX disease-resistant broodstock to industry. NSW DPI used this opportunity to commercially evaluate triploids and measured QX disease resistance and oyster growth performance. Although no QX disease occurred during field evaluations, triploid oysters had significantly faster growth rates compared to a group of selected 2017 year class (YC) families and non-selected oysters.

Sydney Rock Oysters generally require ten weeks of hatchery conditioning to obtain suitable gametes for a successful spawning. Hatchery conditioning requires significant hatchery resources in terms of algae, energy and labour over this period. Reducing the hatchery conditioning period and improving hatchery conditioning reliability with respect to producing ready-to-spawn broodstock reduces the financial impost for Sydney Rock Oyster hatcheries.

Results from three independent trials performed by the University of the Sunshine Coast (USC), revealed that administering the individual forms of buccalin and APGWamide stimulated gonad conditioning in Sydney Rock Oyster. Comparing the stimulatory effect of two different forms of APGWamide, APGWa and RPGWa, USC found that the APGWa form was more potent than the RPGWa form. For buccalin, the buccalin-G form showed a better performance than the buccalin-A form in most of the reproductive activities assessed. Considering the stimulatory effects of APGWa and buccalin-G on Sydney Rock Oyster conditioning, their stimulatory effects appear to be comparable. Hence, either APGWa or buccalin-G can be used on Sydney Rock Oyster broodstock for both breeding runs and commercial spat production. With respect to peptide delivery, we found that using cocoa butter to allow a slow release of peptide was not appropriate as it caused high mortalities. Injection of peptides is therefore preferable and multiple injections are expected to help in maintaining the level of the peptides in the oyster's circulatory system in order to successfully control gonad conditioning. Yet, delivery of peptide by injection is relatively difficult and uncontrollable since different individual oysters could receive different amount of the neuropeptide per injection. To overcome this problem, delivery using other techniques such as, oral delivery by using peptide-encapsulated algae, should be considered and tested in the future.

Spawning inducing factor/pheromone was found to be present in Sydney Rock Oyster sperm by USC. Sydney Rock Oyster sperm was isolated and proteins semi-purified, giving two major extract groups - the intrinsic and extrinsic sperm membrane proteins. Further purification was performed using RP-HPLC, resulting in multiple fractions of sperm membrane proteins. Crude extracts and RP-HPLC fractions were tested in a spawning induction bioassay, in which fully mature oysters were treated with the crude extract or fraction, prior to observation of spawning activity over the period of 2 h. We found that proteins extracted from the extrinsic sperm membrane, but not the intrinsic sperm membrane, could successfully induce spawning in the Sydney Rock Oysters. Further purification of positive extrinsic membrane fraction S3 minute 41-45 led to sub-fractions that were also tested, resulting in two positive sub-fractions (at 6-10 min and 11-15 min). The MS analysis of each revealed 8 proteins, including: aminopeptidase N; calmodulin; 60 kDa SS-A/Ro ribonucleoprotein; protein bark beetle-like; helicase; failed axon connections homolog; nascent polypeptide-associated complex subunit alpha; and, a novel protein. We propose that one, or more of these proteins may play a critical role in stimulating spawning of the Sydney Rock Oyster.

Another objective of this project related to a straightforward method for benchtop storage of Sydney Rock Oyster gametes. This work was done by The University of Newcastle (UoN) and required an understanding of the causes of oocyte degradation and to develop improved storage protocols for oocytes that extend the holding period in vitro after strip-spawning gametes. Specifically, the objective reported on here was to evaluate up to three methods for the improved benchtop storage of Sydney Rock Oyster gametes. This objective was achieved, but further work will continue to evaluate further approaches to gamete storage as part of a continuing PhD project. Typically, Sydney Rock Oyster oocytes show the best fertilisation rates if fertilised within 24 h of stripping; afterwards, the success decreases exponentially. Results in this experiment suggest that vitality can be improved if these characteristics are altered in the media, and when additives are included (such as antibiotics and polyvinylpyrrolidone (PVP)). This is a significant step in extending the life of a cell for breeding purposes, effectively extending the window in which quality testing and fertilisations can be conducted.

Research is ongoing for tetraploidy induction techniques (SCS), neuropeptide delivery to broodstock (USC), spawn inducing factors (USC) and oocyte storage for Sydney Rock Oysters (UoN). Further research is required to incorporate all findings from this research into routine operations of the Sydney Rock Oyster BP. However, outcomes from this work are already providing benefits for this breeding program, notably technical improvements to the fertilisation process. This has increased family production success from 27% to 45%. This outcome is relevant for the Sydney Rock Oyster BP and commercial hatcheries as it reduces the time it takes to create families and the numbers of valuable broodstock required for a breeding or commercial hatchery run.

Reference: Dove, M., Suwansa-ard, S., Elizur, A., Seeto, R., Clulow, J., Gibb, Z., Abramov, T., O'Connor, S., Kent, G. and O'Connor, W. (2020). New technologies to improve Sydney Rock Oyster breeding and production. Final Fisheries Research and Development Corporation report, FRDC Project No. 2016-803, 74 pp. ISBN 978-1-76058-360-6

3.3 Theme 2: Healthy Oysters

3.3.1 FRDC Project No. 2016-804: Advanced understanding of POMS to guide farm management decisions in Tasmania (Principal Investigator, Dr Christine Crawford, IMAS/UTAS).

Executive Summary:

The Tasmanian Pacific Oyster aquaculture industry was severely impacted by an outbreak of the disease ostreid herpesvirus OsHV-1, known as 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' was approved in August 2016 by DIIS, Australian Government to address the disease and production issues in the oyster industry. As part of this program, 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 Future Oysters 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 on-growing 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 to be 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.

Reference: Crawford, C. and Ugalde, S. (2019). Future oysters CRC-P: Advanced understanding of POMS to guide farm management decisions in Tasmania. Final Fisheries Research and Development Corporation report, FRDC Project No. 2016-804, 61 pp. ISBN Print 978-1-922352-09-5

3.3.2 FRDC Project No. 2016-805: Polymicrobial involvement in OsHV outbreaks (and other diseases) (Principal Investigator, Prof. Justine Seymour, UTS).

Executive Summary:

The principal goal of this research was to provide a detailed characterisation of the oyster microbiome and identify links between specific features of the microbiome and oyster disease and mortality events. The conceptual framework for this work is based upon: (i) increasing evidence, across a broad range of species, that the nature of a host organism’s microbiome exerts a fundamental control on host physiology and health; and (ii) the critical paucity in knowledge on the factors contributing to oyster health and the triggers for oyster mortality events and disease outbreaks. The research reported here involved a collaboration between the University of Technology Sydney (UTS) and the NSW Department of Primary Industries (DPI), whereby the UTS members of the team provided expertise in molecular microbial ecology and the DPI team members provided expertise and support in oyster physiology and ecology and aquaculture. The research involved a large-scale screening of the microbiomes of both Pacific Oysters and Sydney Rock Oysters using high-throughput DNA sequencing technologies, providing a characterisation of the microbial communities associated with oysters. The outcomes of this analysis revealed that for both Pacific Oysters and Sydney Rock Oysters, the oyster microbiome is remarkably variable among different oyster families, and over space and time, indicating that both intrinsic physiological features of the oyster host and environmental factors play a role in governing the oyster microbiome. Notably, despite this heterogeneity, a small sub-set of the microbiome was shown to be conserved across oysters within a species, pointing to the existence of a core group of microbes with intrinsic links to oyster ecology and condition. Similarly, a small group of microbes, including members of the Vibrio genus, were consistently associated with diseased or susceptible oysters, indicating a potentially antagonistic role of these microbes. These observations support the hypothesis that the oyster microbiome plays a role in defining oyster health, but also reveal substantial complexities related to the marked heterogeneity of the oyster microbiome over space and time. Appropriately considering this microbiome heterogeneity, while also sharpening focus on the few core microbiome members identified in this research, will be important requisites for future efforts hoping to employ the oyster microbiome for diagnostic purposes.

Background

During the last two decades a number of disease outbreaks have led to mass oyster mortalities and the closure of several oyster-harvesting regions, resulting in multi-million dollar losses. These outbreaks mirror a global pattern of increased aquaculture disease, with disease emergence potentially linked to environmental degradation (pollution) and climate change related processes, such as rising seawater temperature. Within NSW estuaries, multiple microbiological agents have been implicated in oyster diseases, but a clear understanding of the ecological and environmental drivers of disease outbreaks has remained elusive. This means we cannot currently predict when outbreaks will occur, making it very difficult to manage infection events and develop strategies to mitigate future oyster disease events.

Across a wide-range of animal and plant systems, including several benthic marine organisms, there is growing evidence that the structure and function of a host organism's microbiome - the community of microorganisms living in prolonged association with the host macroorganism - plays a fundamental role in the physiology and health of the host and its susceptibility to disease. Shifts in a host organism's microbiome (dysbiosis) can either precede or follow measurable symptoms of syndromes and/or disease, with examples of both microbiome shifts causing disease or occurring in response to disease on-set.

There is a growing recognition for the potential importance of the microbiome in oyster health and physiology (Trabal et al., 2012; Wegner et al., 2013; Lemire et al., 2015; Lokmer and Wegner, 2015; Petton et al., 2015; Lokmer et al., 2016b; de Lorgeril et al., 2018; King et al., 2019b), with emerging evidence suggesting that the oyster microbiome might be directly related to oyster disease dynamics (Wegner et al., 2013; Lokmer and Wegner, 2015; de Lorgeril et al., 2018; Green et al., 2018; King et al., 2019a). However, the factors governing the structure of the oyster microbiome are very poorly resolved, with very little, to no, understanding of the inherent characteristics of a "healthy oyster microbiome" or the identity of core beneficial vs pathogenic microbes within the oyster microbiome. This lack of knowledge currently precludes the use of the oyster microbiome as a diagnostic marker for oyster health or disease status.

Aims/objectives

The three over-arching Objectives of this research were to:

- define microbial communities associated with oysters and identify potential microbial threats;
- link changes in environmental conditions to shifts in the oyster microbiome; and
- better understand the association between the oyster microbiome and disease.

These Objectives gave rise to the following more specific Aims, which evolved as the project progressed:

- Aim 1: Characterise the composition of the Pacific Oyster microbiome across diverse oyster families, including those exhibiting different levels of susceptibility to OsHV-1 μvar disease.
- Aim 2: Define the composition of the Sydney Rock Oyster microbiome across diverse oyster families, including breeding lines generated for resistance to QX disease, and examine spatial and temporal heterogeneity in microbiome structure
- Aim 3: Examine spatial heterogeneity in Pacific Oyster microbiome structure at the individual oyster level and across regional-scales
- Aim 4: Define the Sydney Rock Oyster microbiome associated with QX disease events
- Aim 5: Measure temporal patterns in the Pacific Oyster Microbiome during the Summer OsHV-1 Mortality Period
- Aim 6: Elucidate patterns in Vibrio community diversity and abundance within the microbiomes of oysters subject to disease and mortality events.

Methodology

To address our Aims we focussed our research on two of the major commercial oyster species in Australia, the Pacific Oyster and Sydney Rock Oyster, with a focus on diseases affecting these species, namely OsHV-1 and QX disease respectively.

Our approach involved a tiered characterisation of the oyster microbiome, which included:

- characterising the "base-line" microbiome of Pacific Oysters and Sydney Rock Oysters;
- examining variability in the oyster microbiome across diverse family/breeding lines, including families exhibiting differing levels of susceptibility to OsHV-1 and QX disease;
- defining spatial and temporal variability in Pacific Oyster and Sydney Rock Oysters microbiomes across a continuum of scales, ranging from comparisons across different oyster tissues and between different estuaries;
- measuring patterns in Pacific Oyster and Sydney Rock Oysters microbiomes associated with disease outbreaks and mortality events; and
- targeted screening of oysters for microbiome members putatively involved in oyster disease or mortality.

Throughout the course of this project we characterised the microbiomes associated with Pacific Oysters and Sydney Rock Oysters using 16S rRNA amplicon sequencing, which is currently the optimum approach for defining the diversity and composition of a microbiome. Briefly, this technique involves extraction of microbial DNA from oyster samples, amplification of the bacterial 16S rRNA gene and Illumina miSeq sequencing of the amplified DNA. This technique provides an inventory of the bacterial composition and diversity within a sample (a list of Operational Taxonomic Units; OTUs), allowing for inter-microbiome comparisons and the identification of specific discriminatory or indicator microorganisms. Using a suite of multidimensional statistical analyses we identified patterns in oyster microbiome structure across environments, over time and between different oyster breeding lines. This approach allowed us to both identify members of the "core oyster microbiome" and organisms most responsible for the discrimination of different oyster microbiomes.

Results

The key findings of this research included:

- the identification of a small sub-set of "core members" of the oyster microbiome, including members of the Spirochaetaceae family that were conserved over a continuum of spatial and temporal scales, which may be indicative of key oyster-associates that play a role in oyster physiology and health;
- significant heterogeneity in both the Pacific Oyster and Sydney Rock Oyster microbiomes over space and time, indicating that local environmental factors govern the structure of the oyster microbiome;
- variability in the oyster microbiome across different oyster family-lines, and between different oyster tissue types (e.g. gill, mantle adductor muscle, etc.) indicating that intrinsic genetic and physiological features of the oyster host also govern microbiome structure; and
- sub-sets of the oyster microbiome that were differentially prevalent in Pacific Oyster and Sydney Rock Oyster family-lines with differing levels of susceptibility to OsHV-1 and QX disease respectively, indicating that certain members of the oyster microbiome may either facilitate or protect the oyster from infection.

Implications and Recommendations for Relevant Stakeholders

The outcomes of this research indicate the highly dynamic nature of the Pacific Oyster and Sydney Rock Oyster microbiomes and in some cases point to a potentially significant role of the oyster microbiome in governing oyster health and susceptibility to disease. This, on the one hand, suggests that the oyster microbiome may have substantial utility as a new diagnostic measure of oyster health, but on the other hand, the inherent heterogeneity of the oyster microbiome observed here means that it may be difficult to identify and subsequently use universal community signatures or indicator organisms across oyster microbiomes originating from different environments or genetically dissimilar oyster stocks. We therefore suggest that while the incorporation of characterisation of the oyster microbiome into assessments of oyster condition has substantial promise, care should be taken to ensure that data is collected and interpreted using a context-specific (e.g. environment, oyster genetic stock) approach.

Reference: Seymour, J.R., Labbate, M., O'Connor, W., Jenkins, C., Dove, M., King, W., Siboni, N. and Nguyen, V.K. (2019). Polymicrobial involvement in OsHV-1 outbreaks (and other diseases). Final Fisheries Research and Development Corporation report, FRDC Project No. 2016-805, 225 pp. ISBN ISBN 978-0-646-80891-8

3.3.3 FRDC Project No. 2016-806: Advanced aquatic disease surveillance for known and undefined oyster diseases (Principal Investigator, Dr Marty Deveney, SARDI).

Executive Summary:

This project improved understanding of methods for surveillance for several diseases of farmed oysters. Surveillance is a critical component of biosecurity and aquatic animal health activities. Surveillance is used to assess health status of animal populations, and provides evidence to support claims of freedom from disease or to determine disease prevalence. Effective surveillance also increases the likelihood that a new or emergent disease can be detected early and controlled.

Mollusc diseases are less well understood than terrestrial animal and many finfish diseases and this project sought to develop understanding of three oyster diseases of substantial economic impact in the Australian edible oyster aquaculture industries: POMS, SAMS, and WM.

POMS is caused by the virus OsHV-1 microvariant, and has emerged worldwide since 2008. Information on virus transmission was however lacking, and diagnostic sensitivity and specificity of tests used for OsHV-1 detection were undefined. Improvements in time-to-diagnosis were also required to support management and business continuity when responding to mortalities in OsHV-1 free areas. Development of the National Biosecurity Guidelines for Australian Oyster Hatcheries required rapid diagnostic capacity to provide real-time understanding of risk and surveillance for hatcheries producing OsHV-1 free stock in disease-affected zones. Mortalities of Pacific Oysters in POMS-free areas in South Australia (SA), termed SAMS, have been described since the 1980s but remain poorly understood, with no causative agent or major risk factors identified, despite development of a case definition and investigation of mortality events. WM is a disease of Sydney rock oysters, but despite disease characteristics being described nearly 100 years ago, had no case definition or identified causative agent. The lack of information about SAMS and WM created confusion about what management actions could decrease losses and disruption to business activities, ultimately limiting capacity to act positively to manage these syndromes.

The project included activities with four main aims:

- improving understanding of tests for OsHV-1 and investigation of using these tests for area surveillance;
- development of a low-cost, rapid test for OsHV-1;
- refinement of the case definition and investigation of the cause of SAMS in Pacific Oysters; and

- development of a case definition and improving understanding of the cause of Winter Mortality in Sydney Rock Oysters.

The project provided diagnostic sensitivity (DSe) and specificity (DSp) data for two qPCR tests for OsHV-1 when testing oyster tissue and showed that these tests could also be applied to environmental samples, providing a non-destructive approach for OsHV-1 area surveillance. This information enables improved design and interpretation of surveys for OsHV-1, including improved sampling and testing criteria for hatchery and zone or compartment certification, understanding disease status in OsHV-1 affected areas and monitoring transmission during outbreaks.

A flow cytometry test to detect and quantify OsHV-1 was developed and optimised. The test is particularly applicable to testing seawater. Analysis of infected oysters using this test showed that OsHV-1 concentrations in infected oysters from the Port River are up to $3x10^7$ mL^{-1}, indicating that the viral load released from infected areas would be at least in the 10^{10}-10^{15} viruses per day range. The critical applications of this test are to assess water in hatcheries after intake treatment and to test outflow water prior to discharge. This testing can contribute to making self-declarations of freedom and protect surrounding environments from outbreaks where a hatchery might be the first site of infection and therefore the primary source of virus in a previously free area.

One SAMS event occurred during the study period, hampering investigations. The project identified reference ranges for biochemical values in Pacific Oysters, however, which provide a baseline for understanding 'normal' Pacific Oyster health. These biochemical values also provide information for cases in which mortality or disease is observed but where conventional tools cannot identify a causative agent. SAMS and other mortalities were characterised by few consistent pathological signs, but changes in bacterial communities in oysters were detected during mortalities. SAMS is likely to have multifactorial causes, but the outcomes of this project contribute to refining knowledge about the factors that contribute to SAMS.

Examinations of WM in Sydney Rock Oysters identified a case definition based on historical information and cases investigated during this project:

Mortalities in 18+ month old *Saccostrea glomerata* between July and November, from Port Stephens to the southerly reaches of the range of *S. glomerata*, in water with higher than average salinity. Affected cohorts display gross lesions consisting of brown or yellow pustules on the external surfaces of the gonad, mantle, palps or gills or internally in the adductor muscle or gut epithelium. The lesions are focal and consist of accumulations of haemocytes.

Investigations of these events indicated that microcells such as *Bonamia spp.* and *Mikrocytos spp.* that have been proposed as causative agents were not consistently associated with mortalities, and *Perkinsus spp.* were not detected in any oysters. Prevalent *Vibrio splendidus* isolates from WM cases, however, contain mobile genetic elements that are linked to capacity to cause disease, and the environmental conditions in which WM occurs induces physiological changes in these bacteria. Changes in bacterial communities, however, are inconsistent and may be symptomatic rather than causative. WM is complex and is probably multifactorial, but the case definition and pathology identified in this project facilitate better surveillance and disease investigations, including refining understanding of the contributing factors.

Reference: Deveney, M.R. and Wiltshire, K.H. (eds) (2020). Future Oysters CRC-P: Advanced aquatic disease surveillance for known and undefined oyster diseases. Final Fisheries Research and Development Corporation report, FRDC Project No. 2016-806, 223 pp. ISBN 978-1-876007-24-9

3.4 Theme 3: More Oysters

3.4.1 FRDC Project No. 2016-807: Species diversification to provide alternatives for commercial production (Principal Investigator, Prof. Xiaoxu Li, SARDI).

Executive Summary:

Pacific Oyster Mortality Syndrome (OsHV-1 microvariant; POMS) results in high and rapid mortality in Pacific Oysters (*Crassostrea gigas*) and has been responsible for significant economic loss to oyster industries in Australia and around the world. The diversification of commercial production into different oyster species (native Flat Oysters and Western Rock Oysters), not susceptible to POMS, has been proposed as a way to mitigate the risk of POMS in southern Australia. However, the Australian native Flat Oyster (*Ostrea angasi*) industry is still in its infancy, with knowledge gaps along the production chain. Additionally, Western Rock Oysters (*Saccostrea sp*.) have never been commercially produced in South Australia and translocation policies to move them around the state are non-existent. This project aimed to improve on-farm production of native Flat Oysters and develop safe translocation protocols for Western Rock Oysters in South Australia, in order to help Australian oyster growers diversify into these species.

On farm trials were run in South Australia and Tasmania to develop grow-out methods that maximise survival and growth of juvenile native Flat Oysters. The effect of farm location, site (high and low energy; intertidal and subtidal) and growing height on native Flat Oyster performance was evaluated. A cohabitation study of Western Rock Oysters with Pacific Oysters and native Flat Oysters was run to assess the risk of bringing Western Rock Oysters into existing South Australian oyster growing regions.

The results of the native Flat Oyster farm trials were different between South Australia and Tasmania, however, in both cases growing height had a significant effect on native Flat Oyster growth. In South Australia, subtidal treatments had the slowest growth, whereas in Tasmania, Native Flat Oysters grown at the highest intertidal height had the least growth. With the exception of one South Australian treatment, native Flat Oyster survival was high (> 95%) across the on farm trials in both South Australia and Tasmania.

The high survival and growth rates (less but comparable to Pacific Oysters in South Australia) observed in the on farm native Flat Oyster trials indicate that diversification into this species could be a viable option to mitigate the risk of POMS. The findings from this project will help to advance the native Flat Oyster industry in Australia. Additionally, this project established a native Flat Oyster farmers' network for the sharing of knowledge and methodologies related to native Flat Oyster aquaculture. Preliminary results from the on farm trials have been disseminated to this farmers' network through the three industry workshops held in August – September 2019 in NSW, SA and TAS, including dedicated Angasi Workshops in NSW and SA, which were well attended (30-40 people at each). Native Flat Oyster on farm trials are continuing in Tasmania assessing the effects of stocking density and rotational movement between growing heights on native Flat Oyster growth and condition.

Reference: Li, X., Miller-Ezzy, P., Crawford, C. and Deveney, M. (2020). Future oysters CRC-P: Species diversification to provide alternatives for commercial production. Final Fisheries Research and Development Corporation report, FRDC Project No. 2016-807, 65 pp. ISBN 978-1-876007-27-0

4 Communications and Extension

The researchers associated with the Future Oysters CRC-P disseminated information to the Australian oyster industry from very early in the project until the end, where they contributed significantly to the annual industry conferences in NSW, SA and TAS in August-September 2019. In addition to this, researchers also opportunistically provided presentations at a number of scientific conferences to ensure peer review by their colleagues, with scientific publications now beginning to be flow (more are expected over the next 12-18 months). Most projects released media information on occasion to also keep the wider community aware of the Future Oysters CRC-P and the important role this was playing in delivering the Australian oyster industry's high priority R&D.

To date, 17 scientific papers and reports have been published or are in press, in-review or at a late stage of preparation and 16 posters and/or papers given at national and international scientific conferences. Over 130 industry communications have also taken place, these largely comprising presentations at conferences and workshop, newsletters and short articles and video clips on Oyster Australia's web site. Some 20 media releases have also occurred, these ranging from short radio to television interviews to reasonably lengthy segments on such ABC TV programs such as Landline.

4.1 Scientific Papers and Reports Published, Submitted or In-review

Clarke, S.M and M. Cunningham. (eds). 2020. Future Oysters CRC-P: Final Report (CRC-P50609 – CRC-P Round1). Final Department of Industry, Innovation and Science report. Australian Seafood Industries, Hobart, Tasmania. 65 pp.

Cunningham, M., Kube, P., Trotter, A., Li, X., Kirkland, P., Robson, N., Smith, G., and Carter, C. 2020. Future Oysters CRC-P: Enhancing Pacific Oyster breeding to optimise national benefits. Final Fisheries Research and Development Corporation report, FRDC Project No. 2016-801, 87 pp.

Crawford, C. and Ugalde, S. 2019. Future oysters CRC-P: Advanced understanding of POMS to guide farm management decisions in Tasmania. Final Fisheries Research and Development Corporation report, FRDC Project No. 2016-804, 61 pp.

Deveney, M.R. and Wiltshire, K.H. (eds) 2020. Future Oysters CRC-P: Advanced aquatic disease surveillance for known and undefined oyster diseases. Final Fisheries Research and Development Corporation report, FRDC Project No. 2016-806, 223 pp.

Dove, M., Kube, P., Lind, C. Cumbo, V., Raftos, D. and O'Connor, W. 2019. Accelerated Sydney Rock Oyster (SRO) Breeding Research. Final Fisheries Research and Development Corporation report, FRDC Project No. 2016-802, 33 pp.

Dove, M., Suwansa-ard, S., Elizur, A., Seeto, R., Clulow, J., Gibb, Z., Abramov, T., O'Connor, S., Kent, G. and O'Connor, W. 2020. New technologies to improve Sydney Rock Oyster breeding and production. Final Fisheries Research and Development Corporation report, FRDC Project No. 2016-803, 74 pp.

Green, T.J., Siboni. N., King, W.L., Labbate, M., Seymour, J.R. and Raftos, D. 2019. Simulated marine heat wave alters abundance and structure of Vibrio populations associated with the Pacific oyster resulting in a mass mortality event, Microbial Ecology 77, 736-747.

Heijkoop1, A.C., Mahadevan1, J.J. and Pyecroft, S.B. (In preparation). Towards the development of a biochemical reference range for Pacific Oysters (*Crassostrea gigas*) haemolymph.

Khue, V.N., King, W. L., Siboni, N., Khandaker, M., Dove, M.C., O'Connor, W.A., Seymour, J. R. and Labbate, M. (In preparation). Haemolymph microbiome of the Sydney rock oyster breeding lines for resistance to QX disease from Port Stephens and Wallis Lake. NSW, Australia.

King, W.L., Jenkins, C., Go, J., Siboni, N., Seymour, J. R. and Labbate, M. 2018. Characterisation of the Pacific Oyster microbiome during a summer mortality event. Microbial Ecology 10.1007/s00248-018-1226-9.

King, W.L., Jenkins, C., Seymour, J.R. and Labbate, M. 2019. Oyster disease in a changing environment: decrypting the link between pathogen, microbiome and environment. Marine Environmental Research 143, 124-140.

King, W.L., Siboni, N., Kahlke, T., Dove, M., O'Connor, W., Mahbub, K.R., Jenkins, C., Seymour, J.R., and Labbate, M. (Under review). Multiscale heterogeneity in the *Crassostrea gigas* microbiome.

King, W.L., Siboni, N., Williams, N., Kahlke, T., Khue, V.N., Jenkins, C., Dove, M., O'Connor, W., Seymour, J.R. and Labbate, M. 2019. Variability in the composition of Pacific Oyster microbiomes across oyster family lines exhibiting different levels of susceptibility to OsHV-1 μvar disease. Frontiers in Microbiology 10, 473.

Li, X., Miller-Ezzy, P., Crawford, C. and Deveney, M. 2020. Future oysters CRC-P: Species diversification to provide alternatives for commercial production. Final Fisheries Research and Development Corporation report, FRDC Project No. 2016-807, 65 pp.

Nguyen, V.K., King, W.L., Siboni, N., Mahbub, K.R., Dove, M., O'Connor, W., Seymour, J and Labbate, M. 2019. The Sydney rock oyster microbiome is influenced by local environmental parameters and QX disease resistance. Aquaculture and Marine Biology, 25-26 March, Paris, France.

Seymour, J.R., Labbate, M., O'Connor, W., Jenkins, C., Dove, M., King, W., Siboni, N. and Nguyen, V.K. 2019. Polymicrobial involvement in OsHV-1 outbreaks (and other diseases). Final Fisheries Research and Development Corporation report, FRDC Project No. 2016-805, 225 pp.

Suwansa-ard, S., Dove, M., Wang, T., Sabbatini, Abramov, T., Liang, D., O'Connor, W., Cummins, S. and Elizur, A. (In preparation). Identification of spawning inducing pheromones in the sperm of the Sydney rock oyster, *Saccostrea glomerata*.

4.2 Science Conference Posters and Abstracts

Crawford, C. 2019. Major impacts of POMS disease on Pacific Oyster farming in Australia. Perth, Western Australia.

Cunningham, M. 2018. Mono-seed oyster farming and selective breeding to counter POMS in Australia. Taiwan International Fisheries and Seafood Show/Aquaculture Forum, Taiwan, 21 November 2018.

Deveney, M.R., Perera, R. and Jones J.B. 2016. Aquatic disease biosecurity: moving away from an approach based on pathogens. International Conference on Marine Bioinvasions, Sydney, Australia.

Deveney, M.R., Wiltshire, K.H., Buss, J.J., Lieu, Y.N., Harris, J.O., Speck P., Yeadon, P.J., Ellard, K.A., Mohr P.G., Moody, N.J.G., Bansemer M.S. and Roberts S.D. 2019. OsHV-1 microvariant in the Port River, South Australia: an unnatural history. Australasian Aquatic Animal Health and Biosecurity Conference, Cairns, Queensland.

Deveney, M.R., Wiltshire, K.H. and Ellard K.A. 2017. Biofouling as a long-distance vector for pathogens. Australasian Aquatic Animal Health and Biosecurity Conference, Cairns, Queensland.

Deveney, M.R., Wiltshire, K.H., Giblot-Ducray, D., Roberts S.D., Moody, N., Crane, M., Ellard, K.A. and Livore, J.P. 2018. Disease translocation in a connected world: the anatomy of a mollusc disease outbreak. International Conference on Marine Bioinvasions, Puerto Madryn, Argentina.

Dove, M. and O'Connor, W. 2018. History, status and future of oyster culture in Australia. Rushan International Oyster Forum, 21-23 April, China.

King, W.L., Siboni, N., Williams, N., Kahlke, T., Jenkins, C., Dove, M., O'Connor, W., Seymour, J.R. and Labbate, M. 2016. The effect of microbiological and environmental factors on a summer mortality event in Pacific Oysters. Australian Shellfish Quality Assurance Advisory Committee Conference (ASQAAC).

King, W.L., Siboni, N., Williams, N., Kahlke, T., Jenkins, C., Dove, M., O'Connor, W., Seymour, J.R. and Labbate, M. 2017. Elucidating links between the Pacific Oyster microbiome and summer mortality events. 4th FRDC Australasian Aquatic Animal Health & Biosecurity Scientific Conference.

King, W.L., Siboni, N., Williams, N., Kahlke, T., Jenkins, C., Dove, M., O'Connor, W., Seymour, J.R. and Labbate, M. 2017. Microbiome investigations in a Pacific Oyster summer mortality outbreak in Port Stephens, New South Wales. Australian Microbial Ecology Conference (AUSME).

King, W.L., Siboni, N., Williams, N., Kahlke, T., Jenkins, C., Dove, M., O'Connor, W., Seymour, J.R. and Labbate, M. 2018. Insights into the influence of the Pacific oyster microbiome in oyster disease and resistance. International Society for Microbial Ecology (ISME), 17th International Symposium on Microbial Ecology 2018.

King, W.L., Siboni, N., Williams, N., Kahlke, T., Jenkins, C., Dove, M., O'Connor, W., Seymour, J.R. and Labbate, M. 2018. Characterisation of the Pacific Oyster microbiome during a summer mortality event. 110th Annual Meeting of the National Shellfisheries Association.

Kube, P., Cunningham, M. and Dove, M. 2018. Breeding for resistance to oyster herpes virus. New Zealand, Australia and France POMS Workshop, 31 October 2018, Cawthron Institute, Nelson, New Zealand.

Labbate, M., King, W.L., Siboni, N., Williams, N., Kahlke, T., Jenkins, C., Dove, M., O'Connor, W. and Seymour, J.R. 2018. Is there a role for microbiomes in oyster mortality events? The Australian Shellfish Quality Assurance Program Meeting, University of Technology Sydney, Sydney, New South Wales.

McOrrie, S., Dove, M. and O'Connor, W.A. 2017. Breeding a "better" disease resistant oyster – the trade-offs. 7th International Oyster Symposium, 11-14 September 2017, Bangor, United Kingdom.

Nguyen, K.V., King, W., Siboni, N., Mahbub, K.R., Dove, M., O'Connor, W., Seymour, J.R., Labbate, M. 2019. The Sydney Rock Oyster microbiome is influenced by local environmental parameters and QX disease resistance. The 3rd International Conference on Fish and Shellfish Immunology, 16-20 June, 2019, Las Palmas De Gran Canaria, Spain.

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4.5 Students

4.5.1 PhD Students

Four PhD students were engaged in the Future Oysters CRC-P project with three completed or near completed their thesis and one withdrawing during the final writing up stage. A summary of each of their theses is provided below; their R&D results have been included within the relevant FRDC project reporting. Each student benefited from their involvement in the Future Oysters CRC-P by way of participation in an applied R&D project and greater exposure to the oyster industry through attendance at industry conferences, meetings and workshops and field work that involved visiting the oyster farms of industry representatives.

Ms Rebecca Seeto: PhD (progress to 31/01/2020)

Affiliation: The University of Newcastle

University supervisors: Dr Zamira Gibb and Prof. John Clulow

Industry supervisors: Dr Wayne O'Connor and Dr Michael Dove

PhD thesis title: Development of short-term preservation protocols for Sydney Rock Oyster (*Saccostrea glomerata*) gametes

Future Oysters CRC-P link: This PhD project aligns with FRDC Project No. 2016-803 and the objective to enhance the efficiency of propagation of Sydney Rock Oysters by delaying the onset of programmed cell death in oocytes of the Sydney Rock Oyster and develop a viable storage method to retain genetic diversity. Various abiotic changes and media supplementation were explored to determine the best combinations to retain vitality.

Summary:

In recent years, the Sydney Rock Oyster industry has suffered major population declines due to the occurrence and spread of diseases such as WM, and QX disease. In the early 1990s, a 90% decline in oyster production occurred in the Georges River. It was thought that the disease was limited to northern

NSW due to the warmer temperatures, however an onset of QX disease peaked in 2004 in the Hawkesbury River, resulting in a production decline of 94% by 2006. As a result, oyster research moved from breeding programs aimed at optimising oyster weight and growth rate, to also encompassing disease mitigation and resistance programs.

In addition, climate change factors, such as ocean acidification and increases in temperature due to increased atmospheric carbon dioxide concentrations are predicted to have a constantly increasing effect on oyster production in the coming decades. Therefore, it is imperative that artificial reproductive technologies for oysters and a sound understanding of their physiology is developed to prevent further diminishing of populations and oyster production.

There are many studies which have focused on the storage of cells (such as gametes) and/or tissues from aquatic organisms for use in both commercial and conservation applications. These materials can then potentially be used for genetic engineering (to observe genetic structure and gene expression to generate disease resistance), and in conservation programs for breeding purposes and to retain genetic diversity. Storage can be separated into long-term methods, and short-term methods. Currently, there is no long-term storage protocol for oyster oocytes that doesn't involve cryopreservation – a technique which preserves the structure and viability of cells and tissue using very low temperatures (i.e. -80 to -197 °C) following slow cooling in the presence of a cryoprotectant.

The goal of this project is to delay the onset of programmed cell death in oocytes of Sydney Rock Oysters and develop a viable storage method to retain genetic diversity. Various abiotic changes, and media supplementation will be explored to determine the best combinations to retain vitality.

Most recently, an experiment comparing the InstantOcean® and an Artificial Sea Water (ASW) recipe with a change in: (1) pH, (2) osmolality using mannitol, (3) osmolality using mannitol and sucrose, (4) microorganism content through 1% penicillin/streptomycin, (5) microorganism content through 0.5% penicillin/streptomycin, or (6) viscosity through 3mg/mL PVP. Results suggest that vitality (determined through Eosin Y staining) in base media without alteration showed the greatest decline in oocyte viability (down 51.1 and 47.3% for InstantOcean® and ASW, respectively) whereas all treatments added some degree of increased viability. The treatments where osmolality and PVP were added generally performed the best as did the penicillin/streptomycin when in combination with Instant Ocean® (for InstantOcean® and ASW with 64.4 and 69.5%, respectively). Membrane integrity was also improved when pH was changed (40.01 and 49.8% respectively) and when penicillin/streptomycin was used in combination with Artificial Sea Water (40.6 and 42.0%, respectively.

These preliminary results will be further tested and replicated with certain treatments combined to see if further improvement in viability of oocytes over extended storage periods can be retained. In addition, future studies will combine these treatments with fertilisation trials to determine vitality results correlate with fertilisation. These results are promising for the development of room temperature storage protocols of Sydney Rock Oyster gametes that extend the holding periods of oocytes, prior to fertilisation, above those currently obtainable.

Outcomes to date have been included in the relevant FRDC project report; "New technologies to improve Sydney Rock Oyster breeding and production", Principal Investigator, Dr Michael Dove, NSW DPI. FRDC Project No. 2016-803.

<u>Ms Deborah Gardner: PhD (not completed, withdrew 31/08/2019)</u>

Affiliation: The University of Tasmania

University supervisors: Dr Christine Crawford

Industry supervisors: N/A

PhD thesis title: Development of culture techniques for the native oyster *Ostra angasi* to maximise farm production

Future Oysters CRC-P link: This PhD project aligns with FRDC Project No. 2016-807 and the objectives to develop native Flat Oyster on-farm growing methods that maximise survival and growth in SA and TAS, and establish a native Flat Oyster farmers network to share new techniques and knowledge.

Summary:

Farm trials were undertaken using wild collected native Flat Oyster (*Ostrea angasi*) to investigate the interaction between husbandry and environment. Factors investigated included farm gear (container type), depth the oysters were located within the water, water movement exposure, oyster density and oyster handling across location at two farm sites to optimise grow-out for different life stages and locations, with environmental data (i.e. pH, salinity, temperature, ammonia, phosphate, nitrate, dissolved organic matter and dissolved organic carbon) collected at each site to correlate with oyster survival and growth. Growth and survival was measured on a monthly basis for larger oysters > 20mm and fortnightly for spat < 20mm. Seasonal measures were also conducted for other oyster metrics, including condition, disease monitoring (*Bonamia*) and indicators of stress. The results of this R&D have been incorporated into the Future Oysters CRC-P Project 2016-804 final report.

<u>Mr Viet Khue Nguyen: PhD (completion date 31/07/2020)</u>

Affiliation: University of Technology Sydney

University supervisors: A/Prof. Maurizon Labbatte and Prof. Justin Seymour

Industry supervisors: Dr Wayne O'Connor and Dr Michael Dove

PhD thesis title: Investigation of Sydney Rock Oyster (*Saccostrea glomerata*) microbiomes associated with disease outbreaks

Future Oysters CRC-P link: This PhD project aligns with FRDC Project No. 2016-805 and the objectives to define microbial communities associated with oysters and identify threats, link changes in environmental conditions to changing microbial communities, and better understand the association between microbial communities and disease.

Summary:

The aim of this PhD project was to explore the influence of geographic location, season, genetics, QX disease on the Sydney Rock Oyster microbiome and identify the key taxa of the healthy oyster. To explore the influence of location, season and breeding for QX disease resistance on the oyster microbiome, six SRO families with two categorised as QX-resistant and four as QX-susceptible were deployed to two different locations (Port Stephens and Wallis Lake, NSW, Australia) with sampling conducted on two seasons (Austral summer and winter). To examine the microbiome of infected QX disease oysters, four SRO families with intermediate QX disease resistance level deployed in a known QX disease area with sampling conducted fortnightly from 8 November 2017 to 11 April 2018. Oysters were confirmed as infected with *Marteilia sydneyi* by PCR using the primers LEG1 (5'-CGATCTGTGTAGTCGGATTCCGA) and PRO2 (5'-TCAAGGGACATCCAACGGTC), and the presence of sporulating *M. sydneyi* in the digestive gland examined by using the tissue imprint method. Using16S rRNA (V1 – V3 region) amplicon sequencing, we examined the microbiomes of Sydney Rock Oysters.

Overall, the Sydney Rock Oyster microbiomes dominated by rare taxa belong to the *Candidatus Hepatoplasma*, *Endozoicomonas*, *Mycoplasma* and *Arcobacter* genera and highly distinct from the planktonic microbiome within the surrounding seawater. The Sydney Rock Oyster microbiome was significantly influenced by location and season with members belong to the *Candidatus Hepatoplasma* and *Endozoicomonas* genera identified as significant drivers. Additionally, breeding for QX disease resistance significantly influenced the Sydney Rock Oyster microbiome but only at the winter season with OTUs assigned to the *Mycoplasma*, *Borellia* and *Endozoicomonas* genera were over-represented in QX-resistant Sydney Rock Oyster microbiomes. In contrast, members of the *Pseudoalteromonas*, *Vibrio* and *Candidatus Hepatoplasma* bacteria were over-represented in QXsensitive microbiomes. The Sydney Rock Oyster microbiomes of infected QX disease were significantly different when compared to those of uninfected QX disease with the microbiome shifts associated with QX-infected oysters were principally driven by a relative increase of an OTU assigned to the *Borrelia* genus and drop in the relative abundance of a member belonging to the *Mycoplasma* genus. The Sydney Rock Oyster microbiomes were not significantly different between infected QX with and without mature sporonts in digestive tubule indicating dysbiosis occurred before sporulation in the digestive gland. Finally, the bacterial structures were significantly different across four families had the same the prevalence of QX disease, suggesting the Sydney Rock Oyster microbiome composition may not link to a QX disease outbreak. We identified few core microbiomes that belong to the *Borrelia*, *Mycoplasma* and *Candidatus Hepatoplasma* genus. *Borrelia* genus was identified as core microbiome in pre-QX, uninfected and QX-infected disease oysters. The *Mycoplasma* genus was identified as core microbiome of Sydney Rock Oysters prior but not during QX disease event, implying that there could be an important ecological link between *Mycoplasma* and healthy Sydney Rock Oysters.

<u>Mr William King: PhD (completed 29/02/2020)</u>

Affiliation: The University of Technology Sydney

University supervisors: Prof. Justin Seymour and A/Prof. Maurizon Labbatte

Industry supervisors: Dr Cheryl Jenkins

PhD thesis title: New quantitative tools to decrypt, predict and manage oyster diseases.

Future Oysters CRC-P link: This PhD project aligns with FRDC Project No. 2016-805 and the objectives to define microbial communities associated with oysters and identify threats, link changes in environmental conditions to changing microbial communities, and better understand the association between microbial communities and disease.

Summary:

Oyster aquaculture represents a significant portion of both the Australian, and the global economy, with *Crassostrea gigas* (the Pacific Oyster) representing the most heavily cultivated commercial species. However, infectious diseases have emerged as a major obstacle for the successful growth and sustainability of the oyster aquaculture industry. Oyster diseases are often complex, occurring as a result of disturbance in the synergistic relationship between the host, environment, and pathogen/s. Perturbations of environmental factors (e.g. temperature, salinity, nutrients, pH) can have direct influences on the oyster’s immune system, and can allow for the proliferation and transmission of oyster pathogens. In particular, two major pathogens of *C. gigas*, ostreid herpesvirus 1 (OsHV-1) and *Vibrio* bacteria, are both strongly driven by temperature. One such understudied factor that may influence oyster disease dynamics is the oyster microbiome. Studies in other model systems have shown the involvement of the microbiome in animal health, disease, and behaviour; because of this, it is likely the oyster microbiome also plays a role in oyster disease dynamics. The work presented in this thesis aimed to use a microbiome approach to provide further understanding of oyster diseases.

4.5.2 Graduate and Undergraduate Students

Three undergraduate placements also participated in the Future Oysters CRC-P and greatly benefited from interacting with researchers and industry while participating in applied science:

- Mr Louis Christenson, who was supervised by Dr Christine Crawford, University of Tasmania and assisted with on-farm POMS trials from January 2018 to March 2019, supporting FRDC Project No. 2016-804.
- Ms Natasha Logvinskaia who received a summer scholarship from SARDI and was supervised by Prof. Xiaoxu Li, Dr Penny Ezzy and Dr Stephen Pahl, to investigate the factors impacting the

shelf-life of the native Flat Oyster, *Ostrea angasi*, supporting FRDC Project No. 2016-804. Ms Lonvinskaia deferred the project for one year and subsequently withdrew prior to completion.

- Mr Connor McIvor, who completed a BSc Honours thesis in 2019 titled "Use of flow cytometry as a rapid OsHV-1 detection tool", supporting FRDC Project No. 2016-806. Specifically, he examined the finer elements of the detection test that was initially established during the Future Oysters CRC-P. This included quantifying the detection limits of the flow cytometer, testing various sample incubation temperatures, probe concentrations and sample incubation times. His results strengthen the already established flow cytometric detection test and were incorporated into the Future Oysters CRC-P FRDC Project No. 2016-806 final report.

4.6 Collaboration (including Education and Training)

As a result of the Future Oysters CRC-P, strong relationships were built between researchers from the research organisations involved, and researchers and the four industry participant organisations involved. About ten industry oyster farming companies directly supported components of the research undertaken on-farm and in extending it to the Australian oyster industry, the project outcomes were disseminated to greater than 300 'Small and Medium Enterprises' (SME), primarily oyster farmers.

The seven work experience, graduate and postgraduate students involved in the Future Oysters CRC-P benefited greatly from participating in aspects of the applied research and from opportunity for close interaction with industry, both on farm and at state based industry events.

4.7 Extension

Participants in the Future Oysters CRC-P provided information to populate the Australian oyster industries 'Oysters Australia Web Site' with project descriptions, updates and presentations on project progress and outcomes given at well attended annual industry events in NSW, SA and TAS, with the final 2019 events having about 500 people attend in total.

A diversity of presentations and publications were also produced. Presentations were primarily in the form of project updates and were presented at key industry forums over the life of the Future Oysters CRC-P, although some were given at national and international scientific conferences. Publications were primarily in the form of information "Newsletters' for industry, project updates in popular magazines and in scientific journals (with more expected to be produced over the 12-18 months following the project).

5 Outcomes & Impacts

The key outcomes and impacts of the Future Oysters CRC-P project by theme were:

5.1 Theme 1: Better Oysters

5.1.1 Sydney Rock Oyster Industry

- Acceleration in selective breeding by doubling the number of families produced.

  Annual family line production was increased from about 40 to 80, with the total number of families available to industry raised from about 80 to 250, which greatly increased the gains in commercially valuable traits, bringing forward industries access to key stocks by two years leading to major improvements in productivity.

- Success of fertilisations increased from 27% to 45%.

  The number of selectively bred broodstock that are sacrificed were significantly reduced, which reduced the time required for the schedule of breeding program matings from weeks to days, and reduced the overall time taken in family line production, both leading to cost savings.

- Improved storage techniques for gametes.

  The understanding of gamete viability was increased and this increased the flexibility of procedures used for controlled mating of oysters, which supported the improvement in fertilisation success and provided new opportunities for mating plan designs not previously contemplated.

- Triploid oyster production.

  Some 3 million triploid Sydney Rock Oysters, which were provided to industry for commercial scale production evaluation.

5.1.2 Pacific Oyster Industry

- Establishment of a biosecure breeding facility.

  The IMAS aquaculture facility was greatly advanced by this project through an improved water treatment process and the establishment of an approved biosecurity plan, which has been externally audited and signed off by the TAS Chief Veterinary Officer (CVO). These improvements were fundamental to enabling the ongoing POMS resistant selective breeding by ASI for the TAS oyster industry following the POMS outbreak in this state.

- Improved rates of genetic improvement in the TAS oyster population to fast track POMS resistance.

  ASI through this project has now developed and implemented oyster spat POMS challenge trials that have increased genetic gains (average about 10% per annum), facilitating the fast tracking of establishing POMS resistant oysters for the farmers.

- Improvement in-laboratory oyster POMS challenges.

  These have increased correlations to field survival and a laboratory challenge at IMAS, and arean important precursor to future work on genomics.

- Accelerated maturation.

This has allowed increased usage of one year old oysters as compared to 18-24 month old oysters in the ASI breeding program, which has been shown to offer increased rates of genetic gains in POMS resistance.

- Development of an SNP identification tool.

  This enables differentiation between ASI and non ASI stock and different ASI families, which will, when applied, enable increased confidence as to the source and nature of the seed stock used by farmers.

- Production of POMS resistant family lines in SA.

  Over 160 family lines were produced in SA at SARDI adapted facilities to enable early stage POMS resistant broodstock to be deployed to the SA oyster industry. While the level of POMS resistance in oysters available to SA farmers is much lower than in TAS, a facility now exists in SA to enable gains in POMS resistance to be achieved. So far these gains have been small but because this project developed and initiated another project, which has been funded, the higher level of POMS resistance that now exists in TAS should also exist in SA in three years.

5.2 Theme 2: Healthy Oysters

5.2.1 Sydney Rock Oyster Industry

- Improved understanding of Sydney Rock Oyster Winter Mortality (WM) disease.

  This has established a factually supported case definition to better understand the occurrence of WM. It has also provided estimates of the heritability of WM resistance in Sydney Rock Oysters have for the first time been estimated, which provides a basis for selection of resistance and allows industry to begin to estimate the economic value of this trait within breeding programs. Progeny tests for performance assessment have also been optimised. A greater understanding of the impacts of WM has now allowed reduced testing effort by reducing the time and number of sites required for assessments and provided clearer direction for investigation its cause.

- Identified the potential to address Queensland Unknown (QX) disease of Sydney Rock Oysters by selective breeding.

  Work on the molecular basis for QX resistance has provided an alternative means of selection, in the absence of field evaluations that has opened the pathway to within family selection for QX disease resistant individuals. This overcomes the threat of inconsistent field based outcomes. This project also developed a method to sample oyster tissue for genetic analysis that does not affect oyster reproduction or survival. Seven gene expression markers were identified that could be incorporated into the breeding program once further validation steps are completed. A one-year breeding cycle was successfully introduced for QX resistance, effectively doubling the rate of genetic progress for this trait. This allowed the production of Sydney Rock Oyster families to be advanced so that spat from SOCo families could be challenged to QX during the season following production allowing data to be collected and breeding decisions to be made within 12 months.

- An understanding of the potential to use microbial communities associated with oysters to enhance disease detection.

  The microbial community of oysters was described for different locations, seasons, family lines, and pre and during disease infection. The results suggest microbial communities although influenced by a wide range of factors, can provide capacity to improve selective breeding for disease resistance, offer the chance to improve oyster survival in hatcheries and even to seek methods to improve food quality assurance for consumers.

5.2.2 Pacific Oyster Industry

- Improved understanding of when and where Pacific Oyster Mortality Syndrome (POMS) is of higher risk.

  The key driver of POMS, water temperature >19 °C for one week, and other important environmental factors, water movement and oyster density, were identified by collating and modelling on-farm industry data enabling a good predictive capacity of when and where there is a high risk of POMS occurrence. Collaborative studies with industry also identified that on-farm oyster density had limited effect on mortality rates during the POMS season and that younger and smaller oysters are more susceptible to infection than larger and older juvenile and adult oysters, resulting in industry optimised farm stocking strategies.

- Improved knowledge of POMS surveillance.

  Greater confidence now exists in translocation testing and declarations of areas free from POMS and decreased surveillance costs have been developed. Together, these have resulted in successfully managing the risks of the spread of POMS in SA growing areas and improved the likelihood that the SA industry can remain free from POMS for longer.

- Established a flow cytometry based POMS testing procedure.

  A quicker and more cost effective POMS test has been developed using flow cytometry, which has resulted in decreased surveillance costs for hatcheries, improved confidence about translocations and a faster response to address incursions of POMS.

  With SA's oyster industry value at about $40.1 million in 2016-17 (ABARES 2018; pre-POMS effect on SA spat supplies) and an expectation of a similar level of mortality as occurred in TAS when POMS arrived in the 2016 season (i.e. 40% of production) this equates to a saved industry production value of $16.0 million per annum for each year POMS does not occur.

- A greater understanding of South Australian Mortality Syndrome (SAMS).

  While much remains unknown about SAMS, a better understanding of Pacific Oyster responses to disease in general has been developed along with improved industry sampling and sample transport procedures, and the diagnostic testing protocols at the Veterinary Sciences Laboratories, University of Adelaide. Using this information, future research may be undertaken to further the understanding and management of SAMS to improve oyster production and profitability in SA.

5.3 Theme 3: More Oysters

5.3.1 Native Flat Oyster Industry

- Identified optimal farming techniques.

  Demonstrated in collaboration with industry representatives, that excellent native Flat Oyster, *Ostrea angasi*, survival, growth and product is possible under select conditions using both wild caught and hatchery produced spat.

- Enhanced communication between those in industry interested in *Ostrea angasi* farming.

  A network of those in industry interested in farming this oyster species was established, and through regular liaison with industry R&D collaborators and the holding of workshops to engage with others with interests in this species, the results of the R&D were disseminated along with the challenges and successes experienced by industry in farming this species in the past and now.

- Established a translocation protocol to enable Western Rock Oyster translocation from WA to SA.

  A protocol was established following a review of potential disease and pest issues, some mixed species challenge trials undertaken in SARDI's Aquatic Biosecurity Centre and consultation with SA Government regulators. This enables the SA oyster industry to potentially diversify their businesses by farming another oyster species if they wish to pursue this opportunity that they identified.

  Species diversification using the native Flat Oyster, *Ostrea angasi*, remains of interest to Pacific Oyster farmers to manage business risk and more fully utilise their leases, but at this time they have a cautious approach based on the challenges that a number of them have experienced in the past when developing the farming of this species. While the species continues to demonstrate potential, industry members continue to have different views as to the optimal farming and marketing approach, with growth likely to be slow and niche orientated as the alternative approaches are further evaluated. More industry driven collaborative R&D is recommended.

5.4 Theme 4: Project Management and Communication

5.4.1 Governance

The Future Oysters CRC-P has facilitated advancement of the Australian Oyster Industry resulting in:

- New South Wales

  An industry that is demonstrating new enthusiasm, with considerable improvements occurring and production increasing.

- South Australia

  A much better informed and prepared industry able to detect and address the associated issues if/when it is directly impacted on-farm by POMS.

- Tasmania

  A re-energised and more efficient industry, with production approaching pre-POMS levels (in a survey of TAS oyster farmers 79% of farmers rated the Future Oysters CRC-P research as having a high impact).

- Other

  An early development phase industry, particularly in Western Australia, which is accessing and adapting information to facilitate its growth and reduce risks.

5.4.2 Collaboration

As a result of the Future Oysters CRC-P project, strong relationships were built between researchers from the two research organisations involved, researchers and the four industry participants, and the six supporting universities. The four Honours, two Masters and six PhD students, and three postdoctoral fellows (or equivalent), benefited greatly from the applied research and opportunity for close interaction with industry. Students, researchers and project technical staff, both from research organisations and industry, also participated in a number of training sessions, with the 'YTK Health Training Workshop' the most substantial.

5.4.3 Extension

A wide range of extension activities were undertaken as part of the Future Oysters CRC-P project with many oyster industry members receiving updates as Future Oysters CRC-P information was loaded to the Oysters Australia web site. Many workshop and conference presentations (verbal and posters) were also given, and a number of popular and scientific publications produced, with more underway. About 500 people attended the August-September, 2019 Oyster Industry Conferences held in NSW, SA and TAS at which the final Future Oysters CRC-P results were disseminated.

5.5 Impact Assessment and Benefit to Producers

5.5.1 Nature of Impacts

The beneficial economic impacts of the Future Oyster CRC-P to the Australian edible oyster aquaculture industry were identified as either economically quantifiable or unquantifiable.

The economically quantifiable benefits were in the areas of increased productivity and profitability, which flowed from:

- greater Pacific Oyster survival as a result of enhanced POMS resistance achieved through the ASI breeding programs in South Australia and Tasmania;
- improvements to Pacific Oyster and Sydney Rock Oyster survival as a result of enhanced breeding program methodologies, farm management and oyster production procedures; biosecurity and surveillance protocols; and a better understanding of the influence of the environment and microbiome on oyster health; and
- improvements to Flat Oyster growth as a result of improved farm management / production procedures.

The economically unquantified benefits were identified as increased efficiency and capacity of future Australian edible oyster R&D and enhanced community well-being, which flowed from:

- strengthened Australian edible oyster aquaculture R&D networks across regions and between industry participants and greater knowledge of edible oyster R&D associated with managing oyster diseases and health, including methodologies; and
- flow-on effects of a more productive, profitable and sustainable Australian edible oyster aquaculture industry on the social 'fabric' of Australian regional population centres and the environment where improved marine biodiversity and water quality are in part, respectively, a result of increased infrastructure that oyster farming adds to the marine environment and the filtering effect of the oysters as they feed.

5.5.2 Valuation of Impacts

The Gross Value Production (GVP) for each year assessed was obtained from data published by the Australian Bureau of Agricultural and Resource Economics and Sciences (ABARES). All benefits were discounted by 5% to the year the Future Oysters CRC-P began and a reinvestment rate of 5% was used for estimating the Modified Internal Rate of Return (MIRR) (Hardaker, T. and Chudleigh, P. 2019. Chapter 5. Impact assessment and industry adoption. In Stone, D.A.J., Booth, M.A. and Clarke S.M. (eds) 2019. Growing a profitable, innovative and collaborative Australian Yellowtail Kingfish Aquaculture Industry: Bringing 'White' fish to the market (DAWE Grant Agreement RnD4Profit-14-01-027), Adelaide, South Australia. 896 pp.).

The base analysis used the best available estimates for each variable, notwithstanding a level of uncertainty for many of the estimates. All analyses ran for the project investment period, said to be 2015-16 for simplicity rather than from 2015-16 to 2018-19, the three year life of the project (c.f. https://www.investopedia.com/terms/i/irr.asp), plus 10 years from this time of investment. The Present Value Benefit (PVB) is the discounted sum of the estimated benefit cash flows and should be interpreted as the present value of the expected benefits attributed to the Future Oysters CRC-P (based on the assumptions made) and the Present Value Cost (PVC) is the present value of the RD&E costs of the project. The Net Present Value (NPV) is equal to the PVB less the PVC. The Benefit-Cost Ratio (BCR) is equal to the ratio of the PVB to the PVC and represents the estimated expected return for the investment in the Future Oysters CRC-P. The Internal Rate of Return (IRR) is the rate of discount that produces a NPV of zero (that is the rate that makes the discounted costs equal to the discounted benefits). The MIRR is an alternative to the traditional IRR measure and is calculated assuming that the cash inflows from an investment are reinvested at a specific rate representing the cost of capital (the reinvestment rate).

5.5.3 Impact Analysis Results

The Future Oysters CRC-P impact assessment (Table 5.1) has, based on a range of assumptions and costing only some of the benefits, conservatively estimated that the about $8.3 million total invested produced in present value terms an estimated total discounted benefit of $126.6 million, net present value of $118.3 million, benefit-cost ratio of 15.3:1, internal rate of return of 187%, and a modified internal rate of return of 38%.

It was also estimated (Table 5.2) that if a POMS mortality event did occur across the Pacific Oyster growing areas in SA, the Future Oysters CRC-P would conservatively reduce its impact on the GVP in the year it occurred by about $15.1 million.

5.5.4 Benefits to Producers

The impacts of the Future Oysters CRC-P research on the Australian Pacific Oyster industry have been profound.

In TAS the recovery from POMS has been unparalleled in comparison to other international oyster industries that have been affected by POMS. Also, an intangible outcome of the project was the confidence and mental health that it provided to Pacific Oyster growers in the immediate aftermath of POMS to reinvest in their business knowing that efforts to counter POMS through selective breeding would be accelerated and provide a solution to be able to continue farming. TAS growers have strongly suggested that the Future Oysters CRC-P funded research essentially saved their industry. ASI now provides broodstock to all Pacific Oyster hatcheries with 95% of the seed produced derived from the breeding program. The industry is now reliant on the program for its ongoing sustainability.

Table 5.1. Estimate of impact of the Future Oysters CRC-P on the oyster industry in NSW and TAS ($ values in millions).

	2015-16	2016-17	2017-18	2018-19	2019-20	2020-21	2021-22	2022-23	2023-24	2024-25	2025-26	Total
TAS												
Pacific Oysters GVP ($)*		26.3	28.7	28.2	29.6	31.1	32.6	34.3	36.0	37.8	39.7	
POMS Area Value ($)**		15.8	17.2	16.9	17.8	18.7	19.6	20.6	21.6	22.7	23.8	
ASI CRC-P Protection/improvement (%)		70%	70%	70%	70%	50%	50%	30%	20%	10%	10%	
Future Oysters CRC-P Benefit ($)		11.0	12.1	11.8	12.4	9.3	9.8	6.2	4.3	2.3	2.4	81.6
NSW												
ASI Pacific Oyster triploids GVP ($)*		0	0	0	0	0.5	1	2	2.5	3	4	
Future Oysters CRC-P Benefit ($)		0	0	0	0	0.5	1	2	2.5	3	4	13.0
Sydney Rock Oysters GVP ($)*		45.4	51.8	52.0	54.6	57.3	60.2	63.2	66.4	69.7	73.2	
SOCo Protection/improvement (%)		10%	10%	15%	20.0%	25.0%	30.0%	35.0%	40.0%	45.0%	50.0%	
SOCo benefit ($)		4.5	5.2	7.8	10.9	14.3	18.1	22.1	26.5	31.4	36.6	
CRC-P Contribution (%)		80%	80%	80%	80%	60%	50%	40%	30%	20%	10%	
Future Oysters CRC-P Benefit ($)		3.6	4.1	6.2	8.7	8.6	9.0	8.8	8.0	6.3	3.7	67.1
PVC (total project cost) ($)	-8.3											
Not discounted rate ($) (PVB)		14.7	16.2	18.1	21.2	18.4	19.8	17.0	14.8	11.5	10.0	161.8
Discount Rate												
5% (PVB)		14.0	14.7	15.6	17.4	14.4	14.8	12.1	10.0	7.4	6.2	126.6
Reinvestment Rate												
5%												
Sum of discounted benfits (PVB)		**$126.6**				**(Internal Rate of Return)**	**187%**					
Less initial cost (PVC)		**-8.3**				**MIRR (Modified Internal Rate of Return)**	**38%**					
Net Present Value (NPV)		**$118.3**										
Benefit-Cost Ratio (BCR)		**15.3 :1**										

* based on values published by ABARES (https://daff.ent.sirsidynix.net.au/client/en_AU/search/asset/1030241/0) and pers. comm FRDC for past years and predicted future growth of 5% in both TAS and NSW (approximations based on past industry growth prior to POMS influences and forward projections prior to coroavirus influences)

** based on 60% production from POMS affected areas

Table 5.2. Estimate of the potential impact of the Future Oysters CRC-P on the oyster industry in SA ($ values in millions).

SA		**2016-17**	**2017-18**	**2018-19**	**2019-20**	**2020-21**	**2021-22**	**2022-23**	**2023-24**	**2024-25**	**2025-26**	**Average**
Pacific Oysters	GVP ($)	40.1	20.2	27	29.2	31.5	34.0	36.7	39.7	42.8	46.3	
	Use of POMS resistant oysters in industry (%)	20	30	40	50	60	70	80	90	95	100	
	Protection from POMS resistant oysters (%)	30	30	40	50	60	70	80	85	90	95	
	Total protection benefit to industry (%)	6	9	16	25	36	49	64	76.5	85.5	95	
	Cost of POMS Morts ($)	32.1	14.1	16.2	14.6	12.6	10.2	7.3	4.0	2.1	2.3	
	Potential Industry benefit if POMS occurs ($)	8.0	6.1	10.8	14.6	18.9	23.8	29.4	35.7	40.7	44.0	
	Future Oyster CRC-P contribution (%)	0	0.0	100.0	90.0	80.0	70.0	60.0	50.0	40.0	30.0	
	Potential CRC-P benefit if POMS occurs ($)	1.9	1.3	2.6	3.6	4.5	5.0	4.7	3.0	1.8	2.2	**3.6**

* based on values published by ABARES (https://daff.ent.sirsidynix.net.au/client/en_AU/search/asset/1030241/0) and pers. comm FRDC for past years and predicted future growth of 8% in SA (approximations based on past industry growth prior to POMS influences and forward projections prior to coroavirus influences)

In SA the outcomes of the Future Oysters CRC-P research have provided the opportunity for growers to limit the impacts of POMS if, or more likely when, it affects the oyster growing regions. Hatcheries have been provided with broodstock with partial POMS resistance so that losses from an initial POMS outbreak can be minimised by stocking spat derived from these broodstock. An ongoing breeding program has also been established so that further gains in POMS resistance are achieved and available to industry. The outcomes from the Future Oysters CRC-P research will also allow ASI to mount a more rapid response than occurred in TAS because of the increased POMS resistance in their stock and the methodologies developed as part of this project. SA also has the option, subject to translocation policies, to import highly POMS resistant genetics from TAS to expedite the process. The research has also provided the same intangible outcomes as in TAS.

6 Conclusions: Outcome & Output Achievements by Theme and Project

Identified below are whether the Future Oysters CRC-P theme and project outcomes and outputs were "ACHIEVED", "PARTIALLY ACHIEVED", or "NOT ACHIEVED", as a summary of the more detailed information provided within the previous sections on "Research Project Results", "Communications and Extension", and "Outcomes and Impacts".

6.1 Theme 1: Better Oysters

Outcomes:

- High POMS resistant Pacific Oysters. ACHIEVED
- Improved disease resistant Sydney Rock Oysters. PARTIALLY ACHIEVED (the understanding of WM was improved but a definite cause of these mortalities was not identified)

6.1.1 FRDC Project No. 2016-801: Enhancing Pacific Oyster breeding to optimise national benefits

Outputs and Outcomes:

- Breeding program extended to South Australia to facilitate biosecure access to POMS resistant oysters in unaffected production areas nationally. ACHIEVED
- Biosecurity constraints to effective implementation of breeding program addressed. ACHIEVED
- Methods of producing 'clean' spat from exposed oysters developed, verified and applied in relevant circumstances. ACHIEVED
- Improved laboratory challenge test (higher and more consistant correlation to results from POMS challenges in the wild) to increase selection efficiency for POMS resistance in spat and challenge test extended to larvae. ACHIEVED
- Correlations for POMS survival determined for different life stages of Pacific Oysters enabling more robust predictions of performance. ACHIEVED
- Intervals for selection of POMS resistant oysters shortened from 2 years. ACHIEVED
- Genetic test to identify ASI oysters commercially available. ACHIEVED

6.1.2 FRDC Project No. 2016-802: Accelerating Sydney Rock Oyster (SRO) breeding research

Outputs:

- The number of families produced for the SoCo breeding program to have doubled from 80 to 160 by 2019. ACHIEVED
- A 12 month generation time (reduced from the current 24 months within the breeding program for selection to QX and winter mortality resistance enabling acceleration of genetic gain. ACHIEVED

- Accurate estimate for the heritability of the trait of winter mortality resistance in Sydney Rock Oysters utilised with SoCo's breeding program. PARTIALLY ACHIEVED (a first estimate of the heritability of the trait was obtained but was not as definitive as hoped)
- Recommendations on the incorporation of marker assisted selection utlilising stress markers into the breeding program for Sydney Rock Oysters provided to industry by 2019. ACHIEVED (further validation required)

Outcomes:

- Enhanced breeding program for QX and winter mortality disease resistance enabling a 50% improvement in the rate of gains. ACHIEVED

6.1.3 FRDC Project No. 2016-803: New technologies to improve Sydney Rock Oyster breeding and production.

Outputs:

- Optimised protocols for 4N Sydney Rock Oyster production developed and communicated to industry. NOT ACHIEVED (despite 11 attempts a batch of tetraploid (4N) oysters were not produced that could be made available for commercial hacthery production, however, successful chemical tripolid batches were produced and resulted in the distribution of a few million triploid oysters from QX disease-resistant broodstock to industry)
- A batch of 4N Sydney Rock Oysters for commercial production of triploid Sydney Rock Oysters by 2019. NOT ACHIEVED (see dot point above)
- A commercially applicable gamete storage protocol developed by 2019. ACHIEVED (further validation required)
- A commercially applicable protocol for induction of sexual maturation in Sydney Rock Oysters by 2019. ACHIEVED (further validation required)

Outcomes:

- 20% industry access 3N oysters with a 30% increase in profitability by 2022 ($2.4m). PARTIALLY ACHIEVED (not as planned through the development of protocols to produce tetraploids, but chemical tripolid batches were produced and resulted in the distribution of a few million triploid oysters from QX disease-resistant broodstock to industry)
- Reduce hatchery operation costs by 15% per batch by 2019 ($7.5K/run). ACHIEVED
- Increase spawning success by 50% in selected Sydney Rock Oysters. ACHIEVED

6.2 Theme 2: Healthy Oysters

Outcomes:

- Better farm management strategies. ACHIEVED
- Improved profitability. ACHIEVED

6.2.1 FRDC Project No. 2016-804: Advanced understanding of POMS to guide farm management decisions in Tasmania.

Outputs:

- Period of infection for POMS in TAS, largely based on temperature, is described by May 2017 and refined in 2018 and 2019 as more data becomes available. ACHIEVED
- A predictive framework for POMS which enables forecasting and preparation for danger periods of the disease is prepared by mid 2018. ACHIEVED
- Farm management procedures that are adoptable by industry and reduce mortalities from POMS by 50% are developed by mid 2018. ACHIEVED
- A model is developed, based on an alysis of farm records and on-farm trials, to support oyster farmers developing POMS response strategies. ACHIEVED
- Newsletters providing updates on POMS related information are provided to industry on a regular basis during the summer-autumn POMS infection period, and an annual report is provided to industry after th annual project steering committee meeting. ACHIEVED
- At least one journal publication on OsHV-1 virus in TAS and farm management stratgeies is submitted by 2019. NOT ACHIEVED (expected to be achieved within 12-18 months of completion of the project)
- Annual presentations to TAS oyster farmers at Shellfish Futures conferences, and at least one presentation at an international aquaculture conference on research results. Other presentations as the opportunities arise. ACHIEVED

Outcomes:

- Targeted research in relation to the epidemiology and environmental drivers of POMS, and farm management protocols to minimise the impact of POMS:
    - At least 75% of farmers operating in POMS infected areas use the POMS predictive framework to prepare for POMS outbreaks by 2018, ACHIEVED
    - The production of Pacific Oysters in TAS returns to 70% of pre-POMS production level by June 2019. ACHIEVED
    - No more than 30% of oyster farmers leave the industry due to POMS in TAS over 2017-2019. ACHIEVED

6.2.2 FRDC Project No. 2016-805: Polymicrobial involvement in OsHV-1 outbreaks (and other diseases).

Outputs:

- A new framework for the development of disease resistant oysters based on resolution of the microbiome of Pacific Oysters and Sydney Rock Oysters. NOT ACHIEVED (but concepts have been advanced)
- Recommendations on the design of oyster culture strategies that reduce disease risk based on the understanding of the role of environmental conditions, biogeography and seasonality in structuring the Pacific Oyster microbiome. PARTIALLY ACHIEVED (role of environment is

better understood but the linkage to disease was challenging due to the level of variability experienced)

- Identification of specific members of the Pacific Oyster microbiome that facilitate or cause disease outbreaks, providing capacity to subsequently develop pathogen monitoring and prevention programs. ACHIEVED (but needs further validation)
- Publications/reports defining:
  - oyster microbiome characteristic across species and breeding lines, ACHIEVED
  - links between oyster microbiome characteristics and disease susceptibility/resistance PARTIALLY ACHIEVED (certain microbiome characteristics and taxa were suggested to link to increased probability of disease but a distinct cause and effect has yet to be validated),
  - seasonal and biogeographic drivers of oyster microbiome structure, ACHIEVED
  - links between microbiome characteristics and environmental conditions, ACHIEVED
  - describing putative pathogenic microbes of Pacific Oysters and potential approaches for their detection ACHIEVED, and
  - recommendations for screening oysters for specific microbiobes implicated in disesae outbreaks. ACHIEVED

Outcomes:

- A review and potential redefinition of traits for selection in the Pacific Oyster breeding programs with significantly enhanced response to selection resistance to POMS. PARTIALLY ACHIEVED (a greater knowledge now exists in relation to traits for selection for POMS resistance but further refinement can still be achieved)
- Modifications to the timing and location of Pacific Oyster cultivation practices, leading to reduced occurence of disease outbreaks and significant enhancement of production. PARTIALLY ACHIEVED (TAS oyster farmers have developed a number of strategies to effectively reduce POMS mortalities and improve production but confience will increase with more years of data because of the variability experienced in POMS from year to year during the project)
- Capacity to detect and potentially manage the occurance of microorganisms involved in polymicrobial infections of Pacific Oysters, helping to reduce disease occurance and spreading. PARTIALLY ACHIEVED (detrimental microorganisms within the microbiome, but they have yet to be managed to reduce / prevent mortalities)

6.2.3 FRDC Project No. 2016-806: Advanced aquatic disease surveillance for known and undefined oyster pathogens.

Outputs:

- Update case definition for Winter Mortality disease (WM) in Sydney Rock Oysters. Progress to identifying causes of WM by June 2018. PARTIALLY ACHIEVED (the case definition has been improved and a variety of possible causes of WM discounted; but a definitive cause has yet to be identified)
- Management strategies communicated to key stakeholders by June 2019. PARTIALLY ACHIEVED (final project results were disseminated to industry at the annual industry events in

NSW, SA and TAS in August 2019, but as a definitive cause of WM is unknown management strategies remain general)

- Updated case definition for SAMS in Pacific Oysters by June 2018. ACHIEVED
- Recommended management strategies to avoid SAMS developed and communicated to key stakeholders by June 2019. NOT ACHIEVED (causitive factor(s) still not known)
- Rapid (<24 h turnaround) and cheap (<50% cost of PCR test) flow cytometry based diagnostic test for POMS developed and available for adoption by stakeholders by June 2019. ACHIEVED
- OsHV-1 diagnostic sensitivity and specificity described by June 2019. ACHIEVED
- At least three industry fact-sheets prepared and distributed to industry by June 2019. NOT ACHIEVED (fact-sheets were not considered appropriate at the time as the project was operating behidn schedule, however, a large number of update presentations were provided to industry over the life of the CRC-P, including at the annual industry events in NSW, SA and TAS in August 2019)
- At least five scientific publications submitted by June 2019. NOT ACHIEVED (a number of publications are expected to be completed in the 12-18 months after completion of the CRC-P)
- At least five conferences/workshop presentations made by 2019. ACHIEVED (many more than this were provided – see Communications section)

Outcomes:

- OsHV-1 surveillance informed by accurate understanding of diagnostic sensitivity and specificity and supported by a rapid, lower-cost diagnostic method adopted by all key Pacific oyster producing states. ACHIEVED
- Appropriate surveillance and rapid response to suspect cases of OsHV-1 adopted. ACHEIVED
- Better diagnostic approaches to SAMS and WM adopted and used, with improved production outcomes for industries. PARTIALLY ACHIEVED (better sample submissions and diagnostics were achieved but as a definitive causeof SAMS was not identified improved industry production was not achieved)
- Better confidence about described OsHV-1 free and affected areas. ACHIEVED
- Lower likelihood of transmission to unaffected areas. ACHIEVED
- Improved understanding and management of oyster diseases. ACHEIVED
- Decreased mortality in Pacific Oysters and Sydney Rock Oyster aquaculture. ACHIEVED
- A lower cost method for OsHV-1 testing for surveillance purposes. ACHIEVED
- Improved tools for assessing Sydney Rock Oyster health. ACHIEVED
- Better definition of OsHV-1 free zones. ACHIEVED

6.3 Theme 3: More Oysters

Outcomes:

- More resilient farming systems. PARTIALLY ACHIEVED (field trials provided evidence of the optimum farming conditions for native Flat Oysters, but some industry reprewntatives are still reluctant to change from their past methodolgies to enahnce production and improve marketing)
- Increased production to supply new domestic and global markets. PARTIALLY ACHIEVED (a number of native Flat Oyster farmers that participated in field trials are optomistic about the outcomes of the research, which included increased survival and growth under select conditions, resulting in increased production; however, broader uptake of the these outcomes remain uncertain as they do not align with other industry representatives traditional farming methods or business strategies)

6.3.1 FRDC Project No. 2016-807: Species diversification to provide alternatives for commercial production.

Outputs:

- Research and recommendations on native Flat Oyster production systems and marketing (presented and discussed at stakeholder workshop before June 2019). ACHIEVED
- A native Flat Oyster grower network. ACHIEVED
- Translocation protocols for the safe translocation of Western/Sydney Rock Oysters to SA ACHIEVED.
- Western/Sydney Rock Oysters available for a field trial in SA NOT ACHIEVED (while the tranlocation protocols were established their was insufficient time available to undertake field trials before completion of the CRC-P).
- Report on the relative culture performance of Western/Sydney Rock Oysters based on field trials in SA (subject to agreement by industry and regulators). NOT ACHIEVED (see dot point above)
- At least 2 media releases communicated via web, newspaper and TV by Principal/Co-Investigators and industry partners. ACHIEVED
- At least three presentations at oyster industry annual meetings presenting project progress updates. ACHIEVED
- At least one contribution per year to one or more SAORC/SAOGA, Oyster Tasmania and Oyster Australia newsletters to disseminate project findings to the oyster aquaculture industry. ACHIEVED
- At least two scientific publications on the project findings will be submitted to peer-reviewed journals and/or as postgraduate student theses (by June 2019). NOT ACHIEVED (a number of publications are expected to be completed in the 12-18 months after completion of the CRC-P)
- At least two presentations of scientific findings at national/international conferences. NOT ACHIEVED (but many presentations given at annual industry conferences – see Communications section)

Outcomes:

- Effective mechanisms to share technical and market information among native Flat Oyster growers in Australia. ACHIEVED

- The native Flat Oyster growers are more confident in their business. PARTIALLY ACHIEVED (some growers are more confident, but others are not)
- Option for culturing Western/Sydney Rock Oysters available to farmers in SA to enable diversification of risk related to potential loss of production of Pacific Oysters if POMS outbreaks in SA. PARTIALLY ACHIEVED (translocation protocols achieved but field trials not undertaken - insufficient time)

6.4 Theme 4: Project Management and Communication

6.4.1 FRDC Project No. 2016-800: Future oysters CRC-P: Management and extension.

Outputs:

- Records of meetings and decisions of FOMC. ACHIEVED
- Quarterly and final reports to DIIS. ACHIEVED (some with slight delays)
- Articles and presentation to industry and the international aquaculture community. ACHIEVED
- Communication, extension and education and training plans developed. ACHIEVED

Outcomes:

- Future Oysters CRC-P governance managed to a high standard. ACHIEVED
- Future Oysters CRC-P management Committee provided with information required on progress and outputs of research projects to allow informed recommendations to Oysters Australia R&D Committee. ACHIEVED
- R&D projects achieve milestones, adhere to approved budgets and provide outputs and outcomes needed by industry. ACHIEVED (some minor amendments required)
- Oyster growers in other states (i.e. QLD and WA) benefit from outputs of CRC-P R&D. ACHIEVED

6.4.2 FRDC Project No. 2017-233: Future oysters CRC-P: Communication and adoption.

Outputs & Outcomes:

- Oyster Australia website continually updated and positioned as a trusted communication platform for Future Oysters CRC-P research outputs that can be adopted long term as a primary medium for communication of R&D outputs to industry and stakeholders. ACHIEVED
- A minimum of two specialist industry workshops facilitated ACHIEVED
- A minimum of three travel awards granted. ACHIEVED
- A minimum of two industry connected honours students graduated. ACHIEVED (postgraduate rather than Honours)
- A minimum of ten Future Oysters CRC-P R&D findings are adopted by industry with quantifiable economic outputs. ACHIEVED