FRDC
FISHERIES RESEARCH & DEVELOPMENT CORPORATION

Reinvigorating the Queensland Oyster Industry

Dr. Carmel McDougall

September 2020

FRDC Project No **2018-118**

Version 1.1

1 July 2019

ISBN 978-1-922361-05-9

Reinvigorating the Queensland Oyster Industry
FRDC Project No. 2018-118

2020

Ownership of Intellectual property rights

Creative Commons licence

Disclaimer

Researcher Contact Details

Name: Dr. Carmel McDougall
Address: Australian Rivers Institute,
Griffith University
170 Kessels Road
Nathan QLD 4111
Australia
Phone: 07 3365 3543
Email: c.mcdougall@griffith.edu.au

FRDC Contact Details

Address: 25 Geils Court
Deakin ACT 2600
Phone: 02 6285 0400
Fax: 02 6285 0499
Email: frdc@frdc.com.au
Web: www.frdc.com.au

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

Contents

Tables

Figures

Acknowledgments

The author would like to acknowledge the many people who assisted with oyster collection, including Tony Carlaw, Ben Diggles, John Collison, Brendan McDougall, Tim and Andrew Prowse, Jan Strugnell, Nick Wade and Simon Walker. The author would also like to thank John Healy, Lisa Kirkendale, Sam Nowland, Darryl Potter, Richard Willan and Nerida Wilson for discussions and advice regarding museum collections, oyster identifications and taxonomy.

This FRDC funded project was a subcomponent of a larger Advance Queensland Research Fellowship (AQRF10916-17RD2) from the Queensland Government Department of Science, Information Technology and Innovation, with additional financial contributions from Griffith University and the FRDC and in-kind contributions from the Queensland Museum and Autore R&D Pty Ltd.

Abbreviations

BL	Blacklip oyster
QM	Queensland Museum
qPCR	quantitative polymerase chain reaction
SRO	Sydney rock oyster

Executive Summary

The overall objective of this study is to provide critical background knowledge to support the re-expansion of Queensland oyster aquaculture, which has been experiencing low levels of production since the 1920s. Once the epicentre of the oyster industry in Australia (Schrobback, 2015), Queensland-produced oysters now contribute only 0.8% to the total value of the Australian market (ABARES, 2020). The industry is predominately based on the cultivation of the Sydney rock oyster (SRO), and regularly suffers mass mortality events due to disease outbreaks. One potential course of action for the industry is to investigate the potential of other native species for aquaculture, however this is hampered by the lack of knowledge of the species that exist in Queensland, and of their natural distributions. The project outlined in this report was conducted by researchers from Griffith University with assistance from Queensland Museum (QM) staff and Queensland oyster growers, and was part of a larger Advance Queensland Fellowship directed towards 'reinvigorating the Queensland oyster industry'. Here, we conducted the first comprehensive genetic survey of oyster species along the Queensland coast and found unexpected diversity. We also found one new species and a recent exotic introduction. In the second part of the project we advanced our understanding of the genetics of the 'blacklip oyster' (BL), demonstrating that many of the molecular tools developed for SRO production may be directly transferrable to this closely related species. The findings of this study have already been applied by state government agencies and paved the way for aquaculture of additional native species in Queensland.

Background

Oyster aquaculture in Australia currently has an annual production value of $AUD102 million (ABARES, 2020). Significant gains in product quality and disease resistance have been achieved through the development of a selective breeding program for SROs in NSW (Dove et al., 2012; O'Connor and Dove, 2009). Despite this, regular disease outbreaks (predominately QX disease) remain problematic for Queensland farmers, and the disease resistance of selectively bred SRO lines in central to southern NSW is not experienced when these oysters are grown in Queensland conditions (Tim Prowse, pers. comm.).

In addition to improvements to SRO culture, an alternative solution for the industry is the development of additional species for hatchery production. A number of oyster species have historically been reported in Queensland (Saville-Kent, 1891), however oysters are notoriously difficult to identify based on morphology alone , and shell morphology is insufficient for taxonomy (Sekino and Yamashita, 2016). No systematic molecular study of Queensland oysters has previously been performed.

One particular oyster, distinctive due to its large size and dark inner shell margin, is the tropical blacklip oyster (BL). The taxonomy of this species is confused, and it is referred to in the literature as *Crassostrea/Saccostrea echinata* or *Striostrea/Saccostrea mytiloides*. This species has been identified as having high aquaculture potential in the Pacific and in northern Australia due to its tolerance of environmental fluctuations, fast growth, and acceptance by consumers (Braley, 1984; Coeroli et al., 1984; Nowland et al., 2019a; Southgate and Lee, 1998). However, poor larval survival in culture and poor settlement rates are a current barrier to production (Coeroli et al., 1984; Nowland et al., 2018a; Southgate and Lee, 1998), and the presence of a similar parasite to that which causes QX (Kleeman et al., 2002; Wolf, 1977) indicates that disease mitigation strategies may also be important for this species (however there is some uncertainty regarding the species of oyster examined in these studies).

A further difficulty is that the natural distribution of the BL oyster throughout Queensland is currently unknown. This is problematic for two reasons, 1) the oyster is unlikely to perform well if grown at a location that is outside its natural range, and 2) permission for translocation of BL oysters will not be provided until the risk to natural genetic stock can be assessed.

Aims/objectives

This study had two primary aims:

1. Determine the distribution of *Saccostrea* species around the Queensland coast.
2. Development of molecular tools to facilitate blacklip oyster production.

Methodology

Aim one was achieved by a succession of field trips for oyster collection. Specimens were returned to the laboratory where they were photographed, dissected, and sampled. DNA extractions were performed and partial COI and/or 16S genes amplified by PCR and sequenced. Phylogenetics was conducted on resulting sequences to enable unambiguous identification of species.

Aim two consisted of two components. The first component involved sequencing of all the genes expressed during comparative stages of development of SRO and BL larvae. The technique used, CEL-Seq2, enables assessment of individual larvae and has never before been applied to oysters. The second component involved specific assessment of variation of two antioxidant genes in adult BL gill and mantle tissue by quantitative PCR. These genes have previously been implicated in disease resistance in SROs.

Results/key findings

The genetic survey of Queensland oysters demonstrated the presence of 14 species – a far greater number than previously recognised, and revealed that Queensland's intertidal oysters consist of more than just *Saccostrea*. Species documented included well-known oysters such as the Sydney rock oyster and tropical blacklip, and 10 species previously unreported from Australia. Of these, one appears to be previously undescribed, and another is almost certainly a recent exotic introduction (this latter species has now been deemed 'biosecurity matter' by Biosecurity Queensland). Importantly, this study has also documented the current distributions of these species between Moreton Bay and Cooktown.

The remainder of the study focussed on the blacklip oyster, *Saccostrea lineage J*. Larval gene expression analysis revealed broad similarities between SRO and BL larval stages, indicating that BL larvae grown in culture are reaching a competent state. Despite this, subtle differences in expression of nervous system-related genes were observed, indicating that the genetic circuitry governing settlement induction may differ between species. The genetic resources developed here represent a powerful resource for understanding the developmental biology of each species. Further to this, detailed investigation of the expression levels of two antioxidant genes (EcSOD and Prx6) that have been implicated in disease resistance in SROs showed that wild BL exhibit large variation in expression of these genes in their gills and mantle. This indicates that selective breeding may also yield increased disease resistance in BL should this species become commercialised.

Implications for relevant stakeholders

This study has revealed the intertidal oyster species that are present in Queensland and their distributions. This information is of critical importance to oyster farmers who are considering diversification, as native oyster species are unlikely to perform well outside their natural latitudinal range. The information is also essential for the legislative bodies who govern oyster aquaculture in Queensland (primarily Fisheries Queensland), as information on correct species identities and natural ranges is required to facilitate effective management of the industry. Finally, the results will also be used by industry, government and university personnel to direct future research efforts towards species that are most likely to be commercially viable.

The project has also developed a suite of molecular tools to support the development of the BL oyster as a major aquaculture species. This data is publicly available and will be utilised by researchers to improve production. The analyses presented here demonstrate that differences in settlement induction and in settlement conditions (e.g., salinity, temperature, density) need to be investigated to enable efficient settlement rates of BL within the hatchery. While some differences in biology inevitably exist, the use of comparative genetics presents an opportunity to short-cut the development of advanced techniques for efficient production of this species,by facilitating trials of molecular techniques developed in SROs.

Recommendations

This work has provided required background knowledge for the development of additional rock oyster species for aquaculture in Queensland. In doing so, two additional species were identified to have particular promise; these are *Saccostrea lineages B and G*. It is recommended that hatchery and grow-out trials be conducted for these species to further assess their suitability.

The genetic survey of species conducted here proved invaluable for revealing the diversity of oysters present and assessing their natural distribution. Similar surveys would be of value elsewhere, particularly in states where there is particular interest in expanding oyster aquaculture. The survey also revealed the presence of a recently established exotic oyster species, and the potential impact of this species on the environment and on native oysters should be investigated.

Finally, this study has provided the first insights into the genetic basis of settlement in SROs and BL. Research should continue to investigate the specific pathways that drive settlement in BL oysters as a means to improve hatchery culture.

Keywords

Oysters, Aquaculture, Diversity, Taxonomy, Identification, Gene Expression

Introduction

Saccostrea glomerata (the Sydney rock oyster, SRO) is a major aquaculture species in New South Wales supporting an industry valued at $51 million in 2017-18 (ABARES 2020). SRO farming was once vibrant in Queensland, with the majority of production occurring in Moreton Bay. In the late 1800s it was the largest industry in the region, based upon wild harvest and employing approximately 140 people (Schrobback, 2015). The Moreton Bay estuary still has the largest oyster lease area available of all estuaries along the east coast, however production is now only 0.5% of the total national production of oysters, contributing to the full time employment of only 12 people (Schrobback, 2015).

The decline in oyster production in Moreton Bay has been partly attributable to increased incidence of QX disease, a mortality-inducing condition caused by infection with a protozoan parasite, *Marteilia sydneyi* (Perkins and Wolf, 1976). Within oysters the parasite appears to be specific to SROs, however the life cycle is known to include secondary, non-oyster hosts (Adlard and Nolan, 2015). Declines in water quality are also thought to have played a role in oyster decline (Schrobback, 2015). There is appetite to expand production of oysters in the state as oyster farming is low-impact, of all aquaculture industries it has the greatest potential for approval in environmentally sensitive areas such as the Great Barrier Reef. Molluscan aquaculture has the lowest environmental cost of all animal production sectors (Hilborn et al., 2018), and therefore represents the best option for the development of new, sustainable animal food products.

Before expansion can be realized the significant challenges facing the industry need to be overcome. One potential course of action is to investigate the potential of other native species for aquaculture, however this is hampered by the lack of knowledge of the species that exist in Queensland, and of their natural distributions. One oyster species that is the current focus of investigation is the tropical blacklip oyster (BL), a large species within the *Saccostrea* genus that is currently farmed on a small scale in Bowen, Queensland, and is being investigated as a potential commercial species in Western Australia and the Northern Territory (Nowland et al., 2019a; Schrobback and Rolfe, 2020). This species is an excellent choice for development as, being a tropical species, it can be cultured in parts of Australia that do not currently produce edible oysters. The taxonomic status of the BL is poorly understood, and it is variably reported in the literature as *Crassostrea/Saccostrea echinata* or *Striostrea/Saccostrea mytiloides*. Genetic studies have designated this particular lineage, or species, as *Saccostrea lineage J* (McDougall, 2018; Sekino and Yamashita, 2016), a name that is favoured currently given that molecular diagnosis is unambiguous. There is still relatively little known about this species, and while it is known to have a broad Indo-Pacific distribution (Nowland et al., 2019b; Sekino and Yamashita, 2016), the extent of its Queensland distribution is unknown. This lack of knowledge is a barrier to further development of the industry, as in Queensland approvals for grow-out of oysters will only be given for areas known to naturally host populations of the species (Fisheries Queensland and Biosecurity Queensland, 2019).

The development of a new oyster species for aquaculture is improved by the establishment of hatchery propagation to provide a reliable supply of single seed spat that allows modern oyster farming systems to be used. Initial hatchery trials for the BL were undertaken several decades ago both in Australia and elsewhere (Coeroli et al., 1984; Southgate and Lee, 1998), and more recent trials have been undertaken at the Darwin Aquaculture Centre (Nowland et al., 2018a; Nowland et al., 2018b; Nowland et al., 2019c). While the initial trails experienced low larval survival this has largely been overcome by improved hatchery protocols (Nowland et al., 2018b; Nowland et al., 2019c) . Despite these advances, low settlement rates remain problematic (Nowland et al., 2018a). Settlement induction in molluscan larvae requires that the larvae have reached a certain developmental stage, 'competency', at which they are able to respond to (usually external)

settlement cues (Hadfield et al., 2001). Hatchery production of molluscs often uses the application of particular chemicals to artificially induce settlement (epinephrine in the case of SROs; O'Connor et al., 2008). However, differences in response to these agents have been noted even between closely related species (Joyce and Vogeler, 2018). Investigating the molecular pathways that are involved in settlement induction events may inform the development of more successful species-specific hatchery protocols.

Finally, it is naïve to believe that other species of rock oysters will not also have their own associated disease risks. In particular, a QX-like parasite has been observed in '*Crassostrea echinata'* and '*Striostrea mytiloides'* from Darwin Harbour (Kleeman et al., 2002; Wolf, 1977), however it is unknown whether the parasite can cause disease and mortality in the species, or if the oyster specimen sampled was, indeed, a BL. Within SROs, selective breeding for resistance to QX was found to also confer resistance to other SRO diseases (Green et al., 2008). Comparisons between wild and selectively-bred oysters demonstrated the differential expression of anti-oxidant genes Prx6 and EcSOD, leading to the hypothesis that resistance is mediated via the ability of resistant oysters to generate hydrogen peroxide, a antiparasitic compound, faster and at higher levels than non-selected oysters (Green et al., 2009). This defence mechanism is likely widespread among oyster species, suggesting that there may be the ability to fast-track selective breeding for disease resistance by selecting broodstock with the desired expression levels of these particular genes. Here we investigate this possibility by assessing natural variation of the expression of these genes in the BL.

Objectives

Aim 1. Determine the distribution of *Saccostrea* species around the Queensland coast. Rationale: Identification of *Saccostrea* species is problematic due to their morphological plasticity. Genetic results from Moreton Bay surveys undertaken during the first year of this project revealed the presence of four distinct species, two of which were previously unrecorded. Better knowledge of which species occur around the Queensland coast and where they occur is required to enable Fisheries Queensland to properly assess applications to farm species new to aquaculture, or to establish farms in areas not currently utilised for oyster farming.

Aim 2. Development of molecular tools to facilitate blacklip production. Rationale: There is a solid base of research for production of SROs. By using comparative transcriptomics (=gene expression) we will be able to exploit the close genetic relatedness of the blacklip and SROs to fast- track efficient production of blacklip oysters.

Method

Aim 1. Determine the distribution of *Saccostrea* species around the Queensland coast

This aim consisted of three subcomponents:

1.1. Genetic survey of Queensland oysters

Sampling field trips were conducted in 2018 and 2019 to 19 locations along the Queensland coast. An initial investigation was conducted at each site to determine the number of morphotypes present at each location. Depending on abundance, between one and ten specimens of each morphotype were sampled from each location using a hammer and chisel. Where possible, whole specimens including shells were collected, however very large specimens (>12 cm in shell length) were photographed *in situ* prior to removal of a small tissue sample. Specimens were preserved in 70% ethanol and kept on ice or at 4˚C until processed.

In the laboratory individual oysters were assigned a unique identification code, photographed (external and internal shell), and a small piece of adductor muscle was dissected. DNA was extracted from this tissue using a DNeasy Blood and Tissue kit (Qiagen), with a final elution volume of 30 µL of ultrapure H_2O. To facilitate comparisons with previous studies (Lam and Morton, 2006; Sekino and Yamashita, 2016), both COI and 16S mitochondrial gene fragments were targeted for sequencing. PCR reactions were carried out using NEB Taq polymerase and ThermoPol buffer. COI gene fragments were amplified using the primers LCO1490 and HC02198 (Folmer et al., 1994) and the following thermoprofile: 94°C for 2 min followed by 30 cycles of 94°C for 30 s, 44°C for 30 s and 68°C for 45 s, with a final extension at 68°C for 10 minutes. 16S gene fragments were amplified using the primers 16S_Fwd (Banks et al., 1993) and 16Sbr-H (Palumbi et al., 1991) and the following thermoprofile: 95°C for 30 s, followed by 30 cycles of 95°C for 1 min, 51°C for 1 min and 68°C for 1 min, and a final extension at 68°C for 5 min. PCR products were purified and submitted for Sanger sequencing at Macrogen Inc. (South Korea).

Resulting sequences were manually assessed and trimmed (using the program 4peaks (Griekspoor and Groothuis, 2006)) and aligned against all ostreid COI and 16S sequences downloaded from GenBank (accessed 26 May 2020), using the program AliView (Larsson, 2014). Phylogenetic analyses were conducted separately for each gene using the IQ-Tree webserver (Minh et al., 2020; Trifinopoulos et al., 2016) with 1000 ultrafast bootstrap inferences (Hoang et al., 2018) using automatic model selection (Kalyaanamoorthy et al., 2017). Resulting trees were visualised using FigTree (Rambaut, 2006).

1.2 Interrogate the Queensland Museum collection and validate species designations

Oyster specimens within the wet and dry collections at the Queensland Museum were investigated with the assistance of Dr. John Healy, curator of molluscs. Morphology of specimens was compared to that of those collected in 1.1 above, and collection localities were noted to aid identifications.

1.3 Create a field guide to Queensland oysters

The results from 1.1 and 1.2 were used to create a field guide to Queensland oysters. The guide was compiled in conjunction with Queensland museum staff to facilitate correct species identification by relevant stakeholders. The guide was publicised to stakeholders at the 2020 Queensland Oyster

Growers Association AGM (20th September 2020), provided directly to stakeholders via email in an electronic format, and is available online at www.mcdougall-lab.com/research and in Appendix 3.

Aim 2. Development of molecular tools to facilitate blacklip production

This aim consisted of two subcomponents:

2.1 Identify the molecular basis for poor settlement of blacklip larvae

Larval SRO and BL oyster samples were collected from commercial hatchery runs at Aquafarms Queensland prior to the study (September 2017- March 2020). Hatchery practices largely followed those outlined in (O'Connor et al., 2008). The larval samples (approximately 100 larvae) were taken daily into RNA later and 70% ethanol. Additional samples were taken once larvae were deemed to be competent to settle (by the observation of well-formed gill buds and an active foot), immediately prior to settlement induction via the addition of epinephrine. Further samples were taken 1h, 4h and 24h post settlement induction. All samples were stored at -20˚C until use.

Ten ethanol-preserved larvae from each stage were randomly chosen, mounted on a microscope slide, and imaged. Shell dorso-ventral and anterior-posterior lengths were digitally measured within cellSens software (Olympus). For gene expression analyses individual larvae were transferred to a microscope slide in RNA later, assessed for normal morphology, and imaged. Larvae were then transferred to individual 1.7 mL polypropylene tubes containing 200 µL Tri Reagent (Sigma Aldrich). RNA was extracted following the manufacturer's instructions, but in a reduced volume and adding 0.5 µL RNA-grade glycogen (20 mg/mL) during precipitation. Pellets were resuspended in 4.5 µL of ultrapure H_2O. RNA yield was assessed using 0.5 µL of sample in the Qubit High-Sensitivity RNA Assay (ThermoFisher).

Gene expression was analysed using the CEL-Seq2 protocol (Hashimshony et al., 2016), with primers modified to include a 7 bp unique molecule identifier. 25 ng of RNA (or the entire 4 µL RNA sample if total yield was <25 ng) was used per larva as input for library preparation. Libraries contained 48 individually barcoded samples, and were sequenced on one lane of a NovaSeq 6000 (Ramaciotti Centre for Genomics, UNSW, Sydney). Transcript counts were generated using the CEL-Seq2 analysis pipeline (Hashimshony et al., 2016), with reads mapped against SRO and BL oyster transcriptomes (McDougall, 2018). Normalised counts (counts per million) were generated and differential gene analysis performed using the edgeR package (Robinson et al., 2010) in R (R Core Team, 2014). Gene annotation and ontology (assigning likely functions to genes based on sequence similarity to known genes) and orthologue detection (determining which gene corresponds to which between species) has been performed previously (McDougall, 2018).

2.2 Assess the potential for marker-assisted selection in the blacklip oyster

Previously identified Sydney rock oyster EcSOD and Prx6 genes (Green et al., 2009) were used as query sequences in a tBLASTn search against the SRO and BL oyster transcriptomes (McDougall, 2018). Resulting sequences were aligned with other EcSOD and Prx6 gene family members downloaded from GenBank, using the program AliView (Larsson, 2014). Phylogenetic analyses were conducted in RAxML v8.2.11 (Stamatakis, 2014) using automatic model assignment and 100 rapid bootstrap inferences. Phylogenetic trees were viewed using FigTree (Rambaut, 2006). qPCR primers were designed using Primer3 v0.4.0 (Untergasser et al., 2012) (Table 1).

Table 1. Details of primers used in this study.

Gene	Primer name	Primer sequence	Annealing temp.
ecSODa	qBL_ecSOD_F	ATCACGGACTCCAGATCCAC	56˚C
	qBL_ecSOD_R	TATCGGTGACGTTTCCATGA	
Prx6a	qBL_Prx6_F	GGCCCTGACAAGAAACTGAA	48˚C
	qBL_Prx6_R	ACACTTGTCACCGTCCTTCC	
ELF1α	qBL_ELF1a_F	GAAAGGAAGGAGGGAAATGC	56˚C
	qBL_ELF1a_R	GACGAAGGGCCAAATCTGTA	
β-tubulin	qBL_b_actin_F	AGGATCTGTACGCCAACACC	56˚C
	qBL_b_actin_R	CACCGATCCAGACGGAGTAT	

Adult BL oyster specimens were collected from five locations across Queensland and the Northern Territory (Elizabeth Bay, NT; Buchan's Point, QLD; Finch Beach, QLD; Orpheus Island, QLD; White Lady Bay, QLD). Oysters were immediately dissected and mantle and gill samples taken into RNAlater (Sigma Aldrich). Samples were incubated in RNAlater for approximately 24 h before being stored at -20˚C until use. RNA was extracted using Tri Reagent (Sigma Aldrich) as per the manufacturer's instructions, using and 0.25 mL of isopropanol and 0.25 mL of high salt precipitation solution (0.8 M sodium citrate and 1.2 M sodium chloride) for precipitation. RNA quantity was assessed using a Qubit Broad Range RNA Assay (ThermoFisher), and quality was assessed via agarose gel electrophoresis. RNA was DNAse treated (DNaseI, Invitrogen) and converted to cDNA using the SuperScript™ III First-Strand Synthesis System (Invitrogen), with priming using random pentadecamers. Resulting cDNA was checked via PCR using β-tubulin primers, and no-RT controls were included to confirm the absence of contaminating DNA. Samples that did not produce the expected result in these assays were removed from further analysis.

Expression of Prx6 and EcSOD genes was quantified via qPCR. β-tubulin and ELF1α were chosen as reference genes based upon previous studies (Green et al., 2009; Green et al., 2014), and blacklip oyster β-tubulin and ELF1α homologues (BL_TRINITY_DN39900_c69_g1_i3 and BL_TRINITY_DN25552_c1_g1_i1, respectively) were identified using a BLAST search against the blacklip oyster transcriptome. Primers were designed as outlined above. Quantitative PCR was performed in 10 µL volumes using the QuantiNova SYBR Green PCR kit (Qiagen), using 4.5 µL master mix, 0.2 µL of each primer (from a 2.5 µM working stock) and 2 µL of template DNA. The thermocycle consisted of an initial denaturation at 95˚C for two minutes, followed by 40 cycles of 95˚C for 10 seconds, optimized annealing temperature for 20 seconds, and 72˚C for 20 seconds. PCR annealing temperatures were optimized using a Bio-Rad T100 Thermal Cycler (see Table 1), and qPCR reactions were conducted in a Bio-Rad CFX96 Thermal Cycler, incorporating a melt curve analysis at the conclusion of the program. Primer efficiencies were calculated using a dilution series of a pooled calibrator sample (from 1:10, 1:20, 1:50, 1:100, 1:200, 1:500, 1:1000). Target gene expression was normalized using both reference genes for each sample. Data were analysed within the BioRad CFX Maestro™ software using the ΔΔCq method.

Results, discussion and conclusion

Aim 1. Determine the distribution of *Saccostrea* species around the Queensland coast

COI and/or 16S sequences were obtained for 342 oyster specimens from 19 different locations (Table 2). Phylogenetic analysis revealed the presence of 14 distinct oyster lineages (Figure 1; analysis of COI and 16S produced similar results, only COI is presented here). Of these, only four had previously been recorded from Australia. One of these does not group with any previously sequenced oysters and may represent a newly discovered species. Many of these lineages correspond to those identified in previous studies (Lam and Morton, 2006; Sekino and Yamashita, 2016) and are named according to the convention previously applied (*Saccostrea lineage B,* etc; some previously named lineages such as *S. lineage A* were not found in this survey). Given the phylogenetic separation of these lineages in comparison to the separation observed in accepted species it is likely that each lineage represents a distinct species (Figure 1). Determining the correct species name to assign to these groups is challenging due to the variable morphology of these animals and the difficulty in assigning these to type specimens, this was attempted here only for one species of particular concern (see below). It is important to note that the name '*Saccostrea cucullata'* was previously broadly used to refer to *Saccostrea* specimens from the Indo-West Pacific (Harry, 1985), it has since been demonstrated that *S. cucullata* is an erroneously named superspecies that comprises many of the lineages subsequently designated (Lam and Morton, 2006; Sekino and Yamashita, 2016).

Table 2. Species identified at each location surveyed in this study.

General location	Species identified	Collection sites and GPS co-ordinates
Southern Moreton Bay	*Saccostrea glomerata* *Saccostrea lineage B* *Saccostrea lineage G* *Saccostrea scyphophilla A/B* *Ostrea/Dendostrea sp. 2*	Jacob's Well -27.7845, 153.3729 Colman Road Reserve -27.8214, 153.3790 Macleay Island -27.5812, 153.3613 Wellington Point -27.4647, 153.2401 North Stradbroke Island – Dunwich -27.5079, 153.4092, Amity -27.4025, 153.4372, Frenchman's Beach -27.4267, 153.5441 Goat Island -27.5170, 153.3838
Northern Moreton Bay	*Saccostrea glomerata* *Saccostrea lineage G* *Ostrea equestris* *Talonostrea sp. nov.*	Woody Point -27.2635, 153.1037 Sandgate -27.3218, 153.0811 Ningi Creek -27.0635, 153.1268 Newport, Blue Park -27.1981, 153.1028
Caloundra	*Saccostrea glomerata* *Saccostrea scyphophilla A/B*	Centaur Park -26.8032, 153.1463
Hervey Bay/Fraser Island	*Saccostrea glomerata* *Saccostrea lineage B* *Saccostrea lineage G* *Saccostrea scyphophilla A/B*	Point Vernon -25.2462, 152.8273 Urangan Harbour -25.2960, 152.9088 Fraser Island - Moon Point -25.2281, 152.9928 Big Woody Island -25.299372, 152.9662 Round Island -25.2877, 152.9248
Bundaberg	*Saccostrea lineage B* *Talonostrea sp. nov.*	Burnett Marina -24.7587, 152.3897
Miara	*Saccostrea lineage B* *Saccostrea lineage G*	Miara Holiday Park -24.6679, 152.2017
Seventeen Seventy	*Saccostrea glomerata* *Saccostrea lineage G* *Saccostrea lineage B* *Saccostrea scyphophilla A/B* *Ostrea/Dendostrea sp. 1*	Headland -24.1505, 151.8844 Air Sea Rescue Park -24.1682, 151.8806 Cook's Monument -24.1598, 151.8824 Endeavour Park -24.1633, 151.8844

Turkey Beach	*Saccostrea lineage G* *Saccostrea lineage B* *Saccostrea scyphophilla A/B*	The Esplanade -24.0740, 151.6521
Yeppoon (Rosslyn Bay)	*Saccostrea lineage B* *Saccostrea scyphophilla A/B* *Talonostrea sp. nov.* *Ostrea/Dendostrea sp. 1*	Rosslyn Bay Marina -23.1631, 150.7881 Wreck Point -23.1447, 150.7646
Stanage/Avoid Island	*Talonostrea sp. nov.* *Saccostrea lineage J* *Saccostrea lineage B* *Saccostrea lineage G* *Saccostrea scyphophilla A/B*	Avoid Island (turtle shell) -21.9756, 149.6631 Stanage – Plumtree -22.1369, 150.0309 Stanage - Alligator Bay -22.1384, 150.0631
Percy Islands	*Saccostrea scyphophilla A/B* *Saccostrea lineage G*	Middle Island -21.6525, 150.2484
Whitsunday Islands/Shute Harbour/Newry Island	*Saccostrea lineage J* *Saccostrea scyphophilla A/B* *Saccostrea scyphophilla C* *Saccostrea lineage B* *Saccostrea lineage G* *Saccostrea lineage F*	Whitsunday Island - Dugong Beach -20.2441, 148.9530 Hook Island - Bird Point -20.1196, 148.8867 Hook Island - Nara inlet -20.1589, 148.9024 Shute Harbour -20.2899, 148.7885 Newry Island -20.8533, 148.9230
Bowen	*Saccostrea lineage J* *Saccostrea lineage G* *Saccostrea lineage B* *Saccostrea lineage F* *Talonostrea sp. nov.*	Bowen oyster lease – wild spat -19.9483, 148.1486
Magnetic Island	*Saccostrea lineage J* *Saccostrea lineage B* *Saccostrea lineage G* *Saccostrea scyphophilla A/B* *Talonostrea sp. nov.*	Nelly Bay Harbour -19.1605, 146.8550 Rocky Bay -19.1733, 146.8461 Horseshoe Bay -19.1183, 146.8530 White Lady Bay -19.1066, 146.8627
Orpheus Island	*Saccostrea lineage I* *Saccostrea lineage J* *Saccostrea lineage B* *Saccostrea lineage G* *Saccostrea scyphophilla C* *Dendostrea crenulifera*	Pioneer Bay (Orpheus Island Research Station) -18.6116, 146.4889
Cardwell	*Saccostrea lineage B*	Point Hinchinbrook -18.2764, 146.0483
Cairns	*Magallana bilineata* *Talonostrea sp. nov.* *Saccostrea lineage F* *Saccostrea lineage G* *Saccostrea lineage J* *Saccostrea lineage B* *Saccostrea scyphophilla A/B*	Trinity Blue Water Marina -16.8061, 145.7071 Buchan's Point -16.7360, 145.6644 Cairns Esplanade -16.9186, 145.7793 Cairns Harbour -16.9234, 145.7808
Port Douglas/Cape Tribulation	*Saccostrea scyphophilla A/B* *Saccostrea lineage B* *Magallana bilineata*	Cow Bay -16.2291, 145.4694 Oak Beach -16.5854, 145.5159 Port Douglas Marina -16.4915, 145.4598
Cooktown	*Saccostrea lineage J* *Saccostrea lineage B* *Saccostrea scyphophilla C* *Saccostrea lineage G* *Magallana bilineata*	Finch Beach -15.4717, 145.2656 Cooktown Boat Ramp -15.4609, 145.2492

Although several of these oysters have widespread distributions, some are more restricted (Table 2). *S. lineage B* and *S. lineage G* were found from southern Moreton Bay to the most northern site sampled, Cooktown. Both of these oysters attained reasonably large sizes in the field (observed here to 7.5 cm shell length), and therefore represent potential candidates for aquaculture. *S. glomerata*, the Sydney rock oyster, was found as far north as Seventeen Seventy, whereas *S. lineage J*, the tropical black-lip oyster, was not found south of Stanage (near Rockhampton). *S. lineage I*,

Ostrea/Dendostrea sp. 2, and *Dendostrea crenulifera* are so far known from single specimens, for the *Ostrea/Dendostrea* lineages this is likely because these are subtidal species that were only exposed on a very low tide. *S. lineage I* was collected from mangrove branches high in the intertidal zone and was initially mistaken for a small *S. lineage J*; other specimens of similar appearance were noted on other mangrove branches at this site. It is likely that this oyster would be found elsewhere if looked for specifically.

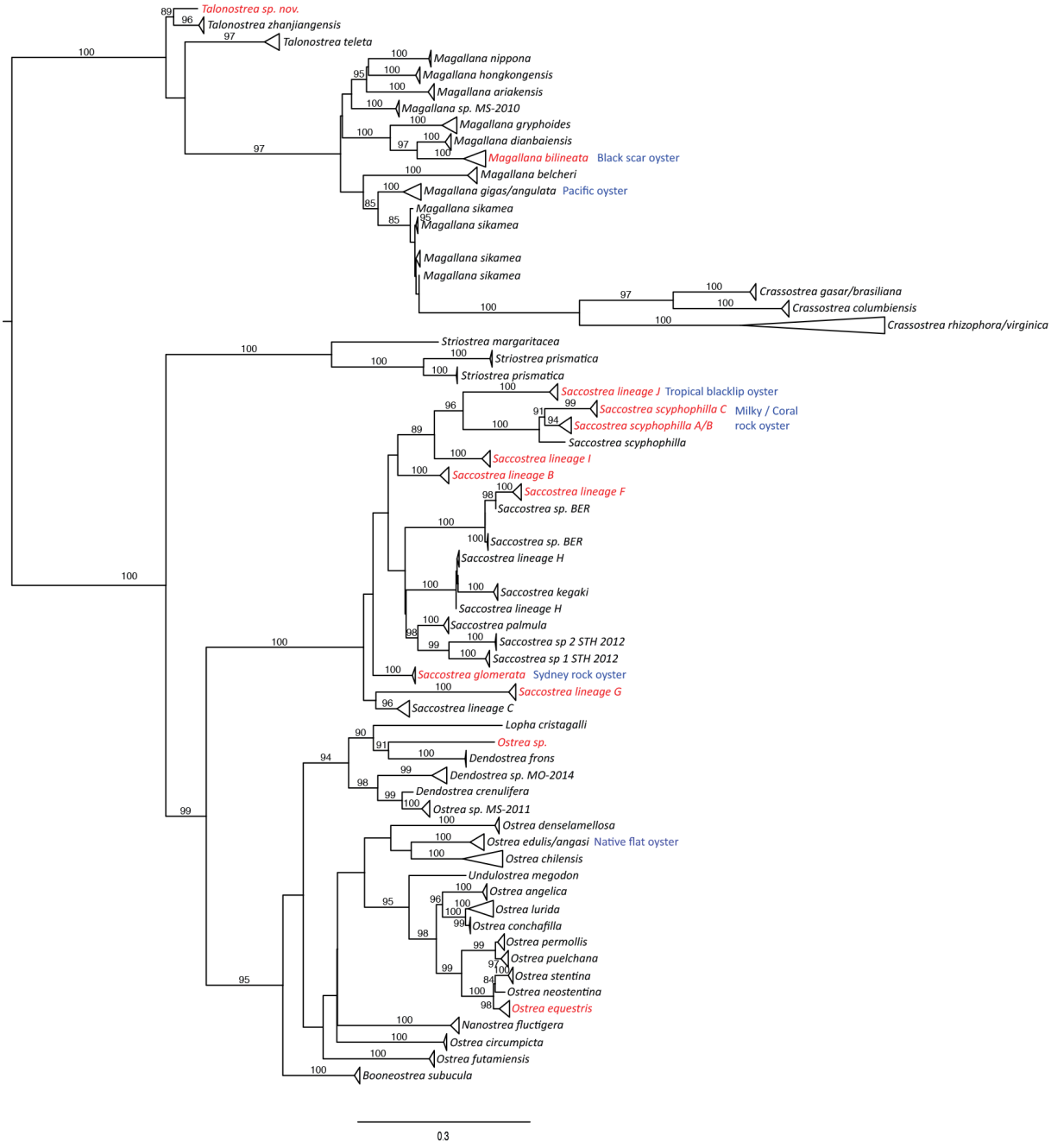

Figure 1. Phylogenetic analysis of oyster COI sequences. Each clade is collapsed for ease of interpretation. Clades containing oysters sequenced in this study are highlighted in red. Common names of key species are indicated in blue. Numbers above branches refer to the percent bootstrap value and are only shown if over 80%. Scale bar indicates substitutions per site.

Two findings warrant specific note. The first is the detection of a small, nondescript oyster that, upon sequencing, appears to represent a previously undescribed species. No other COI or 16S sequences for this species have been lodged within the NCBI database, and no similar oyster specimens can be found in collections at the Queensland Museum. Phylogenetic analysis places this species within the genus *Talonostrea* Li & Qi 1994, and formal description is underway. Given that this oyster has a wide distribution (Moreton Bay to Magnetic Island), is small (< 3.5cm shell length), and has an unremarkable appearance (probably often mistaken as spat of other species), it is likely that the species has remained undetected rather than being a reasonably recent introduction.

On the other hand, the finding of the large and distinctive *Magallana bilineata* (= *Crassostrea bilineata*, *Crassostrea madrasensis, Crassostrea iredalei*) at several sites in far north Queensland is almost certainly the result of the introduction of an exotic species. Specimens of this species are large (found here up to 12 cm in length), deeply cupped when adult, and have a distinctive black muscle scar (Huber, 2010). Shortly after their discovery in this survey (near Cairns) members of the public independently reported the presence of the species to Biosecurity Queensland in both Port Douglas and Cooktown. No previous record of this species in Australia can be found in the literature, and no historic specimens exist in the Queensland Museum or the Museum of Art and Natural History in Darwin (Dr Richard Willan, personal communication). *M. bilineata* is an important aquaculture species in other parts of the world (Chalermwat et al., 2003; Suja et al., 2020), and has been deliberately introduced for this purpose in Fiji (Kinch et al., 2019a; Kinch et al., 2019b). *M. bilineata* is currently deemed to be biosecurity matter under the Queensland *Biosecurity Act 2014* (Queensland Government, 2020). The detection and identification of this species in Queensland has been published (Willan et al., 2020).

The collections at the Queensland Museum were also examined as part of this study. There are 141 registered '*Ostreid*' specimens with a Queensland locality as of November 2019, all but six are identified to at least genus level. No specimens with similarity to *Magallana bilineata* or *Talonostrea sp. nov.* were found within the collection. Within *Saccostrea*, only *S. cucullata*, *S. dactylena*, *S. glomerata* and *S. scyphophilla* are represented. *S. cucullata* specimens have been misidentified (see above), and by morphology could represent any of several of the *Saccostrea* lineages found within this survey. *S. dactylena* is also misidentified (likely *Magallana dactylena*), and specimens in the museum have been collected by dredge, likely explaining why the species was not uncovered in this survey. It is evident that the collection at the museum is incomplete, and representative specimens from this survey will be lodged there for future reference.

To further aid in oyster identification an illustrated 'Guide to Queensland's intertidal oysters' has been produced based upon the findings of this survey. The guide is available online at https://www.mcdougall-lab.com/research, and in Appendix 3.

Aim 2. Development of molecular tools to facilitate blacklip production

2.1 Identify the molecular basis for poor settlement of blacklip larvae

In order to facilitate comparisons between SRO and BL larval development in the hatchery runs sampled in this study, ten ethanol-preserved larvae from approximately every second sampling point for each species were randomly selected, photographed, and measured (Figure 2). From 1-4 days post fertilisation (dpf) BL embryos and larvae were larger than SRO larvae of the same age, however SRO larvae exhibited faster growth rates overall. As a result, SRO larvae developed eyespots earlier than BL larvae (17 vs 20 dpf), however larvae that possessed eyespots were a similar size in the two species (smallest BL larva observed with an eyespot was 256 µm in length, smallest SRO larva was 285 µm). This is the time at which eyespots were observed in the ten larvae randomly selected at

each timepoint, however eyespots were observed in some larvae much earlier in the hatchery (first eyed larvae observed at 14 dpf in SRO run and 16dpf in BL run). Settlement induction took place at 21 dpf for SRO and 24 dpf for BL. Normal settlement rates were observed for SRO, however settlement induction failed for the BL (<1% settlement). We note that hatchery protocols are still in development for the BL oyster and that growth rates obtained within this hatchery run may not be optimal when compared to performance of larvae in other studies using this species (Nowland et al., 2018a; Nowland et al., 2018b; Nowland et al., 2019c). Southgate and Lee (1998) observed that BL settlement occurred between 20 and 25 dpf and varied in response to larval diet, therefore it is possible that nutritional requirements were not adequate during the hatchery run sampled in this study. Despite this, similarly low settlement rates have been observed elsewhere when settlement induction occurred much earlier (Nowland et al., 2018a).

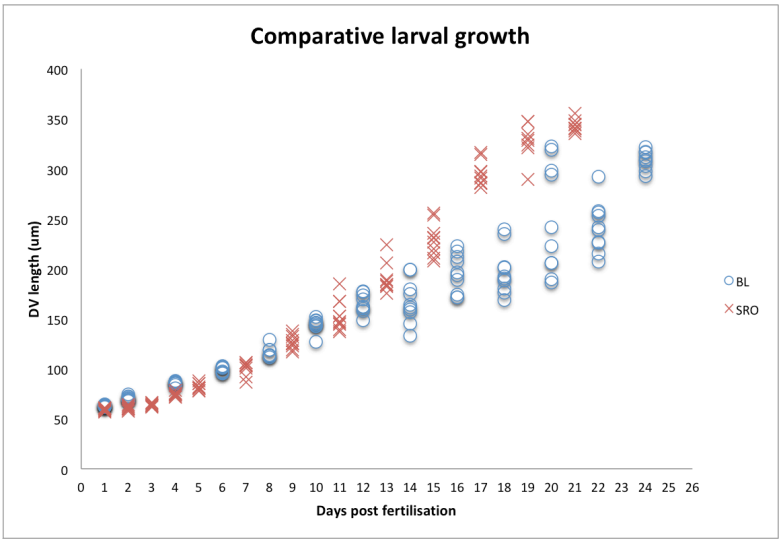

Figure 2. Comparative larval growth rates for blacklip (BL) and Sydney rock oyster (SRO) hatchery runs sampled in this study.

Whole transcriptome data was successfully obtained for between 1 and 4 larvae per time point for each species. Very low counts were obtained for larvae younger than 10 dpf, therefore these samples were omitted from the analysis. An MDS plot was generated for each species based upon filtered whole transcriptome data to visualize the relationship between samples. For SRO (Figure 3a), the samples fell into three main groups. Group one (highlighted in orange) consisting of samples between 10 and 16 dpf, group two (highlighted in blue) consisting of samples from 16 dpf (one specimen) and 21 dpf 4 hours post induction (hpi), and a third group (highlighted in green) consisting of the three 22 dpf 21 hpi specimens. The large separation between groups one and two is of interest, and likely corresponds to precompetent (group 1) and competent (group 2) individuals. Images of the three 16 dpf larvae sequenced (Figure 4 A-C) demonstrated that SRO_16d_3, which falls within group 2, was larger than the other two specimens and had developing gill buds, a morphological sign that the individual was approaching competency (O'Connor et al., 2008). Group three consists of larvae that have clearly metamorphosed (Figure 4 D).

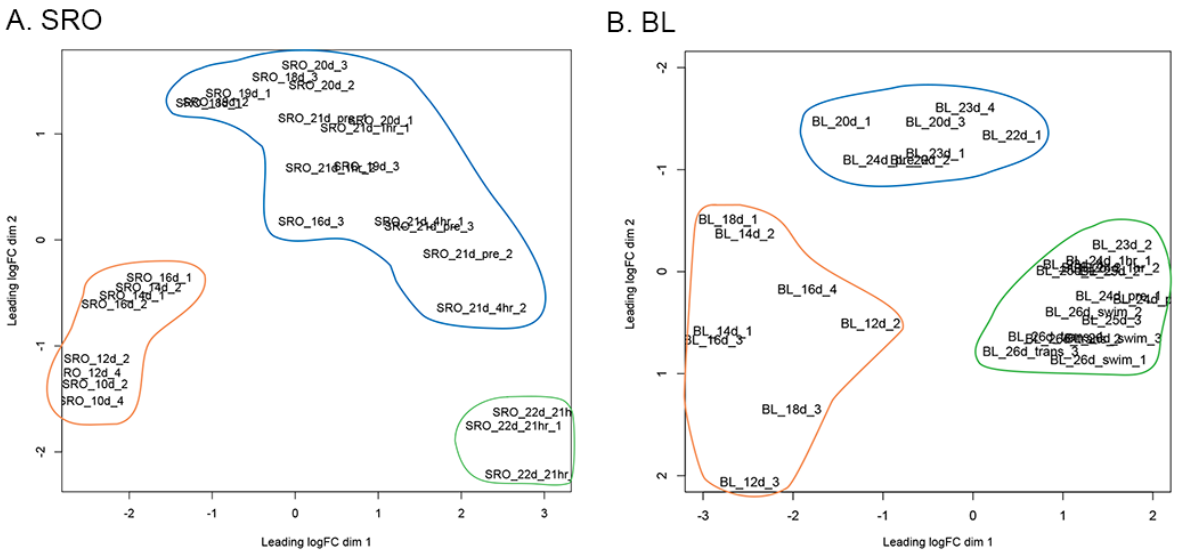

Figure 3. MDS plots of larval sampled profiled using CEL-Seq2. A. SRO larvae. B. BL larvae. Natural groupings of larvae have been indicated by coloured outlines.

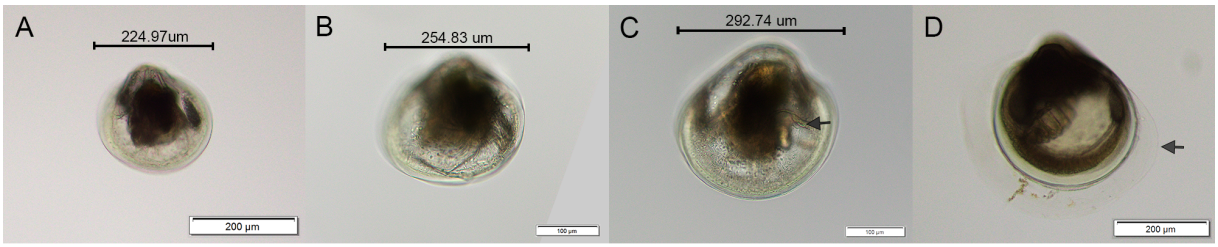

Figure 4. Subset of SRO larvae used for CEL-seq2. A. SRO 16 dpf-1. B. SRO 16 dpf-2. C. SRO 16dpf-3. Arrow indicates the developing gill bud. D. SRO 22 dpf 21 hpi -1. Morphological indication of metamorphosis includes the secretion of adult shell (arrow) and the clearly visible gill.

The MDS plot for the BL samples (Figure 3b) shows roughly similar groupings. Younger larvae (between 12 and 18 dpf) form group 1 (highlighted in orange), individuals between 18 dpf and 24 dpf (one pre-induction individual) can be found in group 2 (blue highlight), and group 3 (green highlight) consists of one 23 dpf individual, two 24 dpf pre-induction individuals, and all post-induction individuals (to 26 dpf 2 dpi). Similar to SRO there is a clear difference between early and late larvae, which we again propose represents the difference between precompetent and competent individuals. There is no observable separation between pre and post-induction larvae, which is congruent with the lack of metamorphosed spat obtained for this species.

Analysis was then performed to identify the genes that are differentially expressed between larvae of different stages for each species. Samples were allocated to three groups based on the MDS plot: precompetent, competent, and post-induction (individuals were allocated to the latter group if they had been exposed to epinephrine regardless of their placement). Pairwise comparisons were performed for each species (Table 3).

Table 3. Numbers of differentially expressed genes identified between stages in each species.

	Differentially expressed genes (FDR corrected p value <0.05)	
Comparison	**SRO**	**BL**
Precompetent vs competent	2406	1182
Precompetent vs post-induction	9641	4894
Competent vs post-induction	4118	2001

Given the failure of settlement induction in BL, genes that were differentially expressed before and after induction in SRO larvae were compared between the two species. Particular attention was placed on genes involved in the nervous system (as assessed by gene ontology annotations), as epinephrine is a neurotransmitter and neural signalling pathways are known to control metamorphosis in many invertebrate larvae including molluscs (Joyce and Vogeler, 2018). Many of the genes that were differentially expressed between competent and post-induction larvae in SROs were also differentially expressed in the same comparison in the blacklip, but there were several exceptions. Figure 5 shows gene expression plots for several genes with nervous system functions in each species. Some genes showed very similar profiles throughout development, whereas others seemed to differ substantially. The exact functions of these genes during settlement and metamorphosis is unknown, but further investigation as to their roles is warranted.

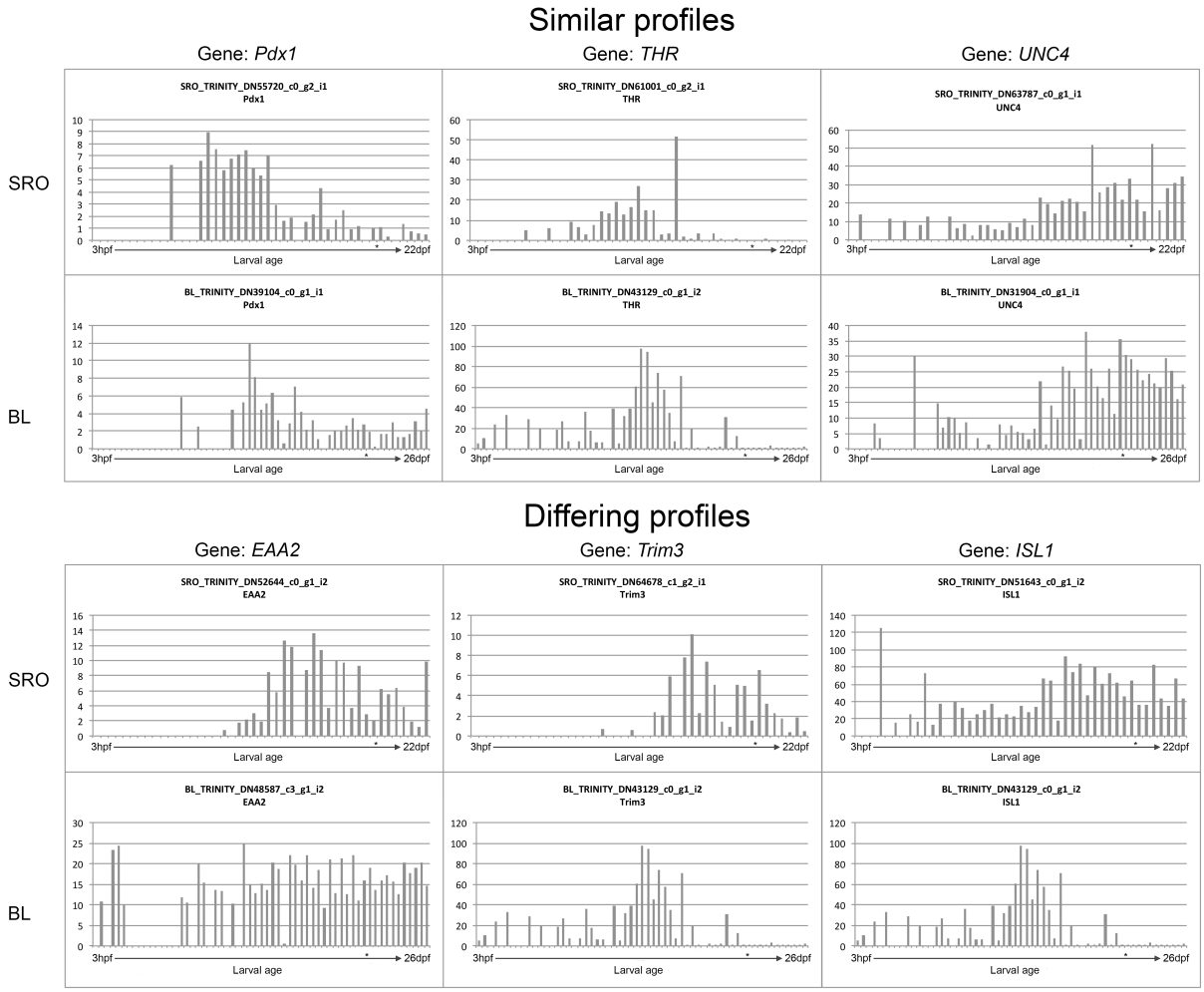

Figure 5. Expression levels of selected nervous system-related genes over development in each species. Genes that have similar profile between the two species are shown in the upper panel, genes with differing profiles are shown in the lower panel. Expression values are normalised counts (counts per million). Larvae younger than 10 dpf are shown in this comparison. The asterisk on the x axis indicates the point of settlement induction.

The vast amounts of data produced during this experiment represent a powerful resource for understanding the developmental biology of both species. From the results outlined here, it appears that BL larvae are attaining a competent state during rearing within the hatchery. The failure of this species to settle and metamorphose at reasonable rates upon exposure to epinephrine, and the differences that are evident in the expression of some nervous system-related genes during the

period of competency, demonstrates that subtle, but important, differences in the induction mechanism exist. Such differences between closely related species are not uncommon for molluscs, and differences in the induction, settlement and metamorphosis processes are likely responsible for the different distributions of oyster species observed in nature.

We note that significant improvements in settlement rates for the BL have been attained using optimized hatchery practices, 0.49% to 4.26% using optimised larviculture protocols (Nowland, 2019) and as high as 10% in recent production (Dr. Sam Nowland, personal communication). This indicates that larval condition affects settlement success. Despite these improvements settlement rates remain significantly lower than those observed for SROs, which is normally between 40 and 70% (O'Connor et al., 2008).

2.2 Assess the potential for marker-assisted selection in the blacklip oyster

Previous research has identified a number of genes for which expression levels are correlated with resistance against a number of different diseases in the Sydney rock oyster (Green et al., 2009). In particular, it was found that two anti-oxidant enzymes, ecSOD and Prx6, were constitutively expressed at higher and lower levels, respectively, in oysters bred for disease resistance. Here we sought to determine whether natural variation in expression of these genes exists within the closely-related tropical blacklip oyster. Searches of oyster transcriptomes and phylogenetic analysis reveals that there are two copies of each of these genes in both species, a complexity not previously realised (Figures 6 and 7). We have designated the Prx6 and ecSOD genes that were investigated in previous studies Prx6a and ecSODa, and the newly identified copies Prx6b and ecSODb. Only Prx6a and ecSODa were investigated further, as it is unknown whether the second copies of these genes also show variable gene expression in Sydney rock oysters.

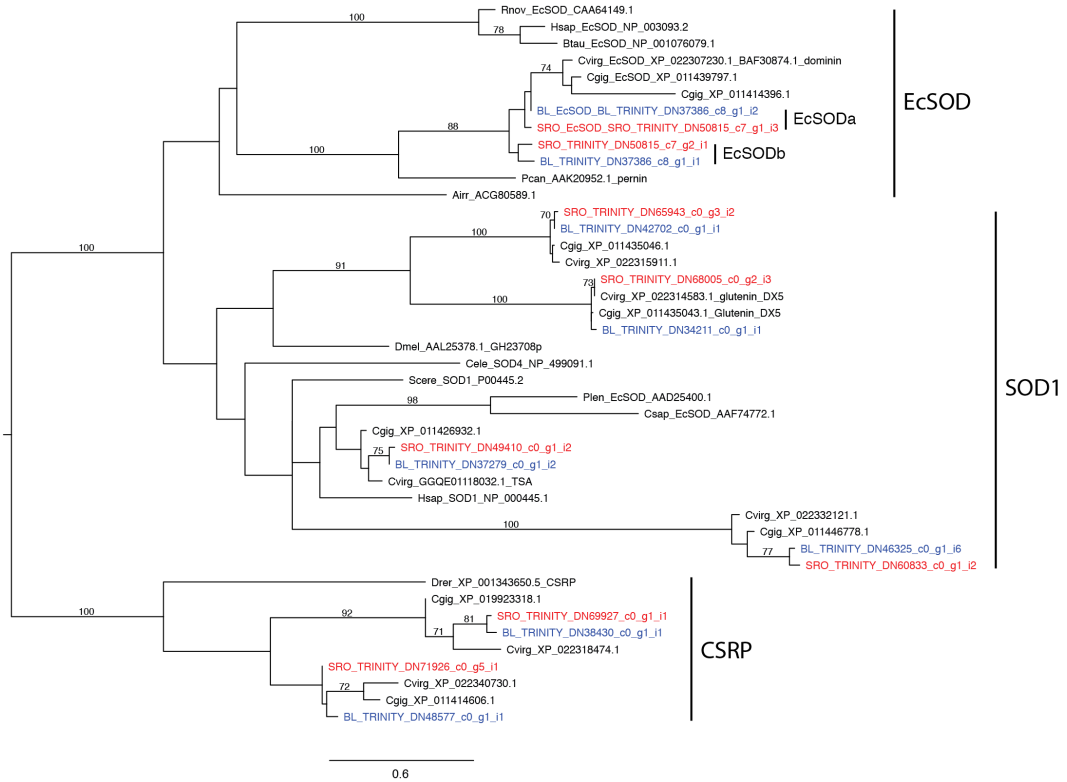

Figure 6. Phylogenetic tree of Sydney rock oyster (red) and blacklip oyster (blue) ecSOD-related transcripts. Both oyster species possess two transcripts that fall within the ecSOD clade. Bootstrap support values are indicated on branches when greater than 70%. The scale indicates the number of

substitutions per site. Labels associated with other taxa (in black) are the GenBank accession numbers for the included sequence.

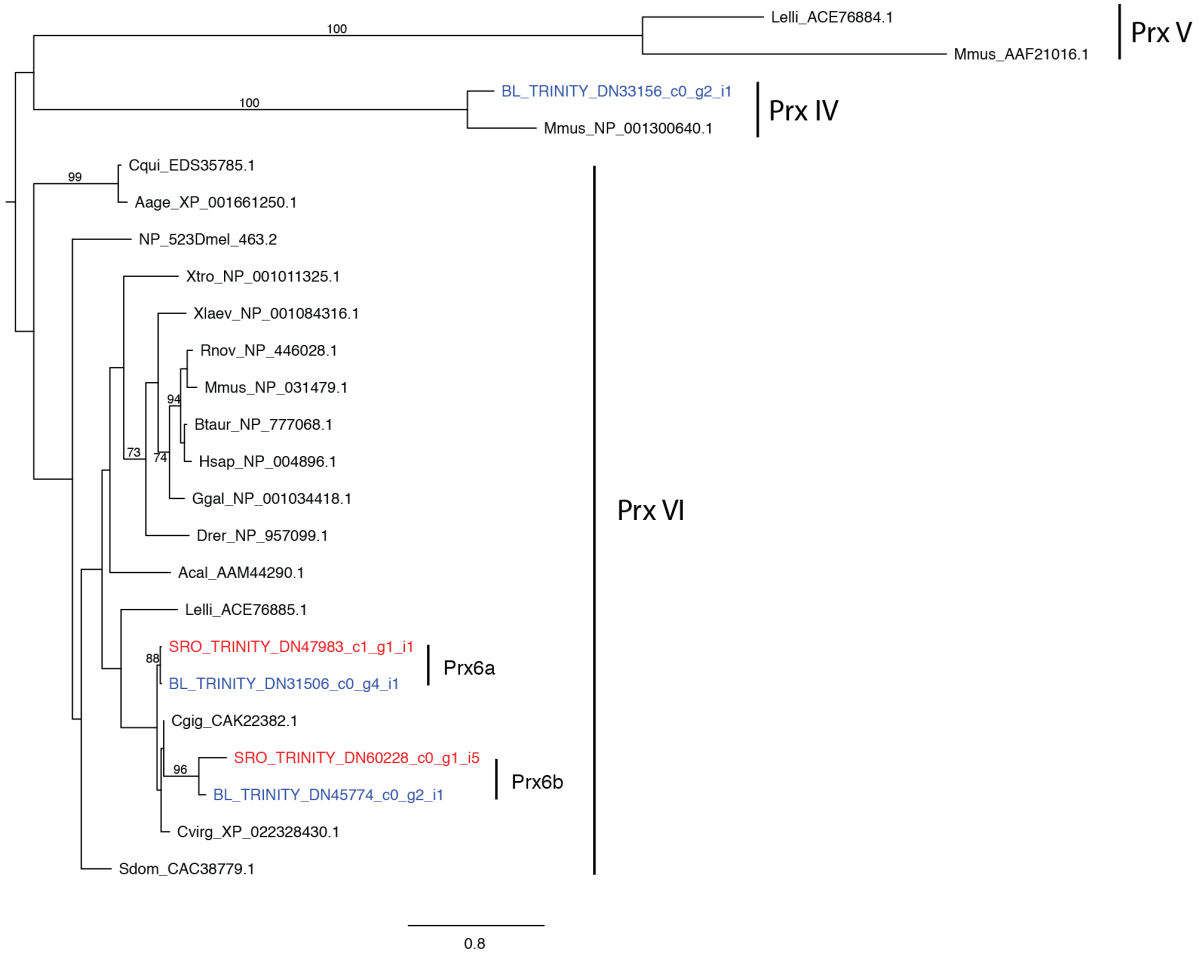

Figure 7. Phylogenetic tree of Sydney rock oyster (red) and blacklip oyster (blue) Prx-related transcripts. Both oyster species possess two transcripts that fall within the Prx VI (Prx6) clade. Bootstrap support values are indicated on branches when greater than 70%. The scale indicates the number of substitutions per site. Labels associated with other taxa (in black) are the GenBank accession numbers for the included sequence.

Adult blacklip oysters were collected from five different localities and the expression levels of ecSODa and Prx6a were assessed in gill and mantle samples by qPCR. Gene expression levels were normalised against two reference genes, ELF1α and β-tubulin. Expression levels were found to vary significantly, with a 68.9 and 17.5-fold difference in expression levels observed in ecSODa and Prx6a, respectively, in gill samples, and a 110.2 and 10.6-fold difference in mantle samples (Figure 8). While population differences in gene expression were not explicitly tested, significant variation was observed in individuals sampled from the same site (for example, from individuals from White Lady Bay, WLB). These results demonstrate that natural variation in Prx6a and ecSODa gene expression exists in wild blacklip oyster populations, suggesting that selective breeding of blacklip oysters may result in increased disease resistance in a similar manner to that observed in Sydney rock oysters.

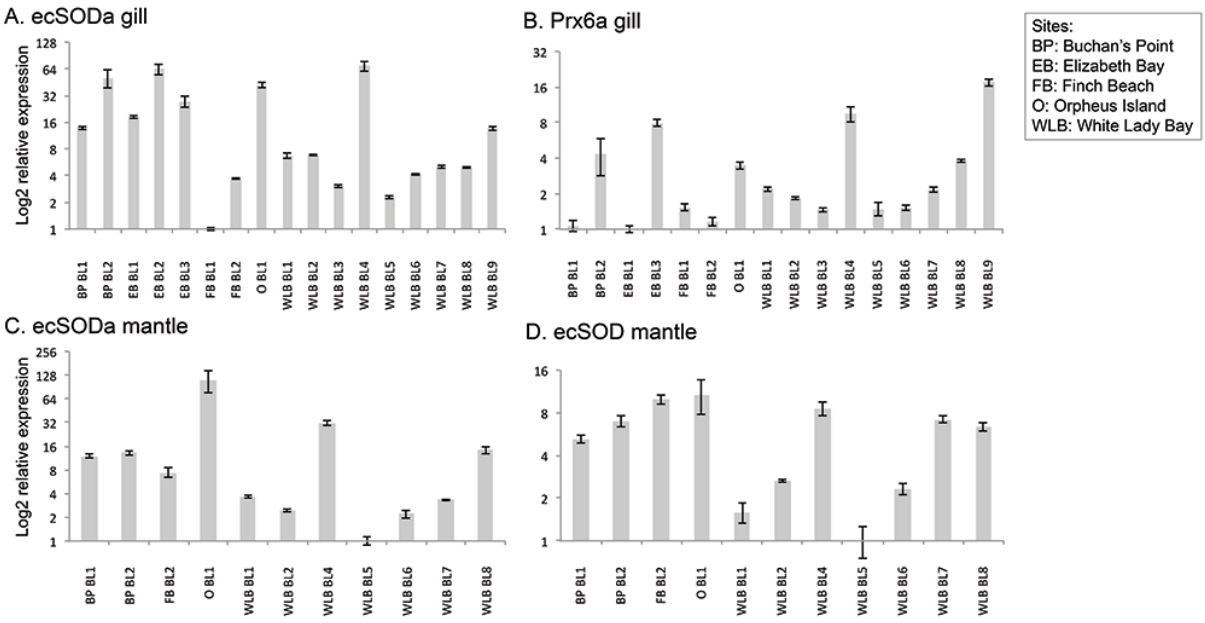

Figure 8. Expression data for ecSODa and Prx6a in the gills and mantle of individual blacklip oysters. Expression values are scaled relative to the sample with the lowest expression levels for each gene and sample type.

Implications

This study has revealed that oyster biodiversity in Queensland is much greater than previously recognised, and that the species identified have different, and overlapping, distributions. The study also demonstrates that the use of genetic techniques is critical for unambiguous identification of oyster species, a finding that has implications for how oyster populations are assessed, regulated, and managed.

Understanding the natural distribution of oyster species is important for oyster farmers who are considering diversification beyond traditionally farmed varieties. The broad distribution of some species, such as *Saccostrea lineages B* and *G*, from Moreton Bay to Cooktown indicates that there are no major barriers to dispersal along the Queensland coast. Rather, the latitudinally restricted distributions of other species likely reflect their differing environmental tolerances, and efforts to farm these species outside their natural range may produce suboptimal results.

Information regarding species distributions is also essential for aquaculture regulators to minimise biosecurity and genetic pollution risks. In Queensland permission to farm a particular oyster species will only be granted within its natural distribution range. Obviously the efficacy of this approach is reliant on accurate information on species identities and distributions.

The project has also produced a suite of molecular tools to support the development of the tropical blacklip oyster as a major aquaculture species. This data is publicly available and will be utilised by researchers to improve production. In other species, molecular approaches have been used to identify factors that induce spawning (In et al., 2016) and gonad maturation (Smith et al., 2019), and to reveal genetic markers for fast growth (Zhang et al., 2019). Further, the molecular analyses conducted here demonstrate that differences in settlement induction need to be investigated to enable efficient settlement rates of BL within the hatchery. Overall, the use of comparative genetics presents an opportunity to short-cut the development of advanced techniques for efficient production of this species by exploring homologues of genes that have demonstrated functional advantages in other species.

Recommendations

This work has provided required background knowledge for the development of additional rock oyster species for aquaculture in Queensland. In doing so, two species in particular were identified to have particular promise. These two species, *Saccostrea lineage B* and *G,* have broad Queensland distributions and were found to attain reasonable sizes in the field. Given their morphological similarity and overlapping distribution with the Sydney rock oyster, *Saccostrea glomerata*, it is possible that these species are already unknowingly grown by farmers where local spat collection is utilised. It is recommended that genetic screens be undertaken to determine whether this is the case, and that hatchery and grow-out trials be conducted for these species to further assess their suitability for culture.

The interest in diversification of rock oyster species for aquaculture is not restricted to Queensland. The genetic survey of species conducted here proved invaluable for revealing the diversity of oysters present and assessing their natural distribution. Similar surveys would also be of value elsewhere in Australia, particularly in states where there is interest in expansion to areas not normally utilised for oyster aquaculture, for example, tropical Western Australia and the Northern Territory, where pilot farming of tropical species is currently taking place. The survey undertaken in this study also revealed the presence of a recently established exotic oyster species, and the potential impact of this species on the environment and on native oysters (natural or otherwise) should be investigated. Distribution mapping will provide important baselines for the evaluation of changes in oyster distribution, native or otherwise, in the future.

Finally, single-larvae transcriptome analysis is a powerful method to understand variation of developmental pathways both within hatchery runs and between different culture conditions. In this study the method has provided the first insights into the genetic basis of settlement in SROs and BL. Research should continue to investigate the specific pathways that drive settlement in BL oysters as a means to improve hatchery culture.

Extension and Adoption

The project and its outcomes have been directly communicated to stakeholders by the following formal presentations and publications:

2020 – Meeting with Rebecca Schofield and John Dexter to discuss the results of the study, implications for oyster aquaculture regulation, and next steps.

2020 – Online publication of 'Guide to Queensland's Intertidal Oysters' and distribution of hard copy to stakeholders including industry, government agencies, other researchers, and Australian museums.

2020 - Willan, R. C., Nenadic, N., Ramage, A, McDougall, C. Molecular confirmation that the large crassostreine oyster newly established in northern Queensland, Australia, is *Magallana bilineata* (Röding, 1798). Scientific publication currently in review at *Molluscan Research*. Audience includes other researchers and relevant government agencies.

2020 – McDougall, C. DNA analysis of potential invasive oyster specimens: a report for Biosecurity Queensland. Audience included government agencies. Project outcome adopted via listing of *Magallana bilineata* as biosecurity matter.

2020 – Presentation and publicity for a draft version of the 'Guide to Queensland's Intertidal Oysters' at the Queensland Oyster Growers AGM. Audience included industry.

2019 Poster presentation – 'Intertidal rock oyster species of Queensland'. NSW Oyster Farmers Conference, Forster, Australia. Audience included industry, other researchers, and relevant government agencies.

2018 Oral presentation – 'A genomic approach to developing tropical oyster aquaculture'. Molluscs 2018 Conference, Wellington, New Zealand. Audience included other researchers.

2018 Invited presentation – 'Genetic studies of oysters in Queensland'. National Tropical Oyster Aquaculture Workshop, Darwin, Australia. Audience included industry, other researchers, and relevant government agencies.

2018 Meeting with John Dexter, Fisheries Queensland, to update results. Audience included government agencies.

2018 Meeting with the Hon. Mark Furner, Minister for Agricultural Industry Development and Fisheries, to discuss the project and results. Audience included government officials.

2018-2020 Honorary position established at the Queensland Museum to facilitate engagement with museum staff and access to collections.

The following communications will be forthcoming:

Scientific publication presenting the results of the genetic survey of oyster species in Queensland.

Scientific publication presenting the results of comparative transcriptome sequencing of Sydney rock and blacklip larvae.

Project coverage

Live Facebook Stream: https://www.facebook.com/whatsonatgriffith/videos/253778349034960
'What's on at Griffith' Sustainability week Oyster Filtration Display with Robbie Porter, OzFish.

Detection of *Magallana bilineata* and listing as biosecurity matter available online
https://www.business.qld.gov.au/industries/farms-fishing-forestry/agriculture/land-management/health-pests-weeds-diseases/pests/invasive-animals/prohibited/black-scar-oyster

Project materials developed

McDougall, C., Nenadic, N., Healy, J. Guide to Queensland's intertidal oysters. Version 1, September 2020. Griffith University, Brisbane, Australia. *Field guide, electronic and printed copies.*

Nenadic, N., McDougall, C. Intertidal oysters of Queensland, Australia. Poster presentation, NSW Oyster Farmers Conference, Forster, Australia.

Willan, R. C., Nenadic, N., Ramage, A, McDougall, C. Molecular confirmation that the large crassostreine oyster newly established in northern Queensland, Australia, is *Magallana bilineata* (Röding, 1798). *Scientific paper under review at Molluscan Research*.

Appendices

Appendix 1: List of researchers and project staff

Dr. Carmel McDougall, Lecturer in Marine Science, Griffith University

Dr. Ali Shokoohmand, Postdoctoral Researcher, Griffith University

Nikolina Nenadic, Research Assistant, Griffith University

Appendix 2: References

ABARES, 2020. Australian Fisheries and Aquaculture Statistics 2018, Canberra.

Adlard, R.D., Nolan, M.J., 2015. Elucidating the life cycle of *Marteilia sydneyi*, the aetiological agent of QX disease in the Sydney rock oyster (*Saccostrea glomerata*). Int. J. Parasitol. 45, 419-426.

Banks, M.A., Hedgecock, D., Waters, C., 1993. Discrimination between closely related Pacific oyster species (*Crassostrea*) via mitochondrial DNA sequences coding for large subunit rRNA. Mol. Mar. Biol. Biotechnol. 2, 129-136.

Braley, R., 1984. Mariculture potential of introduced oysters *Saccostrea cucullata tuberculata* and *Crassostrea echinata*, and a histological study of reproduction of *C. echinata*. Aust. J. Mar. Freshwater Res. 35, 129-141.

Chalermwat, K., Szuster, B.W., Flaherty, M., 2003. Shellfish aquaculture in Thailand. Aquaculture Economics & Management. 7, 249-261.

Coeroli, M., De Gaillande, D., Landret, J., 1984. Recent innovations in cultivation of molluscs in French Polynesia. Aquaculture. 39, 45-67.

Dove, M.C., Nell, J.A., O'Connor, W.A., 2012. Evaluation of the progeny of the fourth-generation Sydney rock oyster *Saccostrea glomerata* (Gould, 1850) breeding lines for resistance to QX disease (*Marteilia sydneyi*) and winter mortality (*Bonamia roughleyi*). Aquac. Res. 44, 1791-1800.

Fisheries Queensland and Biosecurity Queensland, 2019. Health protocol for the movement of live bivalve molluscs. Dapartment of Agriculture and Fisheries, Queensland.

Folmer, O., Black, M., Hoeh, W., Lutz, R., Vrijenhoek, R., 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Mol. Mar. Biol. Biotechnol. 3, 294-299.

Green, T.J., Jones, B.J., Adlard, R.D., Barnes, A.C., 2008. Parasites, pathological conditions and mortality in QX-resistant and wild-caught Sydney rock oysters, *Saccostrea glomerata*. Aquaculture. 280, 35-38.

Green, T.J., Dixon, T.J., Devic, E., Adlard, R.D., Barnes, A.C., 2009. Differential expression of genes encoding anti-oxidant enzymes in Sydney rock oysters, *Saccostrea glomerata* (Gould) selected for disease resistance. Fish Shellfish Immunol. 26, 799-810.

Green, T.J., Montagnani, C., Benkendorff, K., Robinson, N., Speck, P., 2014. Ontogeny and water temperature influences the antiviral response of the Pacific oyster, *Crassostrea gigas*. Fish & Shellfish Immunology. 36, 151-157.

Griekspoor, A., Groothuis, T., 2006. 4Peaks. 1.7.2, Amsterdam.

Hadfield, M.G., Carpizo-Ituarte, E.J., del Carmen, K., Nedved, B.T., 2001. Metamorphic competence, a major adaptive convergence in marine invertebrate larvae. Am. Zool. 41, 1123-1131.

Harry, H.W., 1985. Synopsis of the supraspecific classification of living oysters (Bivalvia, Gryphaeidae and Ostreidae). Veliger. 28, 121-158.

Hashimshony, T., Senderovich, N., Avital, G., Klochendler, A., de Leeuw, Y., Anavy, L., Gennert, D., Li, S., Livak, K.J., Rozenblatt-Rosen, O., Dor, Y., Regev, A., Yanai, I., 2016. CEL-Seq2: sensitive highly-multiplexed single-cell RNA-Seq. Genome Biol. 17, 77.

Hilborn, R., Banobi, J., Hall, S.J., Pucylowski, T., Walsworth, T.E., 2018. The environmental cost of animal source foods. Frontiers in Ecology and the Environment. 16, 329-335.

Hoang, D.T., Chernomor, O., von Haeseler, A., Minh, B.Q., Vinh, L.S., 2018. UFBoot2: Improving the Ultrafast Bootstrap Approximation. Mol. Biol. Evol. 35, 518-522.

Huber, M., 2010. Compendium of Bivalves. ConchBooks, Hackenheim.

In, V.V., Ntalamagka, N., O'Connor, W., Wang, T., Powell, D., Cummins, S.F., Elizur, A., 2016. Reproductive neuropeptides that stimulate spawning in the Sydney rock oyster (*Saccostrea glomerata*). Peptides. 82, 109-119.

Joyce, A., Vogeler, S., 2018. Molluscan bivalve settlement and metamorphosis: Neuroendocrine inducers and morphogenetic responses. Aquaculture. 487, 64-82.

Kalyaanamoorthy, S., Minh, B.Q., Wong, T.K.F., von Haeseler, A., Jermiin, L.S., 2017. ModelFinder: fast model selection for accurate phylogenetic estimates. Nat. Methods. 14, 587-589.

Kinch, J., Vitukawalu, B., Nalasi, U., Waqainabete, P., Bermudes, M., 2019a. Socioeconomic aspects of oyster harvesting in the Rewa River delta area, Fiji., South Pacific Commission Fisheries Newsletter, pp. 45-54.

Kinch, J., Yabakiva, M., Waqainabete, P., Nalasi, U., Pickering, T., Ravunamoce, P., Romeo, A., 2019b. Exploring the market potential for Fiji's Rewa River oysters, South Pacific Commission Fisheries Newsletter, pp. 48-54.

Kleeman, S.N., Le Roux, F., Berthe, F., Adlard, R.D., 2002. Specificity of PCR and in situ hybridization assays designed for detection of *Marteilia sydneyi* and *M. refringens*. Parasitology. 125, 131-141.

Lam, K., Morton, B., 2006. Morphological and mitochondrial-DNA analysis of the Indo-West Pacific rock oysters (Ostreidae: *Saccostrea* species). J. Mollus. Stud. 72, 235-245.

Larsson, A., 2014. AliView: a fast and lightweight alignment viewer and editor for large data sets. Bioinformatics. 30, 3276-3278.

McDougall, C., 2018. Comparative De Novo transcriptome analysis of the Australian black-lip and Sydney rock oysters reveals expansion of repetitive elements in Saccostrea genomes. PLoS ONE. 13, e0206417-0206415.

Minh, B.Q., Schmidt, H.A., Chernomor, O., Schrempf, D., Woodhams, M.D., von Haeseler, A., Lanfear, R., 2020. IQ-TREE 2: New Models and Efficient Methods for Phylogenetic Inference in the Genomic Era. Mol. Biol. Evol. 37, 1530-1534.

Nowland, S.J., 2019. Developing hatchery culture techniques for the black-lip rock oyster, *Saccostrea echinata*, to support Aboriginal economic development in northern Australia, Faculty of Science, Health, Education and Engineering. University of the Sunshine Coast, Sippy Downs.

Nowland, S.J., O'Connor, W., Southgate, P.C., 2018a. Embryonic, larval, and early postlarval development of the tropical black-lip rock oyster *Saccostrea echinata*. J. Shellfish Res. 37, 1-5.

Nowland, S.J., O'Connor, W.A., Southgate, P.C., 2018b. Optimizing stocking density and microalgae ration improves the growth potential of tropical black-lip oyster, *Saccostrea echinata*, larvae. Journal of the World Aquaculture Society. 34, 1-10.

Nowland, S.J., O'Connor, W.A., Osborne, M.W.J., Southgate, P.C., 2019a. Current status and potential of tropical rock oyster aquaculture. Reviews in Fisheries Science and Aquaculture.

Nowland, S.J., Silva, C.N.S., Southgate, P.C., Strugnell, J.M., 2019b. Mitochondrial and nuclear genetic analyses of the tropical black-lip rock oyster (*Saccostrea echinata*) reveals population subdivision and informs sustainable aquaculture development. 1-14.

Nowland, S.J., O'Connor, W.A., Penny, S.S., Osborne, M.W.J., Southgate, P.C., 2019c. Water temperature and salinity synergistically affect embryonic and larval development of the tropical black-lip rock oyster *Saccostrea echinata*. Aquac. Int. 25, 1763-1714.

O'Connor, W.A., Dove, M.C., 2009. The Changing Face of Oyster Culture in New South Wales, Australia. J. Shellfish Res. 28, 803-811.

O'Connor, W.A., Dove, M., Finn, B., O'Connor, S., 2008. Manual for production of Sydney rock oysters (*Saccostrea glomerata*). Port Stephens Fisheries Centre, New South Wales, Australia.
Palumbi, S.R., Martin, A., Romano, S., 1991. Simple Fool's Guide to PCR. University of Hawaii Press, Honolulu.
Perkins, F.O., Wolf, P.H., 1976. Fine structure of *Marteilia sydneyi* sp. n.: haplosporidan pathogen of Australian oysters. J. Parasitol. 62, 528-538.
Queensland Government, 2020. Black scar oyster. https://www.business.qld.gov.au/industries/farms-fishing-forestry/agriculture/land-management/health-pests-weeds-diseases/pests/invasive-animals/prohibited/black-scar-oyster.
R Core Team, 2014. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna. http://www.R-project.org.
Rambaut, A., 2006. FigTree. 1.1.1. University of Edinburgh, Edinburgh.
Robinson, M.D., McCarthy, D.J., Smyth, G.K., 2010. edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. Bioinformatics. 26, 139-140.
Saville-Kent, W., 1891. Oysters and oyster fisheries of Queensland, Brisbane.
Schrobback, P., 2015. Economic analysis of Australia's Sydney rock oyster industry, School of Economics and Finance. Queensland University of Technology, Brisbane.
Schrobback, P., Rolfe, J., 2020. Describing, analysing and comparing edible oyster supply chains in Australia. Final Research Report. Central Queensland University.
Sekino, M., Yamashita, H., 2016. Mitochondrial and nuclear DNA analyses of *Saccostrea* oysters in Japan highlight the confused taxonomy of the genus. J. Mollus. Stud. 82, 492-506.
Smith, M.K., Chieu, H.D., Aizen, J., Mos, B., Motti, C.A., Elizur, A., Cummins, S.F., 2019. A Crown-of-Thorns Seastar recombinant relaxin-like gonad-stimulating peptide triggers oocyte maturation and ovulation. Gen. Comp. Endocrinol. 281, 41-48.
Southgate, P.C., Lee, P.S., 1998. Hatchery rearing of the tropical blacklip oyster *Saccostrea echinata* (Quoy and Gaimard). Aquaculture. 169, 275-281.
Stamatakis, A., 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics. 30, 1312-1313.
Suja, G., Lijo, J., Kripa, V., Mohamed, K.S., Vijayan, K.K., Sanil, N.K., 2020. A comparison of parasites, pathological conditions and condition index of wild and farmed populations of *Magallana bilineata* (Roding, 1798) from Vembanad Lake, west coast of India. Aquaculture. 515, 734548.
Trifinopoulos, J., Nguyen, L.-T., von Haeseler, A., Minh, B.Q., 2016. W-IQ-TREE: a fast online phylogenetic tool for maximum likelihood analysis. Nucleic Acids Res. 44, W232-W235.
Untergasser, A., Cutcutache, I., Koressaar, T., Ye, J., Faircloth, B.C., Remm, M., Rozen, S.G., 2012. Primer3—new capabilities and interfaces. Nucleic Acids Res. 40, e115-e115.
Willan, R.C., Nenadic, N., Ramage, A., McDougall, C., 2020. Molecular confirmation that the large crassostreine oyster newly established in northern Queensland, Australia, is *Magallana bilineata* (Röding, 1798). Molluscan Res. In Review.
Wolf, P.H., 1977. An unidentified protistan parasite in the ova of the blacklipped oyster, *Crassostrea echinata*, from Northern Australia. J. Invertebr. Pathol. 29, 244-246.
Zhang, F., Hu, B., Fu, H., Jiao, Z., Li, Q., Liu, S., 2019. Comparative transcriptome analysis reveals molecular basis underlying fast growth of the selectively bred Pacific oyster, *Crassostrea gigas*. Frontiers in Genetics. 10, 37-14.

Appendix 3: Guide to Queensland's intertidal oysters

FRDC FINAL REPORT CHECKLIST

Project Title:			
Principal Investigators:	XXXX (include all recognised authors -)		
Project Number:	XXXX/XXX		
Description:	Brief one/two paragraph overview of what the project did and achieved.		
Published Date:	XX/XX/XXXX (if applicable)	**Year:**	XXXX
ISBN:	XXXXX (if applicable)	**ISSN:**	XXXXXXXXXXXXX (if applicable)
Key Words:	Needs to include key subject areas and species name (see www.fishnames.com.au)		

Please use this checklist to self-assess your report before submitting to FRDC. Checklist should accompany the report.

	Is it included (Y/N)	**Comments**
Foreword (optional)		
Acknowledgments		
Abbreviations		
Executive Summary		
– **What the report is about**		
– **Background – why project was undertaken**		
– **Aims/objectives – what you wanted to achieve at the beginning**		
– **Methodology – outline how you did the project**		
– **Results/key findings – this should outline what you found or key results**		
– **Implications for relevant stakeholders**		
– **Recommendations**		
Introduction		
Objectives		
Methodology		
Results		
Discussion		
Conclusion		
Implications		
Recommendations		
Further development		
Extension and Adoption		
Project coverage		
Glossary		
Project materials developed		

Appendices		
EXTENSION		
Extension plan developed?		
Extension undertaken?		
If extension was undertaken, who was it undertaken with and was it successful? **(Detail answer in comments section)**		
If No, then is further extension necessary? With who? **How? (detail answer in comments section)**		