

Interrogating the microbiome for  
improved understanding of Pacific  
oyster diseases

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A thesis submitted in fulfillment of the requirements  
for the degree: Doctor of Philosophy

December 2019

University of Technology Sydney  
School of Life Sciences  
Climate Change Cluster

2019

## Certificate of Original Authorship

I, William King, declare that this thesis is submitted in fulfilment of the requirements for the award of Doctor of Philosophy, in the Faculty of Science at the University of Technology Sydney.

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## Acknowledgments

First and foremost, I would like to thank Maurizio Labbate. Including my honours project I have been a student of Maurizio's for nearly five complete years and I really could not have asked for a better supervisor. Maurizio has been absolutely instrumental shaping me into the scientist I am today and without his guidance and support I would not be where I am right now. In fact, without his sassy quirkiness the last few years would have been especially dull. Although I now fear I have picked up a few of his sassy traits. I especially want to point out that Maurizio has always been very welcoming of the occasional unplanned meeting where I was able to just drop by whenever I had a new curious thought. These little meetings have definitely been very important to me not just to improve my scientific learning but by also offering the opportunity to unload some thoughts anytime I needed to talk. These meetings have made me realise the importance of unscheduled meetings and I definitely plan to have the same policy with my own students one day (hopefully).

Next, I would like to thank Justin Seymour. Justin was incredibly patient as I was getting to my feet as I had come from a fairly molecular focused honours project and ventured into the realm of microbial ecology. In addition to Maurizio, Justin has been incredibly supportive over the last four years. He has always nudged me in the right direction and without him I would not have adapted so easily to a new field. One particular thing that Justin has helped me refine is my writing skills. He is an absolute machine when it comes to writing and I have definitely done my best to mould myself to his writing skills to improve my own skillset. Further, our little chats in the gym have been quite important when I needed some unscheduled guidance about navigating my scientific career or when crafting stories for my manuscripts.

Because of these gym chats, Justin's bench press hasn't improved in a few years so I'm sure he is looking forward to me leaving...

Another key figure during my PhD has been Nachshon Siboni. Anyone that knows Nachshon knows that he is legend, he is simply unforgettable! I would like to thank Nachshon for personally teaching me absolutely everything he knows about microbial ecology and I definitely would not be anywhere near as developed as a scientist without his support. Although, I don't think he remembered my name for an entire year! Nachshon's support over the last four years cannot be measured, he has always been available for chats and was always there every single time I needed help. Nachshon is also a really funny guy and can take a joke so our meetings were always full of laughs which always brightened up my days. I will definitely miss having Nachshon around when I inevitably depart on the next stage of my career.

Next, I would like to say a bulk thank you to the Labbate and Seymour laboratory members, those other PhD students that I have interacted with along the way, particularly those in the 'too many flies in the office squad', my family and all of the tech staff. While there are way too many people to individually thank, I would like to specifically thank Rami, Elizabeth, Daniela, Regan and Nathan. Nathan, it has been an absolute joy seeing you develop from the cheeky undergraduate student that I was training during his internship to the independent PhD student you are today. Rami, Elizabeth and Daniela, you've all been supporting me for pretty much my entire PhD project and without our daily gossip sessions I surely would have gotten more work done... kidding! You three, Rami especially, have definitely kept me grounded and chatting to you guys always brightened up my day. I wish you all the best with your future endeavours.

Finally, I would like to thank my partner in crime, Sarah Iwanoczko. Sarah's support during my PhD project has been absolutely invaluable, especially when I have been bogged down by work. Sarah has always helped to keep my chin up and she has nudged me through the harder parts of my PhD. Words can't describe the positive impact Sarah has had on my life and that has definitely reflected on my positive attitude throughout my PhD. No matter what happens in the future, I will always cherish Sarah's support over the last few years and without her my days wouldn't be nearly as bright. I have especially enjoyed our American adventures together and I hope there is many more worldwide adventures to come. You really are amazing Sarah, thank you!

## **Publications associated with this thesis**

### Chapter One

**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. 10.1016/j.marenvres.2018.11.007

### Chapter Two

**King, W.L.**, Jenkins, C., Go, J., Siboni, N., Seymour, J.R., and Labbate, M. (2019). Characterisation of the Pacific Oyster Microbiome During a Summer Mortality Event. *Microbial Ecology*. 2019;77(2):502-12. 10.1007/s00248-018-1226-9

### Chapter Three

**King, W.L.**, Siboni, N., Williams, N.L.R., Kahlke, T., Nguyen, K.V., Jenkins, C., Dove, M., O'Connor, W., Seymour, J.R., and Labbate, M. (2019). Variability in the composition of Pacific Oyster microbiomes across oyster families exhibiting different levels of susceptibility to OsHV-1  $\mu$ var disease. *Frontiers in Microbiology* 10, 473. 10.3389/fmicb.2019.00473

### Supporting Publication One

Green, T.J., Siboni, N., **King, W.L.**, Labbate, M., Seymour, J.R., and Raftos, D. (2018). Simulated Marine Heat Wave Alters Abundance and Structure of *Vibrio* Populations Associated with the Pacific Oyster Resulting in a Mass Mortality Event. *Microbial Ecology*. 10.1007/s00248-018-1242-9

## **Conference presentations associated with this thesis**

**William L King**, Cheryl Jenkins, Jeffrey Go, Nahshon Siboni, Justin R Seymour, Maurizio Labbate. Presentation titled: Microbiome investigations in a Pacific Oyster summer mortality outbreak in Port Stephens, New South Wales, Australia. Australian Microbial Ecology (AusME) meeting.

**William L King**, Cheryl Jenkins, Jeffrey Go, Justin R Seymour, Maurizio Labbate. Presentation titled: The effect of microbiological and environmental factors on summer mortality events in Pacific oysters. Australian Shellfish Quality Assurance Science Day (ASQAAC).

**William L King**, Cheryl Jenkins, Jeffrey Go, Nachshon Siboni, Justin R Seymour, Maurizio Labbate. Presentation titled: Characterisation of the pacific oyster microbiome during a summer mortality event. 110<sup>th</sup> Annual Meeting of the National Shellfisheries Association.

**William L King**, Nachshon Siboni, Michael Dove, Wayne O'Connor, Cheryl Jenkins, Justin R Seymour, Maurizio Labbate. Presentation titled: Polymicrobial involvement in OsHV-1 outbreaks (and other diseases). Oysters Australia Research and Development Meeting

**William L King**, Cheryl Jenkins, Jeffrey Go, Nachshon Siboni, Justin R Seymour, Maurizio Labbate. Presentation titled: Elucidating links between the Pacific Oyster microbiome and summer mortality events. 4th FRDC Australasian Scientific Conference.

Maurizio Labbate, **William L King**, Khue Viet Nguyen, Nachshon Siboni, Michael Dove, Wayne O'Connor, Cheryl Jenkins, Justin R Seymour.

Presentation titled: Is there a role for microbiomes in oyster mortality events?  
Australian Shellfish Quality Assurance Science Day (ASQAAC).

**William L King**, Nachshon Siboni, Cheryl Jenkins, Jeffrey Go, Nathan Williams, Tim Kahlke, Michael Dove, Wayne O'Connor, Maurizio Labbate, Justin R Seymour. Presentation titled: Insights into the influence of the pacific oyster microbiome in oyster disease and resistance. 17<sup>th</sup> International Symposium on Microbial Ecology, International Society for Microbial Ecology (ISME).

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## Abbreviations

<i>C. gigas</i>	<i>Crassostrea gigas</i>
<i>S. glomerata</i>	<i>Saccostrea glomerata</i>
<i>O. edulis</i>	<i>Ostrea edulis</i>
<i>C. virginica</i>	<i>Crassostrea virginica</i>
ROS	Reactive Oxygen Species
OsHV-1	Ostreid herpes virus 1
OsHV-1 $\mu$ var	Ostreid herpes virus 1 microvariant
<i>P. marinus</i>	<i>Perkinsus marinus</i>
<i>H. nelsoni</i>	<i>Haplosporidium nelsoni</i>
<i>M. sydneyi</i>	<i>Marteilia sydneyi</i>
<i>B. roughleyi</i>	<i>Bonamia roughleyi</i>
<i>M. refringens</i>	<i>Marteilia refringens</i>
<i>B. ostreae</i>	<i>Bonamia ostreae</i>
<i>M. mackini</i>	<i>Mikrocytos mackini</i>
<i>R. crassostreae</i>	<i>Roseovarius crassostreae</i>
<i>N. crassostreae</i>	<i>Nocardia crassostreae</i>
MSX	Multinucleate Sphere Unknown X
ROD	Roseovarius Oyster Disease
QX	Queensland Unknown
POMS	Pacific Oyster Mortality Syndrome
<i>V. tubiashii</i>	<i>Vibrio tubiashii</i>
<i>V. splendidus</i>	<i>Vibrio splendidus</i>
<i>V. alginolyticus</i>	<i>Vibrio alginolyticus</i>
<i>V. aestuarianus</i>	<i>Vibrio aestuarianus</i>
<i>V. lentus</i>	<i>Vibrio lentus</i>
<i>V. harveyi</i>	<i>Vibrio harveyi</i>
<i>V. coralliilyticus</i>	<i>Vibrio coralliilyticus</i>

<i>V. crassostreae</i>	<i>Vibrio crassostreae</i>
<i>V. angillarum</i>	<i>Vibrio angillarum</i>
<i>V. diabolicus</i>	<i>Vibrio diabolicus</i>
<i>V. mediterranei</i>	<i>Vibrio mediterranei</i>
<i>V. azureus</i>	<i>Vibrio azureus</i>
<i>V. brasiliensis</i>	<i>Vibrio brasiliensis</i>
<i>V. chagasii</i>	<i>Vibrio chagasii</i>
<i>V. fortis</i>	<i>Vibrio fortis</i>
<i>V. vulnificus</i>	<i>Vibrio vulnificus</i>
<i>V. campbellii</i>	<i>Vibrio campbellii</i>
<i>V. sinaloensis</i>	<i>Vibrio sinaloensis</i>
<i>V. cholerae</i>	<i>Vibrio cholerae</i>
<i>V. parahaemolyticus</i>	<i>Vibrio parahaemolyticus</i>
<i>V. rotiferanus</i>	<i>Vibrio rotiferanus</i>
ASI	Australian Seafood Industries
DNA	Deoxyribonucleic acid
RNA	Ribonucleic acid
rRNA	Ribosomal ribonucleic acid
OTU	Operational Taxonomic Unit
ZOTU	Zero-radius Operational Taxonomic Unit
NSW	New South Wales
DPI	Department of Primary Industries
SRA	Sequence Read Archive
QIIME	Quantitative Insights Into Microbial Ecology
nMDS	Non-metric multidimensional scaling analysis
ANOVA	Analysis of Variance
ANOSIM	Analysis of Similarities
PERMANOVA	Permutational multivariate analysis of variance

CCA	Canonical Correspondence Analysis
SIMPER	Analysis of similarity percentages
PCoA	Principal Coordinates Analysis
RDP	Ribosomal Database Project
GIT	Gastrointestinal Tract
EBV	Estimated Breeding Values
RG	Resistance Group
qPCR	Quantitative Polymerase Chain Reaction
CV	Coefficient of Variation
WGS	Whole Genome Sequencing
FISH	Fluorescence in situ hybridization
MINE	Maximal Information-based Nonparametric Exploration
CR	Clyde River
GR	Georges River
HR	Hawkesbury River
SH	Shoalhaven
PS	Port Stephens
WA	Wapengo
Mt	Mantle
Gl	Gill
Am	Adductor muscle
Dg	Digestive gland
NCBI	National Center for Biotechnology Information
<i>hsp60</i>	Heat shock protein 60
BLAST	Basic Local Alignment Search Tool
NaCl	Sodium Chloride
LB	Lysogeny Broth
nt	Nucleotide

dNTP	Deoxyribonucleotide triphosphate
$\mu\text{L}$	Microlitre
$\mu\text{M}$	Micromolar
km	Kilometre
L	Litre
bp	Base pair
mg	Milligrams
ng	Nanograms

## **Abstract**

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* species, 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 behavior. 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.

## **Thesis prelude – rationale, significance and aims**

The Australian aquaculture industry is valued at \$1.31 billion AUD, representing 97,000 tonnes of production (ABARES, 2017). Of this, the oyster aquaculture industry contributes \$97 million AUD, and 11,300 tonnes of production (ABARES, 2017), making it a valuable contributor to the Australian economy. However, a major hurdle to the continued growth and sustainability of the oyster industry are infectious diseases (Lafferty et al., 2015).

Of the commercially cultivated oyster species, *Crassostrea gigas* (the Pacific oyster) is the most heavily cultivated globally (FAO, 2016a). Despite this, commercial cultivation of *C. gigas* has been continually challenged with disease outbreaks facilitated by viral, bacterial and unknown aetiological agents (Lipovsky, 1972; Paillard et al., 2004; Jenkins et al., 2013; King et al., 2019a). Current efforts to mitigate the impact of *C. gigas* diseases are focused on breeding for disease resistance (Dégremont, 2011; Dégremont et al., 2016b). This usually involves exposing oysters to disease in the field and breeding the surviving oysters (Dégremont, 2011). While breeding for disease resistance has been successful in reducing the impact of these diseases, the mechanism/s behind this protection are poorly understood.

Due to the economic importance of *C. gigas* cultivation, studies have sought to examine the causative factors driving these oyster disease outbreaks, with shifts in the environment (perturbations) often implicated as ‘triggers’ for disease (Burge et al., 2006; Malham et al., 2009; Jenkins et al., 2013; Mortensen et al., 2016; Go et al., 2017). These oyster diseases are complex, often preceding from a disturbance in the synergistic relationship between the host, environment, and pathogen. For example, shifts in environmental

conditions (such as increasing temperature) can drive oyster pathogen transmission and abundance (Petton et al., 2013), while also acting as an immune suppressant to the oyster, as they near their thermal limits (Bougrier et al., 1995). One such host associated factor that may be contributing to oyster disease dynamics is the oyster microbiome.

In recent years, the oyster microbiome has drawn an increasing amount of attention to determine its role in oyster disease dynamics (Lokmer and Wegner, 2015; Petton et al., 2015; Green et al., 2019; King et al., 2019a; King et al., 2019b; King et al., 2019c). However, at the onset of this PhD project, most disease-focused microbiome studies had been culture dependent (Garnier et al., 2007; Petton et al., 2013; Wendling et al., 2014; Lemire et al., 2015; Petton et al., 2015), with only one study employing culture-independent sequencing techniques (Lokmer and Wegner, 2015). Because of this and because of considerable *C. gigas* disease outbreaks in Australia in previous years (Jenkins et al., 2013; Go et al., 2017), this PhD project set out to gain further insight into the oyster microbiome.

This thesis has set out to address five aims, with the overarching goal to provide an improved understanding of oyster diseases using a microbiome approach. Ultimately, the information provided in this thesis will set the framework for future studies by identifying potential probiotic targets in the oyster microbiome and providing new microbiome approaches to oyster diseases for future disease-focused observational studies.

The below aims correspond to chapters one to five accordingly:

**Aim one:** To provide a critical review of oyster diseases, with an emphasis on the environmental drivers and the potential role of the microbiome.

**Aim two:** To use a microbiome approach to investigate a *C. gigas* disease outbreak in Port Stephens.

**Aim three:** To elucidate how breeding *C. gigas* for disease resistance influences microbiome composition and to identify bacteria associated with disease resistance.

**Aim four:** To determine microbiome patterns across geographic locations and tissue-types and identify core taxa innately tied to the *C. gigas* microbiome.

**Aim five:** To develop an amplicon sequencing assay for improved taxonomic resolution of the *Vibrio* community and to apply it to a laboratory *C. gigas* disease event.