Advancement in the SRO Breeding Program -

Families and New Technologies

Australian Government
Department of Industry, Innovation and Science

Business Cooperative Research Centres Programme

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Families and New Technologies

M. Dove, P. Kube, C. Lind, V. Cumbo, W. O'Connor, M. Saowaros, A. Elizur, R. Seeto, Z. Gibb, T. Abramov, D. Raftos, E. Wilkie, SOCo and Southern Cross Shellfish

2019 NSW Oyster Conference, 6-8th August 2019

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Future Oysters CRC-P: Accelerated SRO breeding research

- **Project collaborators:**
  - NSW DPI, CSIRO Macquarie University & SOCo
- **Objectives:**
  - More families
  - Increase QX resistance
  - Understand winter mortality
  - Markers for QX resistance (Vivian Cumbo, Macquarie University)

CSIRO

MACQUARIE University
SYDNEY·AUSTRALIA

select OYSTER COMPANY

Accelerated SRO Breeding Research Outcomes

- **Double family production**
  - Improvements to protocols, facilities, husbandry and training
- **One year breeding cycle for QX resistance**
  - doubles the gain
  - Required understanding of the:
    - Genetic architecture of traits
    - SRO reproduction and the QX disease cycle
    - Logistics with respect to growth and condition

Accelerated SRO Breeding Research Outcomes

Better understanding of winter mortality disease (WM)

- More sites and more tests:
  - Georges and Shoalhaven Rivers
  - Spat and adults
- WM is enigmatic
- No correlation between WM and other traits
- WM is heritable (low to moderate) and can be improved with breeding

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Future Oysters CRC-P: New technologies to improve SRO breeding and production

select OYSTER COMPANY

- **Project collaborators:**
  - NSW DPI, University of the Sunshine Coast, University of Newcastle, SOCO, SCS
- **Objectives:**
  - Increase SRO breeding reliability
  - Reduce conditioning time
  - SRO Tetraploids

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University of the Sunshine Coast
Queensland, Australia

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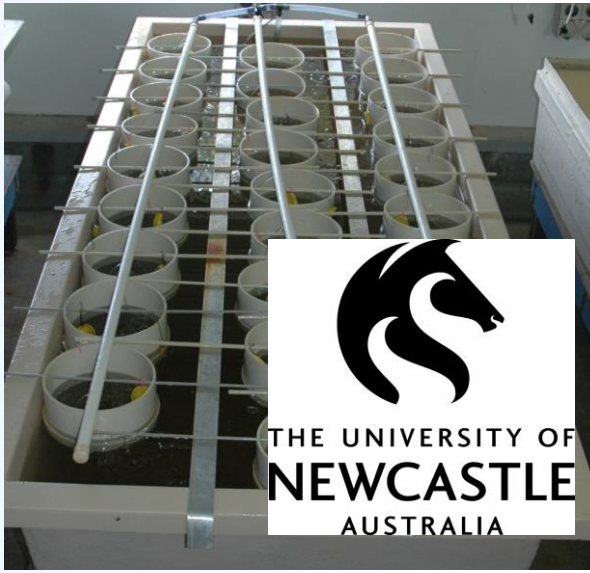
New technologies to improve SRO breeding & production



Improve SRO family success:

- Broodstock conditioning (USC)
  - maturation inducer
- Natural gamete release (USC)
  - spawn inducer
- Preserve gametes when spawning (UoN)
- Tetraploid inductions (Southern Cross Shellfish)

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Queensland, Australia

Preservation of SRO gametes for short term periods.

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- **Provides a backup in the event of failed breeding**
- **Cryopreservation not always practical**
- **Using antioxidants to extend the life span of SRO gametes**
- **Fine tuning a protocol to allow benchtop storage for 5 days**

In vivo bioassay of buccalin, APGW and tachykinin peptides in the Sydney rock oyster, *Saccostrea glomerata*: gonad conditioning

Introduction

Peptides 82 (2016) 109–119

Contents lists available at ScienceDirect

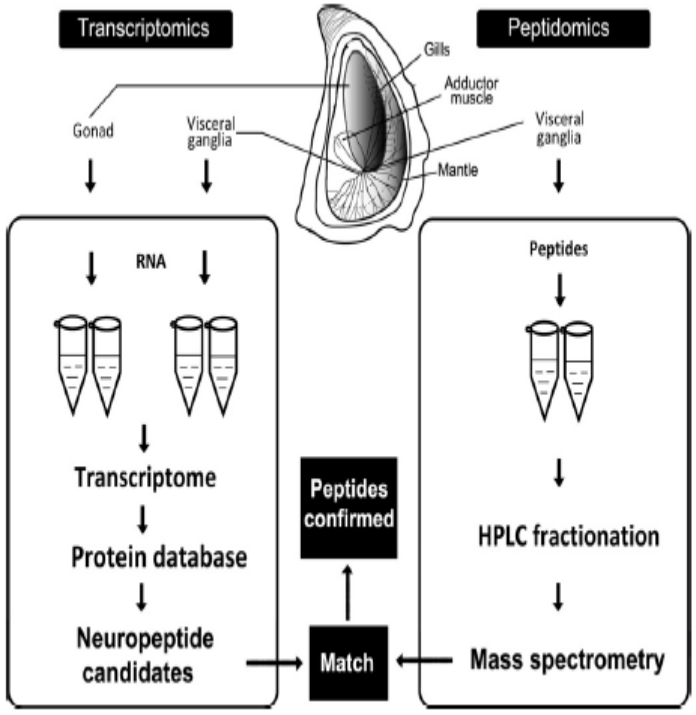
Peptides

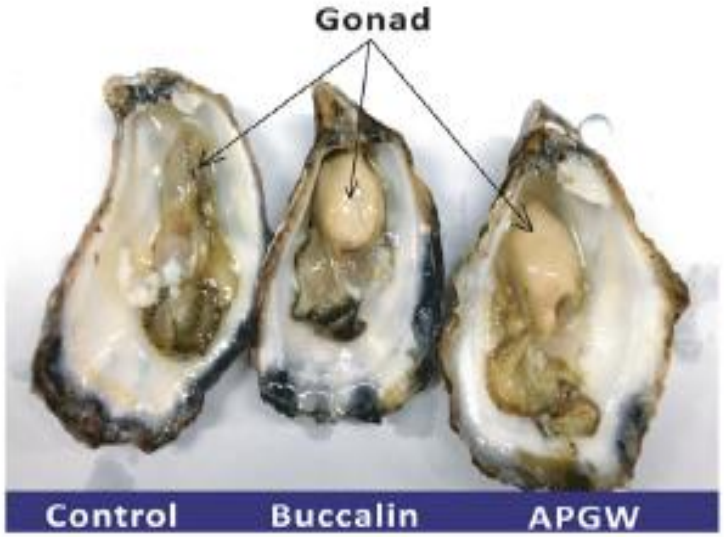
journal homepage: www.elsevier.com/locate/peptides

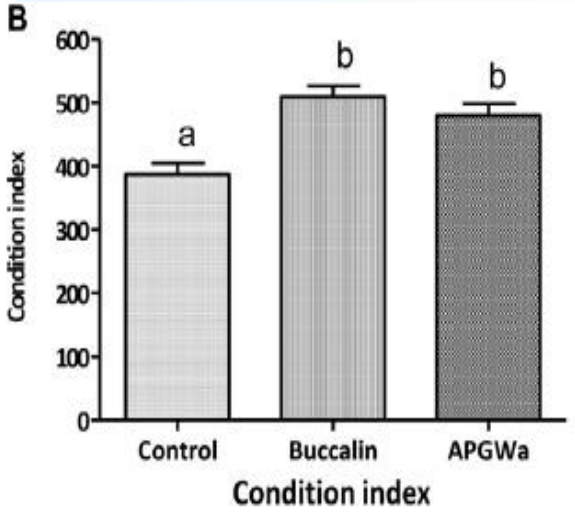
Reproductive neuropeptides that stimulate spawning in the Sydney Rock Oyster (*Saccostrea glomerata*)

Vu Van In[a,b], Nikoleta Ntalamagka[a], Wayne O'Connor[a,c], Tianfang Wang[a], Daniel Powell[a], Scott F. Cummins[a], Abigail Elizur[a,*]

[a] Centre of Genetics, Ecology and Physiology, Univ
[b] Northern National Broodstock Center for Maricu
[c] Industry and Investment NSW, Department of Pr

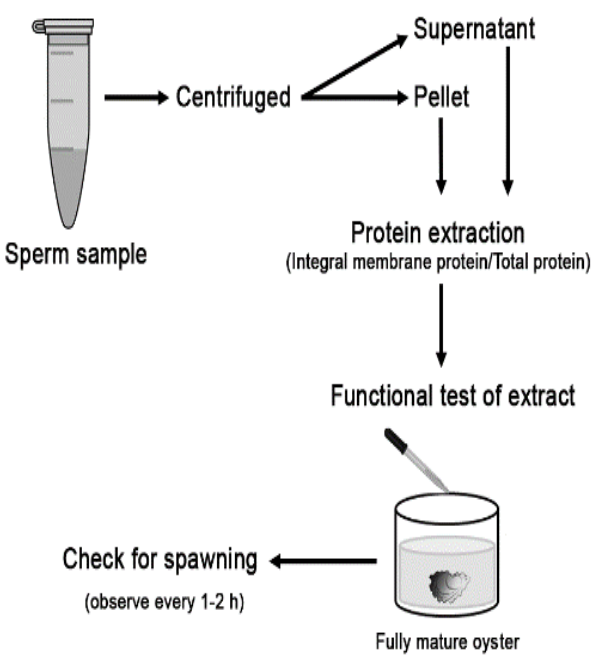


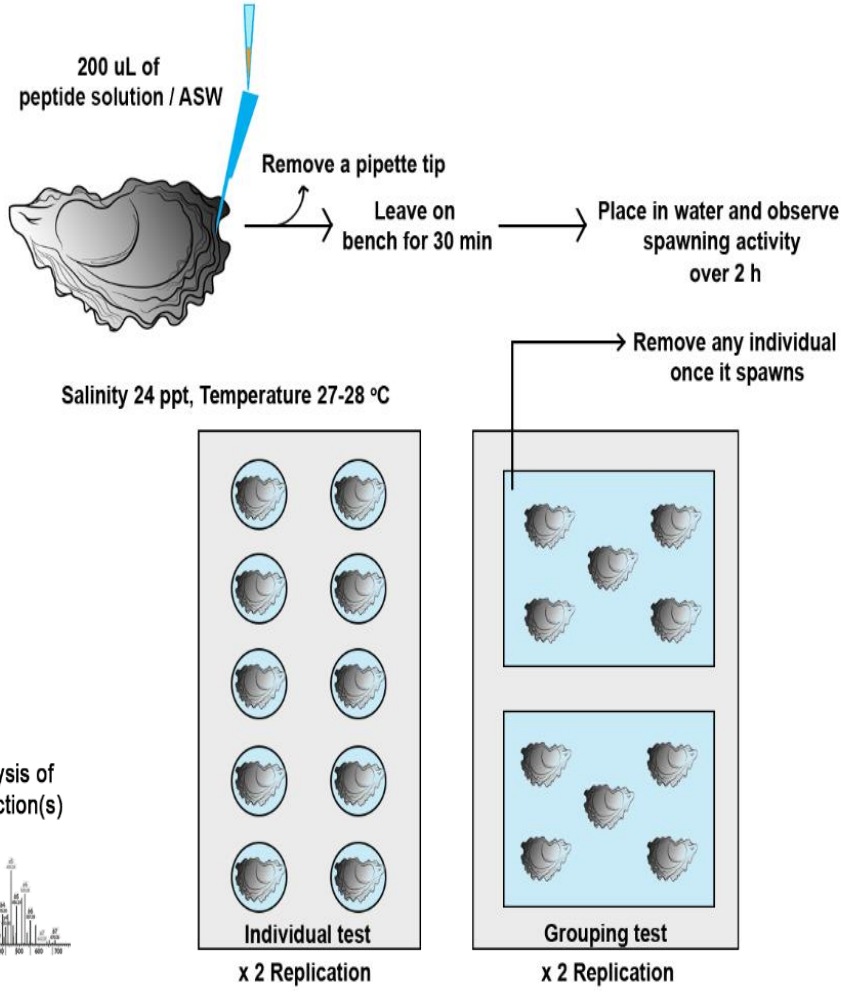


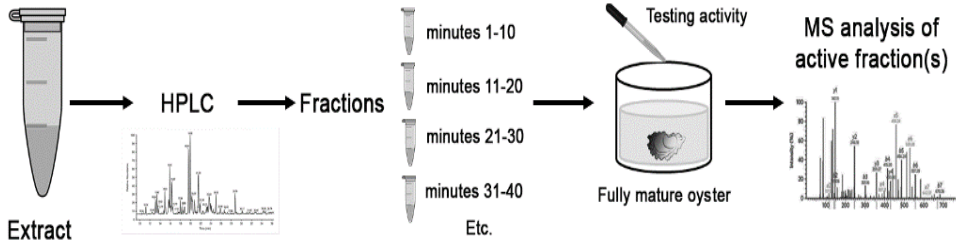


Identification of spawning inducing pheromones in the sperm of the Sydney rock oyster, *Saccostrea glomerata*

Aim: To identify the spawning inducing pheromones (SIPs) in the sperm of the Sydney rock







The Future

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- SRO BP now **operational**

- Further development:
  - improve **family production success**
  - Incorporate:
    - **WM resistance**
    - **Marker assisted selection** for QX resistance

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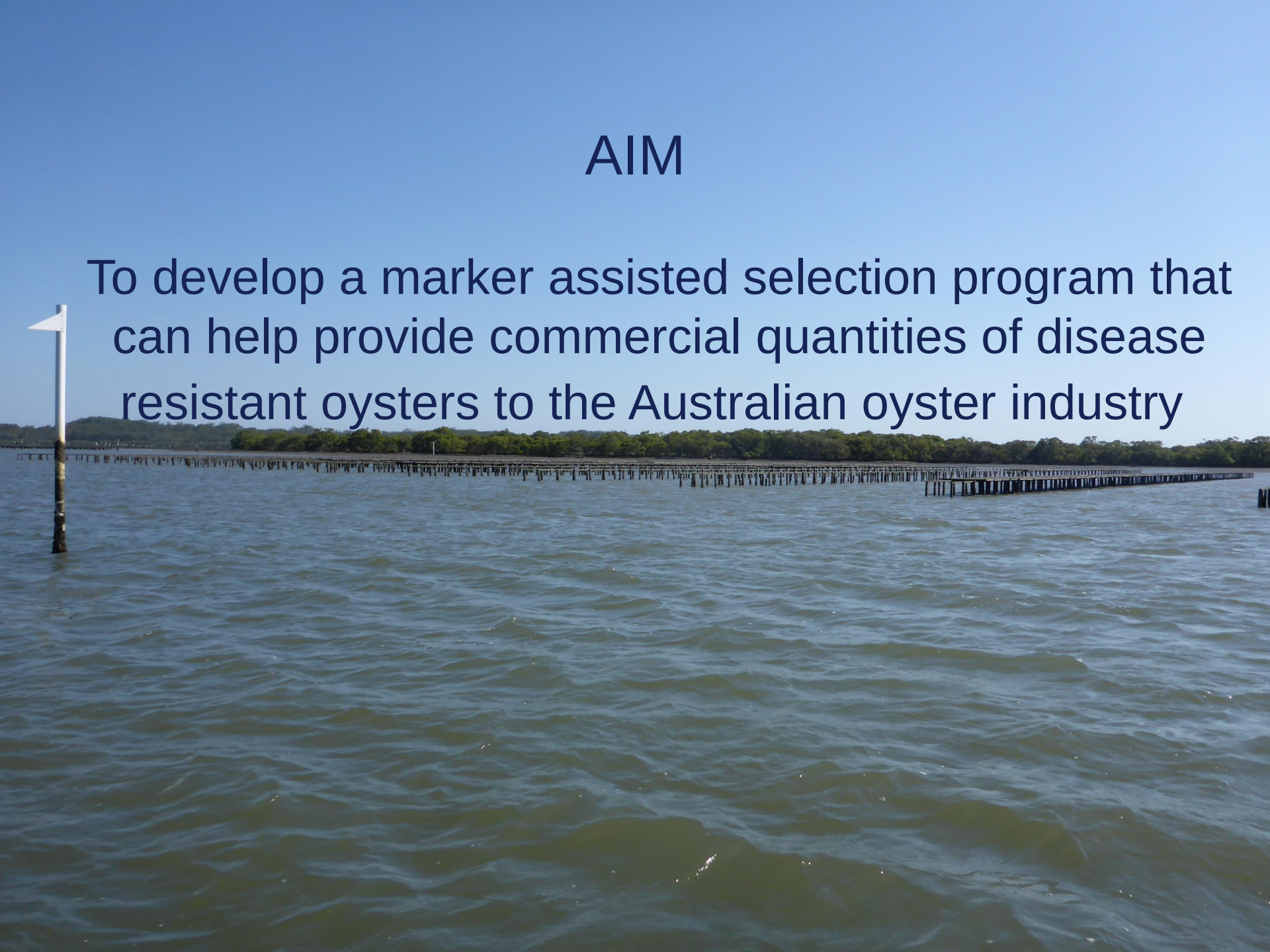
- Breeding program **responsive** to unidentified future problems and needs

- Breeding program that can **ensure long term sustainability** of this industry

AIM

To develop a marker assisted selection program that can help provide commercial quantities of disease resistant oysters to the Australian oyster industry



Experiments

1. Compile a list of genetic markers that could indicate disease resistance

2. Create a method to non-lethally sample oysters

3. Experimentally test markers to determine which indicate disease resistance

Transcriptomic Differences:
Gene Expression Markers

Phenotypic differences between disease resistant and susceptible oysters at the molecular level

qPCR is a fast and cost-effective molecular method for screening large numbers of animals

Marker Selection

Literature search

Genes that were differentially expressed between selectively bred and wild type oysters under ambient conditions

79 genes identified, and 66 chosen for initial analysis


Fish & Shellfish Immunology 26 (2009) 799–810

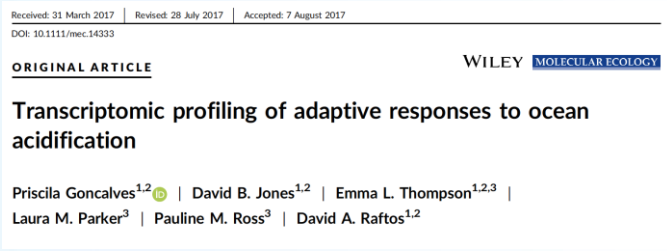
Contents lists available at ScienceDirect

Fish & Shellfish Immunology

journal homepage: www.elsevier.com/locate/fsi

Differential expression of genes encoding anti-oxidant enzymes in Sydney rock oysters, *Saccostrea glomerata* (Gould) selected for disease resistance

Timothy J. Green[a,*], Tom J. Dixon[b,c], Emilie Devic[a], Robert D. Adlard[d,e], Andrew C. Barnes[a,f]


Received: 31 March 2017 | Revised: 28 July 2017 | Accepted: 7 August 2017

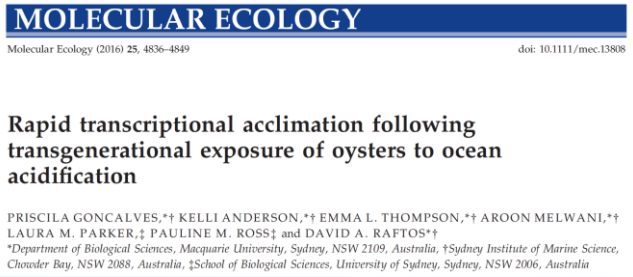
DOI: 10.1111/mec.14333

ORIGINAL ARTICLE

WILEY MOLECULAR ECOLOGY

Transcriptomic profiling of adaptive responses to ocean acidification

Priscila Goncalves[1,2] | David B. Jones[1,2] | Emma L. Thompson[1,2,3] | Laura M. Parker[3] | Pauline M. Ross[3] | David A. Raftos[1,2]

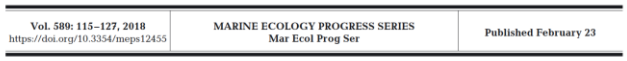

MOLECULAR ECOLOGY

Molecular Ecology (2016) 25, 4836–4849 doi: 10.1111/mec.13808

Rapid transcriptional acclimation following transgenerational exposure of oysters to ocean acidification

PRISCILA GONCALVES,*† KELLI ANDERSON,*† EMMA L. THOMPSON,*† AROON MELWANI,*† LAURA M. PARKER,‡ PAULINE M. ROSS‡ and DAVID A. RAFTOS*†

*Department of Biological Sciences, Macquarie University, Sydney, NSW 2109, Australia, †Sydney Institute of Marine Science, Chowder Bay, NSW 2088, Australia, ‡School of Biological Sciences, University of Sydney, Sydney, NSW 2006, Australia


Vol. 589: 115–127, 2018 https://doi.org/10.3354/meps12455 | MARINE ECOLOGY PROGRESS SERIES Mar Ecol Prog Ser | Published February 23

Intraspecific differences in the transcriptional stress response of two populations of Sydney rock oyster increase with rising temperatures

Dominic McAfee[1,2,*], Vivian R. Cumbo[1], Melanie J. Bishop[1], David A. Raftos[1]

Experiment 1:
Non-lethal Sampling of Oysters

1. Low oyster mortality

2. Remain reproductively active after sampling

3. Enough tissue sampled to obtain for genetic analysis of 66 genes

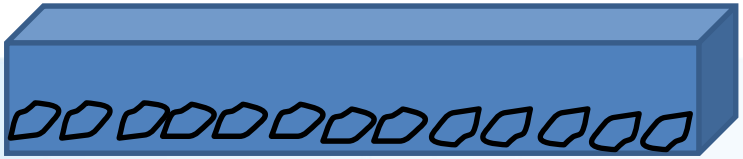
Known disease resistant and disease susceptible family lines from 2015 year class

Susceptible	Survival
2015042	4.0%
2015032	5.3%
2015038	12.0%
2015020	14.6%
2015040	16.0%

Resistant	Survival
2015006	51.3%
2015007	67.0%
2015025	68.3%
2015022	71.0%
2015008	76.0%

40 oysters from each family line acclimated for 2 weeks in 750L tanks

Oysters placed in experimental trays. Air exposed overnight



Treatments:

- Anesthetized + tissue sampled
- Anesthetized only
- Seawater control

Anesthetized oysters mantle tissue biopsied and placed into RNA*later*

10 oysters per family line sampled

1hr

Relaxant bath of $MgCl_2$ water added 16hrs later (50g/L; salinity 32.5, Temp 21ºC)

Oysters placed back into 750L tanks for 1 week

Survival monitored @ 1 week

1 week

Oysters transferred on DPI lease

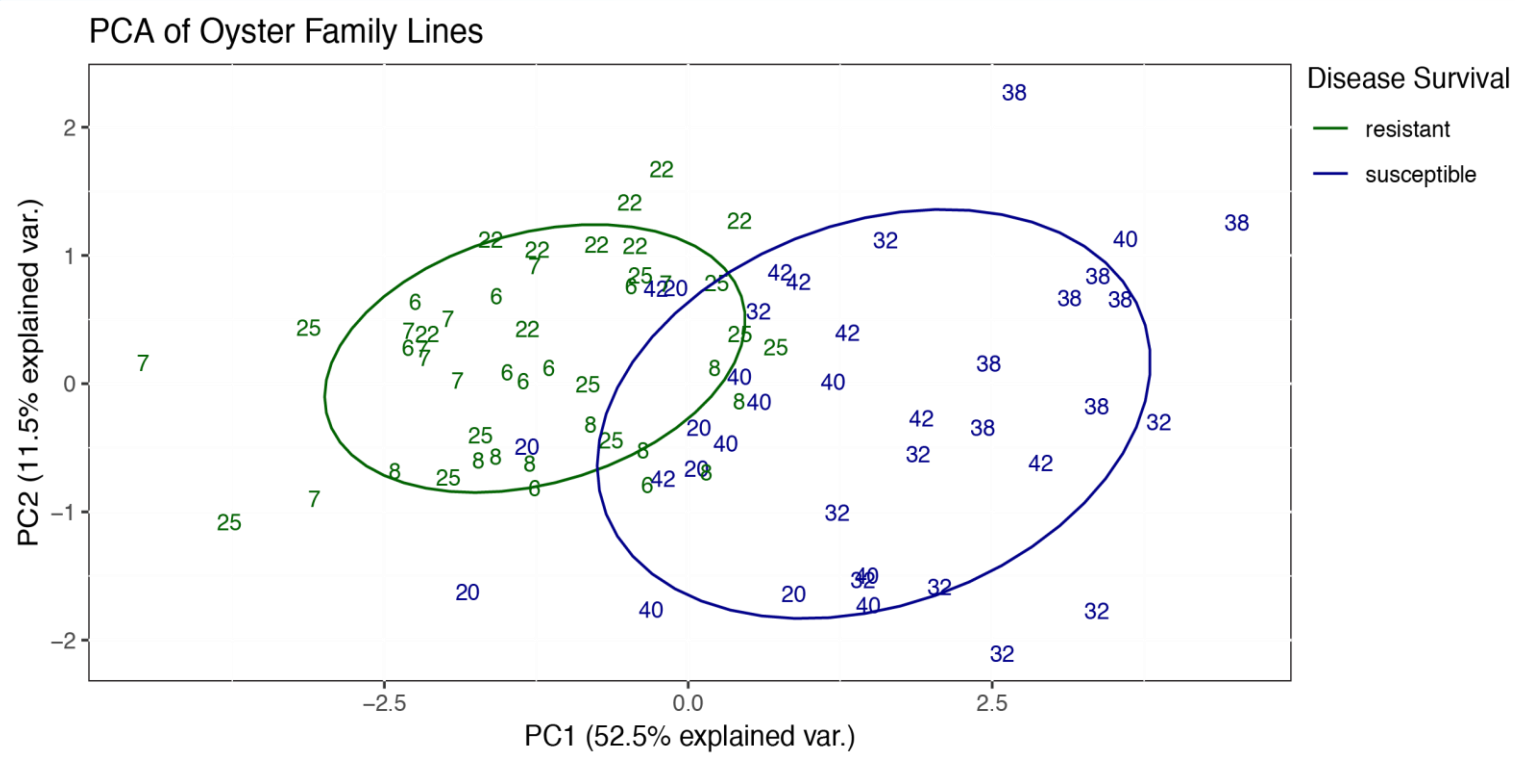
Survival monitored @ 49 days post exposure

Experiment 1:
Non-lethal Sampling of Oysters

1. Low oyster mortality - No mortality detected
2. Remain reproductively active after sampling - Yes
3. Enough tissue sampled to obtain for genetic analysis of 66 genes - Yes

Experiment 2:
Markers for Disease Resistance

- Molecular methods carried out
- Statistically analyzed results and found a combination of 7 gene that separate disease resistant and susceptible oysters



Conclusions

Successful development of a method to sample oyster tissue for genetic analysis that does not affect their reproduction cycle or survival

7 gene expression markers that could be incorporated into the breeding program to accelerate disease resistant selection

Developed a fast and cost-effective method for screening large numbers of oysters

Current limitations

Additional validation steps required:

- Integration of these genetic data into the EBV model
- Consider additional costs associated with screening large numbers of oysters
- Optimal time to screen animals (to get maximum discrimination between susceptible and resistant oysters and have data available for breeding decisions)
- Management of tagged broodstock prior to spawning
- Determine if we can screen sufficient numbers to account for the poor single pair mating success rate

ACKNOWLEDGEMENTS:

- **Future Oysters CRC-P & FRDC**
- **Oyster growers:**

  Bruce Alford, Chris and Dominic Boyton, Alan Brooks, Michael Brooks, Glenn Browne, John and Annette Collison, Gary Collison, Geoff Diemar, Rob Diemar, Bob, Len and Ted Drake, Keith Duggan, Bob Hill, David Maidment, Ewan McAsh, Kevin McAsh, Rob Moxham, Tony Troup, Steve Verdich.
- **Port Stephens Fisheries Institute Hatchery Staff:**

  Stephan O'Connor, Lynne Foulkes, Andrew Parnell, Mitch Elkins, Norm Lawler, Walter Scifleet. Also: John Nell, Ben Perkins and Ben Finn.