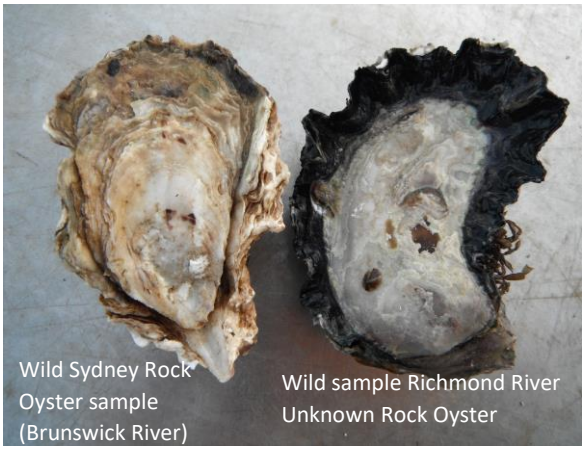
A new Rock Oyster with industry potential ….?

Summary: A local Community Chapter of OzFish Unlimited on the Richmond River (FNC-NSW) working with Dr. Wayne O'Connor's Shellfish team at the Port Stephens Fisheries Institute (PSFI) have monitored and recorded some startling results involving an apparent possible variation to Sydney Rock Oysters *(S.glomerata).*
For two successive years, monitoring was conducted on a single PSFI hatchery run of Richmond River wild oyster brood stock with the propagated stock (now two year old) showing apparent complete immunity to QX disease whilst being held on a lease with high endemic disease rates.

Dr.Jeffrey Go's team at EMAI noted zero sporulating activity within this Unknown Rock Oyster (URO) but saw activity (QX diagnosis) in most of the SRO (*S.glomerata)* that was used each year as a *'Control Set'* set amid the URO on the same lease.
Brunswick River origin SRO *(S.glomerata)* was used the first year as a control set and Wallis Lakes origin SRO *(S.glomerata),* this 2nd year (2020).
Results to date of this oyster in a high QX rated system, definitely needs further investigation.

Background: Forty-five years ago, full cycle (spat to harvest) oyster farming ceased on the Richmond. With changes to the catchment's floodplain and coastal wetland's along with a diverse mix of agriculture, resulting poor water quality has been a consistent trigger for the problematic QX disease. Oyster farming from southern QLD through to south of Sydney continues to suffer from this parasite driven disease, often with severe crop losses and industry downturn.



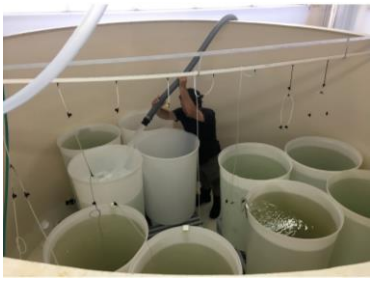
OzFish Richmond River Chapter, while pursuing their mandate to re-in state fish habitat throughout the catchment, which includes oysters and oyster reef restoration on the Richmond, were anxious to work with area oyster growers. By 2015, monitoring records (supported by DPI) witnessed an almost total disappearance of the estuary's Sydney Rock Oyster (*S. glomerata*).

After approaching the Port Stephens Fisheries Institute (PSFI) mid 2016, OzFish were allocated two trial batches from their selectively bred programme, along with a standard SRO control set (~ 9000 oysters in total). After 2 years of close monitoring and care plus two

successive QX outbreaks, all had died albeit the most selected oysters more slowly than the unselected stock. Approximately 2 dozen only of family 2015008 remain today.

By Oct 2017 OzFish monitoring showed evidence of another fresh wild spat fall in the estuary, along with scattered one to two-year-old oysters that had survived the significant floods and poor water quality of summer 2017. An oyster grower, while dismantling his lease, thought these few remaining adults observed by OzFish were a slightly different looking oyster and could be a variety of northern Black-Lip. The oysters had a distinctive pigmentation within the shell beneath the attachment of the adductor muscle, which is not seen in SRO's.
Samples were collected by OzFish and sent to Dr. Wayne O'Connor at PSFI for his team to identify. Reports back suggested they were ***not*** Black Lip but unsure as to the exact species. A year later, Dr. Carmel McDougal's team at Griffith Uni conducted a short sequencing analysis using a common genetic marker (COI) which indicated that these oysters do align genetically with Sydney Rock Oysters (*S. glomerata*)

Early 2018, Dr. Wayne O'Connor's team at PSFI had a small window of opportunity within their quarantine section and requested OzFish send down a number of these wild Richmond adult oysters that were (and still are) proliferating throughout the estuary. These were strip spawned and achieved settlement in their tanks at PSFI three weeks later, last week of March 2018**.**



PSFI quarantine tanks. Staff successfully strip spawned Richmond URO's

Mid July 2018, approximately six thousand were sent back to the Richmond (2-3 mm size) for OzFish to grow out with assistance from a couple of local growers.
Their growth was consistent with Sydney Rock however they had surprisingly low mortality <5% per year.

Under direction of PSFI and a more controlled experiment, a number of healthy adult Sydney Rock from the Brunswick River estuary (traditionally low QX river), were located among the new Unknown Rock Oyster (URO) from the Richmond.

February 2019 saw the normal wet season along with a QX outbreak but ***only*** among the SRO control set (verified Dr. J. Go EMAI Menangle). There was ***no*** sporulating activity among URO hatchery batch (pers comm J. Go 2019) or any apparent QX activity within the estuary wild population as normally happens with each QX outbreak.

Winter and spring 2019 saw continued good growth and health within the URO hatchery stock, again with low mortality. Late 2019 Dr. Wayne O'Connor suggested several dozen SRO from

the Wallis Lakes area be used as a control set. OzFish obtained these (3rd week of December 2019, courtesy Richmond Oysters P/L) and located them on the different cultivation lines/techniques OzFish had been experimenting with.

Following rain in late Feb (2020) there was no immediate QX outbreak but by late April, the Wallis Lakes SRO control set were showing significant mortality and ill health. Samples of both SRO control set and matching URO control set were sent down to EMAI.
Again, SRO control set verified with QX activity (spore activity), but there was ***NO*** activity in the URO control set samples sent down and less than average mortality in remaining URO on the lease.

The above field observations and measurements beg the question as to whether the URO's from the Richmond have ***naturally*** acquired some form of genetic resilience factor to both the Marteilia parasite and poor water quality which could explain their high survivorship. Alternatively, perhaps the oyster is a new species which is not susceptible to *Marteilia sydneyi* infection. Given the extended periods Richmond oysters are under stress from adverse water quality the exact cause of resilience is uncertain at this time.

This is now the question being asked by academics and oyster growers that have been following progress of these URO's on the Richmond. The news of the Richmond River field trials has spread through the industry and raised significant interest particularly in areas which are periodically affected by Qx outbreaks. Two McLeay River farmers have placed orders with a NSW hatchery, who were allocated some of the original propagated hatchery stock from the Richmond brood stock (2nd generation Richmond URO).

OzFish is continuing to report significant wild oyster growth back in the Richmond. OzFish is interested to continue this project further to seek further understanding of the resilience of these oysters which may have wide benefits to the commercial farming industry and to achieving habitat conservation restoration outcomes in estuaries.

Given the reported genetic similarities to *S. glomerata* (McDougal-Griffith), the hatchery batch that are now two years old are similar in appearance taste and colour to traditional SRO's offering promise to further commercial potential.

Collaborating scientists Dr. Carmel McDougal (Griffith), Dr. Kirsten Benkendorff (SCU) along with Dr. Jan Strugnall of James Cook University, have indicated significant interest in pursuing research (funding dependent) to provide further understanding around the following research questions:

1) Are the UROs a genetically distinct strain of SRO?
2) What are the natural recruitment, survivorship and growth rates of the UROs in the Richmond River?

3) How does the growth, survivorship and QX infection rates compare between the URO and SRO?
4) Have the SRO evolved useful adaptive traits from a population bottleneck driven by the high historical mortality due to QX disease and poor water quality?
5) Is the observed black pigment on the UROs in the Richmond an indicator of elevated melanin production resulting from changes in immune gene expression?
6) Is the elevated production of melanin induced in response to an infection?
7) Do the UROs have any molecular markers that confer resistance and are potentially inheritable?

Black pigment at adductor attach points in the oyster

Suggested research avenues (Carmel McDougall, Griffith University; Kirsten Benkendorff, Southern Cross University, Jan Strugnell, James Cook University)

The following research avenues are proposed (funding dependent):

1. **Investigate correlation of pigmentation with QX resistance.**

Initial reports of the wild Richmond River oysters suggested that they have significantly darker pigmentation (of both the shell margin and the adductor muscle scar) than traditionally farmed SROs. As melanisation is known to be associated with immune responses it is possible that there is a link between this pigmentation and reduced mortality. Here, we propose a 1-year honours project to investigate whether there is any correlation between measured levels of pigmentation in SROs and QX incidence. This project will involve monitoring oyster recruitment, growth, pigment product and survivorship using monthly photoquadrats in the field followed by image analysis. Samples of oysters will also be collected each month for detection of QX via histology (digestive gland imprints) and PCR. Water quality (minimally including temperature, salinity and turbidity) will be measured using a portable probe. The student will be based at either Southern Cross University or Griffith University. Potential issue: timing, honours projects typically run from early March until late October and the QX window may be missed. We can ask the student to do some fieldwork before the official start.

Estimated budget: $6,000.00 (note, Griffith U contributes $1,500 towards honours projects)

Travel	9x field trips to Richmond River, overnight accommodation	$1000.00
DNA extractions/PCR	$10/sample, 30 samples per month, 9 months	$3,000.00
General reagents/maintenance	Including histology staining kit	$2,000.00

Supervision (in-kind contribution from University)	*0.05 FTE Kirsten Benkendorff and Carmel McDougall*	*$19,427.00*

2. Population genetics of wild Richmond River oysters

COI barcoding has demonstrated that the Richmond River oysters are *S. glomerata*. It is apparent that this population has undergone a significant bottleneck and is currently re-establishing, which may mean that there are notable genetic differences between this population and those found elsewhere. Here we propose a population genetic study to compare the genetics of the Richmond River population with those found elsewhere. We propose to compare this population with that found in Moreton Bay, the Tweed River, the Clarence River, the Macleay River, and Port Stephens. This will provide a measure of the genetic distance between the Richmond River oysters and those elsewhere, will shed light on whether Richmond River oysters should be considered a subspecies, and will potentially identify molecular markers that can be used to identify Richmond River oysters.

Estimated budget: $70,625.00

Travel	Collection trip to all sites, <1800km via university vehicle. 5 nights accommodation for 2.	$3,125.00
DNA extractions	$5/sample, 30 samples per site, 5 sites	$750.00
Sequencing for population genetics	DArT-Seq of above samples	$18,750.00
Researcher time	Postdoc, casual, equivalent of 4 months full time. Sample processing, analysis, report	$48,000.00
Supervision (in-kind contribution from University)	*0.10 FTE Carmel McDougall and Jan Strugnell*	*$38,854.00*

3. Molecular mechanisms of QX resistance

Even within standard hatchery-bred Sydney Rock Oysters variability is observed in QX infection rates and mortality. Here, we propose to investigate potential molecular mechanisms of QX resistance using a combined transcriptomic and proteomic approach. The Richmond River oyster population (hatchery bred lines if possible) and co-located standard hatchery-bred stock will be monitored for QX using a PCR-based approach. Upon detection of QX, sampling will be conducted to;

1) assess the level of infection of individuals,

2) conduct transcriptomics to investigate gene expression in infected vs non-infected individuals, and
3) proteomics to assess the expression of immune enzymes in infected vs non-infected individuals.

We will also investigate the presence of different isoforms of the prophenoloxidase enzymes that lead to melanin production in the Richmond river oyster compared to other SROs w. This research will identify genes and isoforms that are specific to individuals that are resistant to QX, providing understanding about the mechanism of this resistance and possibly identifying genetic markers for resistance.

Estimated budget: $327,900.00

Travel	9x field trips to Richmond River, overnight accommodation	$1,250.00
QX monitoring (and COI barcoding if needed)	$10/sample, 30 samples per month, 9 months	$3,000.00
Transcriptomics (RNA seq)	$375.00/sample (current AGRF offer), three time points (one prior to QX outbreak), minimum of 5 infected and 5 non-infected oysters from each source per time point, minimum of one tissue (two would be better). Absolute minimum of 60 samples.	$22-45,000.00
Protein/enzyme work	Gels and reagents for electrophoresis, Bradford assay etc plus $50 per sample for sequencing at UQ LEIF facility. Sampling regime as above on gills and hemolymph (min 120). Data processing will use in-house protein pilot software at SCU	$12,500.00
General reagents/maintenance	Collection jars, eppendorfs, pipette tips, slides, stains, RNAlater, buffers, etc.	$6,250.00
Researcher time	2 year postdoc at PhD entry level	$259,900.00
Supervision (in-kind contribution from University)	*0.10 FTE Kirsten Benkendorff, Carmel McDougall and Jan Strugnell*	*$63,510.00*

Proposed funding mechanism

The honours project could potentially be funded directly, however projects 2 and 3 require significant amounts of funding that will need to be leveraged. An ARC Linkage grant may represent the best mechanism for funding an overarching project encompassing all of these research areas.

In summary, ARC Linkages provide:

- between $50,000 and $300,000 per year
- are 2-5 years in duration
- require total cash/in-kind contributions that at least match the requested funding
- must include a cash contribution of at least 25% of the total funding requested, unless all partner organisations are exempt.

Exemptions apply if the partner organisation:

- is an organisation which meets the definition of a charity under the Charities Act 2013, or
- is an organisation which meets the ATO's definition of a non-profit organisation, or
- is an organisation which has fewer than twenty full-time employees.
- Note that even if all partner organisations are exempt a reasonable cash contribution would still be required to make the application competitive.

The ideal situation would be to include funds for a dedicated Postdoctoral Researcher within the project over three years. This would ensure the timely completion of all three projects, with some capacity for additional investigation. This would require $40,000 in cash and in-kind contributions with a total budget of approximately $450,000.00. Note that the host university would normally fund a PhD student as part of an ARC Linkage project of this scale.

If this cash contribution cannot be achieved the project could also be conducted by two PhD students, or a PhD student and part-time research assistant over three years (assuming that one PhD is funded by the host university). This would slow the research and limit outcomes, however would require approximately $22,000 per annum for three years with a total project budget of approximately $260,000.00

Conclusion: OzFish Unlimited, Richmond River Chapter https://ozfish.org.au/chapter/new-south-wales/ have chosen oyster rehabilitation in the Richmond River as a key Chapter mandate. The chapter continues to work closely with Oysters growers of the area that cannot devote the time to a project of this nature.

We are sending you this Richmond River ***Unknown Rock Oyster*** proposal in the hope that you can help us secure funding to help provide more information and industry potential evaluation for this Rock Oyster.

Results to date of this oyster in a high QX rated system, definitely needs further investigation.

Project partners;

OzFish Unlimited Richmond River
Griffith University
Southern Cross University
James Cook University
Port Stephens Fishery Institute (Dr. Wayne O'Connor, Dr. Mike Dove)
Noel Baggaley (Tweed and Brunswick Oysters)
Ian Cardow - Richmond Oysters
(Further partners pending)

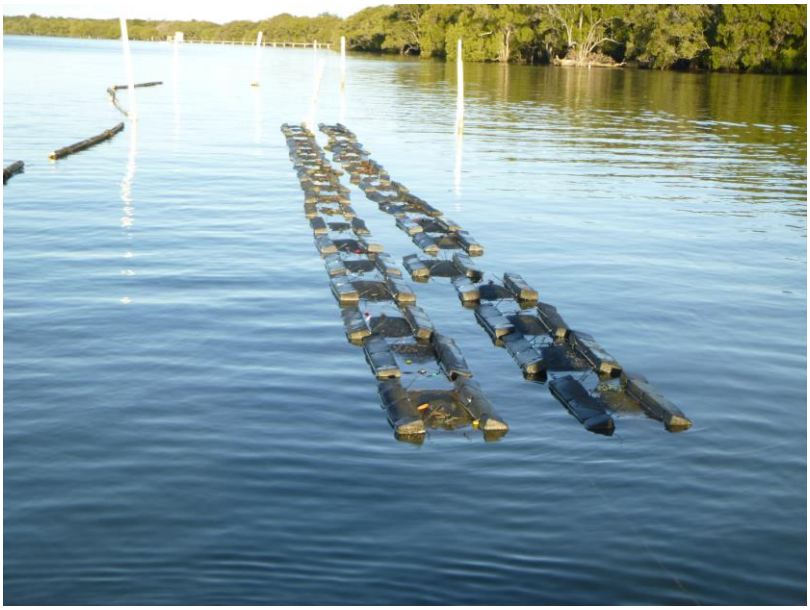
For more information, please call Chapter President - John Larsson (0418 66 1467) or Dr. Carmel McDougal – Griffith University on ……..

URO Spat from Port Stephens Fisheries Institute. First deployed to 1.5 mm Nylon socks for initial grow-out

Dec 2018: Growth assessment conducted monthly for the 1st 12 months

March 2019: Moved to Stanway Tumblers after initial nylon sock grow-out time

April 2019: URO numbers split between fixed Inter-tidal and floating sub-tidal



Mar 2019: URO's Zapco dual float on floating sub-tidal line

Jun 2019: Basket of graded large URO

11th Oct 2020: Basket of washed large URO for closer scrutiny

11th Oct 2020: URO samples

11th Oct 2020: Same three samples

11th Oct 2020: Great tasting …..!