NORTHERN HEALTH SERVICE DELIVERY

TRADITIONAL OWNER-LED DEVELOPMENT

AGRICULTURE & FOOD

Rock oyster identification tools and protocols

A.2.1819053 WA

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Department of Primary Industries and Regional Development

CRCNA DEVELOPING NORTHERN AUSTRALIA

GOVERNMENT OF WESTERN AUSTRALIA

Department of Primary Industries and Regional Development

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Acronyms and Abbreviations

BOLD	Barcode of Life Data System
CO1	Cytochrome c oxidase subunit 1 mitochondrial gene
°C	Degrees Celsius
DNA	Deoxyribonucleic acid
DPIRD	Department of Primary Industries & Regional Development
gL^{-1}	Grams per litre
ID	Identification
L	Litres
NCBI	National Centre for Biotechnology Information (GenBank databases)
$MgCl_2$	Magnesium chloride
µm	Microns or micrometres
mL	Millilitres
mm^3	Cubic millimetre
PCR	Polymerase chain reaction
qPCR	Quantitative polymerase chain reaction
rRNA	Ribosomal ribonucleic acid
SOP	Standard operating procedure

Project Participants

Department of Primary Industries and Regional Development

Maxima Rock Oyster Company Pty Ltd

Athair Aquaculture Pty Ltd

Murujuga Aboriginal Corporation

Executive Summary

This document highlights the species distribution of Western Australian (WA) tropical rock oyster species that have been identified for potential aquaculture development. One of these species, *Saccostrea scyphophilla*, (Milky Rock Oyster), has performed poorly in previous field trials and is not recommended for further research and development. However, its distribution in the wild overlaps with other species of interest that are morphologically similar. Identifying rock oyster species by morphology alone is unreliable and presents challenges for farmers, hatcheries and regulators as the tropical rock oyster industry continues to develop in WA. This document highlights current protocols and processes available to interested parties to identify and manage the farming of several tropical rock oyster species in WA.

Background

As the WA tropical rock oyster industry is still in its infancy, participants are reliant on wild oysters either as spat for on growing or as broodstock for hatchery production. The collection and farming of wild spat and broodstock of unknown species presents challenges for regulators in licensing and translocation, and for commercial hatcheries and growers as they attempt to grow and market a consistent product. While up until recently the nomenclature of WA's rock oysters has been unclear, recent work by Snow (2020) and Department of Primary Industries and Regional Development (DPIRD) staff have provided a good working knowledge of the species that occur in WA and of their geographic distribution.

Within WA, there are four naturally occurring *Saccostrea* lineages (*S. cucullata, S. echinata, S. scyphophilla, S. glomerata*) that have been considered for aquaculture development. The review by Snow (2020) summarises different names allocated to these species in scientific literature. They are presented in Table 1 alongside common and colloquial names used amongst the WA community. Commercial trials with the Milky Rock Oyster at various sites including the Pilbara have proven unsuccessful with slow growth rates and unacceptable shell shape (Fontanini & Bermudes, 2020; Gill, 2020).

Table 1. Different naming conventions for *Saccostrea* rock oyster lineages in Western Australia. Extract of Table 1 (synonyms applied to *Saccostrea* lineages by various authors) from (Snow, 2020), with colloquial names.

Lam and Morton (2006)	Sekino and Yamashita (2016)	Snow (2020)	Colloquial/Common
S. cucullata A	Non-mordax A	*Saccostrea* A	Coral Rock Oyster Western Blacklip Rock Oyster
	Non-mordax J	*Saccostrea* J *S. echinata**	Blacklip Rock Oyster^
S. mordax	*S.* mordax	*S. scyphophilla*	Milky Rock Oyster
S. glomerata	*S. glomerata*	*S. glomerata*	Sydney Rock Oyster Albany Rock Oyster

*Western Australia's *Saccostrea* J confirmed to be from the same genetic grouping as *Saccostrea echinata* in Northern Territory and northern Queensland.
^Registered with Australian Fish Names Standard

The geographic distribution of these species was first described by Snow (2020) in an internal risk assessment prepared for DPIRD and further refined by DPIRD staff through ongoing sampling and anecdotal evidence to reach the current understanding of the distribution shown in Figure 1.

The distribution in Figure 1 provides a practical way to exclude *Saccostrea* A and *S. echinata* (*Saccostrea* J) from each other via selection of appropriate regions for broodstock collection, e.g., collect *Saccostrea* A between Shark Bay and the Pilbara; collect *S. echinata* from the Kalumburu area. However, given the geographical overlap between good and less desirable species (e.g., between *Saccostrea* A and *S. scyphophilla*), hatchery operators and the emerging industry will require practical ways of distinguishing them to select founding broodstock from their native habitat. Wild rock oysters are not always identifiable by morphological characteristics alone. Of the four rock oyster species mentioned above, *S. echinata* is the only species likely to be accurately identified by shell morphology and size (Figure 2). *Saccostrea* A and milky oysters can be difficult to tell apart by their morphological traits alone (Figure 3). Genetic identification is another technique that will give farmers and hatchery managers confidence they are working with their desired species. This document describes several scenarios and protocols to identify *Saccostrea* rock oyster broods and spat lineages to avoid mixed species on farms.

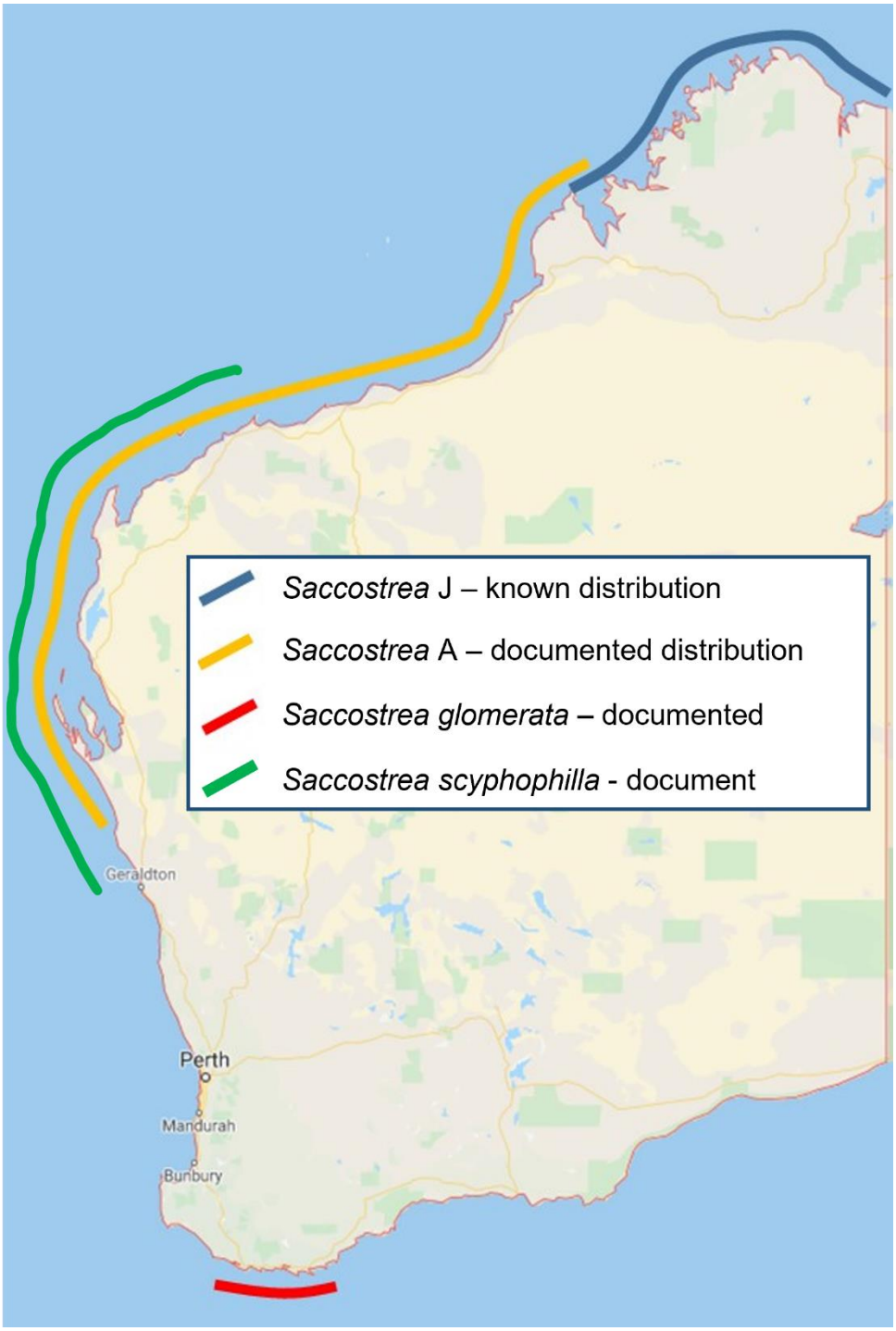

Figure 1. Approximate distribution of *Saccostrea* species in Western Australia.

Figure 2. Wild *Saccostrea* A oysters collected from Karratha in the Pilbara region of WA (top) have a distinctly different morphology to wild *S. echinata* oysters collected from Cone Bay in the Kimberley region of WA (bottom) and can reliably be distinguished by their shell traits and collection areas.

Figure 3. Of seven wild rock oysters collected from Carnarvon, WA, six were identified as *Saccostrea* A and one (circled) was *S. scyphophilla*. It is unreliable to tell these two *Saccostrea* lineages apart by morphology and shell characteristics alone.

Genetic Identification

Extraction, PCR & sequencing

DNA is extracted from the sample using commercial extraction kits following the manufacturer's instructions. Examples of non-destructive and destructive collection of genetic material from rock oysters are detailed below. For species identification, polymerase chain reaction (PCR) is used to amplify a targeted gene region. Mitochondrial cytochrome c oxidase subunit 1 (CO1) region is highly effective in identifying most animal groups. However, mitochondrial 16S ribosomal RNA (rRNA) gene region is more reliable in identification of rock oyster species.

Amplified PCR products were Sanger sequenced. Obtained sequences were used to interrogate (similarity-based searches) Barcode of Life (BOLD) and NCBI GenBank databases to determine species identification. Refer to Snow et al. (2023) for more information regarding this methodology.

Real-time PCR (qPCR)

A real-time PCR (qPCR) assay was developed based on 16S rRNA gene and shown to be able to differentiate the two main *Saccostrea* lineages in WA, *S. scyphophilla* and *Saccostrea* sp. (*cucullata)* complex (Snow and Fotedar, unpublished). However, this method cannot differentiate between species within *S. cucullata* complex (e.g., *Saccostrea* A, *S. echinata*, or *S. glomerata*). Therefore, whilst qPCR provides a rapid scanning tool to identify any *S. scyphophilla*, PCR and sequencing must be undertaken where *S. cucullata* species are identified. As *S. cucullata* lineages have not been shown to share a common geographic distribution in WA (Figure 1), qPCR testing will often be adequate to identify target aquaculture species (*Saccostrea* A, *S. echinata*, and *S. glomerata*) depending on the origin of broodstock in conjunction with morphological characteristics.

Aquaculture scenarios for species identification

Hatchery reared oyster spat

Where farmers want to stock farms with hatchery reared spat, they need to collect wild rock oyster broods. Collectors may be confident in the species identification of the broods they are collecting, or they may be unsure of exactly what species occur within the collection region. Species identification can be made at several stages within the process of producing hatchery reared spat. These options are described in Figure 4.

Low confidence in brood species ID

Where the species of the wild broods is unknown, you may prefer to identify the broods via a non-destructive sampling method before committing to a conditioning and/or spawning regime in the hatchery. Rock oysters can be tagged and immersed in a magnesium bath to relax their abductor muscles. This allows for a swab of DNA to be taken from inside their shell and genetic identification can take place via qPCR and sequencing. Once DNA results are received, the hatchery will discard or separate individuals of different species and work to produce spat of the desired species.

High confidence in brood species ID

If the species identification of the broodstock is assumed to be known, then the hatchery may opt to spawn these animals and collect early-stage larvae for PCR and genetic sequencing. This scenario may also be the most appropriate where the company chooses not to test the species identification of the broods. The risk with these methods lies with the fees incurred for the hatchery's work in rearing larvae whilst waiting for the results of genetic identification. If the larval batch is not pure or of the desired species, the batch will need to be discarded several weeks or months into the culture of the spat. However, in the case of hatchery production of *Saccostrea* A, qPCR enables the testing of batch contamination with Milky Rock Oysters within 48 hours of spawning, hence greatly reducing commercial risk. In the case of *S. echinata*, broodstock selection via morphological traits (e.g., size >150mm) will provide sufficient confidence for hatchery operators. A one-off PCR test of spat is used for commercial genetic validation prior to farm transfer.

Another way to validate brood genetics is via destructive testing of broodstock. If they are no longer required following the spawning, they could be sacrificed to sample abductor muscle and obtain a genetic identification.

Wild collected oyster spat

Farmers may choose to collect oyster spat from the wild instead of purchasing it from a hatchery. A review by Nowland et al. (2020) highlights tropical rock oyster farming operations in 27 countries that use wild collected spat to stock their farms. Of these, 14 are commercial scale operations. In these instances, it is beneficial to have a basic understanding of when your species of interest is in spawning condition, where breeding populations exist, and the hydrodynamics of the region so that you can place spat collectors in an area that is likely to recruit oyster larvae.

To confirm which specie(s) of rock oyster were collected, a sample of spat can be taken whilst stripping the collectors. Oyster spat can be frozen (-20°C) or preserved in 100% ethanol (at an ethanol to meat ratio of at least 4:1) and sent to a laboratory for species identification. Depending on the size of the spat, meat can be stripped from the oyster shells and preserved or alternatively the whole oyster (with the shell) can be preserved. Submitting whole oysters for analysis will incur an additional processing fee. Having spat analysed as they come off the slats will confirm that the target species for cultivation has been collected. With a large enough sample, it will also indicate the proportion of target species compared to other wild oysters.

Collecting wild spat of several species is almost inevitable. This may not be a problem depending on which tropical rock oyster species is being farmed. For example, as wild-collected *S. echinata* spat grow, morphological differences between species on the farm will emerge over time (e.g., colour and shape). This would require a grade out of undesirable species as soon as practical to limit wasting farm resources. It is unlikely to ever be possible to distinguish between other

tropical rock oyster species without genetic identification. This should be considered in any oyster farms operational and marketing strategies.

Collection of wild rock oyster spat was attempted in the Pilbara and Kimberley regions of WA as part of this project, however non-target species outcompeted some target species when settling on spat collectors whilst others were never detected in samples. Although these trials only reflect a small window of settlement during the year, collection of wild spat was determined to likely be unfeasible for farming operations in northern WA with a recommendation that farmers seek spat from hatchery operators (see *Field Trial Report*).

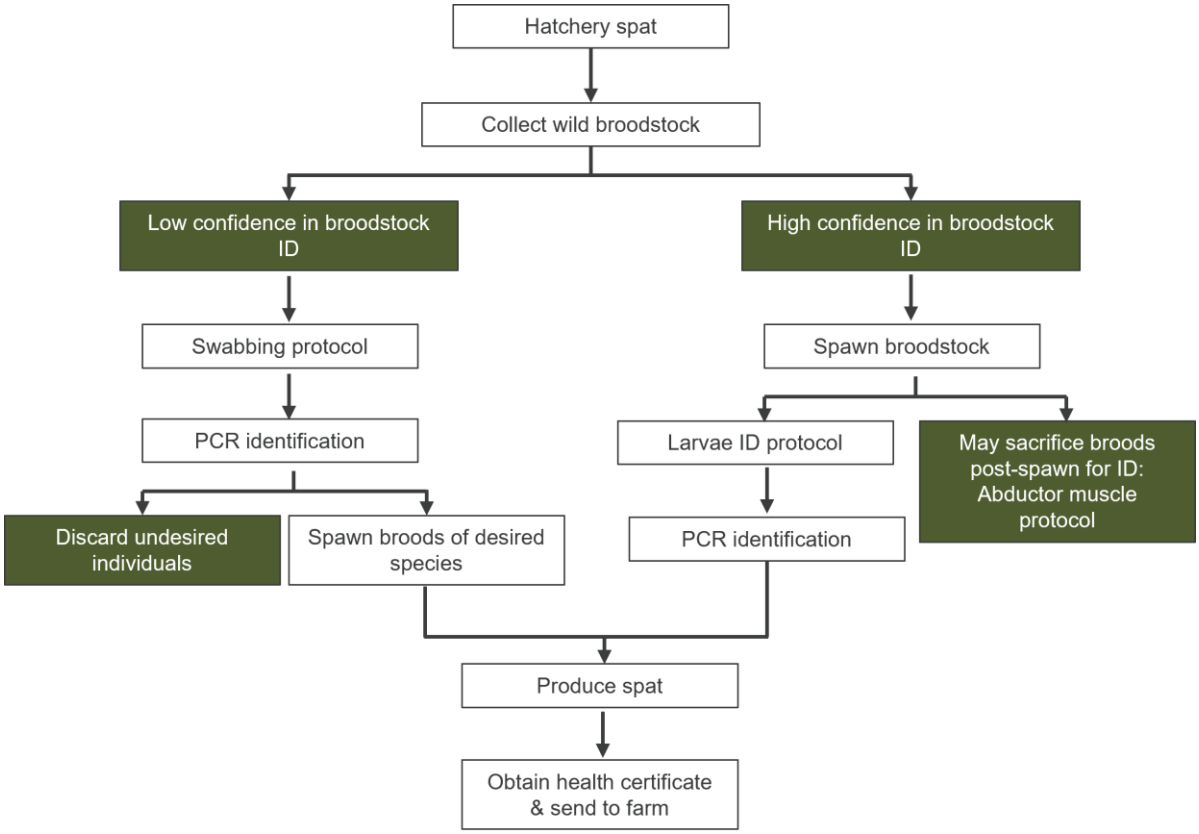

Figure 4. Proposed process of genetic identification for hatchery reared rock oyster spat from wild-collected broodstock in Western Australia.

Non-destructive DNA collection

Swabbing broodstock

It is possible to sample a rock oyster's DNA by swabbing the inner mantle & abductor muscle within their shell. This must be done whilst the oyster is removed from water to avoid contaminating the swab, however it is challenging to keep the shell of rock oysters open once they are removed from water. Whilst it is common to insert a wedge between the top & bottom shell whilst still underwater in other species (e.g. pearl oysters), this is often unsuccessful in wild rock oysters due to the 'frilly', interlocking shape of their top and bottom shells as well as their sensitivity to disturbances that will see them close their shell.

Instead, rock oysters can be bathed in a muscle relaxant to slacken their abductor muscles (Figure 5). Then they can be removed from the water and swabbed in a non-destructive manner (Figure 6). The recipe for the relaxant bath and swabbing regime is described below with DPIRD's standard operating procedure (SOP) attached in the Appendices.

To make the magnesium relaxant, a 1.5L solution of $50gL^{-1}$ $MgCl_2$ is mixed with 1.0L of filtered seawater and 2.0–3.0L of distilled water until a salinity of 34–36ppt is achieved. Oysters are immersed in this solution and begin to relax their abductor muscles within 20–30 minutes. As oysters begin to open their shells, waiting a further 5-10 minutes before removing them from the bath is preferred so that the magnesium relaxant can take full effect. Once oysters are removed

from the bath a flocked swab[1] can be gently passed across the inner mantle and abductor muscle of the oyster (Figure 6). It's important to take care not to disturb the gills and other organs as this may fatally damage the oysters. Place the swab in the capture/transport tube and label the tube before freezing the sample. Return the oyster to filtered seawater where the effects of the relaxant will slowly wear off. Frozen samples should then be sent to a laboratory for PCR and sequencing within a few days. PCR and sequencing can be undertaken by DPIRD's Diagnostic and Laboratory Services for a fee in WA.

Figure 5. *Saccostrea* A oyster relaxing its abductor muscle in a $MgCl_2$ bath.

Figure 6. A rock oyster being swabbed after it was removed from a $MgCl_2$ bath.

In trials conducted at DPIRD's Hillarys Research Hatchery, 90% of oysters opened in response to a magnesium bath, and 86% (n = 83) were able to be successfully swabbed and subsequently identified. It is crucial to match the size of the swab to the oyster to minimise damage to the oysters. As many as 47% (n = 21) of swabbed *S. cucullata* oysters died within a month of being sampled in early trials using large, flocculated swabs. Whilst deceased oysters won't be used as broodstock, it is still valuable to identify the species to understand the wild rock oyster populations from a collection region.

[1] A flocked swab is specimen collection swab with absorbent Nylon® fibers spray-coated on an applicator tip. FLOQSwabs® by Copan manufacturer were used during these trials.

Destructive DNA collection

Tissue sample

Where oyster specimens don't need to be kept alive, they may be sacrificed, and a tissue sample taken for genetic identification. Oysters are opened and a sample of the abductor muscle is collected and preserved in 100% ethanol before being sent to a laboratory for genetic identification. This process kills the oysters and so cannot be used if you intend to use the oysters for breeding. This method is recommended when trying to confirm the genetics of a wild population of oysters or alternatively if oysters have already been used as broodstock to produce spat. When utilising this method, it is recommended that you keep a sample of the specimen and freeze it (-20°C) until you have confirmation from the laboratory that DNA was extracted and sequenced. If the original muscle sample submitted was of poor quality, you will have the option of re-sampling your preserved specimen to try again for genetic identification.

Larval sampling

All larvae and/or spat produced by a research or commercial hatchery can be tested to ensure the purity of the batch. Hatchery operators may collect 0.25-1mL of larvae in an Eppendorf tube and fix the sample in 100% ethanol. Larvae can be collected early in the larval process, at D-stage, 24 hours post spawning. As mentioned above, qPCR testing can distinguish between *S. scyphophilla* and *Saccostrea cucullata* lineages to give results within 48 hours. As *S. scyphophilla* is generally the most likely source of contamination in a WA batch, this is an important tool to use early on in larval culture to conserve hatchery resources.

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Appendices

SOP: Magnesium relaxant bath

Purpose: To sedate oysters and allow for less destructive sampling methods.

Mixing MgCl2

Preparation: Dissolve the $MgCl_2$ at least one day in advance as it is an exothermic reaction and will be too hot to immerse oysters in immediately after mixing.
Ensure oysters have been tagged for individual identification.

Notes: Take precautions when mixing up $MgCl_2$ including using appropriate safety equipment, PPE and completing the procedure slowly.

Procedure:

3L stock solution

1. Collect 3L of distilled water in a Shott bottle and decant ~1.5 - 2L into a glass beaker. Put a magnetic rod in the bottom of the glass beaker.

NB Keep the remaining distilled water in the Shott bottle.

2. Place a magnetic stirrer plate in a fume hood (or somewhere with a protective barrier) and place the beaker of distilled water on top. Turn the stirrer on and pull the fume hood barrier down as low as practicable.
3. Weigh out 150g of $MgCl_2$ salts.
4. Make up a solution of 50g L^{-1} $MgCl_2$ by **slowly** adding the $MgCl_2$ to the glass beaker of water.

NB This is an exothermic reaction and will give off a lot of heat. The solution will hiss and splutter.

The salt must be added to water under the fume hood with the barrier as low as practicable and all PPE must be worn. It is safer to use a glass beaker and not a bottle with a narrow opening such as a Shott bottle to allow heat to dissipate quickly.

5. Once the salt has dissolved you add the remaining water from the Shott bottle to make the solution up to 3L.
6. Allow the solution to cool to room temperature before using.

Seawater sedation

Preparation: Place oysters in a shallow tray before mixing up the sedative solution so you can estimate the total volume needed to cover the immerse the oysters.

Procedure:

7. In a 5L jug combine:
   a. 1.5L of 50g L^{-1} $MgCl_2$ solution;
   b. 1.0L of filtered natural seawater.

   NB At this point, check the salinity of the solution with a salinity meter. Aim to make an isosaline solution of relaxant so that it is the same salinity as the oysters' ambient conditions. The amount of distilled water you add to achieve this may vary slightly each time you use this procedure.

   c. Add 2.0L of distilled water to the jug, mix and check the salinity.
   d. Start to add approx. 250mL of distilled water at a time, mixing and measuring the salinity each time. Stop adding distilled water once you have achieved your desired salinity.

   NB If the salinity is too low, increase it by slowly adding $MgCl_2$.

8. Larger batches of oysters can be sedated by creating more solution. Stick to these ratios.

   Natural Seawater **:** 50 gL^{-1} $MgCl_2$ **:** Distilled water

   2 **:** 3 **:** 4 - 6

9. Place the oysters in a shallow tray and pour in enough relaxant solution in to cover them.
10. Add an air stone with light aeration.
11. Oysters should open within 3 hours, but most will start to open within 20 – 30 minutes.

NB The efficacy of the sedation will wear off over time. Make another batch and try again another day if it hasn't worked after 3 hours.

SOP: Swabbing rock oysters for DNA

Purpose: Obtain a non-destructive DNA sample for species identification.

Procedure:

1. Take a flocked swab and break the seal on the capture tube.
2. Gently collect a sample from the oyster by moving & twisting the swab across the inner mantle and abductor muscle of the top shell.

NB Try to avoid touching the organs held within the bottom shell of the oyster as you're more likely to fatally damage them.

3. Place the swab back inside the capture tube and seal it.
4. Label the capture tube with the following details:
   a. Date of collection;
   b. Individual oyster ID (tag number);
   c. Origin/location of broodstock; and
   d. Any other identifying or noteworthy details.
5. Immediately freeze the capture tube to preserve the sample.
6. Send the frozen samples to DPIRD Diagnostic Laboratory Services within the next few days.
   a. Fill out chain of custody form; and
   b. Arrange courier.

SOP: Tissue sampling

Opening & shucking wild oysters

Purpose: Sample and fix tissue samples from rock oysters for species identification of individuals.

Notes: Wear appropriate PPE including eye protection, steel cap boots and gloves.
This protocol refers to opening wild oysters that often grow in irregular shapes and with much tougher shells than cultivated oysters. Therefore, traditional oyster shucking methods may not work and can often be more dangerous to the shucker when applied to wild oysters. This is an alternate method that minimises risks to staff, however competent shuckers may prefer to use traditional methods.

Procedure:

1. Clean and sterilise an oyster shucking knife and flathead screwdriver. Put safety gloves on to protect your hands.
2. To hold the oyster in position, place the lip under the toe of your (steel cap) boot with the hinge facing out.

NB Do not apply any pressure or crush the oyster. You're just wedging it between your toe and the ground to hold it in place.

3. Place the edge of the flathead screwdriver between the hinge of the oyster shell and lightly tap the top of the screwdriver with a hammer several times until the hinge is dislodged. A small amount of liquid will be evident once the hinge is cracked.
   NB Be careful to start with small taps and gradually increase pressure so that you just knock the hinge open without plunging the screwdriver into the oyster when it opens.
4. Pick the oyster up and hold it in your non-dominant hand with the top shell facing up and the hinge closest to you. Slide the tip of oyster knife blade between the top and bottom shell, about a third of the way up the shell (from the hinge) on the right side.
5. Level the blade so it is horizontal and parallel to the plane of the top shell. Start to move the blade deeper into the top shell with slow back-and-forward movements. This will sever the abductor muscle from the top shell without disturbing the rest of the oyster contained within the cup of the shell.
6. Remove the top shell and slide the oyster knife under the meat of the oyster using the same motion as above to remove the abductor muscle from the bottom shell as well.
7. Clean and sterilise equipment in between shucking oysters.

Collecting and preserving muscle sample

Preparation: Sterilise and label all sample jars and put together a chain of custody to be sent with samples. 2mL sample tubes will be sufficient for a ~5mm^3 muscle sample, however if you're taking a larger sample, choose a vial large enough to maintain a 4-5:1 ratio of ethanol to meat.

Notes: Ensure you are wearing clean disposable gloves whilst handing samples and make sure to get a new pair each time you move to a new oyster to avoid cross-contamination.
Pre-arrange a date for sample submission with DPIRD Diagnostic Laboratory Services (or other DNA

analysis laboratory) so they can advise of workload and ideal sampling window.
Abide by transportation requirements for dangerous goods when transporting samples to DNA analysis laboratory. Please see special provision A180 of the International Civil Aviation Organisation Technical Instructions for the Safe Carriage of Dangerous Goods by Air when sending samples via air.

Procedure:

8. Put on a pair of clean disposable gloves and slide the meat from the shucked oyster into a disposable petri dish.
9. Take a clean, sterile scalpel blade and cut a 5-10mm^3 section of the abductor muscle away from the rest of the meat.
10. Place the muscle sample into the pre-labelled vial and add 1.5mL of 100% ethanol to the vial ensuring you have an ethanol to meat ratio of at least 4-5:1.
11. If you wish to keep the oyster sample (for further testing or in case DNA cannot be extracted from the sample you just took), place it in a specimen jar or bag and label appropriately before freezing (at least -20°C). If you don't need to keep the specimen, discard via your facility's biological disposal procedures.
12. Discard the scalpel (via sharps disposal), gloves, and petri dish and move onto your next oyster sample (repeat from step 8).
13. Replenish your samples within 24-48 hours by decanting off as much ethanol as possible and then topping up the Eppendorf with fresh 100% ethanol.

NB Refrigerating the samples at ~4°C may help with preservation but it is not required. If storing samples for a long time, ensure ethanol is topped up over time as evaporation occurs.

14. Send the samples to DPIRD Diagnostic Laboratory Services (or other provider).
    a. Fill out chain of custody form requesting species level identification,
    b. Arrange courier; and
    c. Abide by transportation requirements for shipping and transporting a class 3 dangerous goods.

SOP: Collecting oyster larval samples for genetic identification

Purpose: Concentrate a sample of larvae and preserve them for qPCR assay to check the purity of the batch.
Notes: Its advantageous to sample larvae for genetic identification as early as possible in a larval run to reduce hatchery associated expenses if the batch is not pure or of the desired species. If you have an excess of hatched larvae after incubation and they cannot all, be stocked into larval tanks, sample as early as day 1.
Pre-arrange a date for sample submission with DPIRD Diagnostic Laboratory Services (or other DNA analysis laboratory) so they can advise of workload and ideal sampling window.
Observe transportation requirements for dangerous goods when transporting samples to DNA analysis laboratory.

Procedure:

1. Pass larvae over 20µm + 60µm stacked nets to catch larvae on the 20µm screen and remove any debris with the 60µm.
2. Rinse the 20µm net with sterilised seawater to concentrate the larvae in the bottom of the net.
3. Use a disposable pipette to collect a concentrated sample of larvae and transfer them to a sterilised 2.5mL Eppendorf tube.
4. Allow larvae to settle before decanting excess water out of the Eppendorf tube.
   NB Larvae are alive, so they will continue to actively swim when in the Eppendorf tube. You may decant some off as you try to remove excess water.
5. Fill the Eppendorf tube with larvae up to or below the 1mL line and remove as much excess water as possible.
6. Aim for at least a 4-5:1 ratio of ethanol:larvae in the Eppendorf tube. Therefore, don't fill larvae past ~0.5mL – 0.6mL within the Eppendorf tube.
7. Add 1.5 - 2.0mL of 100% ethanol to the Eppendorf tube and gently mix the sample.
   NB If there was still a lot of seawater in your tube, you can add some ethanol to the Eppendorf and wait 2-3 minutes for larvae to slowly stop swimming. Then you can decant off excess water/ethanol and replenish the sample with the appropriate amount of 100% ethanol.
8. Replenish the sample within 24-48 hours by decanting off as much ethanol as possible (without disturbing and decanting out larvae) with a disposable pipette and then topping up the Eppendorf with fresh 100% ethanol.
   NB Refrigerating the samples at ~4°C may help with preservation but it is not required.
9. Send the samples to DPIRD Diagnostic Laboratory Services (or other provider).
    a. Fill out chain of custody form requesting species level identification; and
    b. Arrange courier.