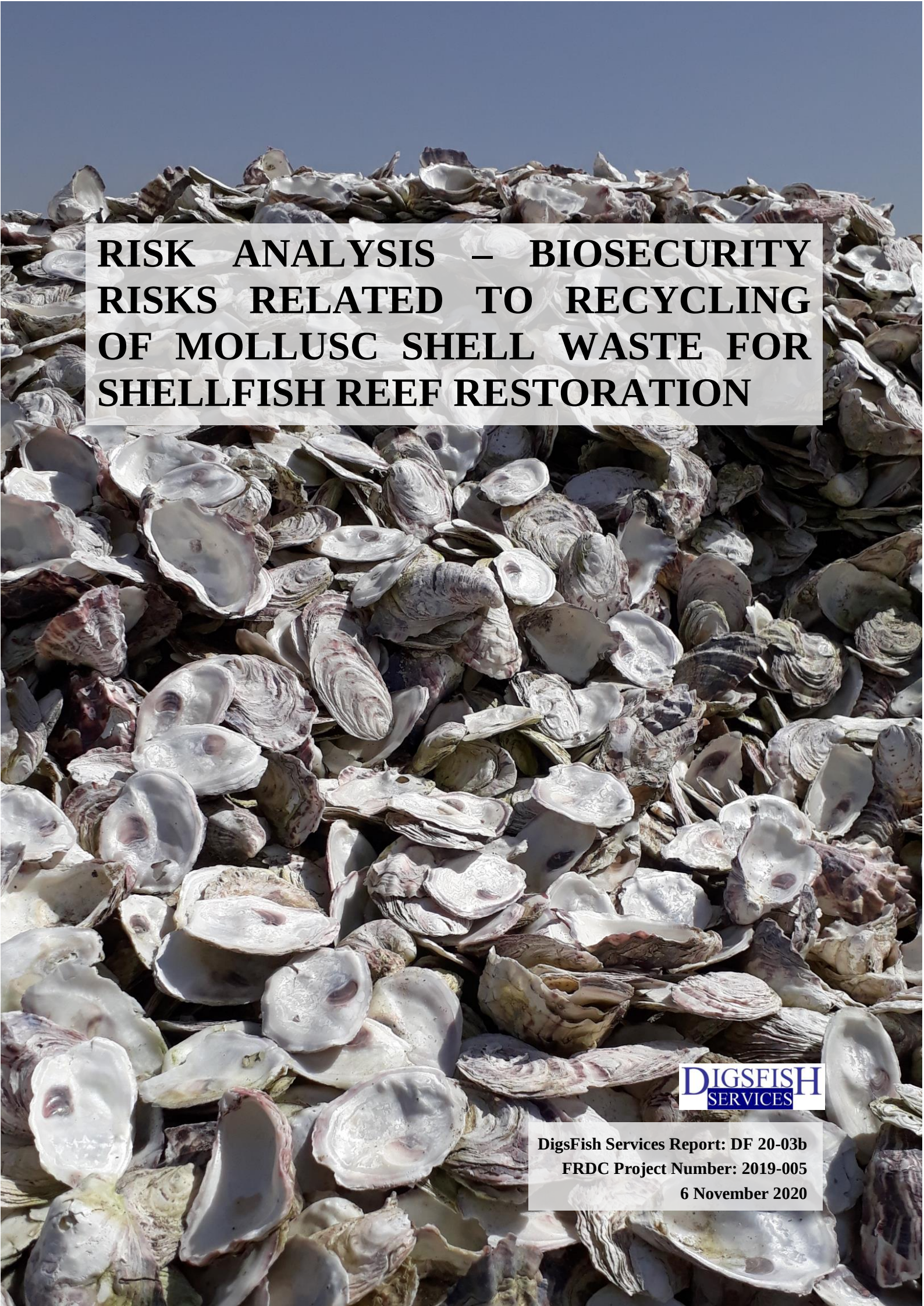

RISK ANALYSIS – BIOSECURITY RISKS RELATED TO RECYCLING OF MOLLUSC SHELL WASTE FOR SHELLFISH REEF RESTORATION

DIGSFISH SERVICES

DigsFish Services Report: DF 20-03b
FRDC Project Number: 2019-005
6 November 2020

RISK ANALYSIS - BIOSECURITY RISKS RELATED TO RECYCLING OF MOLLUSC SHELL WASTE FOR SHELLFISH REEF RESTORATION

Prepared by:

Ben Diggles PhD

DigsFish Services Pty Ltd, Banksia Beach, QLD 4507

Prepared for:

Fisheries Research and Development Corporation FRDC
PO Box 222
Deakin West ACT 2600

Project Number 2019-005

Version Control:

Hazard Identification	1 May 2020
First Draft	19 August 2020
Revised Draft	1 October 2020
Final Report due	6 November 2020

ISBN 978-0-9806995-4-8

Risk Analysis – Biosecurity risks related to recycling of mollusc shell waste for shellfish reef restoration

FRDC Project No: 2019-005

2020

Ownership of Intellectual property rights

This publication (and any information sourced from it) should be attributed to: Diggles BK (2020). *Risk Analysis-Biosecurity risks related to recycling of mollusc shell waste for shellfish reef restoration*. DigsFish Services Report DF20-03b for Fisheries Research and Development Corporation, December 2020. CC BY 3.0

Creative Commons licence

Disclaimer

Researcher Contact Details		FRDC Contact Details	
Name:	Dr. B.K. Diggles	Address:	25 Geils Court
Address:	Banksia Beach QLD, 4507		Deakin ACT 2600
Phone:	07 34088443	Phone:	02 6285 0400
Fax:	07 34088443	Fax:	02 6285 0499
Email:	ben@digsfish.com	Email:	frdc@frdc.com.au
Web:	www.digsfish.com	Web:	www.frdc.com.au

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

Contents

Abbreviations and Acronyms **6**

Non – technical summary **7**

1.0 Introduction **9**

1.1 Commodity description *10*

2.0 Hazard Description **24**

2.1 Elimination of insignificant diseases and marine pests *24*

2.1.1 Viruses 28

2.1.2 Bacteria 29

2.1.3 Protozoa 30

2.1.4 Metazoa 32

2.2 The priority diseases and marine pests of concern to be considered in the RA *32*

3.0 The methodology used for the Risk Analysis **34**

3.1 Release assessment *34*

3.2 Exposure assessment *34*

3.3 Consequence assessment *36*

3.4 Risk estimation *37*

3.5 Risk mitigation *38*

4.0 Risk Assessment **39**

4.1 Infection with AbHV-1 (Abalone viral ganglioneuritis, AVG) *39*

4.2 Iridoviroses of molluscs *44*

4.3 Infection with OsHV-1-µVar (Pacific oyster mortality syndrome, POMS) *48*

4.4 Infection with other malacoherpesviruses *54*

4.5 Infection with Xenohaliotis californiensis *59*

4.6 Infection with Bonamia exitiosa or Bonamia spp. *64*

4.7 Infection with Bonamia ostreae *69*

4.8 Haplosporidosis *73*

4.9 Infection with Haplosporidium nelsoni (MSX disease) *77*

4.10 Infection with Marteilia refringens *81*

4.11 Infection with Marteilia sydneyi (QX disease) *85*

4.12 Infection with Marteilia spp. and Marteilioides spp. *90*

4.13 Infection with Marteilioides chungmuensis *94*

4.14 Infection with Mikrocytos spp. (including M. mackini) *98*

4.15 Infection with Minchinia spp. (including M. occulta) *102*

4.16 Infection with Perkinsus spp. (including P. olseni, P. chesapeaki) *107*

4.17 Infection with Perkinsus marinus *112*

4.18 Akoya oyster disease *117*

4.19 Oyster oedema disease *122*

4.20 Winter mortality (Mikrocytos roughleyi) *124*

4.21 Boring mussels *128*

4.22 Boring sponges *132*

4.23 Invasive barnacles *136*

4.24 Invasive ctenophores (comb jellies) *140*

4.25 Invasive crabs *144*

4.26 Invasive clams *149*

4.27 Invasive mussels *154*

4.28 Invasive oysters *161*

4.29 Invasive polychaetes (including mudworms) *167*

4.30 Invasive seaweeds *173*

4.31 Invasive seastars *179*

4.32 Invasive tunicates/ ascidians/ hydroids *183*

4.33 Invasive whelks/ gastropods/ limpets/ chitons *188*

5.0 Risk Mitigation 193

5.1. Risk Evaluation 193
5.2 Treatment options for risk mitigation 195
5.2.1 Desiccation 196
5.2.2 Heat treatment 197
5.2.3 Freshwater (minimum 24 hours) 197
5.2.4 Acetic acid (4% for 30 minutes) 197
5.3 Summary of suggested risk mitigation methods 198

6.0 Acknowledgements 199

7.0 References 200

Appendix 1. Mollusc shell recycling questionnaire 268

List of Tables

Table 1. Results from questionnaires identifying the bivalve species being recycled in Australia and the relevant sanitary requirements and controlling legislation in various state jurisdictions. Some examples from overseas jurisdictions are also provided for comparative purposes ……. 11

Table 2. The list of diseases and marine pests to be considered during hazard identification ..…… 25

Table 3. The priority list of diseases and marine pests to be considered in the detailed risk analysis ….. 33

Table 4. Nomenclature for the qualitative likelihood estimations used in this RA……. 34

Table 5. Matrix of rules for combining descriptive likelihoods for the release and exposure assessments to arrive at a likelihood of establishment and spread……. 35

Table 6. Definition of terms used to describe consequences of establishment of unwanted disease agents ……. 36

Table 7. Risk estimation matrix showing the ALOP utilized for this RA …….. 38

Table 8. Summary table for risk estimate outcomes from the risk assessment …….. 193

Table 9. Unmitigated risk estimates for pest species of concern which can also act as vectors of disease 194

Table 10. Revised risk estimates for pest species of concern which can also act as vectors of disease ….. 195

Abbreviations and Acronyms

AbHV-1	Abalone Herpesvirus-1 (also known as Haliotid Herpesvirus-1, HaHV-1), a member of the Family Malacoherpesviridae that infects abalone
ALOP	Acceptable (or Appropriate) level of protection
APX	Unknown apicomplexan parasite of New Zealand bluff oysters
AVG	Abalone viral ganglioneuritis (also known as infection with AbHV-1)
AVNV	Acute viral necrosis virus of scallops (a strain of Ostreid herpesvirus 1 OsHV-1)
cPCR	Classical (or conventional) PCR
CRC	Co-operative research centres
Dermo	Dermo disease (also known as infection with *Perkinsus marinus*)
DNA	Deoxyribonucleic acid
GNV	Gill necrosis virus
HIV	Haemocyte infection virus
ISH	*In-situ* hybridisation
JOD	Juvenile disease of eastern oysters (also known as infection by *Roseovarius crassostreae*)
kb	Kilobase = 1000 base pairs of DNA
mJ/cm^2	Millijoules per square centimetre, a measure of UV dose rate. $1\ mj/cm^2 = \frac{\text{UV intensity } (\mu W/cm^2) \times \text{duration of exposure (sec)}}{1000}$
MSX	Multinucleate sphere unknown (also known as infection with *Haplosporidium nelsoni*)
NACA	Network of Aquaculture Centres in Asia-Pacific
1 nm	1 nanometre, = 1 billionth of a metre
1 µm	1 micron, = 1000 nanometres = 1 millionth of a metrer
1 mm	1 millimetre, = 1000 microns = 1 thousandth of a metre
NSW	New South Wales
NA	Northern Australia
NT	Northern Territory
OIE	Office International des Epizooties, the world organisation for animal health
OOD	Oyster oedema disease
ORF	Open reading frame
OsHV-1	Ostreid Herpesvirus-1, a member of the Family Malacoherpesviridae that infects bivalve molluscs (GenBank # AY509253)
OsHV-1 var	A genetic variant of Ostreid Herpesvirus-1 defined by a nucleotide deletion of 2.8 kb in an inverted repeat region compared to the reference genome, a deletion of 200 bp and an insertion of 27 bp in the C region encoding two unknown proteins.
OsHV-1 µVar	A genetic microvariant of Ostreid Herpesvirus-1 defined on the basis of partial sequence data exhibiting a systematic deletion of 13 bp in a microsatellite zone of the ORF 4 of the genome.
OsHV-1-SB	A variant of OsHV-1 with 97.3% similarity to AVNV and 95.2% similarity to the OsHV-1 reference strain, first detected in cultured ark shells (*Scapharca broughtonii*) in China in 2012.
OVV	Oyster velar virus, which causes oyster velar virus disease (OVVD)
PCR	Polymerase chain reaction
POMS	Pacific oyster mortality syndrome, caused by infection with OsHV-1 µVar
qPCR	Quantitative PCR, also known as real time PCR
QLD	Queensland
QPX	Quahog parasite unknown
QX	Queensland unknown (also known as infection with *Marteilia sydneyi*)
RA	Risk Analysis
RFTM	Rays Fluid Thioglycollate Medium
SA	South Australia
SRO	Sydney rock oysters
SSO	Sea side organism (also known as infection with *Haplosporidium costale*)
Tas	Tasmania
TEM	Transmission electron microscopy
TNC	The Nature Conservancy
TRO	Tropical rock oysters
Vic	Victoria
WA	Western Australia
UV	Ultraviolet light, a form of electromagnetic radiation with a wavelength <400nm, which has germicidal properties by inflicting damage to DNA of microorganisms most effectively at c. 254 nm wavelength

Non – technical summary

Restoration of lost shellfish reefs in Australian estuaries is gaining momentum nationwide, with several projects being undertaken in Victoria, SA, NSW, WA and QLD. Most of these projects have utilised recycled bivalve mollusc shell waste (oysters, mussels, scallops) generated by shellfish farmers, processors and retailers which is used as clean spat settlement substrate that is replaced into the water to regenerate intertidal and subtidal shellfish reefs. One barrier to wider use of recycled mollusc shells for shellfish reef restoration is their translocation and reuse underwater poses risks of transfer and spread of disease agents and marine pests of concern. A responsible approach towards restoration of shellfish reefs should aim to only stock restored reefs with shellfish of higher or equal health status to that of shellfish already living in the receiving environment. Prior to this project there had been no thorough analysis of the biosecurity risks involved or best practice biosecurity principles that should be applied to protect Australia's environment, shellfish fisheries and aquaculture industries from diseases or marine pests which may occur in recycled shell waste used for shellfish reef restoration. This risk analysis identified the existing risk mitigation methods being used in Australia to reduce disease translocation risk from recycled mollusc shells, examined the known potential biosecurity hazards (diseases and marine pests) that could be translocated through recycling of mollusc shells, and determined the sanitising methods required to reduce the risk of translocation of each disease and marine pest of concern to an acceptable level.

The initial hazard identification stage identified over 32 disease groups and 64 marine pests of potential concern, including 6 viral diseases, 4 bacterial diseases, 16 protozoan diseases, 3 metazoan diseases and 3 diseases of unknown aetiology, plus invasive pests including 11 species of crabs, 7 species of clams, 7 species of seaweeds, 6 species of mussels, 4 species of oysters, 4 species of barnacles, 4 species of gastropods, 4 species of flatworms, 4 species of tunicates/ascidians/hydroids, 4 species of polychaetes, 2 genera of mudworms, and various species of boring sponges, comb jellies, seastars, boring mussels and pea crabs. When insignificant diseases within the appropriate level of protection (ALOP) were eliminated, this left 20 diseases of concern and 14 groups of marine pests upon which detailed risk assessment was undertaken. The risk assessment process involved release assessment, exposure assessment, and consequence assessment, to arrive at an unmitigated risk estimation as well as risk estimates for a range of shell treatment pathways, including 3, 4 or 6 months desiccation in sunlight, exposure to hot water at 55°C (10 minutes) or 80°C (5 minutes), bathing in freshwater for >24 hours or in vinegar (4% acetic acid) for 30 minutes. The effectiveness of the various treatments as risk management options were then compared, in order to inform development of protocols for treatment of various types of mollusc shells. The ALOP that was adopted in this risk assessment (RA) was expressed in qualitative terms as "**very low**", representing an annual probability between 1 in 20 and 1 in 100 years.

The outcomes from the RA process are summarized in the table below. The assessment indicated that heating recycled mollusc shells in water to 80°C for at least 5 minutes would meet the ALOP for all diseases (despite uncertainly for some disease agents due to lack of information, as indicated by ?), and was within the ALOP for all pests of concern. This method would have limited throughput, however, and thus may be useful only for experimental or pilot scale restoration projects. Desiccation of mollusc shells in air for a minimum of 4 months in sunlight at >20°C was also within the ALOP for all of the pests and disease agents of concern. However, if ambient weather conditions at the recycling facility do not allow the shell pile to dry out or exceed 20°C for several months of the year (such as in temperate parts of Australia), extending the

desiccation period to 6 months with >1 shell turnover is recommended to provide sufficient safety margin to ensure negligible risk (annual probability of occurrence less frequent than 1 in 100 years). Desiccation of mollusc shells can be undertaken at vast scales (e.g. 1000's of tonnes of shell per annum, see Table 1), at minimal cost, and thus appears suitable for shellfish restoration at environmentally meaningful scales.

Summary table for risk estimate outcomes from the risk assessment.

	Unmitigated	Treatment						
Disease agents	No treatment	Desiccation at >20°C			Heat		Salinity	Other
		3 months	4 months	6 months	55°C >10 min	80°C >5 min	0 ppt >24 hrs	4% acetic acid 30 min
Viruses								
AbHV-1 (Abalone viral ganglioneuritis)	H	VL?	N	N	VL?	N	H?	M?
Iridoviroses of molluscs	L	VL	VL	N	VL	N	L	VL
Infection with OsHV1-µVar (POMS)	H	VL	N	N	N	N	H	L
Infection with malacoherpesviruses	M	N	N	N	N	N	M	VL
Bacteria								
Infection with *Xenohaliotis californiensis*	L	VL	N	N	VL?	VL?	L	L?
Protozoa								
Bonamia exitiosa and *Bonamia* spp.	H	N	N	N	M?	VL?	M	L?
Infection with *Bonamia ostreae*	H	N	N	N	M?	VL?	M	L?
Haplosporidosis	L	N?	N	N	VL?	N?	VL?	VL?
Infection with *Haplosporidium nelsoni* (MSX disease)	L	N?	N	N	VL?	N?	VL	VL?
Infection with *Marteilia refringens*	M	VL?	N	N	L?	VL?	L?	L?
Marteilia sydneyi (QX disease)	L	N?	N	N	VL?	N?	VL?	VL?
Marteilia spp. and *Marteilioides* spp.	VL	N?	N	N	N?	N?	N?	N?
Infection with *Marteilioides chungmuensis*	L	N?	N	N	VL?	N?	VL?	VL?
Infection with *Mikrocytos* spp. (including *M. mackini*)	H	N?	N	N	M?	VL?	M?	L?
Minchinia occulta, *Minchinia* spp.	M	VL?	N	N	L?	VL?	L?	L?
Infection with *Perkinsus olseni*, *P. chesapeaki*, *Perkinsus* spp.	H	L	VL	N	VL	VL?	N	L?
Infection with *Perkinsus marinus*	H	L	VL	N	M	VL?	N	L?
Unknown aetiology								
Akoya oyster disease	L	VL?	N	N	L	VL?	L?	VL?
Oyster oedema disease	N	N	N	N	N	N	N	N
Winter mortality (*M. roughleyi*)	M	N?	N	N	L?	VL?	L?	VL?
Invasive marine pests								
Boring mussels	N	N	N	N	N	N	N	N
Boring sponges	N	N?	N	N	N?	N?	N?	N
Invasive barnacles	L	N	N	N	N	N	L	N
Invasive cnidarians/comb jellies	VL	N	N	N	VL?	N	VL	VL?
Invasive crabs*	H	M	N	N	L	N	H	M?
Invasive clams*	H	N	N	N	N	N	H	L
Invasive mussels*	E	N	N	N	N	N	E	M
Invasive oysters*	E	N	N	N	E	N	E	M
Invasive polychaetes*	L	N	N	N	N	N	L	N
Invasive seaweeds	H	L	N	N	N	N	H	N
Invasive seastars	H	N	N	N	VL?	N	N	VL?
Invasive tunicates/ ascidians/ hydroids*	E	N	N	N	L	N	M	L
Invasive whelks/ gastropods/ limpets*	H	N	N	N	M?	N?	H?	H?

N = Negligible risk, VL = very low risk (= ALOP), L = low risk, M = moderate risk, H = high risk, E = extreme risk, ? = uncertainty due to lack of information, * = combined pest + disease risk.

1.0 Introduction

Shellfish reef ecosystems comprised of at least 14 different oyster and mussel species occur in estuarine and coastal areas of sub-tropical and temperate Australia (Gillies et al. 2018, 2020). Globally, at least 85% of shellfish reefs are now considered functionally extinct (Beck et al. 2011), and in Australia there is evidence that reefs formed primarily by *Ostrea angasi* (Australian flat oyster) and *Saccostrea glomerata* (Sydney rock oyster) have undergone massive declines from their pre-European historical distributions (Kirby, 2004, Ogburn et al. 2007, Diggles 2013, Alleway and Connell 2015, Gillies et al. 2015a, 2015b, 2018, 2020, Ford and Hamer 2016, Thurstan et al. 2020). For example, historical records show that Sydney rock oysters were massively abundant in both intertidal and subtidal reefs in water depths up to 15 metres deep in estuaries along Australia's east coast until the early 20th century (Roughley 1939, Ogburn et al. 2007, Diggles 2013, Thurston et al. 2020), dominating these systems ecologically. Shellfish reefs were also important cultural resources for indigenous Australians for thousands of years, as evidenced by middens, but the vast intertidal and subtidal reefs were heavily exploited by early European settlers in the 19th century to make cement for building and as food (Gilles et al. 2015b). This exploitation was soon followed by massive environmental changes to east coast estuaries from sedimentation and eutrophication due to catchment clearing and development, resulting in collapse of subtidal oyster populations due mainly to recruitment failure (Diggles 2013, Thurston et al. 2020), resulting in retraction of the oyster farming industry into intertidal areas (Smith 1985, Nell 2001).

Bivalve molluscs are important ecosystem engineers which enhance fisheries by generating food and habitat for fish and crustaceans, improve water quality via filtration which reduces turbidity and improves seagrass growth, improve uptake of nitrogen, phosphorous and carbon, and protect shorelines (Newell 2004, Grabowski and Peterson 2007, Beck et al. 2011). Loss of extensive three dimensional subtidal shellfish reefs throughout Australia's estuaries and inshore regions has likely resulted in significant declines in subtidal fish nursery surface area and thus fisheries productivity (Diggles 2013, Creighton 2013, McLeod et al. 2019). The need to protect shellfish reefs as vital fisheries nursery habitats in Australian estuaries has been recognised (Creighton 2013), with their restoration being a key component of rehabilitation of depleted inshore fisheries (Beck et al. 2011, Diggles 2013, Alleway and Connell 2015, Gilles et al. 2015a, 2020, Ford and Hamer 2016). A recent assessment of the conservation status of these ecosystems against International Union for Conservation of Nature (IUCN) criteria found them to be classified as Critically Endangered in Australia, suggesting an urgent need for intervention to protect remaining reefs and undertake restoration at suitable sites (Gilles et al. 2020).

Attempts to restore lost shellfish reefs in Australian estuaries and inshore waters have lagged behind those in the USA by around 30 years, but recently they have begun to gain momentum with several restoration projects being undertaken in Vic, SA, NSW, WA and QLD (Gilles et al. 2020). Most of these projects have utilised recycled bivalve mollusc shell waste (oysters, mussels, scallops) generated by shellfish processors and retailers (Diggles 2018, Branigan et al. 2020). The mollusc shell waste is recycled to generate supplies of clean spat settlement substrate that is replaced into the water at appropriate times of year to attract spatfall recruitment and regenerate intertidal and subtidal shellfish reefs. One barrier to wider use of recycled mollusc shells for shellfish reef restoration is the fact that their translocation and reintroduction into the water poses significant risks of spread of disease agents and marine pests of concern (Hine 1996b, Burreson et al. 2000, AFFA 2002, Bushek et al. 2004, Forrest and Blakemore 2006, Mineur et al. 2007, Cohen and Zabin 2009, Ojaveer et al. 2018, zu Ermgassen et al. 2020). Besides microorganisms (viruses, bacteria, protozoa) in bivalve tissue, the marine organisms most likely to survive in recycled mollusc shells are intertidal and supralittoral

organisms that tolerate extended periods out of the water (Cohen and Zabin 2009). A responsible approach towards restoration of shellfish reefs should aim to stock restored reefs with shells of higher/equal biosecurity status to the shellfish already living in the receiving environment (Bushek et al. 2004, NOAA 2013, Jeffs et al. 2019). It is important, therefore, to ensure restoration activities that involve enhancement of wild shellfish reefs with recycled mollusc shells are undertaken with due consideration of biosecurity legislation while respecting best practice biosecurity principles, in order to protect Australia's environment, existing fisheries and aquaculture industries by preventing introduction of unwanted pests and diseases of concern.

This pathogen and pest risk analysis (RA) was undertaken to identify known marine pests and diseases of commercially available molluscs sold in Australia, and assess the risk of translocating these into new locations through shell recycling initiatives developed for shellfish reef restoration projects. The outcomes of this RA may help develop recommendations for biosecurity management frameworks and operating procedures to effectively mitigate the risks of translocating pests and diseases during shellfish reef restoration projects. In developing this RA, an extensive literature review was undertaken to identify scientific and other literature relating to marine pests and diseases associated with oysters (*Saccostrea* spp., *Crassostrea* spp., *Ostrea* spp., *Dendostrea* spp., *Pinctada* spp.), scallops, mussels and other commercially important molluscs in Australia. Some overview documents in these fields include Wolf (1967, 1972, 1977, 1979), Wolf and Sprague 1978, Goggin et al. (1989, 1990), Norton et al. (1993a, 1993b), Humphrey et al. (1998), Hewitt et al. (1999), Hine and Thorne (2000), AFFA (2002), Bushek et al. (2004), Heasman et al. (2004), Hayes et al. (2005), Humphrey and Norton (2005), Forrest and Blakemore (2006), Jones (2007a, 2007b), Summerson et al. (2007), Bearham (2008), Coen and Zabin (2009), Jones et al. (2010), Murphy and Paini (2010), NOAA (2013), Hopkins et al. (2016), Diggles (2017), Bollen et al. (2017), Ojaveer et al. (2018) and Bannister et al. (2019), as well as the various State and Federal government notifiable pest and disease lists (DAWR 2015a, 2019). These potential hazards were then examined within a standardised qualitative risk assessment framework to assess the risks of translocating them via mollusc shell recycling initiatives, so that appropriate biosecurity management arrangements could be identified. Risk analysis includes several steps including hazard identification followed by risk assessment (Diggles and Arthur 2010, OIE 2019). The findings of this RA were based on qualitative assessments of the risks involved with introduction (release), establishment (exposure) and potential spread (consequences) of each hazard through the shell recycling pathway using internationally recognised risk analysis methodologies (Diggles and Arthur 2010, OIE 2019, Diggles 2011a, 2017, 2020a).

1.1 Commodity description

A questionnaire was developed for shellfish reef practitioners to identify the various species of molluscs that are being recycled in Australia for the purposes of shellfish reef restoration, and determine what legislation, if any, was currently being used to oversee the process. The questionnaire (Appendix 1) was to be distributed at the 20th International Conference on Shellfish Restoration at Port Stephens on the 17-20 March 2020. However, the conference was cancelled due to the COVID-19 pandemic, and instead the questionnaire was sent by the author and conference organisers to a selection of international conference participants, as well as each of the project co-investigators including The Nature Conservancy (TNC) and various Australian State and Territory aquatic biosecurity representatives. The questionnaire results were used to compile a list of mollusc species that are being recycled in Australia as well as lists of disease and marine pest hazards covered by legislation within various Australian state jurisdictions. Furthermore, responses from several overseas jurisdictions were also compiled for comparative purposes. These data are summarised in Table 1.

Table 1. Results from questionnaires identifying the mollusc species being recycled in Australia and the relevant sanitary requirements and controlling legislation in various State and Territory jurisdictions. Some examples from overseas jurisdictions are also provided for comparative purposes.

Jurisdiction	Mollusc species permitted for recycling	Diseases and marine pests requiring mitigation P = already present in jurisdiction.	Sanitary requirements applied to shell	Relevant legislation/ Acts controlling recycling	Other comments
Australia					
Queensland	"Oyster shells" (*Saccostrea* spp. *Crassostrea* spp., *Pinctada* spp.) (nb. non-oyster species e.g. mussels, scallops, clams etc. not specifically permitted for recycling)	**Diseases** Abalone viral ganglioneuritis Akoya oyster disease Infection with *Bonamia exitiosa* Infection with *Bonamia ostreae* Infection with *Bonamia* species Infection with *Marteilia refringens* Infection with *Marteilia sydneyi* (P) Infection with *Marteilioides chungmuensis* Infection with *Mikrocytos mackini* Infection with ostreid herpesvirus-1 (OsHV-1) Infection with *Perkinsus marinus* Infection with *Perkinsus olseni* (P) Infection with *Xenohalitotis californiensis* Iridoviroses of molluscs Oyster oedema disease **Marine Pests** American slipper limpet *Crepidula fornicata* Aquarium Caulerpa *Caulerpa taxifolia* (P) Asian bag mussel *Arcuatula senhousia* Asian basket clam *Corbula amurensis* Asian green mussel *Perna viridis* Asian paddle crab *Charybdis japonica* Asian rapa whelk *Rapana venosa* Asian seaweed *Grateloupia turuturu* Asian shore crab *Hemigrapsus sanguineus* Bay barnacle *Balanus improvisus*	1. Oyster shells sourced only from oyster processors or restaurants that are not subject to quarantine restrictions under the Biosecurity Security Act 2014 and its sub-ordinate legislations 2. Cleaned and sterilized using high pressure hose to remove all soft tissue, AND 3. drying in direct sunlight >18°C for at least 4 months OR Boiling in water for >5 min followed by immersion in water >55°C for >10 min	Waste Reduction and Recycling Act 2011 End of Waste Code ENEW07278317 (4 June 2018)	Other acts will apply when replacing shells into the water, e.g. Marine Parks Act, Fisheries Act Steve Wesche, Biosecurity QLD, pers. comm. 4 March 2020.

Jurisdiction	Mollusc species permitted for recycling	Diseases and marine pests requiring mitigation P = already present in jurisdiction.	Sanitary requirements applied to shell	Relevant legislation/ Acts controlling recycling	Other comments
Queensland (con't)		Black striped mussel *Mytilopsis sallei* Brown mussel *Perna perna* Brush-clawed shore crab *Hemigrapsus takanoi, H. penicillatus* Chinese mitten crab *Eriocheir sinensis* Colonial seasquirt *Didemnum* spp. (exotic species) Comb jelly *Mnemiopsis leidyi* European clam *Varicorbula gibba* European fan worm *Sabella spallanzanii* European shore crab *Carcinus maenas* Green macroalga *Codium fragile* ssp. *tomentosoides* Jack-knife clam *Ensis directus* Japanese kelp *Undaria pinnatifida* New Zealand screw shell *Maoricolpus roseus* Northern Pacific seastar *Asterias amurensis* Pacific oyster (*Crassostrea gigas*) Red gilled mudworm *Marenzelleria* spp. Soft shell/long necked clam *Mya arenaria* Strangle weed *Sargassum muticum*			
New South Wales	*Saccostrea glomerata*, *Crassostrea gigas* *Ostrea angasi*	**Diseases** Abalone viral ganglioneuritis Infection with *Bonamia exitiosa* (P) Infection with *Bonamia ostreae* Infection with *Bonamia* species Infection with *Marteilia refringens* Infection with *Marteilia sydneyi* (P) Infection with *Marteilioides chungmuensis* Infection with *Mikrocytos mackini* Infection with *Mikrocytos* (*Bonamia*) *roughleyi* (P) Infection with *Perkinsus marinus* Infection with *Perkinsus olseni* (P)	1. Oyster shells to be heated in water at a minimum of 80°C for a minimum of 5 minutes. This duration of heating is to be timed from the point at which the water bath returns to a temperature of 80°C and not from the time of first entry of the oyster shells into the water bath. Dry heat treatment is not permitted. No whole	Biosecurity Act 2015, Schedule 2, Prohibited Matter, Part 4 and Schedule 2 Biosecurity Regulation 2017, Part 2, Division 5; Part 3; and Schedule 1, Part 2 Biosecurity Order (Permitted Activities)	Other acts will apply when replacing shells into the water, e.g. Marine Parks Act, NSW EPA, Crown Lands Act, Fisheries Management Act, etc. Simon Rowe, Oceanwatch, pers. comm. to Simon Branigan, TNC

Jurisdiction	Mollusc species permitted for recycling	Diseases and marine pests requiring mitigation P = already present in jurisdiction.	Sanitary requirements applied to shell	Relevant legislation/ Acts controlling recycling	Other comments
New South Wales (con't)		Infection with *Xenohalitotis californiensis* Iridoviroses Ostreid herpesvirus-1 µVariant (OsHV-1 µVar) (P) **Marine Pests** American slipper limpet *Crepidula fornicata* Aquarium Caulerpa *Caulerpa taxifolia* (P) Asian bag mussel *Arcuatula senhousia* Asian basket clam *Corbula amurensis* Asian green mussel *Perna viridis* Asian paddle crab *Charybdis japonica* Asian rapa whelk *Rapana venosa* Asian seaweed *Grateloupia turuturu* Asian shore crab *Hemigrapsus sanguineus* Bay barnacle *Balanus improvisus* Black striped mussel *Mytilopsis sallei* Brown mussel *Perna perna* Brush-clawed shore crab *Hemigrapsus takanoi*, *H. penicillatus* Chinese mitten crab *Eriocheir sinensis* Colonial seasquirt *Didemnum vexillum* Comb jelly *Mnemiopsis leidyi* European clam *Varicorbula gibba* European fan worm *Sabella spallanzanii* (P) European shore crab *Carcinus maenas* (P) Jack-knife clam *Ensis directus* Japanese kelp *Undaria pinnatifida* New Zealand chiton *Chiton glaucus* New Zealand green lip mussel *Perna canaliculus* New Zealand half crab *Petrolisthes elongatus* New Zealand screw shell *Maoricolpus roseus* (P) Northern Pacific seastar *Asterias amurensis*	oysters are to be included; any meat left must only be residual tissue. 2. Following heat treatment, all treated shells must be placed in a clean bag away from untreated oysters and equipment.	2019, Part 3 Biosecurity (Pacific Oyster Mortality Syndrome) Control Order (No. 2)	Australia, 12 Dec 2019, and Ben Rampano, NSW DPI, pers. comm. 22 Apr 2020.

Jurisdiction	Mollusc species permitted for recycling	Diseases and marine pests requiring mitigation P = already present in jurisdiction.	Sanitary requirements applied to shell	Relevant legislation/ Acts controlling recycling	Other comments
New South Wales (con't)		Soft shell/long necked clam *Mya arenaria*, *Mya japonica* Strangle weed *Sargassum muticum*			
Victoria	Oysters (*Saccostrea glomerata*, *Crassostrea gigas Ostrea angasi*), mussels (*Mytilus edulis*), and scallops (*Pecten fumatus*, *Mimachlamys asperrima*) and all other domestic oyster, mussel and scallop shells.	**Diseases** Abalone viral ganglioneuritis (P) Infection with *Bonamia exitiosa* (P) Infection with *Bonamia ostreae* Infection with *Bonamia* species Infection with *Marteilia refringens* Infection with *Marteilia sydneyi* Infection with *Marteilioides chungmuensis* Infection with *Mikrocytos mackini* Infection with *Perkinsus marinus* Infection with *Perkinsus olseni* (P) Infection with *Xenohalitotis californiensis* Iridoviroses Ostreid herpesvirus-1 µVariant (OsHV-1 µVar) **Marine Pests** Aquarium Caulerpa *Caulerpa taxifolia* Asian bag mussel *Arcuatula senhousia* (P) Asian green mussel *Perna viridis* European clam *Varicorbula gibba* (P) European fan worm *Sabella spallanzanii* (P) European shore crab *Carcinus maenas* (P) Japanese kelp *Undaria pinnatifida* (P) New Zealand screw shell *Maoricolpus roseus* (P) Northern Pacific seastar *Asterias amurensis* (P) Mudworm (*Boccardia* spp., *Polydora* spp.) (P)	1. Shells washed by restaurants before pick-up to remove organic matter 2. Shells quarantined and cured outdoors for at least 6 months to allow the elements including rain, sun and bacterial processes to break down any remaining tissue and potential disease organisms. 3. Shells to be piled to allow for greater exposure per shell (i.e. minimize height of piles) to render pathogen free. 4. Shells to be turned every 2 to 3 months.	1. Marine & Coastal Act 2018 (as part of the permit application for use and development of coastal crown land section 68 (1) with DELWP) 2. Port Management Act 1995, Regulation 17 of the Port Management (Local Ports Regulation 2015 (as part of the Works Permit application with Parks Victoria).	Simon Branigan, TNC Australia, pers. comm. 14 Apr 2020.

Jurisdiction	Mollusc species permitted for recycling	Diseases and marine pests requiring mitigation P = already present in jurisdiction.	Sanitary requirements applied to shell	Relevant legislation/ Acts controlling recycling	Other comments
South Australia	Oysters (*Crassostrea gigas*)	**Diseases** Abalone viral ganglioneuritis Haplosporidosis Infection with *Bonamia exitiosa* (P) Infection with *Bonamia ostreae* Infection with *Bonamia* species Infection with *Marteilia refringens* Infection with *Marteilia sydneyi* Infection with *Marteilioides chungmuensis* Infection with *Mikrocytos mackini* Infection with *Perkinsus marinus* Infection with *Perkinsus olseni* (P) Infection with *Xenohalitotis californiensis* Iridoviroses Ostreid herpesvirus-1 µVariant (OsHV-1 µVar) (P) **Marine Pests** American slipper limpet *Crepidula fornicata* Aquarium Caulerpa *Caulerpa taxifolia* (P) Asian bag mussel *Arcuatula senhousia* (P) Asian basket clam *Corbula amurensis* Asian green mussel *Perna viridis* Asian paddle crab *Charybdis japonica* Asian rapa whelk *Rapana venosa* Asian seaweed *Grateloupia turuturu* Asian shore crab *Hemigrapsus sanguineus* Bay barnacle *Balanus improvisus* Black striped mussel *Mytilopsis sallei* Brown mussel *Perna perna* Brush-clawed shore crab *Hemigrapsus takanoi*, *H. penicillatus* Chinese mitten crab *Eriocheir sinensis*	1. Shells to be locally sourced only from South Australian oyster farmers. Currently shells from seafood processors or restaurants have not been allowed to be used. 2. Shells quarantined and cured outdoors for at least 6 months to allow the elements including rain, sun and bacterial processes to break down any remaining tissue and potential disease organisms. 3. Shells need to be cleaned using a high pressure hose to remove all sediment and soft tissue.	1. Planning, Development and Infrastructure Act 2016-SECTION 49 & 49A – crown development application required which includes an ecological sustainable risk assessment which covers biosecurity risks. EPA is the referral agency which reviews this application. 2. The EPA regulates the waste and resource recovery using the powers and tools established under the Environment Protection Act 1993 (the Act), including the Environment Protection Regulations 2009 and the Environment Protection (Waste to Resources) Policy 2010 (Waste to Resources EPP). In addition, the EPA has	Not all restoration projects require a development application. For smaller community projects, the SA government has requested the shell provider fills out a statutory declaration stating that the sanitary requirements have been applied to the shell. Anita Nedosyko, TNC Australia, pers. comm. 20 April 2020. The Livestock Act 1997 provides an instrument to manage the disease risk, while depositing aquaculture species into state waters is regulated by the Fisheries Management Act 2007. Shane Roberts, PIRSA, pers. comm. 17 Sept 2020.

Jurisdiction	Mollusc species permitted for recycling	Diseases and marine pests requiring mitigation P = already present in jurisdiction.	Sanitary requirements applied to shell	Relevant legislation/ Acts controlling recycling	Other comments
South Australia (con't)		Colonial seasquirt *Didemnum* (exotic species) Comb jelly *Mnemiopsis leidyi* European clam *Varicorbula gibba* European fan worm *Sabella spallanzanii* (P) European sea squirt *Ciona intestinalis* European shore crab *Carcinus maenas* (P) Green macroalga *Codium fragile* ssp. *tomentosoides* Jack-knife clam *Ensis directus* Japanese kelp *Undaria pinnatifida* Mudworms (*Boccardia knoxi*) New Zealand screw shell *Maoricolpus roseus* Northern Pacific seastar *Asterias amurensis* Red gilled mudworm *Marenzelleria* spp. Soft shell/long necked clam *Mya arenaria*		specific powers in relation to conditions of approval for activities that require approval under the Planning, Development and Infrastructure Act 2016.	
Tasmania	All mollusc species (including abalone).	**Diseases** Abalone viral ganglioneuritis (P) Infection with *Bonamia exitiosa* (P) Infection with *Bonamia ostreae* Infection with *Bonamia* species Infection with *Haplosporidium nelsoni* Infection with *Marteilia refringens* Infection with *Marteilia sydneyi* Infection with *Marteilioides chungmuensis* Infection with *Mikrocytos mackini* Infection with *Mikrocytos* (*Bonamia*) *roughleyi* Infection with *Perkinsus marinus* Infection with *Perkinsus olseni* Infection with *Xenohalitotis californiensis* Iridoviroses Nocardiosis of shellfish Ostreid herpesvirus-1 µVariant (OsHV-1 µVar) (P)	To be announced	Animal Health Act 1995, progressively superseded by the Biosecurity Act 2019. – includes marine pests.	Classified as Biosecurity risk material. Other acts will apply when replacing shells into the water, e.g. EPA, Water and Marine Resources. Kevin de Witte, DPIPWE, pers. comm. 20 March 2020.

Jurisdiction	Mollusc species permitted for recycling	Diseases and marine pests requiring mitigation P = already present in jurisdiction.	Sanitary requirements applied to shell	Relevant legislation/ Acts controlling recycling	Other comments
Tasmania (con't)		**Marine Pests** American slipper limpet *Crepidula fornicata* Aquarium Caulerpa *Caulerpa taxifolia* (P) Asian bag mussel *Arcuatula senhousia* (P) Asian green mussel *Perna viridis* Asian basket clam *Corbula amurensis* Black striped mussel *Mytilopsis sallei* Chinese mitten crab *Eriocheir sinensis* Colonial seasquirt *Didemnum vexillum* European clam *Varicorbula gibba* (P) European fan worm *Sabella spallanzanii* (P) European sea squirt *Ciona intestinalis* European shore crab *Carcinus maenas* (P) Japanese kelp *Undaria pinnatifida* (P) New Zealand green lip mussel *Perna canaliculus* New Zealand screw shell *Maoricolpus roseus* (P) Northern Pacific seastar *Asterias amurensis* (P) Soft shell/long necked clam *Mya japonica* (P)			
Western Australia	All mollusc species (excluding exotic species)	**Diseases** Abalone viral ganglioneuritis Acute viral necrosis in scallops (AVNV) Akoya oyster disease Haplosporidosis Infection with *Bonamia exitiosa* (P) Infection with *Bonamia ostreae* Infection with *Bonamia* species Infection with *Marteilia refringens* Infection with *Marteilia sydneyi* (P) Infection with *Mikrocytos mackini* Infection with *Mikrocytos* (*Bonamia*) *roughleyi*	1. Shells to be locally sourced (within the same bioregion). 2. Shells must be dried under direct sunlight for a minimum of 6 months. DPIRD makes assessments on a case-by-case basis with consideration of the biosecurity and other risks associated with oyster shell		Projects that meet the definition of habitat enhancement also required to meet the published policy "Policy on habitat enhancement structures in Western Australia" and be approved by WA DPIRD. Approvals/requirements from a number of other

Jurisdiction	Mollusc species permitted for recycling	Diseases and marine pests requiring mitigation P = already present in jurisdiction.	Sanitary requirements applied to shell	Relevant legislation/ Acts controlling recycling	Other comments
Western Australia (con't)		Infection with *Perkinsus marinus* Infection with *Perkinsus olseni* (P) Infection with *Xenohalitotis californiensis* Iridoviroses Oyster oedema disease (P) Ostreid herpesvirus-1 µVariant (OsHV-1 µVar) **Marine Pests** Acorn barnacle *Balanus glandula* American oyster *Crassostrea virginica* American slipper limpet *Crepidula fornicata* Aquarium Caulerpa *Caulerpa taxifolia* Asian bag mussel *Arcuatula senhousia* (P) Asian basket clam *Corbula amurensis* Asian green mussel *Perna viridis* Asian oyster *Crassostrea ariakensis* Asian paddle crab *Charybdis japonica* Asian rapa whelk *Rapana venosa* Asian seaweed *Grateloupia turuturu* Asian shore crab *Hemigrapsus sanguineus* Bay barnacle *Balanus improvisus*, *B. eburneus* Black striped mussel *Mytilopsis sallei* Blue crab *Callinectes sapidus* Boring sponge *Cliona thoosina* Brown macroalga *Fucus evanescens* Brown mussel *Perna perna* Brush-clawed shore crab *Hemigrapsus takanoi*, *H. penicillatus* Caribbean barnacle *Chthamalus proteus* Chinese mitten crab *Eriocheir sinensis* Colonial seasquirt *Didemnum* (exotic species) Comb jelly *Beroe ovata*	recycling.		agencies are likely to apply depending on the situation, such as the WA Department of Water and Environmental Regulation, Department of Transport (Marine Works), and Department of Biodiversity, Conservation and Attractions. Katie Webb, DPIRD, pers. comm. 20 April 2020

Jurisdiction	Mollusc species permitted for recycling	Diseases and marine pests requiring mitigation P = already present in jurisdiction.	Sanitary requirements applied to shell	Relevant legislation/ Acts controlling recycling	Other comments
Western Australia (con't)		Comb jelly *Mnemiopsis leidyi* European clam *Varicorbula gibba* European fan worm *Sabella spallanzanii* (P) European shore crab *Carcinus maenas* Green macroalga *Codium fragile* ssp. *fragile* (P) Harris' mud crab *Rhithropanopeus harrisii* Hydroid *Blackfordia virginica* Jack-knife clam *Ensis directus* Japanese kelp *Undaria pinnatifida* Jingle shell *Monia noblis* Mangrove horseshoe crab *Carcinoscorpius rotundicauda* New Zealand green lip mussel *Perna canaliculus* New Zealand screw shell *Maoricolpus roseus* Northern Pacific seastar *Asterias amurensis* Pacific oyster *Crassostrea gigas* Polynesian grapsid crab *Pachygrapsus fakaravensis* Red gilled mudworm *Marenzelleria* spp. Red polysiphonous algae *Polysiphonia setacea* Serpulid tube worm *Hydroides dianthus* Soft shell/long necked clam *Mya arenaria* Strangle weed *Sargassum muticum* Transverse arc clam *Anadara transversa* Variable mussel *Brachidontes pharaonis*			
Northern Territory	No specific species being recycled at present	**Diseases** Abalone viral ganglioneuritis Akoya oyster disease Haplosporidosis Infection with *Bonamia exitiosa* Infection with *Bonamia ostreae* Infection with *Marteilia refringens*	None applied at present	None applied at present	Kitman Dyrting, NT DPI pers. comm. 3 March 2020.

Jurisdiction	Mollusc species permitted for recycling	Diseases and marine pests requiring mitigation P = already present in jurisdiction.	Sanitary requirements applied to shell	Relevant legislation/ Acts controlling recycling	Other comments
Northern Territory (con't)		Infection with *Marteilia sydneyi* Infection with *Mikrocytos mackini* Infection with *Mikrocytos* (*Bonamia*) *roughleyi* Infection with *Perkinsus marinus* Infection with *Perkinsus olseni* Infection with *Xenohalitotis californiensis* Iridoviroses Ostreid herpesvirus-1 µVariant (OsHV-1 µVar) **Marine Pests** American slipper limpet *Crepidula fornicata* Aquarium Caulerpa *Caulerpa taxifolia* Asian bag mussel *Arcuatula senhousia* Asian basket clam *Corbula amurensis* Asian green mussel *Perna viridis* Asian paddle crab *Charybdis japonica* Asian rapa whelk *Rapana venosa* Asian seaweed *Grateloupia turuturu* Asian semelid bivalve *Theora lubrica* Asian shore crab *Hemigrapsus sanguineus* Bay barnacle *Balanus improvisus* Black striped mussel *Mytilopsis sallei* Brown mussel *Perna perna* Brush-clawed shore crab *Hemigrapsus takanoi*, *H. penicillatus* Chinese mitten crab *Eriocheir sinensis* Clubbed tunicate *Styela clava* Colonial seasquirt *Didemnum* (exotic species) Comb jelly *Mnemiopsis leidyi* European clam *Varicorbula gibba* European fan worm *Sabella spallanzanii* European sea squirt *Ciona intestinalis*			

Jurisdiction	Mollusc species permitted for recycling	Diseases and marine pests requiring mitigation P = already present in jurisdiction.	Sanitary requirements applied to shell	Relevant legislation/ Acts controlling recycling	Other comments
Northern Territory (con't)		European shore crab *Carcinus maenas* Green macroalga *Codium fragile* ssp. *fragile* Jack-knife clam *Ensis directus* Japanese kelp *Undaria pinnatifida* Mudworms (*Boccardia proboscidea*, *Polydora cornuta*, *P. websteri*) New Zealand half crab *Petrolisthes elongatus* New Zealand screw shell *Maoricolpus roseus* Northern Pacific seastar *Asterias amurensis* Red gilled mudworm *Marenzelleria* spp. Sabellid polychaete *Euchone limnicola* Soft shell/long necked clam *Mya arenaria*, *M. japonica* Strangle weed *Sargassum muticum*			
Overseas					
California, USA	Not specified	Not specified	1. Oyster shells must be dried out of the water for at least 6 months	California Department of Fish and Game	NOAA (2013)
Maryland, USA	Oyster (primarily *Crassostrea virginica*, but also *C. gigas*, *O. edulis*, etc.), various clam, whelk, scallop, snail/escargot, some mussel shells	Dermo - Infection with *Perkinsus marinus* (P) MSX - Infection with *Haplosporidium nelsoni* (P)	1. Oyster shells must be dried out of the water for at least 12 months. 2. shells cleaned using shell washing machine (tumbler with pressurized water jet system @ ORP/UMCES Horn Point)	Code of Maryland Regulations (COMAR) Sec. 08.02.08.01 "Importation of Shellfish" when collecting from outside of State.	c. 1000 tonnes of shell collected from ~300 restaurants and 70 public drop sites per annum. c. 6600 tonnes collected since 2010. Thomas C Price, oysterrecovery.org, pers. comm 18 Jun 2020.
New York City (NYC), New York, USA	Oyster shells, hard shell clam	Dermo - Infection with *Perkinsus marinus* (P) MSX - Infection with *Haplosporidium nelsoni* (P) SSO - Infection with *Haplosporidium costale* (P)	1. Oyster shells sourced from restaurants in NYC (who are subject to regulation by the	Department of Environmental Conservation's	Other acts by Army Corp of Engineers, DEC, NYC Parks &

Jurisdiction	Mollusc species permitted for recycling	Diseases and marine pests requiring mitigation P = already present in jurisdiction.	Sanitary requirements applied to shell	Relevant legislation/ Acts controlling recycling	Other comments
New York City (NYC), New York, USA (con't)		JOD - Juvenile oyster disease (infection with *Roseovarius crassostreae*) (P) QPX - Quahog parasite unknown (P)	FDA's National Shellfish Sanitation Program and NYS Environmental Conservation Law, Article 13, Sections 13-0309, 13-0319, & 13-0321). 2. Air-cured in piles 3 ft high for 6 months to 1 year (must include summer (May-Sept) following DEC's Acceptable Origin Of Shell & Shellstock in NY Waters). 3. Piles are periodically turned to ensure equal drying out of shell.	Acceptable Origin Of Shell & Shellstock In NY Waters - 6 NYCRR Part 360 Solid Waste Management Facilities General Requirements - 6 NYCRR Part 361-3 Other organics recycling facilities.	Rec, United States Coast Guard, apply when placing oyster shell into New York waters. Charlotte Boesch, Billion Oyster Project, pers. comm 14 Apr 2020.
Oregon, USA	Not specified	Not specified	None required		NOAA (2013)
Rhode Island, USA	Eastern oyster (*Crassostrea virginica*); Surf clam (*Spisula solidissima*)	Dermo - Infection with *Perkinsus marinus* (P) MSX - Infection with *Haplosporidium nelsoni* (P) SSO - Infection with *Haplosporidium costale*	1. Oyster shells must be dried out of the water for at least 6 months and pass visual inspection by an authorized CRMC staff member (the inspection ensures that all remaining oyster tissue has been removed prior to use) 2. Steam-shucked surf clams do not require additional sanitary requirements.	The Rhode Island Coastal Resources Management Council has established an Aquaculture Biosecurity Board that oversees the in-water use of recycled shell.	William Helt, TNC Rhode Island, pers. comm. 8 Apr 2020.

Jurisdiction	Mollusc species permitted for recycling	Diseases and marine pests requiring mitigation P = already present in jurisdiction.	Sanitary requirements applied to shell	Relevant legislation/ Acts controlling recycling	Other comments
South Carolina, USA	"Oyster Shells" (Any species) "Clam Shells" (Any species) "Whelk Shells" (Any species)	Dermo - Infection with *Perkinsus marinus* (P) MSX - Infection with *Haplosporidium nelsoni* (P) SSO - Infection with *Haplosporidium costale* JOD – Juvenile oyster disease (infection with *Roseovarius crassostreae*) Bonamiosis - Infection with *Bonamia exitiosa* (P) QPX – Quahog parasite unknown	1. Oyster shells must be dried out of the water for at least 6 months 2. Shell sourced from out of state cannot be placed in waters of the state without a permit from South Carolina Dept. of Natural Resources.	South Carolina State Code of Laws: Section 50-5-1005; Coastal Zone Management Act; South Carolina Coastal Tidelands and Wetlands Act	South Carolina DHEC-OCRM and USACE also have jurisdiction over shells being placed into the coastal zones critical areas. NOAA (2013) Ben Dyar, South Carolina Department of Natural Resources, pers. comm. 28 May 2020
Washington State, USA	Not specified	Japanese oyster drill (*Ocinebrellus inornatus*) (P)	1. Oyster shells must be kept in a pile at least 200 feet from any body of water. 2. Oyster shells must be dried out for at least 90 days (3 months) before the shells can be moved from one site to another. 3. Shells transported from areas infested with the Japanese oyster drill (*Ocinebrellus inornatus*) must be inspected before they can be planted in an uninfested area.	Washington Department of Fish and Game	NOAA (2013)

2.0 Hazard Description

The next step in the RA process is to develop a list of the relevant hazards to be analysed. The national list of notifiable diseases of molluscs under official control in Australia is available online at http://www.agriculture.gov.au/animal/aquatic/reporting/reportable-diseases#molluscs, while each State has its own list of notifiable diseases of molluscs (Tables 1, 2). Most disease and marine pest lists are also available online, for example for Queensland see https://www.legislation.qld.gov.au/view/html/inforce/current/act-2014-007#sch.1, for NSW see https://www.legislation.nsw.gov.au/#/view/act/2015/24/sch2, for Victoria see https://vfa.vic.gov.au/operational-policy/pests-and-diseases/noxious-aquatic-species-in-victoria, for Tasmania see https://dpipwe.tas.gov.au/conservation/the-marine-environment/marine-pests-and-diseases/pest-identification, for SA see https://www.pir.sa.gov.au/biosecurity/aquatics/aquatic_pests/noxious_fish_list and https://www.pir.sa.gov.au/biosecurity/aquatics/aquatic_diseases, for WA see https://www.agric.wa.gov.au/bam/fish-diseases and http://www.fish.wa.gov.au/Sustainability-and-Environment/Aquatic-Biosecurity/Vessels-And-Ports/Pages/Biofouling-management-tools-and-guidelines.aspx, and for the NT https://nt.gov.au/marine/for-all-harbour-and-boat-users/biosecurity/aquatic-pests-marine-and-freshwater/list-of-noxious-fish. Other State lists of notifiable diseases of molluscs were obtained via direct communication with representatives of biosecurity authorities in each state (e.g. Northern Territory Aquatic Reportable Diseases, M. Barton, personal communication 28/10/2019). In addition to the various listed pest and disease agents, several additional unlisted diseases and marine pests known to occur in molluscs cultured in Australia were also identified as potential hazards which may be relevant to shell recycling in Australia, and these were also included in the initial hazard list for further assessment (Table 2). The various species of molluscs that could potentially be recycled in Australia for shellfish reef restoration were identified from questionnaire responses and the observations of the TNC and the author to include Pacific oysters (*Crassostrea gigas*), Sydney rock oyster (*Saccostrea glomerata*), flat oysters (*Ostrea angasi*), tropical rock oysters (TRO, such as blacklip rock oyster (*Saccostrea echinata*, *Saccostrea* sp.), western or hooded rock oyster (*Saccostrea cucullata*), milky or coral rock oyster (*Saccostrea scyphophilla*), see McDougall et al. 2020), Australian flat oyster (*Ostrea angasi*), mussels (*Mytilus edulis*), scallops (*Pecten fumatus*, *Mimachlamys asperrima*) and pearl oysters (gold lipped pearl oyster (*Pinctada maxima*), blacklip pearl oyster (*Pinctada margaritifera*), Akoya pearl oyster (*Pinctada imbricata fucata*), and Shark Bay pearl oyster (*Pinctada albina*)). In Tasmania, abalone (blacklip abalone (*Haliotis rubra*), greenlip abalone (*Haliotis laevigata*) and hybrids thereof) were considered under shell recycling legislation, and thus pathogens specific to abalone (including AbHV-1 virus that causes AVG and bacteria causing withering syndrome) were also included in the initial hazard list for further assessment.

2.1 Elimination of insignificant diseases and marine pests

Hazard identification for the diseases and pests reported from these hosts identified at least 32 disease groups and 64 marine pests of potential concern (Table 2). These included 6 viral diseases, 4 bacterial diseases, 16 protozoan diseases, 3 metazoan diseases and 3 diseases of unknown aetiology, plus invasive pests including 11 species of crabs, 7 species of clams, 7 species of seaweeds, 6 species of mussels, 4 species of oysters, 4 species of barnacles, 4 species of gastropods, 4 species of flatworms, 4 species of tunicates/ascidians/hydroids, 4 species of polychaetes, 2 genera of mudworms, and various species of boring sponges, comb jellies, seastars, boring mussels and pea crabs (Table 2). Except for flatworms and pea crabs, all of the remaining listed pest species groups were included in the detailed risk assessment process.

Table 2. The list of diseases and marine pests to be considered during hazard identification.

Disease agents	Present in Australia	State where disease occurs[A]	State where disease is listed	Main mollusc hosts	Under official control in Australia[1,2,3,4,5,6]
Viruses					
Infection with AbHV-1 (Abalone viral ganglioneuritis, AVG)	Yes	Vic, Tas	All states	Abalone	Yes
Iridoviroses of molluscs	No	-	All states	Various	Yes
Infection with OsHV1-µVar (Pacific oyster mortality syndrome, POMS)	Yes	NSW, Tas, SA	All states	*Crassostrea gigas*	Yes
Infection with other malacoherpesviruses (incl. AVNV of scallops)	Yes	NSW, WA	QLD, WA	*Ostrea angasi*, scallops	Yes
Intranuclear virus-like inclusions	Yes	WA	-	*Saccostrea cucullata*	No
Viral gametocytic hypertrophy	Yes	QLD, NSW, Tas, SA, WA	-	Various	No
Bacteria					
Infection with *Xenohaliotis californiensis*	No	-	All states	Abalone	Yes
Nocardiosis of shellfish	No	-	Tas	Various	Yes
Rickettsia-like and *Chlamydia*-like organisms (RLOs)	Yes	All states	-	Various	No
Vibro spp., including *V. harveyi*	Yes	All states	-	Various	No
Protozoa					
Ciliates (Ancistrocomids)	Yes	All states	-	Various	No
Coccidians (*Merocystis* spp., *Klossia* spp., *Pseudoklossia* spp.)	Yes	All states	-	Various	No
Infection with *Bonamia exitiosa* and *Bonamia* spp.	Yes	NSW, Vic, Tas, SA, WA	All states	*O. angasi*, *S. glomerata*	Yes
Infection with *Bonamia ostreae*	No	-	All states	*Ostrea edulis*	Yes
Gregarines (*Nematopsis* spp.)	Yes	All states	-	Various	No
Haplosporidosis	Yes	WA, QLD	SA, WA, NT	Various	Yes
Infection with *Haplosporidium nelsoni* (MSX disease)	No	-	Tas, SA, WA, NT	*Crassostrea virginica*	Yes
Infection with *Marteilia refringens*	No	-	All states	*O. edulis*	Yes
Infection with *Marteilia sydneyi* (QX disease)	Yes	QLD, NSW, WA	NSW, Vic, SA, Tas, WA, NT	*S. glomerata*	Yes

[1] http://www.agriculture.gov.au/animal/aquatic/reporting/reportable-diseases#molluscs
[2] https://www.legislation.qld.gov.au/view/html/inforce/current/act-2014-007#sch.1
[3] https://www.legislation.nsw.gov.au/#/view/act/2015/24/sch2
[4] https://dpipwe.tas.gov.au/biosecurity-tasmania/animal-biosecurity/animal-health/notifiable-animal-diseases
[5] https://www.pir.sa.gov.au/biosecurity/aquatics/aquatic_diseases
[6] https://www.agric.wa.gov.au/bam/fish-diseases

Disease agents (con't)	**Present in Australia**	**State where disease occurs[A]**	**State where disease is listed**	**Main mollusc hosts**	**Under official control in Australia[1,2,3,4,5,6]**
Infection with *Marteilia* spp. and *Marteilioides* spp.	Yes	QLD, NSW, WA, NT	WA	*S. echinata*, *O. angasi*	Yes
Infection with *Marteilioides chungmuensis*	No	-	All states	*C. gigas*	Yes
Microsporidians (*Steinhausia mytilovum*, *Microsporidium* spp.)	Yes	All states	WA	Various	Yes
Infection with *Mikrocytos* spp. (including *M. mackini*)	No	-	All states	*C. gigas*	Yes
Infection with *Minchinia* spp. (including *M. occulta*)	Yes	WA	WA	*S. cucullata*	Yes
Infection with *Perkinsus olseni*, *P. chesapeaki*, *Perkinsus* spp.	Yes	QLD, NSW, Vic, SA, WA	All states	Various	Yes
Infection with *Perkinsus marinus*	No	-	All states	Various	Yes
Metazoa					
Cestode metacestodes (*Tylocephalum* spp.)	Yes	All states	-	Various	No
Copepods (*Mytilicola*, *Pseudomyicola* spp.)	Yes	All states	-	Various	No
Digenean sporocysts (*Bucephalus* spp., *Proctoeces* spp.)	Yes	All states	-	Various	No
Unknown aetiology					
Akoya oyster disease	No	-	QLD, WA, NT	*Pinctada fucata*	Yes
Oyster oedema disease	Yes	WA	QLD, WA	*Pinctada maxima*	Yes
Winter mortality (*M. roughleyi*)*	Yes	NSW	NSW, Tas, WA	*S. glomerata*	Yes
Marine Pests	**Present in Australia**	**State where pest group occurs[A]**	**State where pest group is listed**	**Main mollusc host**	**Under official control in Australia[2,3,7,8,9,10,11]**
Boring mussels (*Lithophaga* spp.)	Yes	WA, NT, QLD	-	Various	No
Boring sponges (*Cliona* spp., *Pione* spp.)	Yes	QLD, NSW, WA, NT	WA	Various	Yes
Flatworms (*Enterogonia* spp., *Imogine* spp., *Notoplana* spp., *Stylochus* spp.)	Yes	All states	-	Various	No
Invasive barnacles (*Balanus improvisus*, *B. eburneus*, *B. glandula*, *Chthamalus proteus*)	Yes	QLD, NSW, Vic, WA, NT	QLD, NSW, SA, WA, NT	Various	Yes
Invasive ctenophores (comb jellies, *Beroe ovata*, *Mnemiopsis leidyi*)	No	-	QLD, NSW, SA, WA, NT	Various	Yes

[7] https://vfa.vic.gov.au/operational-policy/pests-and-diseases/noxious-aquatic-species-in-victoria
[8] https://dpipwe.tas.gov.au/conservation/the-marine-environment/marine-pests-and-diseases/pest-identification
[9] https://www.pir.sa.gov.au/biosecurity/aquatics/aquatic_pests/noxious_fish_list
[10] http://www.fish.wa.gov.au/Sustainability-and-Environment/Aquatic-Biosecurity/Vessels-And-Ports/Pages/Biofouling-management-tools-and-guidelines.aspx
[11] https://nt.gov.au/marine/for-all-harbour-and-boat-users/biosecurity/aquatic-pests-marine-and-freshwater/list-of-noxious-fish

Marine Pests (con't)	**Present in Australia**	**State where pest group occurs[A]**	**State where pest group is listed**	**Main mollusc host**	**Under official control in Australia[2,3,7,8,9,10,11]**
Invasive crabs (*Callinectes sapidus, Carcinoscorpius rotundicauda, Carcinus maenas, Charybdis japonica, Eriocheir sinensis, Hemigrapsus penicillatus, H. sanguineus, H. takanoi, Pachygrapsus fakaravensis, Petrolisthes elongatus, Rhithropanopeus harrisii*)	Yes	NSW, Vic, Tas, SA, WA	All states	Various	Yes
Invasive clams (*Anadara transversa, Corbula amurensis, Ensis directus, Mya japonica, M. arenaria, Theora lubrica, Varicorbula gibba*)	Yes	Vic, Tas	All states	Various	Yes
Invasive mussels (*Arcuatula senhousia, Brachidontes pharaonis, Mytilopsis sallei, Perna canaliculus, Perna perna, Perna viridis*)	Yes	Vic, Tas, SA, WA	All states	Various	Yes
Invasive oysters (*Crassostrea ariakensis, C. gigas, C. virginica, Monia noblis*)	Yes	*C. gigas* occurs in NSW, Tas, SA	*C. gigas* is listed in QLD, others in WA	*C. gigas*	Yes
Invasive polychaetes (*Euchone limnicola, Hydroides dianthus, Marenzelleria* spp., *Sabella spallanzanii*) including mudworms (*Polydora* spp., *Boccardia* spp.)	Yes	NSW, Vic, Tas, SA, WA, NT (All states for mudworms)	All states	Various	Yes
Invasive seaweeds (*Caulerpa taxifolia, Codium fragile, Fucus evanescens, Grateloupia turuturu, Polysiphonia setacea, Sargassum muticum, Undaria pinnatifida*)	Yes	NSW, Vic, Tas, SA, WA	All states	Various	Yes
Invasive seastars (*Asterias amurensis*)	Yes	Vic, Tas	All states	Various	Yes
Invasive tunicates /ascidians/ hydroids (*Blackfordia virginica, Ciona intestinalis, Didemnum* spp., *Styela clava*)	Yes	All states	QLD, NSW, SA, Tas, WA, NT	Various	Yes
Invasive whelks/ gastropods/ limpets/ chitons (*Chiton glaucus, Crepidula fornicata, Maoricolpus roseus, Rapana venosa*)	Yes	NSW, Vic, Tas,	All states	Various	Yes
Pea crabs (*Pinnotheres* spp.)	Yes	All states	-	Various	No

[A] At least one of the listed species of disease agent or pest group has been reported from the jurisdiction.

* *Bonamia roughleyi* is considered *nomen dubium* (Carnegie et al. 2014) and it is unclear the role that these microcells play in winter mortality disease in NSW.

The unrestricted risk posed by several disease agents in Table 2 is likely to be either negligible, or within the acceptable level of protection (ALOP), meaning that additional risk management measures would not be required. Section 2.1 contains a brief discussion of the reasons why flatworms, pea crabs and these other disease agents have been excluded from further assessment. However, it must be considered that knowledge regarding the health status of molluscs in Australia is incomplete and that various new diseases will continue to emerge as time goes on (Gaughan 2002). Furthermore, the threat from invasive pest species continues to increase directly in line with increasing volumes of international trade (Diggles 2017). Because of this, it is likely that the hazard list, and this RA, will require regular updating in the future to consider new information on marine pests and diseases potentially associated with Australian molluscs as it becomes available.

2.1.1 Viruses

Molluscs can harbour a range of viruses (Elston 1997). Infections with small RNA viruses were associated with digestive sloughing during mortalities of mussels, scallops and toheroa in New Zealand (Jones et al. 1996, Hine and Wesney 1997). Histological lesions, similar to those in New Zealand, were subsequently reported from scallops (*Pecten alba*) from Port Phillip Bay, Victoria, and pearl oysters in Western Australia (*Pinctada maxima*) and French Polynesia (*P. margaritifera*), but the presence of virus was not confirmed (Pass et al. 1988, Lester 1989, Comps et al. 1999, 2001, AFFA 2002, Humphrey and Norton 2005, Jones 2007b). Hine and Thorne (2000) also found intranuclear virus-like particles in 1 out of 769 *S. cucullata* collected from Exmouth Islands, 1 out of 71 *Pinna bicolor* (including 65 spat) examined from Dampier archipelago, and 1 out of 36 *Isognomon isognomon* during surveys of molluscs in Western Australia. Eosinophilic inclusions occurred in the nuclei of diverticular epithelial cells (Hine and Thorne 2000). The affected bivalves were apparently healthy and the virus particles were not associated with disease. There is some confusion over the identity and significance of these small viruses. Some have been associated with digestive sloughing during outbreaks of mortality (Jones et al. 1996, Hine and Wesney 1997) but sloughing of digestive cells also occurs in the absence of disease. Koch's postulates have not been fulfilled (Hine and Wesney 1997), hence the intranuclear virus-like particles and other poorly characterized viral particles may be incidental findings that are not associated with disease processes (Hine and Wesney 1997). Because it is not clear that these viruses cause significant disease at this time, they are not listed diseases and do not infect mollusc shells, they will not be considered further in this RA.

Viral gametocytic hypertrophy caused by intranuclear papova-like viruses (Choi et al. 2004) has been recorded in an increasing assortment of bivalve hosts in an increasing number of areas around the world (Garcia et al. 2006, Cheslett et al. 2009). These viruses cause massive hypertrophy of individual gametes and gametogenic epithelium by replicating in the host cell nucleus. Host response to infection is usually negligible and the infection is not known to cause disease or reduced fecundity. In Australia viral gametocytic hypertrophy has been reported from Pacific oysters (*Crassostrea gigas*), Sydney rock oysters (*Saccostrea glomerata*), and pearl oysters (*Pinctada maxima*) in locations such as QLD, NSW, WA, SA and Tasmania (AFFA 2002, Norton et al. 1993b, Humphrey et al. 1998, Diggles 2003, Humphrey and Norton 2005, B.K. Diggles, personal observations). In northern Australia, viral gametocytic hypertrophy was observed in the labial palps of 2 of 50 adult *P. maxima* during a routine histological examination of samples from the western Torres Strait region (Norton et al. 1993b). Similar lesions were also observed by Humphrey et al. (1998) in *P. maxima* only from QLD at regional prevalences of 6-50%. It is not known if infection causes disease among wild oysters. In South Australia, viral gametocytic hypertrophy was recorded in cultured *C. gigas* at prevalences of 0.7-1.4%

(Diggles 2003). Apparent increased prevalence of viral gametocytic hypertrophy in recent years in some parts of the world could be due to spread of the disease, increased surveillance of molluscs, increased recognition of the disease by diagnosticians, or a combination of these factors (Garcia et al. 2006). However, as these viruses do not appear to have any significant detrimental effect on the host (Meyers and Burton 2009), infect only the gonad and not mollusc shells, and because they already appear to be widely distributed and are not listed diseases, they will not be considered further in this RA.

2.1.2 Bacteria

All molluscs have a "normal" bacterial flora which is moved whenever the host is translocated. There are also facultative bacterial pathogens such as *Vibrio* spp. that are considered to be ubiquitous in aquatic environments (Pass et al. 1987, Austin and Austin 2007), but certain strains of which can cause disease and mortalities in aquatic animals that are stressed, injured and/or exposed to adverse environmental conditions For example, blacklip pearl oysters sampled from Manihiki Lagoon in the Cook Islands following a period of poor water quality (low oxygen) displayed a broad brown conchiolin deposition on the inside shell nacre, with the deposits lying outside the edge of the retracted mantle in response to infections by *Vibrio harveyi* (see Diggles et al. 2007). This condition was virtually identical to anomalous conchiolin deposition (ACD) described for *Pinctada maxima* from Western Australia associated with the presence of *Vibrio harveyi* (see Dybdahl and Pass, 1985, Pass et al., 1987, Lester 1989, Perkins, 1996). Pass et al. (1987) found that *V. harveyi* infection in *P. maxima* was associated with low water temperatures (19°C) and overcrowding during transport of oysters to leases. *Vibrio* infections are also very common problems in mollusc hatcheries during larval rearing whenever water quality and husbandry parametres are suboptimal (Lester 1989). These data are consistent with disease associated with opportunistic bacteria which invade hosts that are stressed due to unfavorable conditions such as overcrowding, abnormally high or low water temperatures and/or poor water quality. Because of the ubiquitous distribution of *Vibrio* spp., the facultative nature of this disease process, and the fact they are not listed diseases, *Vibrio* spp. including *V. harveyi* will not be considered further in this RA.

Prokaryote rickettsia-like organisms (RLOs) and chlamydia-like organisms (CLOs) are commonly observed in the gills and digestive tract of wild and cultured molluscs worldwide, including in Australia (Humphrey et al. 1998, Hine and Thorne 2000, AFFA 2002, Humphrey and Norton 2005, Jones 2007b, Crockford and Jones 2012). These agents are sometimes associated with disease, especially when they occur in the branchial epithelium of scallops, though most are thought to be benign or facultative pathogens as they are commonly present in apparently healthy molluscs (Humphrey et al. 1998, Hine and Diggles 2002a, Crockford and Jones 2012). Another example are the CLO's found by Crockford and Jones (2012) in pearl oysters *P. maxima* affected by oyster oedema disease (OOD) in WA. Using real time PCR (qPCR), they found the prevalence of these agents (a *Simkania negevensis* -like agent and an uncharacterised bacterium referred to as *maxima*-CLO) was "high". However, both types of CLO were present in both diseased and healthy *P. maxima*, and a causative role in a disease process was not established (Crockford and Jones 2012, Goncalves et al. 2017). Due to their facultative nature and because it is unclear whether these disease agents can cause disease in molluscs in Australia, together with the fact they are not listed diseases and do not infect mollusc shells, RLOs and CLOs will not be considered further in this RA. One exception to this rule is the highly pathogenic prokaryote bacterium *Xenohaliotis californiensis* which infects the digestive tract of various species of abalone in the USA, Chile, Europe, China, Thailand and Japan causing withering syndrome disease (Moore et al. 2001, Balserio et al. 2006, Wetchateng et al. 2010, Kiryu et al. 2013, Crosson et al. 2014). While this disease is

exotic to Australia, because *X. californiensis* causes an internationally notifiable disease that is listed in all Australian states (Table 2), it will be considered further in the detailed risk assessment.

Infections with *Nocardia* -like gram positive bacteria have caused focal necrosis lesions and disease outbreaks during the summer months in Pacific oysters cultured in north America, Europe and Japan (Elston et al. 1987, Friedman et al. 1991, 1998, Engelesma et al. 2008). The main causative agent responsible for nocardiosis disease in North America is *Nocardia crassostreae*, which causes focal necrotic granulomatous lesions and grossly visible yellow-green or brown nodules 2-10 mm in diametre on the mantle (Elston et al. 1987, Friedman et al. 1991, 1998). These bacteria are usually associated with oyster mortality during times of environmental stress, including prolonged high water temperatures and low oxygen levels, often occurring in mixed infections with other *Vibrio* spp. bacteria and viral disease agents such as OsHV-1 (Friedman et al. 2005, Engelesma et al. 2008). Nocardiosis of oysters is a reportable disease in Tasmania (Table 2), however because of the apparent facultative nature of this disease process, and the fact that the disease does not infect mollusc shells and has not been reported from Australian molluscs to date, nocardiosis of shellfish will not be considered further in this RA.

2.1.3 Protozoa

Wild molluscs harbour a range of protozoan parasites, commensals and symbionts including several genera (*Ancistrocoma*, *Sphenophrya*, *Stegotricha*, *Trichodina* and others) of ciliates that occur naturally in the alimentary tract, palps and gills at prevalences that often approach 100% (Bower et al. 1994, Humphrey et al. 1998, Hine and Thorne 2000, Meyers and Burton 2009, Dang et al. 2013, B.K. Diggles, personal observations). These organisms can be spread into new areas whenever molluscs are translocated. Most ciliates infecting molluscs are likely to have direct lifecycles, and thus can be readily translocated into aquatic animal populations in new geographic areas, particularly as these symbionts are likely to have low host specificity. However, these parasites appear ubiquitous in the environment, do not appear to cause any significant harm to their hosts (Meyers and Burton 2009), and do not cause any listed diseases, hence they will not be considered further in this RA.

Apicomplexans of the Subclass Coccidia are intracellular parasites of vertebrates and invertebrates which may parasitize molluscs in the kidneys (*Pseudoklossia* spp., *Klossia* spp.), ovary (*Merocystis* spp.), connective tissues, muscle, haemocytes, haemolymph sinuses (*Margolisiella* spp.) and visceral ganglia (Hine 1997, 2002, Meyers and Burton 2009, Kristmundsson et al. 2011a, 2011b, 2015). Coccidian life cycles incorporate a diverse array of developmental stages representing asexual (merogony forming merozoites) and sexual reproduction (gamogony) which forms oocysts and infective sporozoites (sporogony) which invade other hosts via their digestive tract, after which they enter that hosts cells as trophozoites (Kristmundsson and Freeman 2018). Some species of coccidia have all types of developmental stages in one bivalve host, and these species may be able to complete their entire lifecycle within that one host (monoxenous life cycle) (Kristmundsson et al. 2011a, 2011b). In contrast, others may only have a single developmental stage evident within the bivalve (e.g. Hine 1997, 2002), and these are likely to have two host (heteroxenous) lifecycles that include not only bivalves but fishes or other molluscs (gastropods) as alternate hosts (Meyers and Burton 2009, Kristmundsson and Freeman 2018). Zoites of these parasites may be found at high prevalences (>85% see Hine 1997, 2002) in populations of apparently healthy bivalves, however in some instances high intensity coccidian infections have been associated with disease syndromes including myodegeneration and discolouration of adductor

muscles and gonads in Iceland scallops (*Chlamys islandica*), queen scallops (*Aequipecten opercularis*) and Atlantic scallops (*Placopecten magellanicus*) infected by *Merocystis kathae* (see Kristmundsson et al. 2011a, 2011b, 2015, Inglis et al. 2016, Kristmundsson and Freeman 2018). Furthermore, Hine (1997, 2002) suggested that heavy infections by zoites of an unidentified apicomplexan (APX) in bluff oysters (*O. chilensis*) in New Zealand may predispose its host to infections by *Bonamia exitiosa*. More recent surveys have found the APX agent also infects cultured mussels (*Perna canaliculus*, *Mytilus galloprovincialis*) in New Zealand at prevalences up to 60% (Suong et al. 2019), however its presence in these hosts was not associated with discolouration, reduced growth or mortalities. APX also did not appear to cause mortality in *O. chilensis* held in captivity, which instead died of bonamiosis (Diggles and Hine 2002, Hine et al. 2002b, B.K. Diggles, personal observations). A degree of host specificity has been observed for the coccidians infecting scallops in the North Sea, with apparent disease outbreaks due to *Merocystis kathae* occurring mainly in Iceland scallops and only in areas which are co-habited by large numbers of the alternate host (a gastropod whelk *Buccinum undatum*, see Kristmundsson and Freeman 2018). Although *M. kathae* has been present in diseased scallops, it appears that disease only occurs when these infections reach a high intensity, as low-level infections exist in many apparently healthy scallop populations under normal conditions (Kristmundsson and Freeman 2018). This suggests that *M. kathae* may act as a facultative pathogen in heavily infected scallop hosts, which may possibly be stressed by rising water temperatures in the North Sea and/or changes in the population dynamics of the whelk alternate host (Kristmundsson and Freeman 2018). However, to date it is unknown whether these parasites occur in Australian molluscs. Furthermore, because in other parts of the world they appear to be facultative pathogens which cause disease only under specific circumstances in the presence of appropriate alternate hosts, they do not infect mollusc shells, and do not cause any listed diseases, coccidians will not be considered further in this RA.

Gregarines are another parasite group classified in the Family *Porosporidae* (Class Conoidasida) within the Apicomplexa. Parasitism by gregarines (such as members of the genus *Nematopsis*) is probably ubiquitous in molluscs worldwide (Bower et al. 1994, Bower 2010). Life stages of these parasites occur in molluscs as part of a multi-host lifecycle that is usually completed in the intestinal tract of marine arthropods such as crabs. Sporozoites and oocysts of gregarines occur within phagocytes of molluscs that can move within the connective tissues to most organs, but are most frequently observed in the gut mucosa and gills (Meyers and Burton 2009). Infection with gregarines is usually associated with a focal, benign inflammatory response, without significant tissue damage or health effects and they are common incidental findings in otherwise healthy molluscs (Meyers and Burton 2009). Because of their ubiquitous distribution and benign nature, and the fact they do not infect mollusc shells, and do not cause any listed diseases, these parasites will not be considered further in this RA.

Microsporidia are obligate, intracellular parasites that infect every major group of animals including arthropods, fish, molluscs and mammals (Lom and Dykova 1992, Bower 2010, Cali et al. 2016). *Steinhausia mytilovum* is a microsporidian that has been reported from blue mussels (*Mytilus galloprovincialis*) in Cockburn Sound, WA, but not in other mussel growing areas (Jones and Creeper 2006), leading to the listing of these disease agents as being reportable disease agents in WA (Table 2). *Steinhausia* –like microsporidians have also been recorded from Sydney rock oysters (*Saccostrea glomerata*) in Moreton Bay, QLD (Anderson et al. 1995) and *Crassostrea echinata* from the Northern Territory (Wolf 1977), however the latter parasite reported by Wolf (1977) in retrospect appears more likely to be a *Marteilioides* spp. *Steinhausia mytilovum* is a globally distributed microsporidian parasite which infects the oocytes of blue mussels *Mytilus edulis* and *M.*

galloprovincialis (see Comtet et al. 2004). These parasites can affect the condition index of infected mussels (Rayyan and Chintiroglou 2003) and sometimes induce marked haemocytic infiltration inside affected gonad follicles (e.g. Jones and Creeper 2006, Dang et al. 2013). Dang et al. (2013) found *Steinhausia* sp. at prevalences of 5-28% in Sydney rock oysters (*S. glomerata*) in Moreton Bay, and concluded that this parasite affected the reproductive activity of the host. However, 18 years earlier Anderson et al. (1995) stated the pathogenesis of the infection was unclear and that the low intensity infections they observed in cultured *S. glomerata* were unlikely to significantly reduce host fecundity. Furthermore, in other studies no conclusive evidence has been reported regarding the viability of the infected oocytes, or mortality of the host, and therefore the effect of *Steinhausia* on the reproductive potential of its host remains unclear (Comtet et al. 2004). Their known distribution suggests that *Steinhausia*-like parasites already occur in several species of molluscs throughout Australia, including Sydney rock oysters. This, combined with the fact that it is unclear whether these parasites can cause disease (Meyers and Burton 2009), and the fact they infect gonad and connective tissue, and not mollusc shells, means they will not be considered further in this RA.

2.1.4 Metazoa

Wild molluscs harbour a range of metazoan parasites, commensals and symbionts including larval stages of parasitic helminths that infect fishes as final hosts, such as sporocysts of digenean trematode flukes (*Bucephalus* spp., *Proctoeces* spp., and others), and metacestodes of larval cestodes (*Tylocephalum* spp., and others), flatworms that may predate on oysters (genera *Enterogonia* spp., *Imogine* spp., *Stylochus* spp. and others), and crustaceans such as copepods (*Mytilicola* spp., *Pseudomyicola* spp.) and pea crabs (*Pinnotheres* spp.) that live inside the mantle cavity (Bower et al. 1994, Humphrey et al. 1998, Hine and Thorne 2000, Meyers and Burton 2009, B.K. Diggles, personal observations). These organisms can be spread into new areas whenever molluscs are translocated. The larval digenean trematodes and tapeworms have multi-host lifecycles and use molluscs as an alternate host, multiplying then releasing infective stages into the water (digeneans) or resting within the mollusc tissues until the life cycle is completed when the mollusc is eaten by a fish (cestodes) (Meyers and Burton 2009). In contrast, the flatworms and crustaceans that infect molluscs are generally symbionts that have direct lifecycles, and thus can be readily transferred to aquatic animal populations in new geographic areas, particularly as many of these symbionts are likely to have low host specificity. However, all of these parasites occur in healthy wild oysters, appear ubiquitous in the environment, and with a few notable exceptions (e.g. mudworm disease, which will be subject to detailed assessment), do not occur in mollusc shells or appear to be associated with any significant harm to their hosts (Hine 1997, Meyers and Burton 2009), and do not cause any listed diseases, hence they will not be considered further in this RA.

2.2 The priority diseases and marine pests of concern to be considered in the RA

After elimination of the insignificant diseases from Table 2, the remaining 20 diseases and 14 groups of marine pests listed in Table 3 are considered to be diseases and marine pests of potential concern that are relevant to the recycling of mollusc shells in Australia, and which therefore require detailed risk assessment.

Table 3. The priority list of diseases and marine pests to be considered in the detailed risk analysis.

Disease agent	Present in Australia	State where pest/disease occurs [A]	State where pest/disease is listed	Main mollusc hosts	Under official control in Australia
Viruses					
Infection with AbHV-1 (AVG)	Yes	Vic, Tas	All states	Abalone	Yes
Iridoviroses of molluscs	No	-	All states	Various	Yes
Infection with OsHV1-µVar (POMS)	Yes	NSW, Tas, SA	All states	*C. gigas*	Yes
Infection with other malacoherpesviruses	Yes	NSW, WA	QLD, WA	Various	Yes
Bacteria					
Infection with *Xenohaliotis californiensis*	No	-	All states	Abalone	Yes
Protozoa					
Infection with *Bonamia exitiosa* and *Bonamia* spp.	Yes	NSW, Vic, Tas, SA, WA	All states	*O. angasi*, *S. glomerata*	Yes
Infection with *Bonamia ostreae*	No	-	All states	*O. edulis*	Yes
Haplosporidosis	Yes	WA, QLD	SA, WA, NT	Various	Yes
Infection with *Haplosporidium nelsoni* (MSX disease)	No	-	Tas, SA, WA, NT	*C. virginica*	Yes
Infection with *Marteilia refringens*	No	-	All states	*O. edulis*	Yes
Infection with *Marteilia sydneyi* (QX disease)	Yes	QLD, NSW, WA	NSW, Vic, Tas, SA, WA, NT	*S. glomerata*	Yes
Infection with *Marteilia* spp. and *Marteilioides* spp.	Yes	QLD, NSW, WA, NT	WA	*S. echinata*, *O. angasi*	Yes
Infection with *Marteilioides chungmuensis*	No	-	All states	*C. gigas*	Yes
Infection with *Mikrocytos* spp. (including *M. mackini*)	No	-	All states	*C. gigas*	Yes
Infection with *Minchinia* spp. (including *M. occulta*)	Yes	WA	WA	*S. cucullata*	Yes
Infection with *Perkinsus* spp., including *P. olseni*, *P. chesapeaki*	Yes	QLD, NSW, Vic, SA, WA	All states	Various	Yes
Infection with *Perkinsus marinus*	No	-	All states	Various	Yes
Unknown aetiology					
Akoya oyster disease	No	-	QLD, WA, NT	*P. fucata*	Yes
Oyster oedema disease	Yes	WA	QLD, WA	*P. maxima*	Yes
Winter mortality (*M. roughleyi*)	Yes	NSW	NSW, Tas, WA	*S. glomerata*	Yes
Marine pests					
Boring mussels	Yes	WA, NT, QLD	-	Various	No
Boring sponges	Yes	QLD, NSW, WA, NT	WA	Various	Yes
Invasive barnacles	Yes	QLD, NSW, Vic, WA, NT	QLD, NSW, SA, WA, NT	Various	Yes
Invasive ctenophores (comb jellies)	No	-	QLD, NSW, SA, WA, NT	Various	Yes
Invasive crabs	Yes	NSW, Vic, Tas, SA, WA	All states	Various	Yes
Invasive clams	Yes	Vic, Tas	All states	Various	Yes
Invasive mussels	Yes	Vic, Tas, SA, WA	All states	Various	Yes
Invasive oysters	Yes	NSW, Tas, SA	QLD, WA	*C. gigas*	Yes
Invasive polychaetes (including mudworms)	Yes	All states	All states	Various	Yes
Invasive seaweeds	Yes	NSW, Vic, Tas, SA, WA	All states	Various	Yes
Invasive seastars	Yes	Vic, Tas	All states	Various	Yes
Invasive tunicates /ascidians/ hydroids	Yes	All states	QLD, NSW, Tas, SA, WA, NT	Various	Yes
Invasive whelks/ gastropods/ limpets /chitons	Yes	NSW, Vic, Tas,	All states	Various	Yes

[A] At least one of the listed species of disease agent or pest group has been reported from the jurisdiction.

3.0 The methodology used for the Risk Analysis

This RA is based on a qualitative assessment of the risks involved with translocation of recycled mollusc shell waste. The qualitative RA method addresses risk in a standardised manner (DAFF 2003, 2011, Diggles and Arthur 2010, Diggles 2011a, 2017, 2020a, OIE 2019) utilising a series of risk assessment processes, namely release assessment; exposure assessment; consequence assessment; and risk estimation.

3.1 Release assessment

After defining the hazards of concern (see Tables 1, 2 and 3), the next steps in the RA are to identify the potential pathways of entry and release of these hazards into the receiving environment. After reviewing the existing risk mitigation methods employed during shell recycling in several jurisdictions (Table 1), the 5 pathways that were examined for this risk analysis included introduction of diseases or marine pests via;

1. Reuse of untreated recycled mollusc shells (unmitigated risk)
2. Reuse of mollusc shells that have been desiccated in sunlight for 3, 4 or 6 months
3. Reuse of mollusc shells that have been heat treated to 55°C for >10 minutes or 80°C for >5 minutes
4. Reuse of mollusc shells that have been exposed to freshwater for >24 hours
5. Reuse of mollusc shells that have been exposed to 4% acetic acid (vinegar) for >30 minutes

The likelihood estimations (Table 4) that a hazard would be successfully translocated and released via a particular pathway was determined through qualitative assessments based on information available in the scientific (and other) literature, unpublished data, as well as the professional judgment of the analyst. Indicative annual probability and event horizon estimates are for guidance only. A question mark (?) was used to signify uncertainty due to a lack of information on the effectiveness of a specific risk mitigation pathway for inactivating a hazard. The risk assessment for a particular hazard was concluded if the release assessment determined that the likelihood of release of that hazard via all pathways was negligible (OIE 2019).

Table 4. Nomenclature for the qualitative likelihood estimations used in this RA.

Likelihood	Definition	Annual Probability	Event Horizon
High (H)	The event would be very likely to occur	$0.7 > P \leq 1$	Every 1-2 years
Moderate (M)	The event would occur with an even probability	$0.3 > P \leq 0.7$	Every 2-4 years
Low (L)	The event would be unlikely to occur	$0.05 > P \leq 0.3$	Every 4-20 years
Very Low (VL)	The event would be very unlikely to occur	$0.01 > P \leq 0.05$	Every 20-100 years
Extremely low (EL)	The event would be extremely unlikely to occur	$0.001 > P \leq 0.01$	Every 100-1000 yrs.
Negligible (N)	The event would almost certainly not occur	$0 > P \leq 0.001$	>1,000 years

3.2 Exposure assessment

The exposure assessment examines the likelihood of the environment and aquatic animals in different regions of Australia being exposed to the hazards via the release pathways, and determines the likelihood of the establishment and spread of the hazards. The likelihood of exposure depends on several factors relating to the

capacity of the disease agent or marine pest to survive in the environment, and for disease agents the availability of susceptible hosts, the ease of infection of susceptible hosts, and the likelihood of subsequent transmission of infection to others within a population. In determining the likelihood of establishment and spread, the following key factors were considered relevant:

- Availability and density of susceptible hosts (for diseases)
- Presence of competent vectors, alternate hosts or reservoir hosts
- Climatic and environmental suitability of receiving zone
- Likelihood of early detection/eradication
- Rate of transmission in a population
- Methods of establishment and spread.

Some additional considerations required for assessment of disease agents included:

1. *Route of Infection:* Viable disease agents must be ingested by a susceptible host or otherwise come into contact with susceptible host species. Infection may occur via the digestive tract, through direct contact with contaminated water via the gills or mantle cavity, and/or

2. *Infective Dose:* There must be sufficient quantities of viable disease agents to induce an infection following ingestion or contact with the disease agent.

Once a hazard is released into the environment, the chances of it surviving and/or infecting susceptible hosts and becoming established were expressed qualitatively using the likelihood estimations in Table 4, based on information from the scientific literature, unpublished data, as well as the professional judgment of the analyst. The likelihoods for the release and exposure assessments were combined using the matrix of 'rules' for combining descriptive likelihoods, to arrive at a likelihood of establishment and spread, as shown in Table 5.

Table 5. Matrix of rules for combining descriptive likelihoods for the release and exposure assessments to arrive at a likelihood of establishment and spread.

Likelihood of Release

Likelihood of exposure	**High**	**Moderate**	**Low**	**Very Low**	**Extremely low**	**Negligible**
High	High	Moderate	Low	Very Low	Extremely low	Negligible
Moderate	Moderate	Low	Low	Very Low	Extremely low	Negligible
Low	Low	Low	Very Low	Very Low	Extremely low	Negligible
Very Low	Very Low	Very Low	Very Low	Extremely low	Extremely low	Negligible
Extremely low	Extremely low	Extremely low	Extremely low	Extremely low	Negligible	Negligible
Negligible	Negligible	Negligible	Negligible	Negligible	Negligible	Negligible

The risk assessment for a particular hazard was concluded if the exposure assessment determined that the likelihood of establishment via all pathways was negligible (OIE 2019).

3.3 Consequence assessment

The consequence assessment estimates the likely magnitude of the consequences of establishment and/or spread of a hazard into a new region, including the possible effects of disease agents or marine pests on aquatic animals, the environment, industry and the economy. The qualitative terms that were used to describe the consequences of establishment of an unwanted disease agent in this RA are defined in Table 6.

Table 6. Definition of terms used to describe consequences of establishment of unwanted disease agents.

Consequence	Definition
Extreme	Establishment of a disease agent or marine pest would cause substantial biological and economic harm at a regional or even national level, and/or cause serious and irreversible harm to the environment.
High	Establishment of a disease agent or marine pest would have serious biological consequences (high mortality or morbidity) and would not be amenable to control or eradication. Such organisms would significantly harm economic performance at a regional level and/or cause serious environmental harm which is most likely irreversible.
Moderate	Establishment of a disease agent or marine pest would cause significant biological consequences and may not be amenable to control or eradication. Such organisms could harm economic performance at a regional level on an ongoing basis and/or may cause significant environmental effects, which may or may not be irreversible.
Low	Establishment of a disease agent or marine pest would have moderate biological consequences and would normally be amenable to control or eradication. Such organisms may harm economic performance at a local level for some period and/or may cause some environmental effects, which would not be serious or irreversible.
Very Low	Establishment of a disease agent or marine pest would have mild biological consequences and would be amenable to control or eradication. Such organisms may harm economic performance at a local level for a short period and/or may cause some minor environmental effects, which would not be serious or irreversible.
Negligible	Establishment of a disease agent or marine pest would have no significant biological consequences and would require no management. The organism would not affect economic performance at any level and would not cause any detectable environmental effects.

These descriptions are based on information available in other RAs (Jones and Stephens 2006, Biosecurity Australia 2009, Diggles and Arthur 2010, Diggles 2011a, 2017), the scientific literature, unpublished data, as well as the professional judgment of the analyst. For each hazard of concern, the consequence assessment determined the likelihood of occurrence and the associated impact for each of two main outbreak scenarios.

Either:

1. The disease agent or marine pest becomes established and spreads in the new jurisdiction/zone/bioregion. This scenario assumes that if a hazard were to establish it would eventually spread to its natural geographical limits,

OR;

2. An index case occurs (an animal becomes infected or a marine pest is introduced), but the hazard does not persist in the environment.

Only the first scenario will be considered to represent establishment of the disease agent or marine pest, because the second scenario would most likely go undetected. The risk assessment for a particular hazard was concluded if the consequence assessment determined that the consequences of introduction via all pathways are negligible (OIE 2019).

3.4 Risk estimation

Risk estimation is the final step involved with each assessment and was used to determine whether the extent of the unrestricted risk presented by each disease agent and marine pest to the aquatic animals, environment, industries and community of the new jurisdiction/zone/bioregion would be sufficient to require risk management. 'Unrestricted risk' means the estimated risk if the various hazards were to be translocated with no risk mitigation measures in place (Pathway 1). Risk was assessed using the risk estimation matrix in Table 7 which uses a combination of the qualitative answers given for the combined likelihoods of release and exposure and the significance of the consequences of establishment of a pest or disease agent to provide an estimate of the risk involved, ranging from 'negligible' through to 'extreme'.

The appropriate level of protection (ALOP) that was adopted in this RA was expressed in qualitative terms. as "**very low**" (annual probability between 1 in 20 and 1 in 100 years, see Table 4). This definition of ALOP, and its illustration by way of a risk estimation matrix, is shown in Table 7.

If either the combined likelihood of establishment and spread, or the significance of the consequences of establishment and spread of a hazard were considered negligible, extremely low or very low, the unrestricted risk would be within the ALOP, and there would be no need to implement any additional risk management steps. However, if the unrestricted risk estimation for any hazard was determined to be unacceptable (that is, above very low in the risk estimation matrix shown below), the threats posed by the disease or marine pest were ranked (extreme, high, moderate, low) based on estimates of the relative likelihood of establishment and spread into new regions (i.e. considering the number of pathways/mechanisms/risk factors for entry as well as other relevant aspects such as identity of the hazard, epizootiology, ecological and economic impacts, likely costs of eradication). For any hazards with risk estimation rankings that exceeded the ALOP, additional risk mitigation measures would be required to reduce the risk estimate back to within the ALOP (Table 7).

For the purpose of this risk analysis, to simplify interpretation all risk estimations that fall below the ALOP (green squares to the left of the ALOP in Table 7) were presented as "negligible risk" (i.e. annual probability of occurrence less frequent than 1 in 100 years). The risk estimation tables are colour coded for clarity, using

the following "traffic light" colour scheme: Extreme risk = purple, High risk = red, Moderate risk = orange, Low risk = yellow, at ALOP (i.e. Very low risk) = unshaded, or below ALOP = green (Table 7).

Table 7. Risk estimation matrix showing the ALOP utilized for this RA (white squares = very low risk). Any diseases which fall to the right of the ALOP during the RA will require additional risk management (yellow = Low risk, orange = Moderate risk, red = High risk, purple = Extreme risk).

Combined likelihood of release and exposure	Negligible	Very Low	Low	Moderate	High	Extreme
High	Negligible risk	Very low risk	Low risk	Moderate risk	High risk	Extreme risk
Moderate	Negligible risk	Very low risk	Low risk	Moderate risk	High risk	Extreme risk
Low	Negligible risk	Negligible risk	Very low risk	Low risk	Moderate risk	High risk
Very low	Negligible risk	Negligible risk	Negligible risk	Very low risk	Low risk	Moderate risk
Ext. Low	Negligible risk	Negligible risk	Negligible risk	Negligible risk	Very low risk	Low risk
Negligible	Negligible risk	Negligible risk	Negligible risk	Negligible risk	Negligible risk	Very low risk

Consequences of establishment and spread

3.5 Risk mitigation

For any disease agents or marine pests with risk estimation rankings that exceeded the ALOP of "Very low", additional risk mitigation measures would be required to reduce the risk estimate back to within the ALOP. In other words, if risk of exposure and establishment via a given pathway are considered to exceed an annual probability between 1 in 20 and 1 in 100 years (Table 4), risk mitigation methods will need to be identified to reduce the risk to a probability of occurrence less frequent than 1 in 100 years. These risk mitigation methods (see Section 5 for details) should form the basis of biosecurity protocols and standard operating procedures for mollusc shell recycling initiatives to reduce any such risks to acceptable levels. The additional risk mitigation processes examined as part of this RA process in Section 5 relate only to option evaluation together with an appraisal of the utility of each option for reducing risks to within the ALOP. The options presented in Section 5 could then form the basis of a consultation process that engages governments and shellfish reef restoration practitioners to evaluate the biosecurity risks involved with unrestricted pathways/mechanisms/risk factors for entry with a view towards identifying the most preferred mitigation option(s) that would reduce the risks identified to an acceptable level. The final risk management methods chosen may vary depending on a wide variety of pathway or environmental/jurisdiction-related factors.

4.0 Risk Assessment

4.1 Infection with AbHV-1 (Abalone viral ganglioneuritis, AVG)

4.1.1 Aetiologic agent: Abalone herpesvirus 1 (AbHV-1), also known as Haliotid herpesvirus 1 (HaHV-1), a double stranded DNA virus classified in the genus *Haliotivirus* (syn. *Aurivirus*) within the Family *Malacoherpesviridae* in the Order Herpesvirales (Savin et al. 2010, Adams et al. 2013). At least 6 different AbHV genotypes have been identified to date, including 5 strains (Vic1, Tas1, Tas2, Tas3 and Tas4) found so far in Australia, mostly in Tasmanian waters (Corbeil et al. 2014, 2016).

4.1.2 OIE List: Yes **NACA List:** Yes **Zoonotic:** No

4.1.3 Australia's status: At least 5 variants of AbHV-1 are known to occur in wild and cultured greenlip abalone (*Haliotis laevigata*) and blacklip abalone (*H. rubra*), as well as cultured hybrid *H. laevigata* × *H. rubra* abalone in Tasmania and Victoria (Corbeil et al. 2016, Caraguel et al. 2019). Outbreaks of AVG disease have been recorded in both wild and cultured abalone in Victoria, and in cultured and captive abalone in Tasmania, however to date AVG disease has not been observed in wild abalone in Tasmania.

4.1.4 Epizootiology

Abalone herpesvirus 1 (AbHV-1) was the second described member of the Family *Malacoherpesviridae* after Ostreid herpesvirus (OsHV-1) (see Savin et al. 2010). AbHV-1 is a highly virulent herpesvirus that was first encountered in Australia in late 2005 after outbreaks of abalone ganglioneuritis (AVG) disease in greenlip abalone (*Haliotis laevigata*), blacklip abalone (*H. rubra*) and hybrid abalone (*Haliotis laevigata* × *H. rubra*) farmed in four abalone aquaculture facilities (two land based farms in western Victoria near Portland and Port Fairy, and two sea based farms in Westernport Bay) in Victoria (Victoria DPI 2006, Hooper et al. 2007, Corbeil et al. 2010). Both land based farms pumped raw seawater into their facilities, through their tanks then back out into the ocean via settling ponds without treatment. The farm at Port Fairy continued to discharge water into the adjacent environment for several months during its initial disease outbreak (Hardy-Smith 2006, Gavine et al. 2009). Records show they did not cease discharge or destock until after a second, much larger scale disease outbreak occurred in late March 2006, after which diseased abalone were detected in wild abalone populations on reefs in the bay adjacent to that farm in early May 2006 (Victoria DPI 2006, Gavine et al. 2009). AVG then spread in waves radiating outward from the first affected bay in both easterly and westerly directions along the Victorian coast, initially at rates of up to 5-10 km/month (Prince 2007, Mayfield et al. 2011), significantly impacting wild abalone populations and substantially reducing commercial catches and recruitment in the wild fishery for many years afterwards (Mayfield et al. 2011, Conrad 2015). The fact that wild populations of surviving abalone in Victoria apparently have no resistance to AbHV-1 (Crane et al. 2013) suggests this epizootic was caused by the introduction of a new abalone pathogen into Victorian waters.

Spread of the disease to the first of the sea based farms in Westernport Bay in December 2006 was linked to movement of infected stock from one of the land based farms, however the 4th farm in Westernport Bay, located 640 metres away from the other sea based farm, became infected via the water column in late April 2006, and was depopulated by early May 2006 (Victoria DPI 2006, Hardy-Smith 2006, Gavine et al. 2009). In all cases infection with the virus was associated with inflammation and necrosis of neural ganglia (mainly

those in the cerebral and buccal regions), but also in nerve bundles and pleuropedal ganglia within the foot muscle, and this ganglioneuritis was associated with sudden high levels of mortalities up to 90% within 14 days of onset (Hooper et al. 2007). All sizes of abalone were affected and exhibited signs of disease including loss of righting reflex, swollen mouths, prolapse of the radula, and curling of the foot (Hooper et al. 2007).

Electron microscope and genetic studies confirmed the disease agent causing AVG was a neurotrophic herpes-like virus (Tan et al. 2008, Corbeil et al. 2010, Savin et al. 2010) closely related to herpesviruses responsible for mortalities in abalone in Taiwan (Chang et al. 2005, Corbeil et al. 2010, Savin et al. 2010). Indeed, in retrospect it appears very likely that the herpesvirus responsible for abalone viral mortality (a disease reported since the late 1990's) in China and Taiwan, originated from Australia and was translocated with the large volumes of live *H. laevigata* and *H. rubra* exported into those countries from Australia each year (Chen et al. 2012, OIE 2020c). This is suggested by the fact that the 99% genetic similarity between Australian and Taiwanese isolates of AbHV-1 is within the range of genetic variation evident between the 5 Australian strains (Vic1, Tas1, Tas2, Tas3 and Tas4) (Chen et al. 2012, Cowley et al. 2012, Corbeil et al. 2014, 2016).

In mid-2008, adult wild caught abalone sampled from a commercial land based processing facility in Tasmania that was recording low levels of mortality were positive for AbHV-1 by PCR at a prevalence of 39% (Crane et al. 2009, Corbeil et al. 2010). Further research found that the disease agent occurs naturally at very low prevalences (3 out of 1625 abalone = 0.18% prevalence) in latent, subclinical infections of wild populations of abalone in Tasmania (Corbeil et al. 2010, Ellard et al. 2011), however the sensitivity of the earlier AbHV-1 surveys was confounded by the fact that the PCR test used did not detect certain strain variants of the virus (M.J. Crane, personal communication, Corbeil et al. 2014, 2016). Also, latency characterised by low viral production and/or an abortive viral cycle is well recognised in aquatic herpesvirus infections (LeDeuff et al. 1996, Eide et al. 2011) and in such cases viral particles may not be sufficiently numerous to detect even using PCR (Batista et al. 2007). It is now known that several strains of AbHV (Tas1, Tas2, Tas3 and Tas4) are endemic in wild abalone populations in Tasmanian waters at low prevalences, and another strain of the virus (Vic1) was detected in the coastal waters of western Victoria at high prevalences during the AVG disease outbreak in wild abalone (Crane et al. 2009, Ellard 2011, Corbeil et al. 2010, 2014, 2016). Hence the prevalence of subclinical AbHV-1 infection in wild populations of abalone in Tasmania is likely to be somewhat higher than the earlier surveys suggest, with current scientific consensus suggesting that at least 4 AbHV-1 variants are endemic to Tasmanian waters, with recent work suggesting prevalence levels approximating 7-8% (Department of Agriculture 2014, Caraguel et al. 2019). AbHV-1 is only known to infect abalone at this time, and it has not been reported in wild abalone from any other regions of Australia, although it has been detected in onshore holding tanks in Western Australia following movements of abalone from Tasmanian processors (Ellard et al. 2011). Besides *H. laevigata* and *H. rubra* and their hybrids, other species of abalone known to be susceptible to disease caused by AbHV-1 include Australian brownlip abalone (*H. conicopora*) and several Asian abalone species (Corbeil et al. 2016, OIE 2020c), however it appears that the paua (*Haliotis iris*) from New Zealand is refractory to infection (Corbeil et al. 2017).

AbHV-1 is transmitted horizontally via the water or by contact with mucus trails of infected hosts and infection is by direct exposure of abalone to viral particles (Crane et al. 2009, Corbeil et al. 2012a, 2012b). Elevated water temperatures appear to be the major environmental risk factor associated with disease outbreaks due to herpesviruses in other molluscs (Elston 1997, Renault and Novoa 2004, Garcia et al. 2011, Arzul et al. 2017), and it is likely that rates of AbHV-1 replication increase with increasing water temperature (Corbeil et al. 2016). Other stressors such as reduced water quality, reduced oxygen levels and increased

production of metabolites such as ammonia are also more likely to occur when water temperatures are high. This suggests that in light of the continuing detection of sub-clinical AbHV infections in wild caught abalone held in live holding tanks at abalone processors at water temperatures >15°C (Ellard et al. 2011), the period of greatest risk of AVG outbreaks in abalone farms and holding facilities and in the wild is likely to be during late spring and over the summer months (Corbeil et al. 2016). Indeed, AbHV-1 was detected during AVG disease outbreaks in various abalone processing facilities in Tasmania in the summers of 2008 and 2009, with diseased abalone exhibiting 'hard foot' or tetany, excessive mucus production, abnormal spawning and bloating, but in these cases the outbreaks occurred within closed-loop systems and were therefore contained within the infected premises (Ellard 2011). However, in October/November 2010 outbreaks of AVG occurred in several Tasmanian processing facilities, including one in Bicheno where AbHV-1 contaminated effluent water was released into the environment within 100 metres of the intake of a nearby land-based abalone farm, resulting in an AVG disease outbreak in that farm in January 2011 (Ellard 2011, Ellard et al. 2011). The land based farm was issued with an order to cease discharge by the Tasmanian DPIWE and was emptied of all stock and disinfected (Ellard 2011, Ellard et al. 2011, Diggles 2011b).

Surveillance has shown that AbHV-1 is relatively common in Tasmanian abalone processing facilities (Ellard et al. 2011, Department of Agriculture 2014), suggesting that holding of wild caught abalone in processing plants is a high risk activity likely to stress wild abalone that are latently infected with AbHV-1. The stress of capture and holding increases viral replication and shedding in sub clinically infected wild abalone held at high densities, with the resulting AVG outbreaks quickly amplifying the disease agent to high levels in culture water (Corbeil et al. 2012a, 2012b, 2014). For these reasons, abalone live holding facilities pose a high risk of back spill of AbHV-1 infection of adjacent wild (or nearby cultured) abalone populations wherever untreated effluent water is discharged into the environment (Tasmania DPIW 2007, Ellard 2011, Ellard et al. 2011, Diggles 2011b). Because of this the Tasmanian Government now requires processors who hold abalone to treat their outgoing water so as to achieve a minimum 3 log10 reduction in heterotrophic bacterial levels, and maintain discharge bacterial concentrations of ≤ 999 bacterial colony forming units per mL (Baulch and Ellard 2011, Green 2011, Baulch et al. 2013).

4.1.5 Release assessment

While AbHV-1 infects abalone tissue and not shell material, it is likely that shells from wild and /or cultured abalone that may be procured from abalone processors or retail sale for recycling would retain some residual host tissue in areas such as foot tissue scars, mantle tissue and so on. However, being enveloped viruses, malacoherpesviruses are relatively fragile and hence susceptible to inactivation by high temperatures, chemicals and sunlight/UV irradiation (Renault 2016). It is known that AbHV-1 can survive outside the host in seawater for at least 24 hours at 15-25°C and 5 days at 4°C, but not 12 days at 4°C (Corbeil et al., 2012b), and the virus can be transmitted horizontally at least 640 metres (between two sea based farms in Westernport Bay, see Victoria DPI 2006, Hardy-Smith 2006, Gavine et al. 2009). However, the longevity of AbHV-1 particles inside tissues of dead hosts has not been determined. It is known that other malacoherpesviruses can survive in dead host tissues for longer than they can survive in seawater. For example, Hick et al. (2016) found that OsHV-1 µVar remained viable in seawater at 20°C for 2 days, but remained viable in wet or dry, non-living oyster tissues for at least 7 days at 20°C. Similar data is not available for AbHV-1, however, assuming that malacoherpesviruses in general can survive at least 3.5 times longer within dead host tissues as they can free in the water column, it appears possible from the results of Corbeil et al. (2012b) that AbHV-1 could also survive at least a week in dead host tissues at 15-25°C, and possibly over 17 days (but probably less

than 42 days) at 4°C. This suggests that in the absence of risk mitigation measures there is a significant risk of translocation of viable AbHV-1 in shells obtained from infected abalone.

There appears to be no information available to date on the resistance of AbHV-1 to UV irradiation. However, it is known that herpesviruses in general are susceptible to UV radiation (Wolff and Schneweis 1973, Kasai et al. 2005). In the case of other malacoherpesviruses, Schikorski et al. (2011b) demonstrated that OsHV-1 µVar was inactivated when exposed to an ultraviolet (UV) dose of 972 mJ/cm^2, while Hick et al. (2016) found that free OsHV-1 µVar virus particles in seawater were inactivated by 600 mJ/cm^2 UV irradiation. As for other treatments, Hick et al. (2016) found that OsHV-1 µVar was inactivated by heat (50°C for 5 min), sodium hydroxide (20g/L for 10 min), 0.1% available iodine for 5 min, 10% formalin (30 min), 1% Virkon for 15 min and a quaternary ammonium compound, but the virus survived treatment with chlorine at 200 mg/L for 15 minutes when protected within infected oyster tissues. In the case of treatments against AbHV-1, Corbeil et al. (2012b) found the virus survived treatment with chlorine bleach at 20 mg/L for 10 minutes, but was susceptible to 50 mg/L iodine (buffodine). These data suggest malacoherpesviruses are susceptible to UV irradiation, heat, and iodophors, but not chlorine/bleach, however there appears to be no information available on the susceptibility of these viruses to low salinity and acetic acid. Taking this information into account and considering the likely propagule pressure experienced via the various release pathways examined here, the likelihood estimations for the release of AbHV-1 via the identified release pathways are listed below.

Release assessment for infection with AbHV-1

	Unmitigated	Desiccation			Heat		Salinity	Other
Treatment	None	3 mo.	4 mo.	6 mo.	55°C >10 min	80°C >5 min	0 ppt for >24 hrs	4% acetic acid 30 min
Likelihood of release	High	E low?	Neg	Neg	E low?	Neg	High?	Low?

4.1.6 Exposure assessment

AbHV-1 is transmissible horizontally through the water by co-habitation with infected abalone with 100% mortality observed within 8 days (McColl et al. 2007, Crane et al. 2009). However, infection and establishment of AbHV-1 in new hosts occurs only if sufficient viable viral particles are introduced into an area where susceptible abalone are present (i.e. they receive an infective dose). Crane et al. (2009) used viral homogenates from clinically diseased abalone in a dilution series to find the LD50 by injection into the pedal muscle, and found the LD50 to be $10^{-6.39}$ of the stock solution, suggesting that AbHV is highly virulent for abalone. When AbHV-1 was added to the water, the LD50 increased to around 10^{-2} of the stock solution and after a 3 to 8 day prepatent period, death occurred over a period of 7 to 16 days (Crane et al. 2009). The minimum infective dose for successful horizontal transmission of AbHV-1 is not known, although AbHV-1 is highly virulent for abalone and a 1/100 dilution of viral homogenates from diseased abalone was sufficient to cause infection via the water route (Crane et al. 2009).

During an outbreak of AVG in captive abalone, levels of herpesvirus in the water can get very high because viral levels in infected molluscs can quickly increase to exceed 1 x 10^7 viral DNA copies per mg of tissue (see Corbeil et al. 2012a, 2012b). This means that a single abalone shell containing c. 1 grams of virus infected soft tissue could harbour 1 x 1000 x 1 x 10^7 = >1 x 10^{10} viral DNA copies, which (if the viral particles remain viable long enough to be transferred into the water with the shell during a reef restoration event) is

theoretically enough virus to kill around 1000 other abalone assuming a minimum lethal dose of 1 x 10^7 viral DNA copies per abalone (a likely lethal dose based on the results from Corbeil et al. (2012b)). This massive viral replication in diseased abalone explains why AVG spreads so rapidly in populations of confined abalone. Furthermore, all AbHV-1 variants tested to date (Vic1, Tas1, Tas2, Tas3 and Tas4) cause disease and mortality in all native abalone stocks tested (greenlip, blacklip and brownlip) (Corbeil et al. 2016) without any signs of resistance developing (Crane et al. 2013). These data demonstrate that AbHV is highly virulent, and that abalone can become infected via the water once exposed to relatively low concentrations of the virus (Corbeil et al. 2012a, 2012b). Together, these data suggest that if abalone shells infected with AbHV-1 were recycled and subsequently used as substrate during shellfish reef restoration projects without mitigation, they would pose a significant risk of infection of nearby abalone populations. There is already evidence that AbHV-1 has been spread by anthropogenic movements of infected molluscs into new locations (e.g. from Tasmania to Victoria). Given that various pathways exist to allow AbHV-1 to enter the environment through recycled abalone shells, environmental conditions are suitable for disease transmission and suitable hosts are likely to occur in the wild in areas where shellfish reefs are being restored, the overall likelihood of exposure and establishment of AbHV-1 via recycled mollusc shells is considered to be **High**.

4.1.7 Consequence assessment

Experience has shown that outbreaks of AVG disease due to AbHV-1 in wild abalone are not able to be controlled or eradicated, and that AVG epizootics can spread widely from their epicentre resulting in significant mortality, ecological damage and economic damage to aquaculture and abalone fisheries. Since AVG is a notifiable disease listed by the OIE and in all states of Australia, any introductions of AbHV-1 into new areas would also significantly impact trade. Taking these factors into consideration, the consequences of establishment of AbHV-1 in wild abalone near shellfish restoration sites are considered to be **High.**

4.1.8 Risk estimation

The risk estimation for AbHV-1 is determined by combining the likelihood of release and exposure (from Table 5) with the consequences of establishment (Table 6) to arrive at a risk estimation (Table 7). The risk estimate for AbHV-1 exceeds the ALOP for some release pathways, suggesting that additional risk management is required for this disease agent.

Risk estimate for infection with AbHV-1

	Unmitigated	Desiccation			Heat		Salinity	Other
Treatment	None	3 mo.	4 mo.	6 mo.	55°C >10 min	80°C >5 min	0 ppt for >24 hrs	4% acetic acid 30 min
Combined likelihood of release and exposure	High	E low?	Neg	Neg	E low?	Neg	High?	Low?
Consequences of establishment	High	High	High	High	High	High	High	High
Risk estimation	High	V low?	Neg	Neg	V low?	Neg	High?	Mod?

4.2 Iridoviroses of molluscs

4.2.1 Aetiologic agent: Iridoviroses are infections of molluscs caused by iridoviruses, such as gill necrosis virus (GNV), haemocyte infection virus (HIV) and oyster velar virus (OVV), which are large (220-380 nm) icosahedral double stranded DNA viruses with affinities with the Family *Iridoviridae*.

4.2.2 OIE List: No **NACA List:** No **Zoonotic:** No

4.2.3 Australia's status: Not recorded. Iridoviruses have not been reported from molluscs in Australia to date, and are considered exotic.

4.2.4 Epizootiology

Iridoviruses have been reported in diseased bivalve molluscs in various parts of the northern hemisphere. These viruses are easily recognised by their icosahedral shape, relatively large size and distinctive mode of replication (Elston 1997). The first instance of iridoviral infection of oysters was a disease of adult Portuguese oysters (*Crassostrea angulata*) called "Maladie des branchies" (gill necrosis), which first emerged in the mid 1960s in France (Comps 1969), and was caused by gill necrosis virus (GNV). GNV was associated with mortalities of around 70% of *C. angulata* in France during the summer of 1967, with gross signs of the disease including the appearance of necrotic yellow lesions on the gills and labial palps (Comps 1969, Elston 1997, Renault and Novoa 2004). The yellow lesions increased in size and developed brown centres as the tissue died, leaving a hole in the gill structure surrounded by a massive haemocytic infiltration (Comps 1988), often leading to complete destruction of the gills (Renault and Novoa 2004). Histopathology found basophilic inclusions in the cytoplasm of enlarged necrotic cells, while electron microscopy found numerous large icosahedral viral particles (350-380 nm capsid diametre) scattered throughout the cytoplasm of large polymorphic cells and necrotic gill cells (Comps 1988). In 1968, Portuguese oyster populations in France began to show signs of recovery from the GNV outbreak, however in the summer of 1970, epizootic mortalities of 90% of oysters were again reported in French *C. angulata*, this time associated with the detection of another irido-like virus which was named haemocyte infection virus (HIV) (Comps 1988). The HIV agent had a similar icosahedral viral morphology, but exhibited different pathological effects, affecting mainly haemocytes instead of the gills. HIV-affected Portuguese oysters exhibited atrophy and weakness of the adductor muscle; but no other clinical signs were noted (Comps 1988). The most characteristic pathological change was an acute inflammatory response associated with the presence of atypical virus infected haemocytes in the connective tissue and an increase in the number of brown cells (Comps 1988, Grizel and Héral 1991). The disease remained enzootic until 1973 and led to the virtual disappearance of the Portuguese oyster, driving that species to commercial extinction in French coastal waters (Comps 1969, 1988, Grizel and Héral 1991).

When Pacific oysters (*Crassostrea gigas*) were introduced into France to replace *C. angulata* in the early 1970's, they appeared largely resistant to the iridovirus infections as well as *Marteilia refringens*, however a virus similar to GNV was reportedly associated with sporadic disease in *C. gigas* populations in the Bay of Arcachon long after the disappearance of the Portuguese oyster (Comps and Bonami 1977). It has been

speculated that the most likely source of the original GIV infections may have been from vertical transmission following importation of *C. angulata* adults from Portugal (Grizel and Héral 1991).

Then, in the late 1970's, a new disease was reported from hatchery-reared larval Pacific oysters (*C. gigas*) from Washington State on the west coast of North America (Elston 1979, Elston and Wilkinson 1985). The disease was due to lesions causing necrosis and detachment of the velum once pediveliger larvae cultured at 25 to 30°C for around 10 days had reached around 150-170 µm shell length. Investigations using electron microscopy found the affected (rounded and necrotic) epithelial cells on the velum and oesophagus had numerous icosahedral viral particles (230 nm capsid diametre) in the cytoplasm (Elston 1979). Viral inclusion bodies (1.2 to 2.4 µm in diametre) within the cytoplasm of infected cells were basophilic using haematoxylin and eosin histopathology, feulgen positive and stained green with acridine orange, indicating the presence of viral DNA (Elston and Wilkinson 1985). Infected cells were hypertrophied and devoid of cilia, leading to reduced larval locomotory activity and causing the "blebs" or "blisters" on the velum which characterise the gross appearance of this disease (Elston and Wilkinson 1985, Renault and Novoa 2004). Due to the involvement of the velum as the main target organ for viral infection (which usually resulted in complete sloughing of the velar epithelial cells), the disease was called oyster velar virus disease (OVVD). Over an 8-year period, losses of up to 50% of hatchery production during the April to May spawning period were attributed to OVVD (Elston and Wilkinson 1985). The seasonal nature of the disease outbreaks (spring) suggested that the virus had some reservoir host (most likely adult oysters) which allowed it to reinfect the oyster larvae each year (Elston and Wilkinson 1985). However, the virus was never visualized in post-metamorphic juvenile or adult oysters using electron microscopy (Elston 1997). Isolation of larvae from water containing broodstock and destruction of infected larvae and broodstock and sterilization of tanks and equipment were considered to be the main steps available to reduce the risk of OVVD outbreaks (Elston and Wilkinson 1985). As previously occurred for the iridoviral agents that infected *C. angulata* in France, disease outbreaks in *C. gigas* on the west coast of North America due to iridoviral disease agents apparently ceased and have no longer been reported in the scientific literature for many years, despite the development of iridovirus -specific molecular diagnostic tools (Sudthongkong et al. 2002) which have been used to screen for OVVD during mass mortality events in bivalve species around the world (Kim et al. 2019).

4.2.5 Release assessment

Iridoviruses have not been recorded from Australian molluscs and are considered exotic, however they are included in this risk analysis as they are listed as notifiable disease agents that could cause serious harm if they were introduced at some point in the future. Little is known regarding the epidemiology of iridoviral infections of molluscs. From descriptions of the earlier disease outbreaks, however, it is evident that transmission occurred horizontally through the water via exposure to infective viral particles shed by nearby infected hosts, while vertical transmission from broodstock oysters was also suspected (Elston and Wilkinson 1985, Grizel and Héral 1991). Much more is known about iridoviral infections of finfish, such as red seabream iridoviral disease (RSIVD), epizootic haematopoietic necrosis virus (EHNV) and infectious spleen and kidney necrosis virus (ISKNV), for which experimental studies have shown horizontal transmission occurs via cohabitation with infected hosts and contact with free viral particles in water (Go and Whittington 2006, Whittington et al. 2010, Rimmer et al. 2017). For iridoviral infections of fish, the unenveloped iridovirus particles are known to be quite resistant and can remain viable in the water outside the host for some time, at least 48 hours at 25°C in the case of ISKNV (Fusianto et al. 2019), while Langdon (1989) found that EHNV was extremely resistant to desiccation, retaining infectivity in dried tissue culture medium after 113 days (3.8

months), but not after 200 days (6.5 months) at 15°C. Iridoviral agents infecting finfish can also remain viable in frozen muscle tissue for extended periods (OIE 2020b) whilst inactivation of ISKNV occurs following heating to 65 °C for 20 min, exposure to pH 3 or pH 11 for 30 min, exposure to 1% Virkon™ for 10 min; exposure to 1000 ppm sodium hypochlorite for 30 min, and 650 mg/L benzalkonium chloride for 10 min (Fusianto et al. 2019). For the purposes of this RA, it will be assumed that iridoviruses that infect molluscs will have similar inactivation characteristics to those infecting finfish.

Hine and Thorne (2000) found virus-like inclusions in 1 out of 769 *Saccostrea cucullata* sampled from the Exmouth Islands, Western Australia (prevalence 0.13%). The inclusions reported by Hine and Thorne (2000) were intranuclear, however, unlike the cytoplasmic inclusions found in oysters infected with GNV, HIV and OVVD iridoviruses. This suggests that the inclusions reported by Hine and Thorne (2000) were instead related to other groups such as the intranuclear papova-like viruses or herpesviruses (Elston 1997). So, in summary, iridoviruses are not known to occur in Australian molluscs, but unidentified virus-like agents are known to occur in several mollusc species in Australia and thus the presence of iridoviruses cannot be ruled out at this time. Furthermore, it appears possible that exotic iridoviruses could be introduced into Australia via infected hosts translocated in ballast water, or as biofouling on the hulls or sea chests of commercial shipping, fishing vessels and pleasure craft (Howard 1994, Deveney et al. 2017), or in imported mollusc products used as bait or burley (Diggles 2017). Outbreaks of iridovirus disease in molluscs occur in the summer months (Renault and Novoa 2004), usually when water temperatures are 25°C or higher (Elston 1979, Elston and Wilkinson 1985), suggesting that conditions in many parts of Australia would be permissive for transmission of iridoviral infections either seasonally or year round. Taking this information into account and considering the likely propagule pressure experienced via the various release pathways examined here, the likelihood estimations for the release of iridoviruses via the identified release pathways are listed below.

Release assessment for iridoviroses of molluscs

	Unmitigated	Desiccation			Heat		Salinity	Other
Treatment	None	3 mo.	4 mo.	6 mo.	55°C >10 min	80°C >5 min	0 ppt for >24 hrs	4% acetic acid 30 min
Likelihood of release	Moderate	Low	V low	E low	V low	Neg	Mod	V low

4.2.6 Exposure assessment

While iridioviruses infect oyster tissue and not shell material, it is likely that shells from wild and /or cultured oysters procured from oyster processors or retailers for recycling would retain some residual host tissue in areas such as retractor muscle scars, mantle tissue and so on. The survival of mollusc iridoviruses in dead host tissue is unknown, as is the minimum infective dose required for their transmission horizontally via the water to new hosts, however it is known that some other aquatic iridoviruses are extremely resistant to desiccation (Langdon 1989). On the other hand, it appears that iridoviruses that infect bivalve molluscs have relatively high host specificity (Comps and Bonami 1977, Grizel and Héral 1991), hence it is unclear whether introduced iridoviruses released via various pathways could come in contact with susceptible host species. Nevertheless, environmental conditions in many parts of Australia would appear suitable for transmission and establishment of iridoviruses, hence infection could occur if an infective dose was introduced into an area where susceptible hosts were present. Given that various pathways exist to expose shellfish reef restoration projects in Australia

to iridoviruses via recycled oyster shells, environmental conditions are suitable for disease transmission and suitable hosts may occur in the wild in areas where shellfish reefs are being restored, the risk of exposure and establishment is non-negligible, and the overall likelihood of exposure and establishment of iridoviruses via recycled mollusc shells is considered to be **Low.**

4.2.7 Consequence assessment

The environmental impacts of introduction of iridoviruses are difficult to determine, however establishment of iridoviruses usually results in mass mortalities during larval rearing or growout of cultured oysters. This indicates that establishment of mollusc iridoviruses in new geographical areas could potentially result in significant bottlenecks in hatchery production which could affect oyster aquaculture industries as well as oyster reef restoration activities. Iridoviruses of molluscs are not listed by the OIE or NACA, hence the presence of iridovirus in wild or cultured oysters would be unlikely to have significant international trade implications. Nevertheless, because these viruses are listed as notifiable disease agents under official control in Australia, an iridovirus outbreak in an oyster farm or hatchery in Australia would require intervention by government authorities. This would likely cause disruption to the affected facilities and could affect normal trade in mollusc commodities by commercial aquaculture and fisheries, mollusc gathering by recreational fishers and shellfish reef restoration as authorities to try to limit potential spread into uninfected areas. Unless these viruses were detected in an enclosed system, there would appear to be little chance of eradication, which could have devastating effects if wild oysters are susceptible (e.g. *C. angulata* in France). However, the impacts of these disease agents lasted only around a decade both in France and along the west coast of the USA, and there may be opportunities for hatchery breeding programmes to select hosts for resistance. Taking all of these factors into consideration, the consequences of establishment of iridoviruses via recycled mollusc shells are considered to be **Moderate.**

4.2.8 Risk estimation

The risk estimation for iridoviroses is determined by combining the likelihood of release and exposure (from Table 5) with the consequences of establishment (Table 6) to arrive at a risk estimation (Table 7). The risk estimate for iridoviroses of molluscs exceeds the ALOP for some release pathways, suggesting that additional risk management is required for these disease agents.

Risk estimate for iridoviroses of molluscs

	Unmitigated	Desiccation			Heat		Salinity	Other
Treatment	None	3 mo.	4 mo.	6 mo.	55°C >10 min	80°C >5 min	0 ppt for >24 hrs	4% acetic acid 30 min
Combined likelihood of release and exposure	Low	V low	V low	E low	V low	Neg	Low	V low
Consequences of establishment	Moderate	Mod.	Mod.	Mod.	Mod.	Mod.	Mod.	Moderate
Risk estimation	Low	V low	V low	Neg	V low	Neg	Low	V low

4.3 Infection with OsHV-1-µVar (Pacific oyster mortality syndrome, POMS)

4.3.1 Aetiologic agent: Infection with a microvariant of Ostreid Herpesvirus 1 (OsHV-1 µVar), a double stranded DNA virus classified in the genus *Ostreavirus* within the Family *Malacoherpesviridae* in the Order Herpesvirales (Davison et al. 2005, 2009). OsHV-1 µVar is a genetic microvariant of OsHV-1 defined in part on the basis of partial sequence data exhibiting a systematic deletion of 13 bp in a microsatellite zone (C region) of the Open Reading Frame 4 (ORF4) of the genome (Segarra et al. 2010).

4.3.2 OIE List: Yes **NACA List:** No **Zoonotic:** No

4.3.3 Australia's status: OsHV-1 µVar was first recorded in Australia in Pacific oysters (*Crassostrea gigas*) in Woolooware Bay, Georges River estuary in Botany Bay in late November 2010 (AusVet 2011). The disease was also detected in *C. gigas* in the Paramatta River, Sydney Harbour in January 2011 (Jenkins et al. 2013), and then was spread 50 km north to the Hawkesbury River in January 2013 causing severe mortalities (Paul-Pont et al. 2014). Then in late January 2016 OsHV-1 µVar caused mass mortalities of cultured *C. gigas* in Tasmania (de Kantzow et al. 2017), followed by mortalities of feral *C. gigas* in the Port River, Adelaide in February 2018 (Deveney et al. 2019). There is evidence that the virus is spread within Australia by biofouling on domestic shipping (Deveney et al. 2017).

4.3.4 Epizootiology

In 1991, outbreaks of disease in Pacific oysters *Crassostrea gigas* due to herpes-like viruses were reported in France (Nicolas et al. 1992) and New Zealand (Hine et al. 1992). Subsequently a virus isolate obtained during a disease outbreak in *C. gigas* larvae in France in 1995 was characterised as a member of the Herpesviridae family and named Ostreid herpesvirus 1 (OsHV-1) (Davison et al. 2005). This virus was later reclassified in the Order Herpesvirales as the first member of the Family *Malacoherpesviridae* (Davison et al. 2009). Several genetic variants of OsHV-1 have continued to be associated with disease during the summer months in larval and juvenile *C. gigas* and a range of other bivalves around the world (Renault et al. 2001, Arzul et al. 2001a,b,c, Renault and Novoa 2004, Batista et al. 2007, Garcia et al. 2011, Renault 2011, Martenot et al. 2011, 2015, Keeling et al. 2014, Mineur et al. 2015, Barbieri et al. 2019, Kim et al. 2019). One variant of OsHV-1 (OsHV-1 var) was found in *C. gigas*, *R. philippinarum* and *P. maximus* in various French mollusc hatcheries (Arzul et al. 2001a,b,c). This OsHV-1 variant was characterized by a nucleotide deletion of 2.8 kb in an inverted repeat region compared to the reference genome, a deletion of 200 bp and an insertion of 27 bp in the C region encoding two unknown proteins (Arzul et al. 2001a,b). A few years later, a new highly virulent genetic microvariant of OsHV-1 (OsHV-1 µVar) was associated with particularly severe mortalities of *C. gigas* in France in 2007 and 2008 (Sauvage et al. 2009, Segarra et al. 2010, Renault 2011, Martenot et al. 2011, AusVet 2011). The µVar strain of OsHV-1 was defined on the basis of partial sequence data exhibiting a systematic deletion of 13 bp in a microsatellite zone of the open reading frame 4 (ORF 4) of the genome (Segarra et al. 2010, EFSA 2010, Martenot et al. 2011). Both OsHV-1 types (reference and µVar) were detected during the 2008 epizootics in France, but only the µVar strain was detected in 2009 (Renault 2011, Schikorski et al. 2011b). The µVar strain was not found in samples archived from 1995-2007 in France or the USA, all of which contained only OsHV-1 reference strain (Segarra et al. 2010). Furthermore, a sample collected in China in 2002, presented the main deletion typical of the OsHV-1 µVar as well as all other

polymorphisms reported for the µVar strain from France, except for two substitutions in ORF 4 and 100% homology with the OsHV-1 reference strain in ORF 43 (Segarra et al. 2010). More recent phylogeographic analyses suggest that the variants of OsHV-1 that were originally described from France probably originated from East Asia, where the greatest range of genotypes for these viruses are found in several bivalve species (Bai et al. 2015, 2016, 2017, 2019, Mineur et al. 2015, Xia et al. 2015). Indeed, the variant that emerged in New Zealand in late November 2010 (OIE 2010a,b,c, OIE 2011a,b, Johnston et al. 2011, Muellner et al. 2011) was genetically grouped with strains present in Japan at that time, suggesting spread via international shipping (Renault et al. 2012, Bai et al. 2015, Arzul et al. 2017). This mode of viral introduction would follow that of its host, as *C. gigas* was first recorded in New Zealand in 1971, after presumably being introduced via ballast water or as hull fouling on ships (Dinamani 1971, Smith et al. 1986, Deveney et al. 2017). Given the gene sequence of the first Australian OsHV-1 µVar isolates were closest to isolates from New Zealand and Japan (Jenkins et al. 2013), it seems probable that the first outbreak of Pacific oyster mortality syndrome (POMS) in Botany Bay/Sydney Harbour from late November 2010 onwards (OIE 2011c,d, Frances et al. 2011, AusVet 2011) was also due to introduction of OsHV-1 µVar via biofouling on shipping originating from Asia, or New Zealand.

The outbreak of POMS in *C. gigas* in Woolaware Bay, Georges River Estuary (Botany Bay), New South Wales began in late November 2010 (OIE 2011c,d, Frances et al. 2011, AusVet 2011). There, unhealthy cultured *C. gigas* were observed after environmental stress from exposure to a plume of discoloured water following rainfall. The presence of OsHV-1 µVar in diseased oysters was confirmed by PCR testing, while histological lesions were non-specific and viral particles could not be detected by transmission electron microscopy (OIE 2011c,d). Mortality in the affected *C. gigas* spat (2.2 mm) was 100%, 95% for market sized oysters, and die offs of wild *C. gigas* in the nearby area were also observed (OIE 2011c,d). Die offs of wild *C. gigas* in the Paramatta River, Sydney Harbour associated with OsHV-1 µVar infection were also observed in January 2011 (AusVet 2011). In contrast, mortalities were not observed in other mollusc species, including nearby Sydney rock oysters (*Saccostrea glomerata*) (OIE 2011c,d), even though OsHV-1 µVar DNA was detected in asymptomatic *S. glomerata* and several other invertebrates using PCR (Frances et al. 2011, Jenkins et al. 2013, Evans et al. 2017a). Within 2 years the disease had been spread 50 km north causing epizootics in *C. gigas* cultured in the Hawkesbury River in January 2013 (Paul-Pont et al. 2014), while in January 2016 OsHV-1 µVar was spread to Tasmania causing mass mortalities of *C. gigas* near Hobart (de Kantzow et al. 2017). The fact that *C. gigas* infected with OsHV-1 µVar were detected in South Australia on barges originating from NSW (Deveney et al. 2017), then the virus subsequently became established and caused mortalities in feral populations of *C. gigas* in the Port River, Adelaide in February 2018 (Whittington et al. 2018, Deveney et al. 2019) re-emphasises the high risk of movement of this disease agent via biofouling on shipping. Once introduced into an area, there is evidence that OsHV-1 and its microvariants have also been translocated with movements of covertly infected bivalve seed spat (e.g. EFSA 2010, Dundon et al. 2011).

While the main host species for OsHV-1 µVar worldwide is *C. gigas*, various other species of bivalves are known to be susceptible to this variant. Indeed, unlike herpesviruses from vertebrate hosts, herpesviruses in bivalve molluscs appear less host specific (Bai et al. 2019). The OsHV-1 µVar has also been detected in *Crassostrea angulata*, mussels (*Mytilus edulis* and *Mytilus galloprovincialis*), clams (*Donax trunculus*) and diseased scallops (*Argopecten irradians*) (see Martenot et al. 2015, Bai et al. 2016, Kim et al. 2019). Many species of bivalves may therefore be susceptible to disease or act either as true carriers, or mechanical vectors and/or reservoirs of infection, including cupped oysters like *S. glomerata* (see Frances et al. 2011, Evans et al.

2017a), clams, and scallops as well as blue mussels (*Mytilus galloprovincialis*) and hairy mussels (*Trichomya hirsuta*) (Meyers et al. 2009, Burge 2010, EFSA 2010, Burge et al. 2011, Bai et al. 2015, Evans et al. 2017a).

All life history stages of *C. gigas* appear susceptible to infection with OsHV-1 µVar, including planktonic larvae, spat (settled oysters < 5 grams in weight), juveniles (oysters 5-40 grams in weight) and adults (oysters over 40 grams in weight). Susceptibility to disease associated with OsHv-1 µVar decreases with increasing oyster size, as spat and early juvenile oysters suffer proportionally much higher mortality rates compared to adult oysters (Paul-Pont et al. 2014, Whittington et al. 2015b, de Kantzow et al. 2016, 2017, Hick et al. 2018). Diseased *C. gigas* larvae infected with herpes-like viruses cease feeding, growth becomes retarded, and they exhibit velar lesions (detached parts of the velum are observed free in the water). Acute mortalities generally begin to occur between days 4 and 8 post-fertilization (Hine et al. 1992, Nicolas et al. 1992, LeDeuff et al. 1996), with mortalities up to 100% occurring within 11 to 14 days of fertilization (Hine et al. 1992). Larvae of flat oysters (*Ostrea chilensis*) may be infected with herpes-like viruses as little as 4 hours after exiting the brood pouch, with abundant virogenesis evident in several organs within 2 days and mass mortalities occurring in 3 - 5 days at 16 to 18°C (Hine et al. 1998). In New Zealand prevalence and intensity of OsHV-1 µVar in farmed *C. gigas* spat and juveniles increased to detectable levels 5 days after being stocked, with mortality commencing at day 6 and continuing till day 15 post stocking (Johnston et al. 2011).

Healthy juvenile and adult *C. gigas* can be asymptomatic carriers of herpes-like viruses and in these cases infected oysters may display no clinical or pathological signs of disease (Hine and Thorne 1997, Arzul et al. 2002, Dundon et al. 2011, Evans et al. 2017b). Indeed, viruses in the Family *Herpesviridae* are known to persist in their host in asymptomatic latent infections, with virus expression and active replication being associated with exposure of carrier hosts to stressful conditions (Eide et al. 2011). Latency is characterised by low viral production and/or an abortive viral cycle (LeDeuff et al. 1996, Eide et al. 2011), and in such cases viral particles may be too few to detect using TEM (Arzul et al. 2002, OIE 2011c,d) or even by cPCR (Batista et al. 2007). The main pathological changes sometimes observed in *C. gigas* larvae infected with herpes-like viruses are abnormal basophily of connective tissue cells, and presence of hypertrophic nuclei with unusual marginated chromatin (Nicolas et al. 1992, Renault et al. 1994, LeDeuff et al. 1996). Cowdry type A-like intranuclear inclusions within tissues and haemocytes infected with herpes-like viruses have been recorded in larval (Hine et al. 1992) and juvenile *C. gigas* (see Meyers et al. 2009), but histopathological lesions may not be present in acutely infected spat, juvenile or adult oysters (Friedman et al. 2005). The development of sensitive molecular tests has confirmed that herpesvirus DNA can be present in apparently healthy spat, juvenile and adult oysters in the absence of clinical signs or pathological lesions (Arzul et al. 2002), which is why sensitive and specific molecular diagnostic techniques (cPCR, qPCR) are required for confirmatory diagnosis (Renault et al. 2000b, Batista et al. 2007).

There is evidence that OsHV-1 can be transmitted both horizontally through the water and vertically between generations. Vertical transmission or pseudo-vertical transmission of OsHV-1 is known to occur (Hine et al. 1998, Arzul et al. 2002) and was demonstrated by the persistence of the virus in 3 successive generations of *C. gigas* (Barbosa-Solomieu et al. 2005). Viral DNA was detected by cPCR in 2-day-old larvae, indicating that, even if "true" trans-ovum vertical transmission does not occur, infection of progeny takes place very soon after fertilization (Barbosa-Solomieu et al. 2005). On the other hand, Barbosa-Solomieu et al. (2005) noted that the detection of viral DNA in parental oysters did not necessarily correspond to a productive infection or result in

successful transmission to the progeny - infected parents did not always produce infected larvae, and failure to detect OsHV-1 amongst parents did not always correspond to production of non-infected larvae.

Outbreaks of disease due to OsHV-1 occur almost exclusively during hotter summer periods when water temperatures exceed 16-19°C (LeDeuff et al. 1996), leading to OsHV-1 outbreaks becoming synonymous with "summer mortality" events in *C. gigas* in many parts of the world (Renault and Novoa 2004, Friedman et al. 2005, Burge et al. 2006, EFSA 2010, Schikorski et al. 2011b). Mortality events have been observed at seawater temperatures of 16–24 °C in Europe (Petton et al. 2013, Renault et al. 2014), and at 19–24 °C in Australia (de Kantzow et al. 2016, Whittington et al. 2019), however high water temperatures in the absence of the virus do not induce mortality (Dégremont et al. 2010b). Higher water temperatures induce expression of disease by promoting viral replication with the 1-2 day delay often observed between a temperature increase and mortality reflecting the time required for the virus to initiate an intense replication phase in latently infected oysters, after which a rapid spread of the disease and mortality occurs (Sauvage et al. 2009). The evidence suggests that OsHV-1 is a necessary cause of oyster mortality, but may not be sufficient by itself to cause mass mortality without other contributing factors, chiefly elevated water temperatures (EFSA 2010).

Production of specific pathogen free spat and development of OsHV-1 resistant oyster populations appear to be the most promising methods for facilitating bivalve aquaculture in regions where OsHV-1 is enzootic (Renault 2011, Dégremont 2011, AusVet 2011, Whittington et al. 2019). French researchers have shown that resistance against summer mortality is highly heritable (Dégremont et al. 2007, 2010a, 2010b, Dégremont 2011). The virus can be excluded from aquaculture establishments by aging water for over 48 hours or filtration to less than 5µm (Whittington et al. 2015a), further suggesting that it is particle or vector associated in the wild (Evans et al. 2016, Whittington et al. 2018, Liu et al. 2020).

4.3.5 Release assessment

It is known that OsHV-1 µVar occurs in most of Australia's main Pacific oyster production areas including some estuaries in NSW (Botany Bay, Sydney Harbour, the Hawkesbury River), Tasmania and the Port River in South Australia (AusVet 2011, Jenkins et al. 2013, Paul-Pont et al. 2014, de Kantzow et al. 2017, Deveney et al. 2019). It is also known that *S. glomerata* in NSW are susceptible to infection and are a potential vector that could translocate OsHV-1 µVar into new regions (Frances et al. 2011, Jenkins et al. 2013, Evans et al. 2017a). The identity of the intranuclear virus-like inclusions found by Hine and Thorne (2000) in 1 out of 769 *Saccostrea cucullata* sampled from the Exmouth Islands, Western Australia (prevalence 0.13%) remains to be determined. Given their location in the affected cells, it is possible that the viral inclusions reported by Hine and Thorne (2000) are due to herpesviruses, but they could also be related to other groups such as the intranuclear papova-like viruses (Elston 1997). This information suggests that Pacific oyster shells as well as other oyster species obtained from oyster processors or retailers in Australia for recycling are likely to have been exposed to OsHV-1 µVar.

Herpesviruses have a lipid-containing envelope, so OsHV-1 variants are likely to be relatively fragile and less resistant to inactivation compared to some other viruses of aquatic animals (Renault 2011). Hick et al. (2016) found that OsHV-1 µVar remained infectious in seawater for only 2 days at 20°C, but in contrast the virus remained viable in wet or dry, non-living oyster tissues for at least 7 days at 20°C. Hick et al. (2016) also found that free OsHV-1 µVar virus particles in seawater were inactivated by 1% Virkon after 15 min, but the

virus survived treatment with chlorine at 200 mg/L for 15 minutes when protected within infected oyster tissues. OsHV-1 µVar was also inactivated by heat (50°C for 5 min), sodium hydroxide (20g/L for 10 min), 0.1% available iodine for 5 min, 10% formalin (30 min) and a quaternary ammonium compound (Hick et al. 2016). Herpesviruses are known to be susceptible to UV radiation (Wolff and Schneweis 1973, Kasai et al. 2005) and Schikorski et al. (2011b) demonstrated that OsHV-1 µVar was inactivated when exposed to a very high ultraviolet (UV) dose of 972 mJ/cm^2, while Hick et al (2016) found it was also inactivated by a lower UV dose of 600 mJ/cm^2. OsHV-1 survives freezing at -20°C for at least 2 days (Schikorski et al. 2011b), however, successive freezing/thawing cycles reduced OsHV-1 DNA detection by PCR (Vigneron et al. 2004), suggesting that OsHV-1 is damaged by freeze/ thawing. Taking this information into account and considering the likely propagule pressure experienced via the various release pathways examined here, the likelihood estimations for the release of OsHV-1 µVar via the identified release pathways are listed below.

Release assessment for infection with OsHV-1 µVar

	Unmitigated	Desiccation			Heat		Salinity	Other
Treatment	None	3 mo.	4 mo.	6 mo.	55°C >10 min	80°C >5 min	0 ppt for >24 hrs	4% acetic acid 30 min
Likelihood of release	High	E low	Neg	Neg	Neg	Neg	High	V low

4.3.6 Exposure assessment

While OsHV-1 µVar infects oyster tissue and not shell material, it is likely that shells from wild and /or cultured oysters that may be procured from oyster processors or retailers for recycling would retain some residual host tissue in areas such as retractor muscle scars, mantle tissue and so on. Given that OsHV-1 µVar can survive in dead host tissue for at least 7 days at 20°C, viral particles would highly likely be present in residual host tissues if recycled oyster shells were placed back in the water. Herpes-like viral diseases of bivalves can be transmitted by cohabitation with conspecifics and other species of bivalves (Hine et al. 1998, Arzul et al. 2001a,b, Schikorski et al. 2011a,b). The route of infection by this method is horizontal via the water as viral particles are detectable in water surrounding infected bivalves (Vigneron et al. 2004). The minimum infective dose required for horizontal transmission via the water is not known, though the 1 x 10^8 viral DNA copies/L in the seawater used in the cohabitation experiments of Schikorski et al. (2011a) was calculated by those authors to equate to an infective dose of 2.5 x 10^7 viral DNA copies per oyster, while a dose of 1.5 x 10^7 viral DNA copies per oyster was used to successfully transmit OsHV-1 µVar via intramuscular injection (Schikorski et al. 2011a,b).

The viral burden in moribund *C. gigas* juveniles ranges from 3 x 10^7 to 1 x 10^8 viral DNA copies per mg of adductor muscle, mantle or digestive gland (Sauvage et al. 2010, Burge et al. 2020). If 5 grams (5 x 10^3 mg) of infected oyster tissue were retained on an oyster shell, this equates to 3 x 10^{10} to 5 x 10^{11} viral DNA copies per moribund oyster, which (assuming a minimum lethal dose of 1-2 x 10^7 viral DNA copies per oyster, see Schikorski et al. 2011a,b) means that residual tissue on the shell of one moribund *C. gigas* theoretically contains sufficient virus to provide infective doses for 1500 to 20000 other *C. gigas*, assuming the viral particles remain viable long enough to be transferred to the water after the death of the host. Water chemistry influences viability with higher alkalinity reducing viral survival (Burge et al. 2020). Nevertheless, this massive viral replication explains why the virus can cause acute mass mortalities when susceptible bivalves

are held at high density (Renault 2011). In contrast, for OsHV-1 and OsHV-1 µVar, respectively, an average of 1×10^3 (Pepin et al. 2008) or 1×10^4 (Evans et al. 2017b) viral DNA copies per mg of oyster tissue represented a sub clinical carrier state. Similarly, Dundon et al. (2011) found that healthy *C. gigas* juveniles in the latent carrier state may carry very low levels of OsHV-1 µVar (8×10^1 -3.6×10^2 viral DNA copies/mg of mantle tissue), which is 5 to 6 orders of magnitude fewer viral particles than observed in diseased oysters and likely to be insufficient to generate an infectious dose sufficient to transmit the virus from oyster shells to new hosts. Given that various pathways exist to expose shellfish reef restoration projects in Australia to OsHV-1 µVar via recycled oyster shells, environmental conditions are suitable for disease transmission and suitable hosts are likely to occur in the wild in areas where shellfish reefs are being restored, the risk of exposure and establishment is non-negligible, and the overall likelihood of exposure and establishment of OsHV-1 µVar via recycled mollusc shells is considered to be **High.**

4.3.7 Consequence assessment

Introduction and establishment of OsHV-1 µVar into new regions of Australia would have highly significant ramifications, not only for mollusc aquaculture but also for wild populations of susceptible molluscs which may suffer mortalities when water temperatures exceed 20°C. Since OsHV-1 µVar is under official control, its detection in a new area would require intervention by government authorities and disruption to aquaculture as well as mollusc fisheries and shellfish reef restoration if attempts were made to contain the infection and prevent its further spread into uninfected areas. Because this disease agent is listed by the OIE (OIE 2020a), its presence in new regions may also have significant trade implications, and once this virus is detected in wild mollusc populations there is virtually no chance of eradication. Taking all of these factors into consideration, the consequences of establishment of OsHV-1 µVar via recycled mollusc shells is considered to be **High.**

4.3.8 Risk estimation

The risk estimation for OsHV-1 µVar is determined by combining the likelihood of release and exposure (from Table 5) with the consequences of establishment (Table 6) to arrive at a risk estimation (Table 7). The risk estimate for infection with OsHV-1 µVar exceeds the ALOP for some release pathways, suggesting that additional risk management is required for this disease agent.

Risk estimate for infection with OsHV-1 µVar

	Unmitigated	Desiccation			Heat		Salinity	Other
Treatment	None	3 mo.	4 mo.	6 mo.	55°C >10 min	80°C >5 min	0 ppt for >24 hrs	4% acetic acid 30 min
Combined likelihood of release and exposure	High	E low	Neg	Neg	Neg	Neg	High	V low
Consequences of establishment	High	High	High	High	High	High	High	High
Risk estimation	High	V low	Neg	Neg	Neg	Neg	High	Low

4.4 Infection with other malacoherpesviruses

4.4.1 Aetiologic agent: Other herpesvirus diseases of molluscs are caused by Ostreid Herpesvirus 1 (OsHV-1), which are double stranded DNA viruses classified in the genus *Ostreavirus* within the Family *Malacoherpesviridae* in the Order Herpesvirales (Ren et al. 2013). This section will examine malacoherpesviruses of bivalves other than OsHV-1 µVar (POMS).

4.4.2 OIE List: No **NACA List:** Yes (AVNV) **Zoonotic:** No

4.4.3 Australia's status: Malacoherpesviruses have been previously recorded from bivalves in Australia including hatchery reared flat oysters (*Ostrea angasi*) from near Albany in WA (Hine and Thorne 1997), and SRO (*S. glomerata*) in Port Stephens, NSW (P. M. Hine, personal communication, cited in Jenkins et al. 2013), however other malacoherpesvirus strains infecting bivalves such as acute viral necrosis in scallops (AVNV) and OsHV1-SB have not been recorded from Australia.

4.4.4 Epizootiology

Herpesviruses and herpes-like viruses have been reported from a wide variety of bivalve molluscs in many parts of the world. Herpes-like viruses were first recorded in bivalve molluscs in 1972 when adult American oysters *Crassostrea virginica* suffering high mortality (52%) in the east coast of the USA (Marsh River, Maine) were shown to be infected with a herpes-like virus (Farley et al. 1972). The Pacific oyster *Crassostrea gigas* originates from Japan, and the phenomenon known as "summer mortality" first began along the Japanese Pacific coast in the 1940s (Sauvage et al. 2009). Features common to summer mortality episodes in Japan were elevated water temperature, full maturation or spawning and eutrophic conditions (Koganezawa, 1974). After years of investigation (in the absence of knowledge of the existence of bivalve herpesviruses and without todays molecular diagnostic techniques), it was concluded that summer mortality in Japan was due to a "physiological disorder and metabolic disturbance derived by heavy gonad formation and massive spawning under high water temperature and eutrophication" (Koganezawa, 1974). However, herpesviruses were eventually reported from *C. gigas* in Japan (Moss et al. 2007), suggesting that it is possible that herpesviruses were associated with the earlier summer mortality events in that country.

In the late 1950s, summer mortalities of *C. gigas* were also noted on the west coast of USA after *C. gigas* had been imported there from Japan for aquaculture production (Sauvage et al. 2009), a practice that occurred from 1902 until 1989 (Friedman 1996, Burreson et al. 2000). Over 20 years later, the first reports of herpes-like virus infections in bivalves came from the east coast of the USA in *C. virginica* in Maine (Farley et al. 1972). It is important to note that live *C. gigas* from the USA west coast were translocated to the east coast of the USA (including Maine) and the Gulf of Mexico on many occasions between the 1930's and the 1970's (Burreson et al. 2000), resulting in the introduction of pathogens such as the protozoan *Haplosporidium nelsoni*, cause of MSX disease (see Section 4.9), which was introduced in the mid 1950's and has caused disease problems in *C. virginica* on the east coast ever since (Friedman 1996, Burreson et al. 2000, Kamaishi and Yoshinaga 2002). Hence it is possible that the herpes-like virus discovered by Farley et al. (1972) in *C. virginica* and that recorded by Meyers (1981) in *C. virginica* in the thermal effluent of a power station in

Maine were also translocated to the east coast of the USA in *C. gigas* from the west coast, after originating from Japan or Asia.

The early history of summer mortality events in *C. gigas* in the USA revolved around the occasional isolation of several different bacterial pathogens, but none of them alone could explain the onset of summer mortality (Elston 1997, Friedman et al. 1991, 1998). Then, in 1991, outbreaks of disease in *C. gigas* due to herpes-like viruses were reported in France (Nicolas et al. 1992) and New Zealand (Hine et al. 1992). A virus isolate collected from French *C. gigas* larvae in 1995 was characterised as a herpesvirus and named Ostreid herpesvirus 1 (OsHV-1) (Davison et al. 2005). Since then, modern molecular diagnostic techniques have subsequently confirmed that OsHV-1 had also been involved in summer mortality episodes in *C. gigas* along the USA west coast in California (Friedman et al. 2005, Burge et al. 2006, 2011, Meyers et al. 2009, Burge 2010). In hindsight it is possible that the herpes-like virus originally reported from *C. virginica* in Maine by Farley et al. 1972 and rediscovered by Meyers (1981) was also OsHV-1, and it would be interesting to see if surveys in the USA with modern molecular diagnostic techniques can confirm this (Meyers et al. 2009).

In New Zealand and Australia, herpes-like viruses (other than OsHV-1 µVar and AbHV-1) have been reported from flat oysters (*Ostrea* spp.), *C. gigas* and SRO (*S. glomerata*) (see Jenkins et al. 2013). Malacoherpesviruses were first recorded in the southern hemisphere in 1991 during mass mortalities of *C. gigas* spat in hatcheries at Mahurangi, New Zealand (Hine et al. 1992). Larvae of flat oysters (*Ostrea chilensis*) in Wellington Harbour were also found to be susceptible to herpes-like viruses using TEM after co-habitation experiments with *C. gigas* larvae (Hine et al. 1998). In Australia, Hine and Thorne (1997) used TEM to visualise herpes-like viruses within intranuclear inclusions in haemocytes and connective tissues of adult flat oysters (*Ostrea angasi*) from Oyster Harbour near Albany, Western Australia, while larvae of clams (*Katelysia scalarina*) from Tasmania were also reported to be infected by herpes-like viruses by Handlinger (personal observation, reported in EFSA 2010). Herpesviruses were also visualised using TEM in hatchery reared SRO larvae experiencing mortalities in Port Stephens in NSW in the late 1990's (P. M. Hine, personal communication, cited in Jenkins et al. 2013). The identity of the viral inclusions described by Hine and Thorne (2000) in 1 out of 769 *Saccostrea cucullata* sampled from the Exmouth Islands, Western Australia (prevalence 0.13%) remains to be determined, but given their intranuclear location in the affected cells, it is possible that these were also due to herpesviruses.

Bivalves in Asia are now known to harbour several strains of malacoherpesviruses. Since the mid 1990's, a new and serious disease emerged in China causing acute mortalities of farmed zhikong scallops *Chlamys farreri* during the summer months when water temperatures rose above 23-25°C (Xiao et al. 2005, Bai et al. 2015, Arzul et al. 2017). Unlike mortalities in larvae and spat of *C. gigas* infected with OsHV-1, the disease of *C. farreri* only occurred in adult scallops 2 or more years old, resulting in epizootic mortalities of over 90% within 5-8 days of first onset of disease (Ren et al. 2013, Bai et al. 2015). Diseased scallops exhibited clinical signs including slow reactions, weak shell closing reflex, mucous accumulation in the mantle cavity, shrunken or dislodged mantle, gill erosion, adductor muscle ulceration, and an enlarged digestive gland (Tang et al. 2010, Ren et al. 2013). Since 1997 the disease reoccurred each summer and was eventually found to be due to a virus that was called acute viral necrosis virus (AVNV) (Song et al. 2001, Liu et al. 2002, Zhang et al. 2010), or acute viral necrobiotic virus (e.g. Tang et al. 2010). The enveloped virus particle was 130-170 nm in diametre with large numbers of virus particles found near the basement membrane of cells in the digestive gland, kidney, mantle, intestine and gills of scallops dying of the disease (Liu et al. 2002). The occurrence of

epizootic disease during the summer months, the morphology of the virus as well as the associated pathological changes, including evidence of intranuclear inclusion bodies and nuclei with marginated chromatin, suggested that AVNV may be related to OsHV-1 (Ren et al. 2013). Subsequent genetic characterisation of AVNV isolates from *C. farreri* found the DNA sequence of AVNV was 97% identical to the reference strain of ostreid herpesvirus 1 (OsHV-1), and that the variants from *C. farreri* form a distinct separate clade within the ostreid herpesviruses (Bai et al. 2019). The genomic organization of AVNV was similar to OsHV-1, consisting of two unique regions (170.4 kb and 3.4 kb, respectively), each flanked by two inverted repeats (7.6 kb and 10.2 kb, respectively), with a third unique region (1.5 kb) situated between the two internal repeats (Ren et al. 2013).

The AVNV variant causing disease in Chinese scallops (*C. farreri*) in China has not been detected in any other bivalve species (Bai et al. 2015, 2019). However, a new malacoherpesvirus variant, termed OsHV-1-SB with 97.3% similarity to AVNV and 95.2% genome similarity to the OsHV-1 reference strain, was first detected in populations of cultured ark shells (*Scapharca broughtonii*) in China that had experienced disease outbreaks in 2012 once water temperatures exceeded 18°C (Xia et al. 2015, Bai et al. 2016, 2017, 2019, Xin et al. 2018, 2020). Since 2012 the OsHV-1-SB variant has subsequently been detected in not only *Scapharca broughtonii*, but several other species of bivalves, including scallops *Chlamys farreri*, *Platinopecten yessoensis*, clams *Ruditapes philippinarum* and *Meretrix meretrix*, and oysters *Crassostrea hongkongensis* (see Bai et al. 2015). Because of their low host specificity, OsHV-1 variants such as OsHV-1-SB may be able to infect several species of bivalves that occur in Australian waters. However, it is also known that some species of bivalves are not susceptible to AVNV infection, including bay scallop (*Argopecten irradians*, see Bai et al. 2015) and potentially other species that occur in northern Australia including black lip pearl oyster (*Pinctada margaritifera*, see Tan et al. 2015).

Unlike herpesviruses from vertebrate hosts, there is evidence that herpesviruses in bivalve molluscs may be less host specific, as OsHV-1 was detected in a range of bivalve species, including cupped oysters *C. gigas*, *C. virginica*, *C. ariakensis* and *C. sikamea*, flat oysters (*O. edulis*) as well as clams (*R. philippinarum*), mussels (*Mytilus galloprovincialis*) and scallops (*Pecten maximus*) (see Arzul et al. 2001a,b,c, Burge 2010, EFSA 2010, Burge et al. 2011, Bai et al. 2015). Recent phylogeographic analyses suggest that not only OsHV-1, but the other variants of OsHV-1 that were originally described from France, probably originated from East Asia where the highest range of genotypes for these viruses are found in several bivalve species (Bai et al. 2015, 2019, Mineur et al. 2015). Most epidemiological information relating to infection with malacoherpesviruses has been determined for OsHV-1 µVar in *C. gigas* (see Section 4.3). However, given the close relatedness of the various other strains of malacoherpesviruses from bivalves, it is reasonable to assume that many of the key epidemiological aspects of infections (e.g. role of temperature in disease process, routes of transmission, latency), will be similar to those described for OsHV-1 µVar in Section 4.3.

4.4.5 Release assessment

It is known that malacoherpesvirus variants other than OsHV-1 µVar occur in several parts of Australia including some estuaries in NSW (Port Stephens), Tasmania, and in Western Australia (Hine and Thorne 1997, 2000, J. Handlinger -personal observation, reported in EFSA 2010, P. M. Hine, personal communication, cited in Jenkins et al. 2013). This information suggests that mollusc shells obtained from processors or retailers in Australia for recycling have potentially been exposed to other malacoherpesviruses.

As previously mentioned in Sections 4.1 and 4.3, herpesviruses have a lipid-containing envelope, and because of this they are likely to be relatively fragile and less resistant to inactivation compared to some other viruses of aquatic animals (Renault 2011). The resistance of the AbHV-1 and OsHV-1 µVar strains of malacoherpesviruses to desiccation, UV irradiation and various chemical treatments have been discussed previously in Sections 4.1 and 4.3, and it is likely that other malacoherpesvirus strains are likely to be inactivated in a similar manner to AbHV-1 and OsHV-1 µVar. Taking this information into account and considering the likely propagule pressure experienced via the various release pathways examined here, the likelihood estimations for the release of other malacoherpesviruses via the identified release pathways are listed below.

Release assessment for other malacoherpesviruses

	Unmitigated	Desiccation			Heat		Salinity	Other
Treatment	None	3 mo.	4 mo.	6 mo.	55°C >10 min	80°C >5 min	0 ppt for >24 hrs	4% acetic acid 30 min
Likelihood of release	High	E low	Neg	Neg	Neg	Neg	High	V low

4.4.6 Exposure assessment

While malacoherpesviruses infect oyster tissue and not shell material, it is likely that shells from wild and /or cultured oysters or other bivalves that may be procured from processors or retailers for recycling would retain some residual host tissue in areas such as retractor muscle scars, mantle tissue and so on. Given that some malacoherpesvirus strains OsHV-1 µVar can survive in dead host tissue for at least 7 days at 20°C, and possibly over 17 days (but probably less than 42 days) at 4°C in the case of AbHV-1 (see Section 4.1), viral particles would highly likely be transmitted from residual host tissues if recycled mollusc shells were placed back in the water. Transmission is horizontal and direct via the water (Hine et al. 1998, Arzul et al. 2001a,b, Vigneron et al. 2004, Schikorski et al. 2011a,b), and it will be assumed here that both the minimum infective dose required for horizontal transmission via the water and the length of time other malacoherpesviruses remain viable in the water column are likely to be similar to those values known for OsHV-1 µVar (Schikorski et al. (2011a, b, see Section 4.3). However, in the case of other malacoherpesvirus strains, their host specificity may be lower than for OsHV-1µVar, as shown by their wider host ranges (Arzul et al. 2001a,b,c, Burge 2010, EFSA 2010, Burge et al. 2011, Bai et al. 2015). Furthermore, the range of water temperatures permissive for transmission of these strains may also be broader, given that AVNV requires water temperatures around 23-25°C to cause disease, while related variants such as OsHV-1-SB cause disease at lower temperatures (e.g. above 18°C), with viral replication ceasing above 29°C (Xin et al. 2020). AVNV can also be detected in both water and plankton (Zhang et al. 2010), and like OsHV-1 µVar they may be able to be spread by planktonic mechanical vectors (Whittington et al. 2015a, 2015b, 2016, 2018, Evans et al. 2016, 2017a, 2017b). These data together suggest that other malacoherpesvirus strains released through various pathways are likely to contact susceptible host species, and that environmental conditions conducive for transmission are likely to occur throughout many parts of Australia. If an index case occurred, these disease agents would likely persist and establish in the host population via vertical transmission. There is already evidence that malacoherpesviruses have been spread around the world via shipping and become established in wild and cultured populations of molluscs in new locations such as New Zealand and southern Australia.

Given that various pathways exist to expose shellfish reef restoration projects in Australia to other malacoherpesviruses via recycled mollusc shells, environmental conditions are suitable for disease transmission and suitable hosts may occur in the wild in areas where shellfish reefs are being restored, the risk of exposure and establishment is non-negligible, and the overall likelihood of exposure and establishment of OsHV-1 µVar via recycled mollusc shells is considered to be **High.**

4.4.7 Consequence assessment

Introduction and establishment of other malacoherpesviruses into new regions of Australia would have significant ramifications, potentially resulting in significant bottlenecks in hatchery production which could affect bivalve aquaculture industries as well as shellfish reef restoration activities. Some of these other malacoherpesvirus strains (e.g. AVNV) are listed disease agents under official control in Australia (Tables 1, 2) and internationally (NACA), hence their detection in a new area may impact trade and necessitate intervention by government authorities and disruption to aquaculture as well as mollusc fisheries and shellfish reef restoration if attempts were made to contain the infection and prevent its further spread into uninfected areas. Unless these viruses were detected in an enclosed system, there would appear to be little chance of eradication. Taking all of these factors into consideration, the consequences of establishment of other malacoherpesviruses via recycled mollusc shells are considered to be **Moderate.**

4.4.8 Risk estimation

The risk estimation for other malacoherpesviruses is determined by combining the likelihood of release and exposure (from Table 5) with the consequences of establishment (Table 6) to arrive at a risk estimation (Table 7). The risk estimate for infection with other malacoherpesviruses exceeds the ALOP for some release pathways, suggesting that additional risk management is required for these disease agents.

Risk estimate for malacoherpesviruses

	Unmitigated	Desiccation			Heat		Salinity	Other
Treatment	None	3 mo.	4 mo.	6 mo.	55°C >10 min	80°C >5 min	0 ppt for >24 hrs	4% acetic acid 30 min
Combined likelihood of release and exposure	High	E low	Neg	Neg	Neg	Neg	High	V low
Consequences of establishment	Moderate	Mod	Mod	Mod	Mod	Mod	Mod	Moderate
Risk estimation	Moderate	Neg	Neg	Neg	Neg	Neg	Mod	V low

4.5 Infection with *Xenohaliotis californiensis*

4.5.1 Aetiologic agent: Withering syndrome (WS) disease in abalone is caused by infection with *Xenohaliotis californiensis*, an obligate intracellular prokaryote bacterium which is closely related to the *Neorickettsia* genus as the most ancestral form of the Family *Anaplasmataceae* within the Order Rickettsiales (Cicala et al. 2017).

4.5.2 OIE List: Yes **NACA List:** Yes **Zoonotic:** No

4.5.3 Australia's status: *Xenohaliotis californiensis* has not been recorded from Australia (Handlinger et al. 2005) and is considered exotic.

4.5.4 Epizootiology

Withering syndrome was first observed in black abalone (*Haliotis cracherodii*) populations on the south shore of Santa Cruz Island, California, USA, in 1985 shortly after the strong 1982−1983 El Niño-Southern Oscillation (ENSO) event (Haaker et al. 1992, Lafferty and Kuris 1993). The disease was initially reported by commercial abalone fishers who had noted large numbers of empty abalone shells and weak abalone with shrunken, withered foot muscle in an area where fisheries yields were rapidly declining (Lafferty and Kuris 1993). Over the next few years the mortality syndrome spread to nearby islands (Anacapa, Santa Rosa, Santa Barbara, San Miguel) between 50 and 100 km away from the initial focus, but the cause of the mortalities remained undetermined with coastal development, pollution, a coccidian parasite and starvation of abalone caused by environmental variations all being considered potential causes (Lafferty and Kuris 1993, Friedman et al. 1997, 2002), and overharvesting by the fishery being ruled out (Lafferty and Kuris 1993). Abalone fishers and some scientists suspected a chronic disease process may have been responsible for the decline, due to the fact that mortalities occurred only during warmer periods (>16.5°C) when affected abalone displayed consistent morphological signs described by fishers as withering syndrome (WS) for which the most prominent feature was the atrophied and withered foot that allowed fishers to easily detach weakened abalone from the substrate (Lafferty and Kuris 1993). By 1992 the disease had devastated the commercial fishery for black abalone near the original focus and was being reported for the first time in black abalone from San Nicolas Island, over 100 km away from the initial focus (Van Blaricom et al. 1993). Then in 1994 WS was observed in wild red abalone (*Haliotis rufescens*) from San Miguel Island, around 40 km from the initial focus of disease (Friedman et al. 2003). During the 1997–1998 El Niño event it was noted that there was a direct relationship between WS mortality rate of cultured red abalone and the intensity of an infection of a conspicuous rickettsia-like organism (RLO) in the digestive tract of affected animals (Moore et al. 2000). Being an obligate intracellular bacterium and due to the lack of molluscan cell lines, the WS-RLO could not be grown in culture, however subsequent work lead to the development of molecular diagnostic tools (Andree et al. 2000, Antonio et al. 2000) and laboratory transmission trials (Friedman et al. 2002) which eventually confirmed a pathogenic role of the withering syndrome Rickettsia like organism (WS-RLO), which was named *Candidatus* Xenohaliotis californiensis by Friedman et al. (2000).

Studies of the wild population of black abalone at San Nicolas Island showed their populations declined by around 95% within 2-3 years of the arrival of the WS-RLO at that location, eventually reducing to 99.2%

within 9 years (i.e. by 2001, see Van Blaricom et al. 1993, Crosson et al. 2014). Then between 2002 and 2012, abalone densities increased via recruitment over 200% to bring the population back to around 2% of the original biomass, suggesting that a small number of black abalone survived and were able to reproduce, indicating that resistance may eventually occur over time (Crosson et al. 2014). However, another factor that may explain this evolving host-pathogen relationship off the coast of California has been the increased prevalence over time of a phage-infected WS-RLO variant (RLOv) (Friedman and Crosson 2012, Friedman et al. 2014, Cruz-Florez et al. 2016, 2018) and an unrelated stippled RLO (ST-RLO) that were associated with reduced WS-RLO infection loads and associated mortality in some (but not all) abalone species (Friedman et al. 2014, Vater et al. 2018). The ST-RLO was first observed in the mid-1990s, but it wasn't until 10-15 years later that phage infected RLOv was confirmed after studies found that it appears to modulate the effects of WS-RLO infection (Friedman et al. 2014, Crosson and Friedman 2018).

The WS RLO is thought to be able to infect all members of the genus *Haliotis*, with highly susceptible species including black abalone, red abalone, white abalone (*Haliotis sorenseni*), pinto abalone (*H. kamtschatkana*), pink abalone (*H. corrugata*), and Japanese black abalone (*H. discus discus*) (Kiryu et al. 2013, Crosson and Friedman 2018, OIE 2020d). In contrast, green abalone (*H. fulgens*) appear to have some resistance to disease provided water temperatures remain below 20°C (Moore et al. 2009, Crosson et al. 2014), European abalone (*H. tuberculata*), and small abalone (*H. diversicolor supertexta*) may be infected but do not show signs of disease (Balseiro et al. 2006, Wetchateng et al. 2010, OIE 2020d), and Japanese abalone (*H. discus hannai*) may be resistant to infection (Gonzalez et al. 2014). It appears that abalone species with cool water evolutionary histories are most susceptible to WS (Crosson and Friedman 2018). The susceptibility of other *Haliotis* species to infection with *X. californiensis* has not been assessed, however, until the full range of host susceptibility has been determined, it is reasonable to assume that Australian species of *Haliotis* are susceptible. Until recently the WS-RLO has not been identified in any non-haliotid hosts (e.g. limpets and snails) cohabiting with WS-RLO infected abalone, however Crosson et al. (2020) cited an unpublished report of WS-RLO inclusions in an intertidal snail belonging to the Family *Turbinidae* in Japan, and the bacterium can be isolated from filter feeding organisms which may act as reservoirs (OIE 2020d).

Temperature is important in both pathogen transmission and disease expression. Susceptible species of abalone maintained at cool water temperatures (e.g. 15°C for red abalones) do not usually show signs of disease, however exposure to elevated seawater temperatures (e.g. >17°C for red, black and white abalones) typically results in clinical disease (Moore et al. 2000, Friedman et al. 2000, Friedman et al. 2002, Kiryu et al. 2013). Local water temperature variations may influence rates of disease, with more abalone surviving WS infections in areas or microhabitats exposed to cooler waters (Friedman and Finley 2003, Moore et al. 2011). The prepatent incubation period of WS is prolonged and typically ranges between 3 and 7 months (Friedman et al. 1997, Moore et al. 2000, Friedman et al. 2002, Braid et al. 2005, Balseiro et al. 2006). Clinical disease is characterised by large numbers of WS-RLO inclusions in the epithelia of the digestive tract, and morphological changes in the digestive gland including degeneration (atrophy of tubules, increase in connective tissues and inflammation) and/or metaplasia of the digestive tubules in the form of replacement of terminal secretory/absorptive acini with absorptive/transport ducts similar in appearance to the post-oesophagus (OIE 2020d). These morphological changes reduce the hosts ability to digest food resulting in anorexia, depletion of glycogen reserves, catabolism of the foot muscle as an energy source causing 'withering' of the foot, which impairs its ability to adhere to substrates and makes affected abalone vulnerable to predation and/or eventual death (Friedman et al. 1997, Moore et al. 2000, Friedman et al. 2002, Braid et al. 2005,

Balseiro et al. 2006). Surviving abalone remain infected and act as carriers, and their translocation can spread the pathogen into new geographic areas (Friedman and Finley 2003). Indeed, it appears that anthropogenic movements of abalone (particularly California red abalone *Haliotis rufescens*) for fisheries and aquaculture enhancement have spread *X. californiensis* from California to many countries including Canada, Chile, China, France, Iceland, Ireland, Israel, Japan, Mexico, Spain, Thailand, and Taiwan (Wetchateng et al. 2010, Kiryu et al. 2013, OIE 2020d).

Transmission of *X. californiensis* is horizontal and occurs *per-os* via the water or biofilms (Crosson et al. 2014, 2020) and through the faecal-oral route (Moore et al. 2001, Friedman et al. 2002). Exposure of abalone to seawater containing infectious material is sufficient for transmission of the bacterium, and no direct animal to animal contact is required (Moore et al. 2000, Friedman et al. 2002, Braid et al. 2005, Crosson et al. 2020). There is no evidence of vertical transmission and larval abalone are non-feeding and hence refractory to infection (OIE 2020d). Crosson et al. (2020) found that *X. californiensis* was inactivated after 48-72 hours in seawater at 14-18°C in the dark, and measured between 2 and 90 *X. californiensis* DNA copies/ml in abalone farm effluent water, which was 2 to 3 orders of magnitude lower concentration than the dose they found was required to infect 50% of abalone. The data of Crosson et al. (2020) suggested that while abalone farm effluent may be a source of WS infective particles, the distance from the farm outlet at which direct infection of wild abalone stocks was likely to occur was likely to be relatively localised and nowhere near as extensive as the 20 km claimed by Lafferty and Ben-Horin (2013). Post-larval abalone of all sizes appear susceptible to infection, including 6-week-old abalones 1–2 mm in shell length, but the prolonged prepatent period means WS is usually observed in abalone more than one year old (OIE 2020d). Experiments have shown that treatment with oxytetracycline (OTC) by intramuscular injection (12 x 4 mg/kg over 24 days), orally via the feed (33.3 g OTC/kg feed for 2 weeks or 90 mg/kg of OTC daily for 20 days) or via bath immersion (400-500 mg/L for 1-24 hours each day for 7-21 days) are all effective for controlling WS in infected abalone (Friedman et al. 2003, 2007, Winkler et al. 2018) and can result in complete elimination of the bacterium at higher doses (Moore et al. 2019). However, the slow metabolism of abalone means prolonged retention of antibiotics in tissue and in the digestive gland up to 6 months post-treatment, indicating an excessively long withdrawal period before human consumption of treated abalone (Braid et al. 2005, Friedman et al. 2007, Moore et al. 2019).

4.5.5 Release assessment

Xenohaliotis californiensis has not been recorded from Australian molluscs and is considered exotic, however it is included in this risk analysis as it is listed as a notifiable disease agent that could cause serious harm if it were introduced at some point in the future. The bacterium infects abalone gastrointestinal tissue only and not foot muscle or shell material (OIE 2020d), hence it is unlikely that shells from wild and /or cultured abalone that may be procured from abalone processors or retail sale for recycling would retain bacteria in residual host tissue in areas such as foot tissue scars, mantle tissue and so on. The stability of *X. californiensis* in dead host tissue has not been published. Crosson et al. (2020) found that outside host tissues *X. californiensis* was inactivated after 48-72 hours in seawater at 14-18°C in the dark, however because *X. californiensis* is an obligate intracellular organism, it is considered likely to be able to survive and remain viable for much longer periods within dead host gastrointestinal tissues. Resistance of *X. californiensis* to heat, desiccation or acetic acid has also not been published, but it is known that exposure of seawater containing WS-RLO to >10 mg/L

calcium hypochlorite and disinfection of equipment in a bath of 1% iodine in freshwater for 1 hour were effective disinfectants (OIE 2020d).

So, in summary, *X. californiensis* is not known to occur in Australian molluscs at this time, however there is a risk that exotic bacteria could be introduced into Australia via infected hosts translocated in ballast water, or as biofouling on the hulls or sea chests of commercial shipping, fishing vessels and pleasure craft (Howard 1994, Deveney et al. 2017), or in imported mollusc products used as bait or burley (Diggles 2017). Taking this information into account and considering the likely propagule pressure experienced via the various release pathways examined here, the likelihood estimations for the release of *X. californiensis* via the identified release pathways are listed below.

Release assessment for infection with *Xenohaliotis californiensis*

	Unmitigated	Desiccation			Heat		Salinity	Other
Treatment	None	3 mo.	4 mo.	6 mo.	55°C >10 min	80°C >5 min	0 ppt for >24 hrs	4% acetic acid 30 min
Likelihood of release	Low	E low	Neg	Neg	E low?	E low?	Low	V low?

4.5.6 Exposure assessment

Xenohaliotis californiensis infects host gastrointestinal tissue hence its unlikely that shells from wild and /or cultured abalone that may be procured from abalone processors or retail sale for recycling would retain bacteria in residual host tissue in foot muscle scars or mantle tissue. The survival of *X. californiensis* in dead host tissue is unknown, however it is known that water temperatures below 13°C limit transmission of the bacterium (i.e. less than 1% transmission) relative to those held at ~18°C (72–94% transmission) (Braid et al. 2005), and that transmission can occur at temperatures of at least 25°C and possibly up to 29°C (OIE 2020d). Crosson et al. (2020) developed a standardized protocol for exposing abalone to *X. californiensis* and calculated the dose required to generate 50% infection prevalence (ID50) via the water was 2.3 x 10^6 *X. californiensis* DNA copies/L, which was between 100 and 1000 times higher than the WS-RLO DNA quantities they found in abalone farm effluent water (0.2-9.3 x 10^4 DNA copies/L). They also found that *X. californiensis* was inactivated after 48-72 hours in seawater at 14-18°C in the dark (Crosson et al. 2020), suggesting that there is a relatively narrow window of horizontal infection via the water under natural conditions where the bacterium would also be subject to sunlight/UV irradiation and microbial processes. Outbreaks of WS disease in molluscs occur in the summer months, usually when water temperatures are 17°C or higher (Moore et al. 2000, Friedman et al. 2000, Friedman et al. 2002, Kiryu et al. 2013), and the wide range of water temperatures permissive for transmission of this bacterium (15 to >25°C) suggests that if it were introduced into this country, conditions in many parts of Australia would be permissive for transmission of *X. californiensis* infections either seasonally or year round. Given that there may be pathways to expose shellfish reef restoration projects to *X. californiensis* via recycled abalone shells, environmental conditions are suitable for disease transmission and suitable hosts may occur in the wild in areas where shellfish reefs are being restored, the risk of exposure and establishment is non-negligible, and the overall likelihood of exposure and establishment of *X. californiensis* via recycled mollusc shells is considered to be **Low**.

4.5.7 Consequence assessment

Introduction and establishment of *X. californiensis* via shell recycling would have highly significant ramifications. Experience overseas has shown that outbreaks of WS disease in wild abalone result in significant mortality, long term ecological damage and destruction of abalone fisheries. Since this disease agent is under official control, its detection in a new area would necessitate intervention by government authorities and disruption to aquaculture, and commercial and recreational abalone fisheries if attempts were made to contain the infection and prevent its further spread into uninfected areas. As *X. californiensis* is a notifiable disease agent, there would likely be significant impacts on international trade, and once this bacterium was detected in the wild there would appear to be little chance of control or eradication. Taking all of these factors into consideration, the consequences of establishment of *X. californiensis* in wild abalone near shellfish restoration sites is considered to be **High.**

4.5.8 Risk estimation

The risk estimation for *X. californiensis* is determined by combining the likelihood of release and exposure (from Table 5) with the consequences of establishment (Table 6) to arrive at a risk estimation (Table 7). The risk estimate for infection with *X. californiensis* exceeds the ALOP for some release pathways, suggesting that additional risk management is required for this disease agent.

Risk estimate for infection with *Xenohaliotis californiensis*

	Unmitigated	Desiccation			Heat		Salinity	Other
Treatment	None	3 mo.	4 mo.	6 mo.	55°C >10 min	80°C >5 min	0 ppt for >24 hrs	4% acetic acid 30 min
Combined likelihood of release and exposure	V low	E low	Neg	Neg	E low?	E low?	V low	V low?
Consequences of establishment	High	High	High	High	High	High	High	High
Risk estimation	Low	V low	Neg	Neg	V low?	V low?	Low	Low?

4.6 Infection with *Bonamia exitiosa* or *Bonamia* spp.

4.6.1 Aetiologic agent: Parasites of the genus *Bonamia* are small (2-3µm) protozoan microcells which are members of the Phylum Endomyxa, Order Haplosporida (Adl et al. 2019). Unlike most other haplosporidians *Bonamia* sp. have a direct life cycle and mainly infect the haemocytes of flat oysters (Reece et al. 2004, Engelsma et al. 2014, Buss et al. 2019). *Bonamia exitiosa* was originally described from flat oysters (*Ostrea chilensis*) in New Zealand (Hine et al. 2001a, Berthe and Hine 2003).

4.6.2 OIE List: Yes **NACA List:** Yes **Zoonotic:** No

4.6.3 Australia's status: *Bonamia exitiosa* has been found in at least 7 oyster species worldwide including flat oysters (*Ostrea angasi*) and Sydney rock oysters (*Saccostrea glomerata*) in Australia (Corbeil et al. 2006, Hill et al. 2014). In Australia *B. exitiosa* has been recorded from NSW, Victoria, Tasmania, South Australia and Western Australia (Heasman et al. 2004, Jones and Creeper 2006, Corbeil et al. 2006, Carnegie et al. 2014, Crawford 2016, Bradley et al. 2017, Buss et al. 2017, 2019, 2020a, 2020b), while *Bonamia*-like microcells have also been visualised in cultured *C. gigas* in South Australia (Diggles 2003). *Bonamia roughleyi* from *S. glomerata* in NSW has recently been considered *nomen dubium* (Carnegie et al. 2014).

4.6.4 Epizootiology

Beginning in 1960, significant mortalities occurred in populations of bluff oysters (*Ostrea chilensis*) in the dredge fishery in Foveaux Strait, New Zealand (Cranfield et al. 2005). By 1964 the original disease outbreak had abated, but another more severe epizootic between 1985 and 1992 reduced the oyster population to less than 9% of the pre-disease level (Cranfield et al. 2005). The epizootic was due to infection by a microcell similar to *Bonamia ostreae* (see Section 4.7) (Dinamani et al. 1987, Hine 1991a, 1991b, Hine and Jones 1994). The new *Bonamia* sp. caused a wave of mortality that moved through the oyster population from west to east over several years (Hine 1991a, 1991b, Hine and Jones 1994, Cranfield et al. 2005). A fishing moratorium was enacted to protect the stock but as the oyster population began to recover, another epizootic occurred between 1999 and 2004 (Cranfield et al. 2005). The parasite responsible was described as a new species of *Bonamia*, namely *Bonamia exitiosa* (formerly *B. exitiosus* Hine et al. 2001a, Berthe and Hine 2003). Retrospective study of archived materials suggested that *B. exitiosa* was present in the Foveaux Strait fishery during the earlier mortality event between 1960 and 1964 (Hine and Jones 1994, Hine 1996a, Cranfield et al. 2005). A *Bonamia* sp. was subsequently found in Australia in *Ostrea angasi* from Port Phillip Bay, Victoria in 1991, Tasmania in 1992, and soon after (1993-4) in southwest Western Australia in *O. angasi* from Oyster Harbour (Hine and Jones 1994, Hine 1996a, Hine and Thorne 1997, Adlard 2000, Jones and Creeper 2006). This parasite was also reported by Heasman et al. (2004) in *O. angasi* in NSW and the NSW, Victorian and South Australian parasites in *O. angasi* were later confirmed to be *B. exitiosa* (see Corbeil et al. 2006, 2009, Carnegie et al. 2014, Crawford 2016, Bradley et al. 2017, Buss et al. 2017).

A *Bonamia* sp. is also known to be present in the waters of southern WA in *O. angasi* from Oyster Harbour (Hine and Thorne 1997), but its specific identity remains to be fully described using modern molecular diagnostic techniques. In contrast, the prevalence of *B. exitiosa* in *S. glomerata* in NSW is quite low. Carnegie et al. (2014) examined 608 *S. glomerata* from various locations and found *B. exitiosa* in only 1

oyster from the Georges River (prevalence 0.2%). Spiers et al. (2014) examined *S. glomerata* from the Georges and Shoalhaven Rivers using cPCR and did not detect any *Bonamia* positive oysters from the Shoalhaven River, but prevalence averaged 3% (peak 10% in September 2010) in samples taken from the Georges River. Of interest was the fact that histology detected microcells in the same oyster samples at prevalences of up to 50% (Spiers et al. 2014). However, ISH did not detect an active *Bonamia* infection in the tissues of any of the cPCR positive oysters from the Georges River (Spiers et al. 2014), suggesting that these microcells possibly represented infection by another haplosporidian (an organism genetically 99.3% similar to *Haplosporidium costale* was detected in a number of oysters from the Georges, Pambula and Shoalhaven Rivers) or even a related group of new protists (Spiers et al. 2014, Carnegie et al. 2014).

Bonamiosis is easily spread through oyster translocations, as unlike other haplosporidian oyster pathogens (e.g. *Haplosporidium* sp.) and Paramyxida (e.g. *Marteilia* sp.) which have multi host lifecycles (Haskin and Andrews 1988, Powell et al. 1999, Audemard et al. 2002, Arzul and Carnegie 2015), *Bonamia* can be transmitted directly from oyster to oyster via waterborne microcells (Hine 1996a, Buss et al. 2020a). Infection occurs via the gills and palps then the microcells are engulfed by haemocytes and distributed via the haemolymph through various organs as they divide within the haemocyte until it bursts, liberating many microcells that infect more haemocytes (Hine and Jones 1994, Hine 2020). Viable microcells are released from the gonad, kidneys, gills, and gut of infected oysters (via diapedesis), and oyster death facilitates rapid release of parasites from decaying tissue (Hine and Jones 1994, Buss et al. 2020a). If intermediate hosts or non-oyster reservoirs exist in the *Bonamia* spp. life cycle, they may have a role in transmission and environmental persistence (Buss et al. 2020a), however they are unnecessary to explain the spread of this disease through oyster populations. *Bonamia* spp. proliferates rapidly when oysters are held at high densities in captivity, especially when they are stressed (Hine et al. 2002b, Buss et al. 2020a), and it is known that *B. ostreae* can be detected in freshly spawned larval *O. edulis*, suggesting that vertical or pseudo-vertical transmission can occur (Arzul et al. 2011). However, older, larger flat oysters usually display the heaviest levels of infection (Engelesma et al. 2014, Buss et al. 2020b), but it is not clear whether other species (e.g. adult *S. glomerata*) are actively infected with *B. exitiosa* or whether they simply act as mechanical vectors (Spiers et al. 2014, Carnegie et al. 2014). These parasites occur in waters above 20 ppt salinity (Audemard et al. 2008) and the annual pattern of infection of *B. exitiosa* is related to water temperature and the maturation cycle of the oyster, with prevalence and intensity of infection highest during late summer during resorption of gonad material after spawning (Hine 1991a, 1991b, Hine and Jones 1994, Carnegie et al. 2008). The presence of *B. exitiosa* in Florida, USA (Laramore et al. 2017) and the discovery of a tropical *Bonamia* spp. in *Dendostrea sandvicensis* in Hawaii (Engelesma et al. 2014, Hill-Spanik et al. 2015), demonstrates that *Bonamia exitiosa* and other *Bonamia* spp. could also occur in northern Australia, especially as its known that *B. exitiosa* can cause disease at water temperatures of at least 25°C (Audemard et al. 2008, Carnegie et al. 2008).

By the early 2000's it was assumed that *B. ostreae* occurred in the northern hemisphere and *B. exitiosa* occurred in the southern hemisphere (Engelsma et al. 2014). However, recent studies have complicated that simple picture since the detection of *B. ostreae* in New Zealand (Lane et al. 2016) and discovery of *Bonamia exitiosa* and *B. exitiosa*-like microcells in many locations around the world in several host species including flat oysters in Spain (Abollo et al. 2008), Chile (Lohrmann et al. 2009), Tunisia (Hill et al. 2010) and England (Laing et al. 2014), as well as in moribund *Crassostrea ariakensis* in North Carolina (Burreson et al. 2004, Bishop et al. 2006, Audemard et al. 2008, 2014, Hill et al. 2010, Hill et al. 2014) and apparently healthy *C. virginica* and *Ostrea stentina* as far south as Florida (Laramore et al. 2017). It now appears that *B. ostreae* is

mainly a host specialist (in *O. edulis*) (Culloty et al. 1999, Hill et al. 2014, but see Lane et al. 2016), while *B. exitiosa* is a host generalist, having been recorded from at least 7 species of flat and cupped oysters around the world, including *S. glomerata* in NSW (Hill et al. 2014). The possibility of international translocation of *B. exitiosa* and *B. ostreae* through routes such as oyster fouling on the hulls of shipping and/or via ballast water (see Howard 1994, Bishop et al. 2006, Lane et al. 2016, Deveney et al. 2017) appears a probable method by which *B. exitiosa* could have been spread around the world via shipping (Azevedo and Hine 2016), possibly even through dispersal of a common host *Ostrea stentina* (see Hill-Spanik et al. 2015). Furthermore, it is known that some of the oysters in Oyster Harbour in WA are actually *Ostrea edulis* (see Morton et al. 2003), possibly established there from the practice of cleaning/defouling of early sailing ships after their voyages from Europe and North America. If this was indeed the case, the specific identity of the *Bonamia* sp. in *O. angasi* from Oyster Harbour (Hine and Thorne 1997) needs to be re-examined using modern molecular methods to determine whether it is *B exitiosa* or *B. ostreae*, or whether both parasites occur there.

Besides *B. ostreae* and *B. exitiosa*, there are 2 other described species of *Bonamia* as well as undescribed ones. *Bonamia roughleyi* (originally named as *Mikrocytos roughleyi*) was reported from *S. glomerata* in NSW associated with winter mortality disease (Farley et al. 1988, Cochennec-Laureau et al. 2003, see also Section 4.20), however recent evidence suggests that *S. glomerata* in NSW is instead infected with *B. exitiosa* and that *B. roughleyi* is *nomen dubium* (doubtful name) (Carnegie et al. 2014). The second species is the unique, spore forming *Bonamia perspora*, from *O. stentina* (= *Ostreola equestris*) in southeastern USA (Carnegie et al. 2006, Laramore et al. 2017). While *B. perspora* retains the basal haplosporidian characteristic of spore formation, an undescribed species of tropical *Bonamia* recently found in *Dendostrea sandvicensis* in Hawaii (Engelesma et al. 2014, Hill-Spanik et al. 2015), like all other known members of the genus, apparently does not form spores (Hill et al. 2014). The taxonomic affinities of the *Bonamia* spp.-like microcells visualised in cultured *C. gigas* in South Australia (Diggles 2003) are currently unknown. Since *C. gigas* is known to carry *Bonamia* sp. (see Lynch et al. 2010), the parasite could be a *Bonamia* sp., but other microcells could also be implicated including *Mikrocytos* and *Paramikrocytos* (see Farley et al. 1988, Bower et al. 1997, Abbott et al. 2014, Hartikainen et al. 2014b). The *Bonamia*-like parasites sometimes observed in pearl oysters in WA are probably uninucleate stages of the haplosporidian *Minchinia occulta* (see Bearham et al. 2008c, 2009).

4.6.5 Release assessment

In Australia bonamiosis has caused significant mortalities in farmed *Ostrea angasi* in Victoria, but fewer mortalities in Tasmania, SA, WA and NSW (Corbeil et al. 2009, Bradley et al. 2017, Buss et al. 2020a, 2020b). The other unidentified microcell parasites reported in *C. gigas* in SA were also not associated with disease (Diggles 2003). Using histology, the mean prevalence of low intensity *Bonamia* sp. infections in apparently healthy wild flat oysters *O. angasi* at 5 sites in southern NSW was 26% (range 12.8-44.1%) (Heasman et al. 2004), however histology is about half as sensitive for detecting *Bonamia* infections compared to molecular diagnostic techniques (Diggles et al. 2003), and the actual prevalence of *Bonamia* sp. in wild flat oysters in NSW therefore may be closer to 50%, which is similar to the ~50% overall prevalence (maximum 88%) of *B. exitiosa* infections in SA *O. angasi* populations (Buss et al. 2017, 2020b). In healthy Tasmanian *O. angasi*, *B. exitiosa* is usually encountered in histological surveys at prevalences of 10% or less[12]. Overall prevalence of the *Bonamia*-like microcell in a histological survey of healthy *C. gigas* from SA was 16%, ranging between 3.3-66.4% at different sites (Diggles 2003).

[12] http://www.fish.wa.gov.au/Documents/aquatic_animal_health/australian_flat_oyster_with_bonamia_parasites_jan_mar_2006.pdf

Spores have been detected in only one *Bonamia* species, *B. perspora* from North Carolina, USA (Carnegie et al. 2006). Spores have not been observed in all other *Bonamia* species, including the tropical *Bonamia* spp. from Hawaii (Engelesma et al. 2014, Hill-Spanik et al. 2015), for which the microcell is known to act as the infective stage, with the disease being transmitted horizontally by cohabitation (Diggles and Hine 2002, Hine et al. 2002b, Audemard et al. 2014, Buss et al. 2020a). *Bonamia* spp. microcells can survive in dead oyster tissues for at least 24 hours, and 40% can survive in the water column for a minimum of 2 days and possibly up to 1 week at 15-18°C (Arzul et al. 2009, Arzul and Carnegie 2015), with 50% survival after 4 days at 4°C (B.K. Diggles and P.M. Hine, personal observations, Diggles and Hine 2002). Buss et al. (2020c) found *B. exitiosa* microcells were sensitive to high doses of chlorine (40 g/L for 10 min) and iodine (2g/L for 1 min), but their resistance to desiccation, heat or acetic acid has not been determined. It is thus known that *Bonamia exitiosa* and/or *Bonamia* spp. are present in several parts of Australia, in not only flat oysters (often at high prevalence) but also cupped oysters, suggesting that mollusc shells obtained from processors or retailers in Australia for recycling could potentially have been exposed to *Bonamia exitiosa* and/or *Bonamia* spp. Taking into account the likely propagule pressure experienced via the various anthropogenic introduction pathways, the likelihood estimations for the release of *Bonamia exitiosa* or *Bonamia* spp. via the identified risk pathways are listed below.

Release assessment for infection with *Bonamia exitiosa* or *Bonamia* spp.

	Unmitigated	Desiccation			Heat		Salinity	Other
Treatment	None	3 mo.	4 mo.	6 mo.	55°C >10 min	80°C >5 min	0 ppt for >24 hrs	4% acetic acid 30 min
Likelihood of release	High	Neg	Neg	Neg	Low?	E low?	Low	V low?

4.6.6 Exposure assessment

While *Bonamia exitiosa* and *Bonamia* spp. infect oyster tissue and not shell material, it is likely that shells from wild and /or cultured oysters that may be procured from processors or retailers for recycling would retain some residual host tissue in areas such as retractor muscle scars, mantle tissue and so on. It is known that *B. exitiosa* and *Bonamia* spp. can be transmitted to new hosts horizontally and directly via the water (Hine 1996a, Diggles and Hine 2002, Arzul et al. 2011, Buss et al. 2020a). Experimental exposure to 10^3 - 10^5 *B. exitiosa* via the water causes mortalities of 25 to 40% of *O. chilensis* within 18 weeks, and the 18-week LD50 for *B. exitiosa* was experimentally determined to be approximately 2 x 10^5 microcells per oyster (Diggles and Hine 2002). Heavily infected adult *O. chilensis* with terminal *B. exitiosa* infections contain on average around 5 x 10^8 microcells (Diggles and Hine 2002, Cranfield et al. 2005), and this large difference between the infective dose and parasite burdens in moribund oysters (a single oyster dying of *B. exitiosa* could theoretically release enough infective stages to kill up to 2500 nearby oysters), suggests *Bonamia* spp. infections pose a serious risk to confined oyster populations. Bivalves are efficient filter feeders and therefore are also efficient particle collectors, and can collect spores and infective stages of a wide range of bivalve disease agents (Barber and Ford 1992, Ford et al. 2009). Because of this, oysters are particularly susceptible to infection by protozoan infective stages delivered via the water, and this is one of the reasons why movements of infected oysters are highly likely to result in exposure and establishment of oyster pathogens in new areas, as has been demonstrated several times in other parts of the world (Elston et al. 1986, Friedman and Perkins 1994, Howard

1994, Bishop et al. 2006). As there may be pathways to expose shellfish reef restoration projects to *Bonamia exitiosa* and *Bonamia* spp. via recycled mollusc shells, environmental conditions are suitable for disease transmission and suitable hosts may occur in the wild in areas where shellfish reefs are being restored, the risk of exposure and establishment is non-negligible, and the overall likelihood of exposure and establishment of *Bonamia exitiosa* and *Bonamia* spp. is considered to be **Moderate**.

4.6.7 Consequence assessment

Although *Bonamia exitiosa* and *Bonamia* spp. are already present in populations of wild and cultured oysters in several regions of southern Australia, other regions of Australia remain free of infection at this time. Furthermore, it is currently unclear whether there are 2 (or more) species of *Bonamia* in Australia at this time, and the likely impacts of introduction and spread of new species or strains of *Bonamia* into areas where other strains are already endemic are unclear (Lane et al. 2016). There is evidence that once introduced, *Bonamia* can cause major disease outbreaks and long term (60+ years), possibly irreversible impacts on populations of wild and cultured oysters (Burreson et al. 2004, Cranfield et al. 2005, Audemard et al. 2008). These disease agents are listed by the OIE and NACA, and they are also reportable disease agents in all Australian States, hence their detection in new locations within Australia may result in intervention by government authorities, which could cause disruption to aquaculture, fisheries or shellfish reef restoration if attempts were made to prevent its further spread into uninfected areas. Once these parasites are detected in the wild, there is virtually no chance of eradication. Taking all of these factors into consideration, the overall consequences of establishment of *B. exitiosa* or *Bonamia* spp. via recycled mollusc shells is considered to be **High.**

4.6.8 Risk estimation

The risk estimation for *B. exitiosa* or *Bonamia* spp. is determined by combining the likelihood of release and exposure (from Table 5) with the consequences of establishment (Table 6) to arrive at a risk estimation (Table 7). The risk estimate for infection with *B. exitiosa* or *Bonamia* spp. exceeds the ALOP for some release pathways, suggesting that additional risk management is required for these disease agents.

Risk estimate for infection with *Bonamia exitiosa* or *Bonamia* spp.

	Unmitigated	Desiccation			Heat		Salinity	Other
Treatment	None	3 mo.	4 mo.	6 mo.	55°C >10 min	80°C >5 min	0 ppt for >24 hrs	4% acetic acid 30 min
Combined likelihood of release and exposure	Moderate	Neg	Neg	Neg	Low?	E low?	Low	V low?
Consequences of establishment	High	High	High	High	High	High	High	High
Risk estimation	High	Neg	Neg	Neg	Mod?	V low?	Mod	Low?

4.7 Infection with *Bonamia ostreae*

4.7.1 Aetiologic agent: Parasites of the genus *Bonamia* are small (2-3µm) protozoan microcells which are members of the Phylum Endomyxa, Order Haplosporida (Adl et al. 2019). *Bonamia ostreae* was originally described from flat oysters (*Ostrea edulis*) in France after probable translocation of the parasite originally from the west coast of the USA (Comps et al. 1980, Elston et al. 1986, Friedman et al. 1989).

4.7.2 OIE List: Yes **NACA List:** Yes **Zoonotic:** No

4.7.3 Australia's status: *Bonamia ostreae* has not been recorded from Australia and is considered exotic.

4.7.4 Epizootiology

The first recorded instance of a microcell disease in flat oysters was in populations of the European flat oyster (*Ostrea edulis*) cultured in California, USA, which suffered epizootic mortalities in the late 1960's associated with infection by an intrahaemocytic microcell which caused stunted growth and cumulative mortalities up to 100% at some locations (Katkansky et al. 1969, Katkansky and Warner 1974). Originally the microcells in *O. edulis* were thought to be the same as those observed earlier in Pacific oysters (*C. gigas*) at Denman Island, British Columbia from 1960, which were later described by Farley et al. (1988) as *Mikrocytos mackini* (see Section 4.14). However, at locations in California cohabited by both *C. gigas* and *O. edulis*, microcells were only observed in diseased *O. edulis* using histology, suggesting host specificity (Katkansky and Warner 1974). Movements of flat oysters from the US east coast may have introduced the microcells to the west coast (Azevedo and Hine 2016), subsequent movements of flat oysters from California to Maine on the east coast of the USA and also to France probably spread the flat oyster disease agent to other areas of the east coast of the USA (Friedman and Perkins 1994) as well as Europe (Elston et al. 1986). Subsequent outbreaks of disease in *O. edulis* in France in the late 1970's lead to the description of the first species of *Bonamia*, described as *Bonamia ostreae* by Comps et al. (1980). Later studies confirmed *B. ostreae* was indeed originally present in California, highlighting the biosecurity risk of these translocations (Friedman et al. 1989).

Since then, *Bonamia ostreae* together with another parasite *Marteilia refringens* has contributed to significant mortalities and over 90% reduction in production of cultured *O. edulis* in France from 20,000 tonnes per year in the 1970's to 1,800 tonnes in 1995 (Grizel 1985, Laing et al. 2014). Bonamiosis is considered the most serious impediment to flat oyster production in Europe as *B. ostreae* was rapidly spread with movements of live shellfish into many countries including Spain, England, Ireland, Denmark, the Netherlands and Morocco (Elston et al. 1986, Laing et al. 2014). Bonamiosis is easily spread through oyster translocations, as unlike other oyster pathogens within the orders Haplosporida (e.g. *Haplosporidium* sp.) and Paramyxida (e.g. *Marteilia* sp.) which have multi host lifecycles (Haskin and Andrews 1988, Powell et al. 1999, Audemard et al. 2002, Arzul and Carnegie 2015), transmission of *Bonamia ostreae* is direct from oyster to oyster via waterborne uninucleate microcells (Hine 1996a, Reece et al. 2004, Engelsma et al. 2014, Buss et al. 2019, Buss et al. 2020a). Lynch et al. (2007) found 8 species of benthic macroinvertebrates and 19 zooplankton samples gave positive results for *B. ostreae* DNA, and transmission of *B. ostreae* was detected in two naïve oysters cohabiting with the brittle star *Ophiothrix fragilis*, suggesting that invertebrates may act as reservoirs and vectors. If intermediate hosts or non-oyster reservoirs exist in the *B. ostreae* life cycle, they may have a

role in transmission and environmental persistence of the parasite, however they are unnecessary to explain the spread of *B. ostreae* through oyster populations. It is also known that *B. ostreae* can be detected in the epithelium surrounding the visceral cavity in freshly spawned larval *O. edulis*, suggesting that vertical or pseudo-vertical transmission can occur (Arzul et al. 2011).

While *B. ostreae* appears highly pathogenic to flat oysters, many species of cupped oysters appear tolerant, or even resistant to infection. For example, Pacific oysters (*C. gigas*) are highly resistant to infection with *B. ostreae* (see Renault et al. 1995b), though they may still act as carriers (Lynch et al. 2010). At the cellular level, *C. gigas* haemocytes are able to phagocytose and destroy the parasite, unlike flat oysters in which the phagocytosed parasite survives and divides within a parasitophorous vacuole within the host haemocyte (Chagot et al. 1992). Resistance to *B. ostreae* can develop in exposed populations of wild flat oysters, though this process takes many generations and selective breeding appears necessary in order to prevent natural resistance being diluted by cross breeding with susceptible oysters (Flannery et al. 2014, Morga et al. 2017). Around 30 years after the introduction of *B. ostreae* into the UK, there have been early signs of resistance developing in a very few wild *O. edulis* populations in that country (Laing et al. 2014). If the experience following the introduction of *Haplosporidium nelsoni* into the east coast of the USA is any guide, development of meaningful resistance to *B. ostreae* following its introduction into wild oyster populations may take 50 years or more (Carnegie and Burreson 2011, Ford and Bushek 2012).

By the early 2000's it was assumed that *B. ostreae* infected flat oysters in the northern hemisphere and *B. exitiosa* infected flat oysters in the southern hemisphere (Engelsma et al. 2014). However, more recent discoveries have complicated that simple picture, especially since the detection of *B. ostreae* in New Zealand (Lane et al. 2016), which was the first time this parasite has been recorded from the southern hemisphere. It appears that *B. ostreae* infects mainly *O. edulis* (see Culloty et al. 1999, Hill et al. 2014), but it can also infect *O. chilensis* (see Grizel et al. 1982, Lane et al. 2016), *Ostrea puelchana* (see Pascual et al. 1991), and *Ostrea angasi* (see Bougrier et al. 1986), and can even establish mild infections in *Crassostrea ariakensis* (see Azevedo and Hine 2016) and *C. gigas* (Renault et al. 1995b, Hine 2020). The possibility of international translocation of *B. exitiosa* and *B. ostreae* through routes such as oyster fouling on the hulls of shipping and/or via ballast water (see Howard 1994, Bishop et al. 2006, Lane et al. 2016, Deveney et al. 2017) appears a probable method by which *B. ostreae* could have been spread around the world via shipping (Azevedo and Hine 2016). Due to the many similarities between *B. ostreae* and *B. exitiosa* in their morphology and ecology of the infectious agent, transmission, and expression of disease, for more details on the epizootiology of this disease agent readers are referred to Section 4.6.

4.7.5 Release assessment

Bonamia ostreae has not been recorded from Australian molluscs and is considered exotic, however it is included in this risk analysis as it is listed as a notifiable disease agent that could cause serious harm if it were introduced at some point in the future. Furthermore, it is known that some of the oysters in Oyster Harbour in WA are actually *Ostrea edulis* (see Morton et al. 2003), possibly established there from the practice of cleaning/defouling of early sailing ships after their voyages from Europe and North America. If this was indeed the case, the specific identity of the *Bonamia* sp. in *O. angasi* from Oyster Harbour (Hine and Thorne 1997) needs to be re-examined using modern molecular methods to determine whether it is *B exitiosa* or *B. ostreae*, or whether both parasites occur there. *Bonamia ostreae* microcells can survive in dead oyster tissues

for at least 24 hours, and 40% can survive in the water column for a minimum of 2 days at 15-18°C at normal seawater salinity (Arzul et al. 2009), while 50% of *B. exitiosa* can survive outside the host for at least 4 days at 4°C (B.K. Diggles and P.M. Hine, personal observations, Diggles and Hine 2002), but their resistance to desiccation, heat or acetic acid has not been determined. Taking into account the likely propagule pressure experienced via the various anthropogenic introduction pathways, the likelihood estimations for the release of *Bonamia ostreae* via the identified risk pathways are listed below.

Release assessment for infection with *Bonamia ostreae*

	Unmitigated	Desiccation			Heat		Salinity	Other
Treatment	None	3 mo.	4 mo.	6 mo.	55°C >10 min	80°C >5 min	0 ppt for >24 hrs	4% acetic acid 30 min
Likelihood of release	High	Neg	Neg	Neg	Low?	E low?	Low	V low?

4.7.6 Exposure assessment

While *Bonamia ostreae* infects oyster tissue and not shell material, it is likely that shells from wild and /or cultured oysters that may be procured from processors or retailers for recycling would retain some residual host tissue in areas such as retractor muscle scars, mantle tissue and so on. It is known that *B. ostreae* can survive in dead oyster tissue for at least 24 hours and can be transmitted to new hosts horizontally and directly via the water (Arzul et al. 2011) where it may survive for up to 1 week at salinities above 25 ppt and temperatures below 15°C (Arzul et al. 2009, Arzul and Carnegie 2015). Experimental exposure to 10^2 - 10^6 *B. ostreae* via injection causes mortalities of 17- 69% of *O. edulis* within 4 months, and the 50% infectious dose for *B. ostreae* was experimentally determined to be approximately 8 x 10^4 microcells for a 3 year old oyster (Hervio et al. 1995). Bivalves are efficient filter feeders and therefore are also efficient particle collectors, and can collect spores and infective stages of a wide range of bivalve disease agents (Barber and Ford 1992, Ford et al. 2009). Because of this, oysters are particularly susceptible to infection by protozoan infective stages delivered via the water, and this is one of the reasons why movements of infected oysters are highly likely to result in exposure and establishment of oyster pathogens in new areas, as has been demonstrated several times in other parts of the world (Elston et al. 1986, Friedman and Perkins 1994, Howard 1994, Bishop et al. 2006). As there may be pathways to expose shellfish reef restoration projects to *Bonamia ostreae* via recycled mollusc shells, environmental conditions are likely to be suitable for disease transmission in many parts of Australia and suitable hosts may occur in the wild in areas where shellfish reefs are being restored, the risk of exposure and establishment is non-negligible, and the overall likelihood of exposure and establishment of *Bonamia ostreae* is considered to be **Moderate**.

4.7.7 Consequence assessment

Although *Bonamia exitiosa* is already present in populations of wild and cultured oysters in many regions of southern Australia, other areas are free from infection, whilst *B. ostreae* has never been recorded here and is considered exotic. There is evidence from New Zealand that the introduction of *B. ostreae* into cultured oyster populations already affected by *B. exitiosa* results in significant increases in mortality rates (Lane et al. 2016), suggesting that co-infections of *B. exitiosa* together with an incursion of *B. ostreae* would likely have a catastrophic effect on the affected oyster population (Culloty et al. 2019). These disease agents are listed by

the OIE and NACA, and they are also reportable disease agents in all Australian states, hence their detection in new locations within Australia may result in intervention by government authorities, which could cause disruption to aquaculture, fisheries or shellfish reef restoration if attempts were made to contain the infection and prevent its further spread into uninfected areas. The discovery of *B. ostreae* in oysters in Australia would likely pose a significant obstacle to future investment and growth of such industries. However, once these parasites are detected in the wild, there is virtually no chance of eradication (Engelsma et al. 2014), and development of resistance in the host population may take 50 years or more. Taking all of these factors into consideration, the overall consequences of establishment of *B. ostreae* via recycled mollusc shells is considered to be **High.**

4.7.8 Risk estimation

The risk estimation for *B. ostreae* is determined by combining the likelihood of release and exposure (from Table 5) with the consequences of establishment (Table 6) to arrive at a risk estimation (Table 7). The risk estimate for infection with *B. ostreae* exceeds the ALOP for some release pathways, suggesting that additional risk management is required for this disease agent.

Risk estimate for infection with *Bonamia ostreae*

	Unmitigated	Desiccation			Heat		Salinity	Other
Treatment	None	3 mo.	4 mo.	6 mo.	55°C >10 min	80°C >5 min	0 ppt for >24 hrs	4% acetic acid 30 min
Combined likelihood of release and exposure	Moderate	Neg	Neg	Neg	Low?	E low?	Low	V low?
Consequences of establishment	High	High	High	High	High	High	High	High
Risk estimation	High	Neg	Neg	Neg	Mod?	V low?	Mod	Low?

4.8 Haplosporidosis

4.8.1 Aetiologic agent: Parasites of the genus *Haplosporidium* are another group of protozoans which are members of the Order Haplosporida in the Phylum Endomyxa (Adl et al. 2019). Species of *Haplosporidium* infect mainly connective tissues and epithelia and have been described infecting not only bivalves but also gastropods and abalone as well as a range of other invertebrates including tunicates, annelid worms and crustaceans (Burreson and Ford 2004, Urrutia et al. 2019). Most *Haplosporidium* species develop spores 3-12 x 2-5 µm in dimension with an apically hinged operculum and other ornaments (Azevedo and Hine 2016). Those that infect molluscs have unknown, probably indirect life cycles, possibly requiring alternate (planktonic) hosts (Haskin and Andrews 1988, Powell et al. 1999, Hartikainen et al. 2014a).

4.8.2 OIE List: No **NACA List:** No **Zoonotic:** No

4.8.3 Australia's status: Haplosporidosis of pearl oysters has been reported in WA where *Pinctada maxima* spat can be infected with *Haplosporidium hinei* (see Hine and Thorne 1998, Bearham et al. 2008a, 2008b). The *Haplosporidium* sp. reported from western or hooded rock oysters *Saccostrea cucullata* in WA by Hine and Thorne (2002) was later reclassified as *Minchinia* sp. based on molecular data (Bearham et al. 2007), and described as *M. occulta* Bearham et al. (2008c) (see Section 4.15). Another study found a *Haplosporidium* sp. in *S. glomerata* from the Georges River, Port Stephens and Pambula River at high prevalences (31.8- 87.5%) using cPCR, however there was no histological evidence of infection by recognizable *Haplosporidium* sp. life stages in any of the material examined (Carnegie et al. 2014).

4.8.4 Epizootiology

There are at least 34 described species of *Haplosporidium*, but molecular analyses suggest the genus is paraphyletic (Burreson and Ford 2004, Arzul and Carnegie 2015, Azevedo and Hine 2016, Catanese et al. 2018). Infection of molluscs by *Haplosporidium* spp. parasites has resulted in economically and ecologically significant mass mortalities in many parts of the world (Burreson and Ford 2004). Most recently, mass mortalities of fan mussels (*Pinna noblis*) in the western Mediterranean Sea were attributed to infections by *Haplosporidium pinnae* (see Catanese et al. 2018). However, the best known example is from the USA where the haplosporidian parasite *Haplosporidium nelsoni* causes MSX disease (see Section 4.9), which has caused massive epizootics in eastern oysters (*Crassostrea virginica*) in high salinity (> 15 ppt) areas along the east coast of the United States since 1957 (Andrews 1966, 1968, 1996, Ford and Haskin 1982, Haskin and Ford 1982, Burreson et al. 2000). In *Crassostrea gigas* in China (Wang et al. 2010a) and *C. virginica* growing in water > 25 ppt salinity on the east coast of the USA, *H. nelsoni* sometimes occurs in mixed infections with the closely related *Haplosporidium costale*, which exhibits seasonal infections (Andrews 1982) and has also been detected in *C. gigas* on the US west coast following translocation of oysters (Burreson and Stokes 2006). Other molluscan hosts include gastropods - for example, a new haplosporidian parasite emerged in cultured abalone (*Haliotis iris*) in New Zealand resulting in mortalities of up to 90% (Diggles et al. 2002, Hine et al. 2002a). The New Zealand abalone parasite (NZAP) contained rickettsiales-like prokaryotes in its cytoplasm (Hine et al. 2002a) and molecular and ultrastructural analysis suggest that it falls at the base of the Phylum Haplosporidia (Reece et al. 2004, Hine et al. 2009, Arzul and Carnegie 2015, Azevedo and Hine 2016, Urrutia et al. 2019). The inability to transmit infection horizontally or directly through inoculation (Diggles et al.

2002) suggested that an alternate host was required for completion of the lifecycle of the NZAP. The lack of subsequent reports of the NZAP in other abalone culture facilities or wild abalone over 17 years later suggests that it is either extremely rare, and/or that abalone may not be a normal host of the NZAP (B.K. Diggles and P.M. Hine, personal observations). Infections by haplosporidians have also caused disease in limpets (Di Giorgio et al. 2014, Ituarte et al. 2014), mussels (Molloy et al. 2012) and crustaceans, including wild caught crabs (Stentiford et al. 2004, 2013), jelly prawns (Diggles 2020b), amphipods (Urrutia et al. 2019) and aquacultured prawns (Utari et al. 2012).

In Australia, haplosporidians of the genus *Haplosporidium* and *Minchinia* have caused sporadic but heavy mortalities in hatchery reared pearl oysters (*Pinctada maxima*) and wild western or hooded rock oysters (*Saccostrea cucullata*) in Western Australia (Hine and Thorne 1998, 2000, 2002, Jones and Creeper 2006). The *Haplosporidium* sp. parasite in pearl oysters was first found in 6 out of 106 (5.6%) of *P. maxima* spat 5-10 mm in shell height from a hatchery at Oyster Creek, Canarvon in northern WA in the early 1990s (Hine and Thorne 1998). By the time the presence of the infection was detected, however, the remaining spat had been moved to a grow-out area, where they apparently all died (Hine and Thorne 1998). Subsequent studies found it difficult to detect the parasite again, until a second occurrence in December 1995 found the *Haplosporidium* sp. at a prevalence of 4.6% in a sample of 150 *P. maxima* spat taken 6 weeks after their deployment to a nursery area at Cascade Bay in King Sound north of Broome (Jones and Creeper 2006, Bearham et al. 2008b). By the time the oysters were destroyed 15 days later, the prevalence had increased to 10% (Jones and Creeper 2006). The parasite was later described as *Haplosporidium hinei*, a pathogen that is considered to represent a serious risk to the pearl industry (Bearham et al. 2008b, 2009). The second parasite originally observed by Hine and Thorne (2000), in samples of diseased *S. cucullata* from northern WA in 1993-94 has been associated with mortalities of up to 80% in wild hooded rock oysters around Exmouth Island (Hine and Thorne 2000, Bearham et al. 2007) and was eventually described as *Minchinia occulta* (see Bearham et al. 2007, 2008a, 2008c and Section 4.15). Mixed infections of *M. occulta* and *H. hinei* have also been recorded in hatchery reared *P. maxima*, with *M. occulta* occurring at prevalences up to 26.7% during disease outbreaks (Bearham et al. 2009).

Infections by haplosporidians are usually systemic and terminal (Hine and Thorne 1998, 2002, Diggles et al. 2002). Haplosporidian vegetative stages proliferate within host connective tissues forming masses of multi-nucleated plasmodia that are too large to be phagocytosed by host haemocytes (Hine 2020). Eventual host death is thought to be due to overwhelming numbers of parasites interfering with normal organ functions. Sporulation usually occurs within the epithelia of the digestive tract (Bearham et al. 2008b). It appears unlikely that haplosporidian vegetative cells can survive freezing, however the freeze tolerance of spore stages remains unknown (Diggles 2011a). The inability to transmit haplosporidians directly by cohabitation or injection of spores suggests they have an indirect lifecycle requiring an alternate host. The earliest vegetative stages of *Haplosporidium nelsoni* are found in the epithelia of the gills and palps, suggesting that the infective stage is waterborne and can be easily spread (Haskin and Andrews 1988). Neither the infective stage nor the mode of transmission has ever been identified (Powell et al. 1999, Sunila et al. 2000), although it is known that the infective stage for *H. nelsoni* can pass through a 1 mm filter (Sunila et al. 2000), and a 150 µm filter, but not a 1 µm filter followed by UV irradiation at a dose of 30 mJ/cm^2 (Ford et al. 2001).

4.8.5 Release assessment

Haplosporidosis due to infection with *Haplosporidium hinei* has caused sporadic but significant mortalities in hatchery reared *P. maxima* in northern WA, however infections by *H. hinei* in *P. maxima* have only been recorded a handful of times, at relatively low prevalence (<10%) (Hine and Thorne 1998, Jones and Creeper 2006, Bearham et al. 2008b, 2009). To date no *Haplosporidium* sp. have been reported from oysters of the genera *Ostrea*, *Crassostrea*, *Saccostrea* in Australia. Given the paucity of information on the health status of oysters in many parts of Australia, and the fact that filter feeding bivalves are efficient particle collectors which can concentrate haplosporidian life stages which may occur in the environment (Ford et al. 2009), it is possible that there are other haplosporidians in Australian oysters that are currently undescribed. The emergence of new haplosporidian diseases in Australian oyster species such as *S. cucullata* and *P. maxima* and occurrence of *Bonamia*-like microcells in species such as *C. gigas* in SA (Diggles 2003) (microcells which could also be uninucleate stages of haplosporidians, see Bearham et al. 2007, 2008a, 2008b, 2009) demonstrate that the full range of haplosporidian infections present in the various species of Australian molluscs remains to be determined. Haplosporidian infective stages are undescribed, but it is known that they are likely to be susceptible to UV radiation with transmission prevented by exposing infected water to a 1 µm filter followed by UV irradiation at a dose of 30 mJ/cm^2 (Ford et al. 2001). However, their resistance of infective or vegetative stages to desiccation, heat or acetic acid has not been determined. Taking into account the likely propagule pressure experienced via the various anthropogenic introduction pathways, the likelihood estimations for the release of haplosporidosis via the identified risk pathways are listed below.

Release assessment for haplosporidosis

	Unmitigated	Desiccation			Heat		Salinity	Other
Treatment	None	3 mo.	4 mo.	6 mo.	55°C >10 min	80°C >5 min	0 ppt for >24 hrs	4% acetic acid 30 min
Likelihood of release	Moderate	E low?	Neg	Neg	Low?	E low?	Low?	V low?

4.8.6 Exposure assessment

While haplosporidians infect oyster tissue and not shell material, it is likely that shells from wild and /or cultured oysters that may be procured from processors or retailers for recycling would retain some residual host tissue in areas such as retractor muscle scars, mantle tissue and so on. *Haplosporidium* spp. may proliferate rapidly when oysters are held at high densities in captivity (Hine and Thorne 1998, Sunila et al. 2000), however there is little evidence that *Haplosporidium* spp. can be transmitted horizontally through the water or vertically between generations. Spread of haplosporidians via contaminated recycled mollusc shells would occur only if sufficient viable infective stages were introduced into areas where susceptible alternate hosts and/or oyster final hosts were present under environmental conditions suitable for transmission.

Because unknown alternate host(s) are presumably needed in order to complete the lifecycle of haplosporidians (Haskin and Andrews 1988, Ford et al. 2001, Diggles et al. 2002), the exposure pathway required for transmission remains unknown, as does important information such as the minimum infective dose required for an index case to occur, though being a parasite, theoretically infection by just one infective stage can result in successful transmission. If an index case did occur, these disease agents are highly pathogenic and it would be likely that the infected bivalve would become diseased, after which transmission and further spread from the index case may occur. The apparent restricted distribution of these parasites may

be due to inadequate knowledge of the disease status of molluscs in Australia, but also could be due to the fact that their alternate hosts may also be restricted in distribution. However, the fact that some haplosporidians (e.g. *Haplosporidium nelsoni*) have been translocated and established infections in new regions (Friedman 1996, Burreson et al. 2000, Renault et al. 2000a), suggests that some of the presumptive alternate hosts may be widespread and/or ubiquitous (e.g. planktonic copepods, Hartikainen et al. 2014a), or that these parasites may have lower host specificity for the alternate host. As there may be pathways to expose shellfish reef restoration projects to haplosporidians via recycled mollusc shells, environmental conditions are likely to be suitable for disease transmission in many parts of Australia and suitable hosts may occur in the wild in areas where shellfish reefs are being restored, the risk of exposure and establishment is non-negligible, and the overall likelihood of exposure and establishment of haplosporidians is considered to be **Low.**

4.7.7 Consequence assessment

Although haplosporidian parasites are already present in populations of wild molluscs in some regions of Australia, other regions may be free from infection at this time. There is evidence that haplosporidians can cause major disease outbreaks and significant impacts on populations of both wild and cultured oysters and other molluscs in Australia and overseas. Haplosporidosis is no longer listed by the OIE and NACA, but these disease agents remain listed as a reportable disease in SA, WA and the NT (Table 3). Hence the spread of haplosporidian parasites to new areas is likely to adversely impact trade and could potentially cause economic harm to aquaculture and/or fisheries together with significant environmental effects for wild oyster populations. Furthermore, once these disease agents are detected in the wild, there would appear to be little chance of eradication. Taking all of these factors into consideration, the consequences of establishment of haplosporidosis via recycled mollusc shells is considered to be **Moderate.**

4.8.8 Risk estimation

The risk estimation for haplosporidosis is determined by combining the likelihood of release and exposure (from Table 5) with the consequences of establishment (Table 6) to arrive at a risk estimation (Table 7). The risk estimate for haplosporidosis exceeds the ALOP for some release pathways, suggesting that additional risk management is required for these disease agents.

Risk estimate for haplosporidosis

	Unmitigated	Desiccation			Heat		Salinity	Other
Treatment	None	3 mo.	4 mo.	6 mo.	55°C >10 min	80°C >5 min	0 ppt for >24 hrs	4% acetic acid 30 min
Combined likelihood of release and exposure	Low	E low?	Neg	Neg	V low?	E low?	V low?	V low?
Consequences of establishment	Moderate	Mod	Mod	Mod	Mod	Mod	Mod	Moderate
Risk estimation	Low	Neg?	Neg	Neg	V low?	Neg?	V low?	V low?

4.9 Infection with *Haplosporidium nelsoni* (MSX disease)

4.9.1 Aetiologic agent: Multinucleate sphere X (MSX) disease in American eastern oysters is caused by *Haplosporidium nelsoni*, a member of the Order Haplosporida in the Phylum Endomyxa (Adl et al. 2019). This life cycle of this parasite is unknown, but probably requires at least one alternate (possibly planktonic) host (Haskin and Andrews 1988, Powell et al. 1999, Hartikainen et al. 2014a).

4.9.2 OIE List: No **NACA List:** No **Zoonotic:** No

4.9.3 Australia's status: Other species of *Haplosporidium* occur in various regions of Australia (see Section 4.8), however *Haplosporidium nelsoni* has not been recorded from Australia and is considered exotic.

4.9.4 Epizootiology

In 1957 catastrophic mortalities were observed in American eastern oysters *Crassostrea virginica* within the first 6 weeks of them being laid down on oyster beds in Delaware Bay (Haskin et al. 1966, Ford and Haskin 1982). The disease subsequently spread through oyster populations in the higher-salinity (greater than 10 ppt) areas of affected estuaries up and down the east coast of the USA, emerging in Chesapeake Bay by 1959 and in many other locations throughout the 1960's, 70's and 80's (Ford and Haskin 1982, Ewart and Ford 1993). A new parasite was observed in the tissues and haemal spaces of affected oysters, and in areas where salinity was greater than 15 ppt, the parasite was highly pathogenic, killing oysters within 3 weeks of becoming visible in tissue sections of the gills, often resulting in mortalities of 90 to 95% (Haskin et al. 1966, Ford and Haskin 1982). By 1960 the organism had become known as multinucleate sphere X, or MSX, and in 1966 the parasite was provisionally identified as the plasmodial stage of a haplosporidian (Andrews 1966, Perkins 1968), first named *Minchinia nelsoni* (Haskin et al. 1966). Later it was redesignated by Sprague (1978) as *Haplosporidium nelsoni*. Today this parasite infects wild and cultured *C. virginica* populations along the entire eastern seaboard of the USA as far north as Nova Scotia, Canada, often at prevalences between 30-50% (Ewart and Ford 1993, Burreson and Stokes 2006, Marquis et al. 2020), but the parasite apparently does not occur in the Gulf of Mexico (Ford et al. 2011). Infections of the same parasite also occur at a much lower prevalence (< 5%) in *Crassostrea gigas* in Japan, Korea, California and France. It is thought that *H. nelsoni* was probably translocated from Japan to the east coast of the USA, then onto France, through imports of live *C. gigas* spat (Friedman 1996, Burreson et al. 2000, Renault et al. 2000a, Kamaishi and Yoshinaga 2002).

These translocations occurred despite the fact *H. nelsoni* has an unknown, possibly indirect lifecycle that probably requires at least one alternate host for transmission (Haskin and Andrews 1988, Barber and Ford 1992, Ford et al. 2001, 2018). Attempts at experimental transmission by proximity, feeding, injection, and tissue transplantation have all failed (Ford and Haskin 1982, ICES 2010, Ford et al. 2018). Spores are presumed to be a resistant transmission stage that infects a hypothetical alternative host. Spores are observed only rarely in adult oysters, but commonly occur in juvenile oysters less than one year old with advanced infections (Barber et al. 1991a, Burreson 1994). This may be due to reduced immune competence in smaller, younger oysters (Hine 2020). Modelling (and the fact that the parasite is first observed in gill tissue) suggests that the infective stage of *H. nelsoni* which infects the oyster is water borne, and most likely acquired by

feeding (Ford and Haskin 1982, Haskin and Andrews 1988). The infective stage for *H. nelsoni* is smaller than 150 µm, but larger than 1 µm, and susceptible to UV radiation at a dose of 30 mJ/cm^2 (Ford et al. 2001).

Various studies have found that infections of *H. nelsoni* in *C. virginica* on the east coast of the USA vary both seasonally and annually. One major infection window occurs during late spring and early summer when oysters increase activity after water temperatures rise from their winter lows of around 5°C (Andrews 1982, Ford and Haskin 1982). Yearly cycles of infection became apparent in Delaware Bay and elsewhere in the initial decades after introduction of the parasite, with peaks of infection pressure occurring every 6-8 years (Andrews 1982, Ford and Haskin 1982, Ewart and Ford 1993, Carnegie and Burreson 2011). Marked reductions of *H. nelsoni* infection pressure 1-2 years after extremely cold winters suggested these fluctuations may be related to how environmental variables (temperature, salinity) influence the parasite (Ford 1985) and the dynamics of host (or intermediate host) populations (Ford and Haskin 1982, Ewart and Ford 1993, Burreson and Ford 2004). Indeed, a series of extreme, multi-year droughts since the early 1980s increased salinities in many estuaries along the east coast of the USA, and this was thought to have permitted the spread of *H. nelsoni* into new areas where it had previously been absent (Burreson and Ford 2004).

The requirements for the hypothetical intermediate host to conform with the observed effects of *H. nelsoni* on oyster populations are as follows: 1. It must be capable of releasing large number of infective particles rapidly and continuously during the warm months; 2. normal temperature and salinity variation cannot affect it; 3. it must be affected by cold winters, but capable of recovery within a year or two; 4. it must produce infective particles independently of *H. nelsoni* levels in the oyster population; and 5. it must exist at relatively high salinity (Powell et al. 1999, Burreson and Ford 2004). Studies have found *H. nelsoni* DNA in many potential reservoir hosts including 60-70% of tunicates (*Didemnum* spp., *Styela* spp.), 30% of plankton samples and gastropods in areas where diseased *C. virginica* are cultured (Messerman and Bowden 2016). Occurrence of parasite DNA in these other species was highest in the summer months when prevalence of *H. nelsoni* in oysters was highest, however their role in the disease process as true hosts or reservoirs/mechanical vectors remains unclear (Messerman and Bowden 2016). It has been pointed out that movements of alternative or reservoir hosts by ballast water or shipping could be an important mechanism of spread of *H. nelsoni* to new locations (Burreson et al. 2000, Messerman and Bowden 2016, Table 9). Absence of hypothetical intermediate host(s) may also explain the apparent absence of *H. nelsoni* in the Gulf of Mexico (Ford et al. 2011, 2018).

A gradual increase in the resistance of *C. virginica* populations along the east coast of the USA to *H. nelsoni* infection has been observed in the 60+ years since the introduction of the parasite. The initial high mortality rates in Delaware Bay quickly decreased from 90-95% down to around 50% by 1960 due to strong selection within the first 2 or 3 generations of surviving oysters (Ford and Haskin 1982). After the initial selection process, survival in the wild oyster population plateaued for several decades, probably because the majority of unselected oysters still inhabited the upper bay, contributing to recruitment downriver while being protected from *H. nelsoni* infection by low salinity (Carnegie and Burreson 2011, Ford and Bushek 2012). Only after a series of extreme, multi-year droughts in the early 1980s were these upper estuary oysters also exposed to and thus selected to survive *H. nelsoni* infection, after which population resistance increased further. After 50 years post-incursion, prevalences in *C. virginica* populations rarely exceeded 20-30% and mortality rates remain relatively low except in exceptional circumstances (Carnegie and Burreson 2011, Ford and Bushek 2012). Nevertheless, unselected *C. virginica* still experience high mortalities when exposed at high salinity locations (Barber et al. 1991b), confirming that the high virulence of *H. nelsoni* along the east coast of the USA was due to introduction of an exotic pathogen (Burreson et al. 2000).

It appears that resistance to MSX disease is due to physiological ability of the oyster to compensate metabolically for the energetic burden posed by the parasite infections (Barber et al. 1991b). Heavy infections with *H. nelsoni* cause a significant decrease in the feeding rate of the oyster (Newell 1985, Barber et al. 1991b), and it is this loss of condition, together with organ dysfunction, which is likely to be the cause of eventual mortality of heavily infected oysters (Newell 1985, Barber et al. 1991b).

4.9.5 Release assessment

Haplosporidium nelsoni has not been recorded from Australian molluscs and is considered exotic, however it is included in this risk analysis as it is listed as a notifiable disease agent that could cause serious harm if it were introduced at some point in the future. Haplosporidian infective stages that infect oysters are undescribed, but they are smaller than 150 µm and their transmission is prevented by exposing infected water to a 1 µm filter followed by UV irradiation at a dose of 30 mJ/cm^2 (Ford et al. 2001). It is known that *H. nelsoni* does not tolerate freshwater (Ford 1985), however, it appears the resistance of infective or vegetative stages of the parasite to desiccation, heat or acetic acid has not been determined. Taking into account the likely propagule pressure experienced via the various anthropogenic introduction pathways, the likelihood estimations for the release of *Haplosporidium nelsoni* via the identified risk pathways are listed below.

Release assessment for infection with *Haplosporidium nelsoni*

	Unmitigated	Desiccation			Heat		Salinity	Other
Treatment	None	3 mo.	4 mo.	6 mo.	55°C >10 min	80°C >5 min	0 ppt for >24 hrs	4% acetic acid 30 min
Likelihood of release	Moderate	E low?	Neg	Neg	Low?	E low?	V low	V low?

4.9.6 Exposure assessment

While *H. nelsoni* infects oyster tissue and not shell material, it is likely that shells from wild and /or cultured oysters that may be procured from processors or retailers for recycling would retain some residual host tissue in areas such as retractor muscle scars, mantle tissue and so on. *Haplosporidium nelsoni* may proliferate rapidly when oysters are held at high densities in captivity (Sunila et al. 2000), however there is little evidence that it can be transmitted horizontally directly through the water or vertically between generations. Spread of *H. nelsoni* via contaminated recycled mollusc shells would occur only if sufficient viable infective stages were introduced into areas where susceptible alternate hosts and/or oyster final hosts were present under environmental conditions suitable for transmission. Because unknown alternate host(s) are presumably needed in order to complete the *H. nelsoni* lifecycle (Haskin and Andrews 1988, Ford et al. 2001, 2018), the exposure pathway required for transmission remains unknown, as is important information such as the minimum infective dose required for an index case to occur, though being a parasite, theoretically infection by just one infective stage can result in successful transmission. These disease agents are highly pathogenic, however *H. nelsoni* appears to have relatively high host specificity infecting predominately *C. gigas* and *C. virginica* (see Burreson and Ford 2004) and it is not clear whether transmission and/or further spread from an index case would occur in Australian native oyster species. It would, however, persist in areas of Australia where *C. gigas* is present. The absence of *H. nelsoni* in some parts of the range of *C. virginica* may be due to a restricted distribution of alternate hosts (Ford et al. 2011, 2018). However, the fact that *H. nelsoni* has been

translocated and established infections in new regions (Friedman 1996, Burreson et al. 2000, Renault et al. 2000a), suggests that some of the presumptive alternate hosts may be widespread and/or ubiquitous (e.g. planktonic copepods, Hartikainen et al. 2014a), or that these parasites may have lower host specificity for the alternate host. As there may be pathways to expose shellfish reef restoration projects to haplosporidians via recycled mollusc shells, environmental conditions are likely to be suitable for disease transmission in many parts of Australia and suitable hosts may occur in the wild in areas where shellfish reefs are being restored, the risk of exposure and establishment is non-negligible, and the overall likelihood of exposure and establishment of *H. nelsoni* is considered to be **Low.**

4.9.7 Consequence assessment

Although other haplosporidian parasites are already present in populations of wild molluscs in some regions of Australia, *H. nelsoni* has never been recorded here and is considered exotic. There is evidence that *H. nelsoni* can cause major disease outbreaks in wild and cultured native oysters in some countries, but it appears to cause minimal problems in *C. gigas* and exhibits host specificity such that it is unclear whether Australian oysters would be susceptible to infection. This disease agent is no longer listed by the OIE (OIE 2009) or NACA, but it remains listed as a reportable disease in Tasmania, SA, WA and the NT (Table 3). Hence its detection in Australia may result in intervention by government authorities, which could cause disruption to aquaculture, fisheries or shellfish reef restoration if attempts were made to contain the infection and prevent its further spread into uninfected areas. If *H. nelsoni* was detected in the wild, there would appear to be little chance of eradication, and it would likely take at least 50 years before the new host oyster population may be able to adapt and become resistant to it. Taking all of these factors into consideration, the consequences of establishment of *H. nelsoni* via recycled mollusc shells is considered to be **Moderate.**

4.9.8 Risk estimation

The risk estimation for *H. nelsoni* is determined by combining the likelihood of release and exposure (from Table 5) with the consequences of establishment (Table 6) to arrive at a risk estimation (Table 7). The risk estimate for infection with *H. nelsoni* exceeds the ALOP for some release pathways, suggesting that additional risk management is required for this disease agent.

Risk estimate for infection with *Haplosporidium nelsoni*

	Unmitigated	Desiccation			Heat		Salinity	Other
Treatment	None	3 mo.	4 mo.	6 mo.	55°C >10 min	80°C >5 min	0 ppt for >24 hrs	4% acetic acid 30 min
Combined likelihood of release and exposure	Low	E low?	Neg	Neg	V low?	E low?	V low	V low?
Consequences of establishment	Moderate	Mod	Mod	Mod	Mod	Mod	Mod	Moderate
Risk estimation	Low	Neg?	Neg	Neg	V low?	Neg?	V low	V low?

4.10 Infection with *Marteilia refringens*

4.10.1 Aetiologic agent: Aber disease (or marteiliosis) is caused by *Marteilia refringens*, a protozoan parasite classified in the Order Paramyxida within the Phylum Endomyxa (Adl et al. 2019). Members of the Paramyxida are parasites of invertebrates (Ward et al. 2016) which are characterised by their distinctive "cell within cell" development (Carrasco et al. 2015). *Marteilia refringens* infects a range of bivalve species as well as planktonic copepods *Paracartia grani*, females of which can become infected through contact with spore stages (see Audemard et al. 2001, 2002, 2004, Boyer et al. 2013).

4.10.2 OIE List: Yes **NACA List:** No **Zoonotic:** No

4.10.3 Australia's status: Other species of *Marteilia* occur in various regions of Australia (see Sections 4.11, 4.12), however *Marteilia refringens* has not been recorded from Australia and is considered exotic.

4.10.4 Epizootiology

The Paramyxea are parasites of marine invertebrates that are characterised by the formation of spores via internal cleavage of sporangia within plasmodia (Desportes and Perkins 1990). Paramyxean parasites of the genus *Marteilia* have caused significant disease and economic impacts in aquaculture and fisheries targeting oysters and clams in several regions of the world (Berthe et al. 2004, Carrasco et al. 2015). In 1968, mass mortalities of European flat oysters (*Ostrea edulis*) were first described in the upstream reaches of the Aber Wrach River in Brittany, France (Comps 1970, Herrbach 1971). Affected oysters displayed reduced growth, weight loss, yellowish pallor of the digestive gland and gaping prior to death (Comps 1970). Wet preparations and histology of oysters affected with "Aber disease" found large numbers of various developmental stages of a new spherical refringent parasite in the epithelium of the digestive gland tubules (Herrbach 1971). The parasite appeared to divide by internal division inside its own primary (mother) cells, producing up to 8 secondary cells (sporonts) per primary cell, eventually displacing most of the digestive gland tissue, leading to sporulation followed by the death of the host due to starvation (Grizel et al. 1974, Berthe et al. 2004).

The new parasite was originally aligned taxonomically with fungi, then microsporidia, however further ultrastructural detail from the description of *Marteilia refringens* by Grizel et al. (1974) and others lead to its placement in the Haplosporidia (Perkins 1976a). It was subsequently moved again following the erection of the Phylum Paramyxea (Desportes and Perkins 1990), and with the advent of small sub unit rDNA phylogenetic analysis *M. refringens* was finally settled within the Paramyxea, now known as the Order Paramyxida (reviewed by Berthe et al. 2004, Lester and Hine 2017) within the Phylum Endomyxa (Adl et al. 2019). During this time, Aber disease had spread throughout populations of *O. edulis* in Western Europe, devastating the flat oyster industry in France with mortality rates between 50 and 90% which reduced production to less than 10% of pre epizootic levels (Grizel et al. 1974, Alderman 1979, Grizel 1985). The disease has since spread south to be recorded throughout France as well as in Spain, Portugal, Italy Croatia, Greece and other areas of the Mediterranean Sea (Alderman 1979, Balouet 1979, Kerr et al. 2018). Aber disease occurs in the summer months, with sporulation and mortalities occurring once water temperatures exceed 17°C (Balouet 1979, Grizel 1985, Audemard et al. 2001, 2004). It appears that the parasite enters the oyster via the gills, palps and anterior gut during a relatively short (3 month) summertime window of infection

(Audemard et al. 2004). Once inside the oyster the parasite divides and bicellular stages are liberated into the surrounding connective tissue and haemolymph spaces (Grizel et al. 1974). Following systemic dissemination, the parasite infiltrates the digestive gland epithelium where secondary (daughter) cells are formed, and sporulation occurs with the spores being released via the faeces (Perkins 1976a, Grizel 1985). The massive proliferation of parasites results in extensive destruction of the digestive gland epithelium and host death (Balouet 1979, Grizel 1985).

Several attempts over 30 years to directly infect oysters with *M. refringens* by feeding or injecting infected homogenized digestive gland, by cohabitation with infected stock or by introduction of oysters into the field after a disease event all failed to produce infections (Balouet et al. 1979, Grizel 1985, Berthe et al. 1998), suggesting at least one alternative host is required to compete the life cycle. Eventually, the development of molecular diagnostic tools allowed close study of the disease process in semi-closed claire ponds in France which had a much reduced biodiversity of potential alternate hosts (Audemard et al. 2001). These studies generated a breakthrough when they found *M. refringens* DNA in various invertebrates including cnidarians and nematodes, but *M. refringens* DNA and developmental stages were also found in the planktonic copepod *Paracartia grani*, where it infected the female gonad (Audemard et al. 2002). Subsequent work confirmed that copepods can become infected through contact with spore stages released by infected oysters (Audemard et al. 2002, 2004, Boyer et al. 2013), confirming their role in the lifecycle. Ingestion of the developmental stages from the copepod by *O. edulis*, however, did not infect the oysters (Audemard et al. 2002, 2004), suggesting that the full life cycle may require other intermediate hosts, or that *P. grani* could be a dead end host for *M. refringens* infecting *O. edulis*. Subsequent studies in Thau Lagoon in southern France found that Mediterranean mussels (*Mytilus galloprovincialis*) could ingest eggs and nauplii of *M. refringens* infected *P. grani*, and that the digestive epithelium and alimentary canal of both male and female copepod could become infected (Boyer et al. 2013). Boyer et al. (2013) did not rule out the possibility of a copepod-mussel-copepod lifecycle for *M. refringens* in mussels.

Marteilia refringens was first observed to naturally infect *O. edulis*, but experiments infected other flat oysters including *O. angasi*, *O. puelchana*, and *O. chilensis* (see Berthe et al. 2004). The parasite was also detected in blue mussels (*Mytilus edulis*) and Mediterranean mussels (M. *galloprovincialis*) in northern Europe (Longshaw et al. 2001, Berthe et al. 2004, Kerr et al. 2018). *Marteilia refringens* has also been found infecting Pacific oysters (*C. gigas*, see Balouet 1979), American oysters (*C. virginica*, see Renault et al. 1995a) and clams (*Solen marginatus*, *Chamelea gallina*, *Ruditapes decussatus*, see Lopes-Flores et al. 2008a, 2008b, Boyer et al. 2013) in the absence of disease. The situation with infections of mussels is interesting as *Marteilia maurini* was originally described in blue and Mediterranean mussels from France, but was later synonymised with *M. refringens* when no strict correlation of *Marteilia* types to mussels or oysters could be established (Longshaw et al. 2001, Berthe et al. 2004). However, later studies found the two parasites could be differentiated by DNA sequencing and lifecycle studies using alternate host *P. grani* found that copepod infection patterns were different depending on whether they were infected with spores from oysters or mussels (Carrasco et al. 2008). They found that "M type" *M. refringens* (= *M. maurini*) did not proliferate in *P. grani*, while "O type" *M. refringens* (= *M. refringens sensu stricto*) rapidly proliferated in infected copepods (Carrasco et al. 2008), suggesting biologically significant differences between the two genotypes of parasite. Subsequent studies found that only "M type" parasites were detected in *Mytilus* spp. in northern France, the UK, Sweden and Norway, while both "M Type" and "O Type" parasites were detected in mixed infections in both *O. edulis* and *Mytilus* spp. from central and southern Europe (Kerr et al. 2018). This led to the

redescription of the "M type" as *Marteilia pararefringens* sp. nov. which infects mussels in the UK, Sweden, and Norway, as well as mussels and *O. edulis* in France, with no evidence of *M. refringens* north of France (Kerr et al. 2018).

4.10.5 Release assessment

Marteilia refringens has not been recorded from Australian molluscs and is considered exotic, however it is included in this risk analysis as it is listed as a notifiable disease agent that could cause serious harm if it were introduced at some point in the future. The parasite has an indirect life cycle and it is known that spore stages of *M. refringens* can infect planktonic copepods (Audemard et al. 2002, 2004), however the infective stages that infect oysters have not been described. It is possible that eggs and nauplii of planktonic copepods infected with *Marteilia* may be able to transmit parasite DNA to mussels (Boyer et al. 2013), however it is possible the parasite in that study was *M. pararefringens*, not *M. refringens*, and the relevance of the findings of Boyer et al. (2013) for transmission of *M. refringens* remains uncertain. Certainly, many other species of filter feeding invertebrates which may be attached to oyster shells are likely to temporarily accumulate *Marteilia* spores in their digestive tract (see Audemard et al. 2002), and hence these could also act as mechanical vectors. Unfortunately, the resistance of infective or vegetative stages of the parasite to desiccation, freshwater, heat or acetic acid hava apparently not been determined. Taking into account the likely propagule pressure experienced via the various anthropogenic introduction pathways, the likelihood estimations for the release of *M. refringens* via the identified risk pathways are listed below.

Release assessment for infection with *Marteilia refringens*

	Unmitigated	Desiccation			Heat		Salinity	Other
Treatment	None	3 mo.	4 mo.	6 mo.	55°C >10 min	80°C >5 min	0 ppt for >24 hrs	4% acetic acid 30 min
Likelihood of release	Moderate	E low?	Neg	Neg	Low?	E low?	V low?	V low?

4.10.6 Exposure assessment

While *M. refringens* infects oyster tissue and not shell material, it is likely that shells from wild and /or cultured oysters that may be procured from processors or retailers for recycling would retain some residual host tissue in areas such as retractor muscle scars, mantle tissue and so on. Since there is little evidence that *M. refringens* can be transmitted horizontally directly through the water or vertically between generations, spread of the parasite via contaminated recycled mollusc shells would occur only if sufficient viable infective stages were introduced into areas where susceptible alternate hosts and oyster final hosts were present under environmental conditions suitable for transmission. The full range of planktonic alternate host(s) required in order to complete the *M. refringens* lifecycle (Audemard et al. 2002, 2004, Boyer et al. 2013) is unknown, as is the exact exposure pathway required to complete the lifecycle. The minimum infective dose required for an index case to occur is also unknown, though being a parasite, theoretically infection by just one infective stage can result in successful transmission. *Marteilia refringens* is highly pathogenic to flat oysters, and it is known to cause mortality in *O. angasi*, while *C. gigas* is susceptible to infection but does not become diseased, thus susceptible hosts are known to occur in Australia. As there may be pathways to expose shellfish reef

restoration projects to *M. refringens* via recycled mollusc shells, environmental conditions are likely to be suitable for disease transmission in many parts of Australia and suitable hosts may occur in the wild in areas where shellfish reefs are being restored, the risk of exposure and establishment is non-negligible, and the overall likelihood of exposure and establishment of *M. refringens* is considered to be **Low.**

4.10.7 Consequence assessment

Although other paramyxid parasites are already present in populations of wild molluscs in some regions of Australia, *M. refringens* has never been recorded here and is considered exotic. There is evidence that *M. refringens* can cause major disease outbreaks in wild and cultured flat oysters, and *O. angasi* is known to be susceptible to infection. The known alternate hosts for *M. refringens* are planktonic copepods which are widespread and thus the indirect lifecycle may not necessarily restrict dispersal of the parasite through movements of molluscs or their shells. This disease agent is listed by the OIE, and is a reportable disease in all states (Table 3). Hence its detection in Australia may have significant trade implications, and would likely result in intervention by government authorities. Such intervention could cause disruption to aquaculture, fisheries or shellfish reef restoration if attempts were made to contain the infection and prevent its further spread into uninfected areas. Nevertheless, if *M. refringens* was detected in the wild, there would appear to be little chance of eradication. Taking all of these factors into consideration, the consequences of establishment of *M. refringens* via recycled mollusc shells is considered to be **High.**

4.10.8 Risk estimation

The risk estimation for *M. refringens* is determined by combining the likelihood of release and exposure (from Table 5) with the consequences of establishment (Table 6) to arrive at a risk estimation (Table 7). The risk estimate for infection with *M. refringens* exceeds the ALOP for some release pathways, suggesting that additional risk management is required for this disease agent.

Risk estimate for infection with *Marteilia refringens*

	Unmitigated	Desiccation			Heat		Salinity	Other
Treatment	None	3 mo.	4 mo.	6 mo.	55°C >10 min	80°C >5 min	0 ppt for >24 hrs	4% acetic acid 30 min
Combined likelihood of release and exposure	Low	E low?	Neg	Neg	V low?	E low?	V low?	V low?
Consequences of establishment	High	High	High	High	High	High	High	High
Risk estimation	Moderate	V low?	Neg	Neg	Low?	V low?	Low?	Low?

4.11 Infection with *Marteilia sydneyi* (QX disease)

4.11.1 Aetiologic agent: QX disease is caused by *Marteilia sydneyi*, a protozoan parasite classified in the Order Paramyxida within the Phylum Endomyxa (Adl et al. 2019). *Marteilia sydneyi* infects Sydney rock oysters *Saccostrea glomerata* (see Perkins and Wolf 1976) and some polychaete annelids, including *Nephtys australiensis* (see Adlard and Nolan 2015).

4.11.2 OIE List: No **NACA List:** No **Zoonotic:** No

4.11.3 Australia's status: *Marteilia sydneyi* infections have been reported in *S. glomerata* from QLD (Wolf 1972, Perkins and Wolf 1976), NSW (Adlard and Ernst 1995) and WA (Hine and Thorne 2000).

4.11.4 Epizootiology

Marteilia sydneyi is responsible for QX disease (Queensland unknown disease) that has caused massive losses (up to 99% mortality) in wild and cultured Sydney rock oyster *Saccostrea glomerata* populations along the east coast of Australia from Great Sandy Straits in QLD to the NSW/Victoria border since the late 1960's (Wolf 1972, Perkins and Wolf 1976, Lester 1989, Roubal et al. 1989, Adlard and Ernst 1995, Wilkie et al. 2013, Reid and Bone 2020). The extent of the problems highlighted by this disease are shown by the fact it is not uncommon for aquaculture production of *S. glomerata* in estuaries where QX outbreaks have become problematic to be reduced to less than 1% of historic production within 20 years of its emergence (Reid and Bone 2020). Outbreaks of QX disease were first problematic in South East Queensland then later emerged in rivers further to the south (Wolf 1979), suggesting that the disease agent was possibly translocated into more southern estuaries via movements of infected oysters (Adlard and Ernst 1995, Kleeman et al. 2004). Then in the early 1990's an apparently identical parasite was found in *S. glomerata* near King Bay in the Dampier Archipelago, northern WA at very low prevalence (1 out of 933) in surveys undertaken between 1992 and 1994 (Hine and Thorne 2000), as well as in subsequent surveys (1 of 411 *S. glomerata*) undertaken in 1995 between Canarvon and the Dampier Archipelago (Jones and Creeper 2006).

QX disease is caused by a massive infection of the digestive gland with *M. sydneyi* developmental stages (Kleeman et al. 2002a, 2002b). Like for *M. refringens* (see Section 4.10), *M. sydneyi* infective stages enter the oyster via the gills or labial palps during feeding, followed by extrasporogenic proliferation into the surrounding connective tissues and haemolymph (Kleeman et al. 2002a). This initial proliferation leads to systemic infection with further development in the digestive gland where primary cells develop secondary cells with between 8 and 16 secondary cells produced within each primary cell, often followed by sporulation and death (Perkins and Wolf 1976, Kleeman et al. 2002a, 2002b). Oysters appear to be exposed to the infective stage for only a short period, usually after heavy rainfall in the summer months (Lester 1989, Roubal et al. 1989, Wesche 1995) with infection ceasing once water temperatures drop below 21.5°C (Rubio et al. 2013), The infection window varies from as little as 3 weeks (Wesche 1995) to as long as 18 weeks starting from November to February, with the onset of the window probably depending on various location, water temperature and other environmentally specific variables (Rubio et al. 2013). Under suitable conditions for disease development, oyster deaths increase from late summer into autumn (Lester and Hine 2017). However, the parasite remains present in healthy oyster populations at low prevalences in most infected estuaries (Adlard

and Nolan 2008), and in estuaries where QX disease occurs, outbreaks do not occur every year (Butt et al. 2006, Butt and Raftos 2007, Rubio et al. 2013). This is probably because the onset of QX disease is related to a combination of the presence of mud which harbours the potential alternate hosts (Adlard and Nolan 2008, 2015, Diggles 2013), as well as immunosuppression of the oyster host (Peters and Raftos 2003, Butt and Raftos 2007), due to reduced salinity (Butt et al. 2006, Green and Barnes 2010) and unidentified waterborne contaminants carried in summer rain runoff (Butt and Raftos 2007, Rubio et al. 2013). The first recognised epizootics of QX disease were documented in Pumicestone Passage in 1968 (Wolf 1972) following dieoffs of seagrasses and invertebrates (including mud crabs *Scylla serrata*) after aerial spraying of herbicides during development of pine plantations in the adjacent catchment during the mid 1960's (Harry Sunderland, quoted in Clarke (2020), supported by information from interviews of other long term local residents by B.K. Diggles). Similarly, the original outbreak of QX disease in the Hawkesbury River in 2004 occurred after a period of poor water quality following large scale glyphosate herbicide spraying to control aquatic weeds during the 2003/04 *Salvinia* sp. bloom which covered over 88km of river (Coventry 2006). The conditions leading up to the *Salvinia* sp. outbreak and its dispersion may have been the "transient stressor" identified by Butt and Raftos (2007) that precipitated the original QX disease outbreak in that river system.

Like *Marteilia refringens*, the lifecycle of *Marteilia sydneyi* cannot be completed by direct transmission pathways and is likely to be indirect (Roubal et al. 1989, Wesche et al. 1999, Berthe et al. 2004). Recent evidence suggests that *M. sydneyi* may require passage through polychaete worms of the Family *Nephtyidae* as one (of possibly several) alternate host (see Adlard and Nolan 2008, 2015, Cribb 2010). *Marteilia sydneyi* DNA was detected in around 6% of 1247 samples of various species of benthic polychaetes (Families *Cirratulidae*, *Lumberineridae*, *Magelonidae*, *Nephtyidae*, *Sabellidae*, *Trichobranchidae*, *Spionidae*) in the Hawkesbury River, and of these only the polychaete *Nephtys australiensis* harboured developmental stages of *M. sydneyi* in the form of two differing morphological forms: a 'primordial' cell that contained a well-defined nucleus but had little differentiation in the cytoplasm, and a 'plasmodial' cell that showed an apparent syncytial structure (Adlard and Nolan 2015). *Nephtys australiensis* (now taxonomically revised to *Aglaophamus australiensis* by Ravara et al. 2010) grows to 85 mm and is described as being more common in muddy rather than sandy sediments (Rainer and Hutchings 1977). The increased virulence of *M. sydneyi* in degraded estuaries compared to historical times may therefore be due to a combination of increased immunosuppression of the host due to declining water quality together with increased abundance of polychaete alternate hosts that are favored by sedimentation, eutrophication and other anthropogenic changes derived from catchment development (Saville-Kent 1891, Green et al. 2011, Diggles 2013). This observation is supported by the fact that: 1). oysters that carry low levels of *M. sydneyi* infections can shed the parasite and apparently make a full recovery under suitable environmental conditions (Roubal et al. 1989), and 2). *M. sydneyi* remains at extremely low prevalences in the absence of disease in *S. glomerata* sampled from remote, undeveloped locations in northern WA (Hine and Thorne 2000, Jones and Creeper 2006).

Besides the availability of alternate hosts, other environmental variables such as seasonal factors related to temperature and salinity are the main drivers that influence the lifecycle of *Marteilia sydneyi* (see Rubio et al. 2013). Epizootics due to this parasite occur mainly after summer floods have immunosuppressed oyster hosts and helped drive the lifecycle to completion by increasing the quantity of mud and sediment in the river system (and perhaps at the same time liberating infective stages from alternate hosts, see Diggles 2013). Selective breeding can develop some resistance to QX disease within 2 (Nell and Hand 2003) to 4 (Dove et al. 2013) oyster generations in small scale trials. However, the emergence of severe QX disease is often followed

by long term, terminal declines in aquaculture production of *S. glomerata* in affected estuaries (Reid and Bone 2020). The failure of QX resistant oysters to improve *S. glomerata* survival in commercial aquaculture in certain estuaries (e.g. Dang et al. 2013), suggests that QX disease is a symptom (but not the underlying cause) of the ongoing decline of environmental quality in those estuaries (Diggles 2013, Reid and Bone 2020).

4.11.5 Release assessment

Marteilia sydneyi has been detected at high prevalence in wild and cultured *S. glomerata* in many areas of QLD and NSW (Adlard and Ernst 1995, Wilkie et al. 2013). The same disease agent has also been recorded at low prevalence (1 out of 117 *S. glomerata* (prevalence 0.9%) from the Dampier Archipelago, see Hine and Thorne (2000), and 1 out of 411 *S. glomerata* (prevalence 0.24%) from the same region, see Jones and Creeper 2006) in the absence of disease in northern WA. *Marteilia sydneyi* is also known to infect at least one group of alternate hosts - polychaetes within the Family *Nephtyidae*, while many other species of filter feeding invertebrates in estuaries (including spionid mudworms on mollusc shells), are likely to temporarily accumulate *Marteilia* spores in their digestive tract (see Audemard et al. 2002, Adlard and Nolan 2015). The earliest vegetative stages of *M. sydneyi* are found in the epithelia of the gills and palps, suggesting that the infective stage for the oyster is planktonic and can be easily spread (Kleeman et al. 2002a). Neither the infective stage nor the mode of transmission have been identified to date, however at certain times of year in areas where *M. sydneyi* is endemic, large numbers of infective stages and spores must occur in the water column and sediments (Roubal et al. 1989) and these can be concentrated within the digestive tract of filter feeding bivalves, which are efficient particle collectors, as well as many other species of filter feeding invertebrates (see Audemard et al. 2002, Adlard and Nolan 2015), which could act as mechanical vectors. Polychaetes are also known natural hosts for other species of paramyxids, such as *Paramyxa nephtys* in *Nephtys caeca* (see Larsson and Koie 2005, Ward et al. 2016), and crabs may also be hosts for these parasites (Ward et al. 2016), therefore it is possible that polychaetes and crabs associated with mollusc shells may also harbour infective stages.

Spores of *M. sydneyi* survive freezing, with 20% survival after 7 days and 5.8% of spores viable after 220 days at -20°C (Wesche et al. 1999). Spores can also survive in the water for up to 35 days at 15°C and 34 ppt salinity (Wesche et al. 1999), but do not survive passage through the gut of fish or birds (Wesche et al. 1999). Unfortunately, the resistance of infective or vegetative stages of *M. sydneyi* to desiccation, freshwater, heat or acetic acid has apparently not been determined. Taking into account the likely propagule pressure experienced via the various anthropogenic introduction pathways, the likelihood estimations for the release of *M. sydneyi* via the identified risk pathways are listed below.

Release assessment for infection with *Marteilia sydneyi*

	Unmitigated	Desiccation			Heat		Salinity	Other
Treatment	None	3 mo.	4 mo.	6 mo.	55°C >10 min	80°C >5 min	0 ppt for >24 hrs	4% acetic acid 30 min
Likelihood of release	Moderate	E low?	Neg	Neg	Low?	E low?	V low?	V low?

4.11.6 Exposure assessment

While Sydney rock oysters along Australia's east coast are already at risk from natural exposure to *M. sydneyi*, translocation of infected oyster shells from areas where *M. sydneyi* is enzootic into other estuaries via shellfish reef restoration projects could transport these parasites to new regions. *Marteilia sydneyi* infects oyster tissue and not shell material, however it is likely that shells from wild and /or cultured oysters that may be procured from oyster processors or retailers for recycling would retain some residual host tissue in areas such as retractor muscle scars, mantle tissue and so on. However, since there is little evidence that *M. sydneyi* can be transmitted horizontally directly through the water or vertically between generations, spread of the parasite via contaminated recycled mollusc shells would occur only if sufficient viable infective stages were introduced into areas where susceptible alternate hosts and oyster final hosts were present under environmental conditions suitable for transmission.

The full range of alternate host(s) required in order to complete the *M. sydneyi* lifecycle is unknown (Adlard and Nolan 2015), as is the exact exposure pathway required to complete the lifecycle. The minimum infective dose required for an index case to occur is also unknown, though being a parasite, theoretically infection by just one infective stage can result in successful transmission. At this time *Marteilia sydneyi* is only known to infect *S. glomerata* and polychaetes of the Family *Nephtyidae* which occur in sediments, however the latter hosts are ubiquitous in the Australian environment (Rainer and Hutchings 1977), meaning that the indirect lifecycle of *M. sydneyi* may not be a barrier to its wider dissemination through movements of oysters. *Marteilia sydneyi* is only known to be pathogenic to *S. glomerata*, and the disease agent is present in many estuaries in the absence of disease. Nevertheless, environmental conditions are suitable for disease transmission in many parts of Australia, particularly in estuaries with developed catchments and near urbanized areas where QX disease would be favoured because of the ongoing decline of environmental quality in those estuaries (Diggles 2013, Reid and Bone 2020). As there may be pathways to expose shellfish reef restoration projects to *M. sydneyi* via recycled mollusc shells, and suitable hosts may occur in the wild in areas where shellfish reefs are being restored, the risk of exposure and establishment is non-negligible, and the overall likelihood of exposure and establishment of *M. sydneyi* is considered to be **Low.**

4.11.7 Consequence assessment

Although *M. sydneyi* is already present in populations of wild rock oysters in some regions of Australia, it tends to only cause disease in degraded estuaries where environmental quality has undergone significant decline. Nevertheless, many other regions appear free of infection at this time. The known alternate hosts for *M. sydneyi* are widespread and therefore the indirect lifecycle may not restrict dispersal of the parasite through movements of oysters or their shells. There is evidence that *M. sydneyi* can cause major disease outbreaks and significant impacts on populations of both wild and cultured rock oysters under certain conditions. *Marteilia sydneyi* is no longer listed by the OIE and NACA, but this disease agent remains listed as a reportable disease in NSW, Victoria, Tasmania, SA, WA and the NT (Table 3). Hence the spread of *M. sydneyi* to new areas could adversely impact trade and may result in intervention by government authorities. Such intervention could cause disruption to aquaculture, fisheries or shellfish reef restoration if attempts were made to contain the infection and prevent its further spread into uninfected areas. Nevertheless, once *M. sydneyi* is detected in the wild, there would appear to be little chance of eradication. While selective breeding over many generations may be able to develop strains of oysters resistant to QX, it appears likely that this will take

several decades to achieve meaningful increases in survival of cultured populations. In contrast, wild oyster populations will likely be even slower to acquire natural resistance due to genetic dilution by cross breeding with susceptible oysters. Taking all of these factors into consideration, the consequences of establishment of *M. sydneyi* via recycled mollusc shells is considered to be **Moderate.**

4.11.8 Risk estimation

The risk estimation for *M. sydneyi* is determined by combining the likelihood of release and exposure (from Table 5) with the consequences of establishment (Table 6) to arrive at a risk estimation (Table 7). The risk estimate for infection with *M. sydneyi* exceeds the ALOP for some release pathways, suggesting that additional risk management is required for these disease agents.

Risk estimate for infection with *Marteilia sydneyi*

	Unmitigated	Desiccation			Heat		Salinity	Other
Treatment	None	3 mo.	4 mo.	6 mo.	55°C >10 min	80°C >5 min	0 ppt for >24 hrs	4% acetic acid 30 min
Combined likelihood of release and exposure	Low	E low?	Neg	Neg	V low?	E low?	V low?	V low?
Consequences of establishment	Moderate	Mod	Mod	Mod	Mod	Mod	Mod	Mod
Risk estimation	Low	Neg?	Neg	Neg	V low?	Neg?	V low?	V low?

4.12 Infection with *Marteilia* spp. and *Marteilioides* spp.

4.12.1 Aetiologic agent: *Marteilia* spp. and *Marteilioides* spp. are protozoan parasites classified in the Order Paramyxida within the Phylum Endomyxa (Adl et al. 2019). This section includes *Marteilioides* spp. and *Marteilia* spp. (other than *Marteilia sydneyi* that causes QX disease in Sydney rock oysters (see Section 4.11) and *Marteilioides chungmuensis* (see Section 4.13)).

4.12.2 OIE List: No **NACA List:** No **Zoonotic:** No

4.12.3 Australia's status: A paramyxid resembling *Marteilia lengehi* was found in *Saccostrea cucullata* from near Exmouth in northern WA at a prevalence of 0.08% (Hine and Thorne 2000). In southern NSW an unidentified protozoan reminiscent of *Marteilia* spp. was observed in histological sections of flat oysters (*O. angasi*) sampled from Bermagui and Narooma at prevalences of around 1% (Heasman et al 2004). Species of *Marteilioides* have also been described in the ovary of *Saccostrea echinata* from the NT and the gills of *S. glomerata* from northern NSW (Anderson and Lester 1992, Hine and Thorne 2000).

4.12.4 Epizootiology

Paramyxids are parasites of marine invertebrates (molluscs, annelids, crustaceans) that are characterised by the formation of spores via internal cleavage of sporangia within plasmodia (Desportes and Perkins 1990, Ward et al. 2016). Paramyxean parasites of the genus *Marteilia* and *Marteilioides* have caused significant disease and economic impacts on oyster culture in several regions of the world (Berthe et al. 2004, Carrasco et al. 2015). For a summary of epizootiological information related to the most significant notifiable paramyxid disease agents (*Marteilia refringens*, *M. sydneyi* and *Marteilioides chungmuensis*), see Sections 4.10, 4.11 and 4.13. While these three oyster pathogens have generated the majority of knowledge about paramyxid parasites to date, recent studies have found evidence of several new species of *Marteilia* in the northern hemisphere. For example, Kerr et al. (2018) found that *M. refringens* outbreaks in Europe are in fact likely to have been due to two different parasite species, *M. refringens sensu stricto* (previously "O type"), and *M. pararefringens* (previously "M type"). In Spain, *Marteilia cochillia* emerged and caused mass mortalities in common cockles (*Cerastoderma edule*), leading to the collapse of that fishery (Carrasco et al. 2013, Villalba et al. 2014), while another species, *Marteilia octospora* was also found in razor clams in the absence of disease (see Ruiz et al. 2016). Interestingly, Stentiford et al. (2017) found that *M. cochillia* was itself infected with the hyperparasitic microsporidian *Hyperspora aquatica*. In Asia, new species of paramyxids discovered in the last decade include *Marteilia* (*Eomarteilia*) *granula* and *Marteilia tapetis* from Manila clams (*Ruditapes philippinarum*) (see Itoh et al. 2014, Ward et al. 2016, Kang et al. 2019), with the latter found at low prevalence (c. 2%) in the absence of disease despite being most closely related to *M. sydneyi* (89.9% similarity in 18S rDNA sequence, see Kang et al. 2019). This section will not, however, consider other paramyxid parasites such as the genera *Paramyxa* and *Paramyxoides* which are found in polychaetes (Lester and Hine 2017), except to note the likely involvement of polychaetes in the lifecycle of some species of oyster-infecting *Marteilia* (see Adlard and Nolan 2015). The remaining paramyxid genus, namely *Paramarteilia*, is known to infect crustaceans, such as *P. canceri* which was found at a prevalence of 1-3% in the hepatopancreas of European edible crabs *Cancer pagurus* (see Feist et al. 2009), and *P. orchestiae* which infects the testes and ovaries and modifies the sexual status of the amphipod *Orchestia gammarellus* (see Ward et al. 2016, Pickup and Ironside 2018). Again, the

involvement of crustaceans in the lifecycle of some species of oyster-infecting *Marteilia* which also infect the gonads of planktonic copepods (see Audemard et al. 2002, 2004, Boyer et al. 2013), is notable.

In contrast to the many recent discoveries in the northern hemisphere, there are only a few recorded instances of detection of paramyxid species other than *M. sydneyi* in Australia. Wolf (1977) described a *Marteilioides*-like parasite in the ovary of 57% of female *Saccostrea echinata* sampled from Darwin Harbour in the Northern Territory. Hine and Thorne (2000) also recorded the presence of a *Marteilioides* sp. in the ovary of 9 out of 94 (prevalence 9.6%) *S. echinata* sampled from the same location. A paramyxid resembling *Marteilia lengehi* was found in *Saccostrea cucullata* sampled between 1992 and 1994 from near Exmouth in northern WA (Hine and Thorne 2000). The parasite occurred within the epithelium of the distal and part of the proximal digestive diverticulae (but not in the main digestive tract) of 1 out of 26 *S. cucullata* sampled from the Montebello Islands off Exmouth (prevalence 3.8%). This was the only oyster infected in the 769 oysters sampled from near Exmouth (prevalence 0.1%), and after examination of a total of 1254 *S. cucullata* from various locations throughout WA, this suggests that the parasite was rare (overall prevalence 0.08%, Hine and Thorne 2000). *Marteilia lengehi* was originally described by Comps (1976) from *S. cucullata* sampled from the Persian Gulf, where it was found to infect the epithelium of the digestive gland, but in the absence of transmission electron microscopy and molecular data, the identity of the *Marteilia* spp. recorded by Hine and Thorne (2000) near Exmouth remains undetermined.

Sydney rock oysters *S. glomerata* from the Clarence River, northern NSW, were also found to be infected by a new species of *Marteilioides* during a QX disease outbreak in June 1991 (Anderson and Lester 1992). The parasite infected focal (1-2 mm diametre) areas of the gills causing hyperplasia and haemocytosis as well as grossly visible discolouration of the gill lamellae (Anderson and Lester 1992). The parasite was described as a new species, *Marteilioides branchialis*, based on its location in the gills and different sporulation characteristics compared to *Marteilioides chungmuensis* (see Anderson and Lester 1992). In a later study a *Marteilia*-like paramyxid was detected in the epithelium of the digestive gland of two *O. angasi* (overall prevalence 0.4%) during a survey of flat oysters from NSW in 2002/03 (Heasman et al. 2004). One oyster from Bermagui (prevalence 1.1%) had a moderate infection while another from Narooma (prevalence 1%) had a heavy infection with all tubules in section infected. Both affected oysters had very pale digestive glands. Primary cells in the digestive gland epithelium contained 1 to >10 secondary cells (Heasman et al. 2004). Sporogenesis was observed in both infected oysters, but was particularly apparent in the heavily infected oyster from Narooma, with up to 14 acid fast spore-like bodies observed in sections through secondary cells within the tubule epithelium (Heasman et al. 2004). Other records of paramyxid infections in Oceania that are of relevance to Australia include observations of *Marteilia*-like parasites in the kidney of giant clams (*Tridacna maxima*) from Fiji (Norton et al. 1993a). The lifecycle of all these other species of *Marteilia* and *Marteilioides* are unknown. However, given the situation with the better known members of the genus (see Sections 4.10, 4.11), their lifecycles are likely to be indirect (Roubal et al. 1989, Wesche et al. 1999, Audemard et al. 2001, 2002, 2004, Adlard and Nolan 2008, 2015, Boyer et al. 2013) requiring at least one alternate host which possibly could be polychaetes and/or copepods.

4.12.5 Release assessment

Marteilioides branchialis and several unidentified *Marteilia* spp. and *Marteilioides* spp. are already known to occur generally at low prevalences in Australian oysters, although Wolf (1977) reported a *Marteilioides*-like

parasite at prevalence of 57% in apparently healthy female *S. echinata* from the Northern Territory (43% overall prevalence). Furthermore, the occasional detection of new paramyxids in Australian oysters demonstrates that the full range of paramyxid infections present in the various species of Australian molluscs remains to be determined. Because of this, there is a non-negligible risk that *Marteilia* spp. or *Marteilioides* spp. may occur in molluscs that are recycled for shellfish reef restoration in Australia. Neither the infective stage nor the mode of transmission for any of these parasites have been identified to date, however there is little evidence to suggest that paramyxids can be transmitted horizontally and directly through the water or vertically between generations. The available evidence suggests that polychaetes, copepods and amphipods that may be associated with mollusc shells must be considered potential alternative hosts for paramyxids (see Larsson and Koie 2005, Ward et al. 2016). Unfortunately, the resistance of infective or vegetative stages of these parasites to desiccation, freshwater, heat or acetic acid has not been determined. Taking into account that these parasites are known to occur in wild populations of oysters in Australia, and given the likely propagule pressure experienced via the various anthropogenic introduction pathways, the likelihood estimations for the release of *Marteilia* spp. and *Marteilioides* spp. *M. sydneyi* via the identified risk pathways are listed below.

Release assessment for infection with *Marteilia* spp. and *Marteilioides* spp.

	Unmitigated	Desiccation			Heat		Salinity	Other
Treatment	None	3 mo.	4 mo.	6 mo.	55°C >10 min	80°C >5 min	0 ppt for >24 hrs	4% acetic acid 30 min
Likelihood of release	Moderate	E low?	Neg	Neg	Low?	E low?	V low?	V low?

4.12.6 Exposure assessment

While rock oysters around Australia are already at risk from natural exposure to *Marteilia* and/or *Marteilioides* spp., translocation of infected mollusc shells from areas where these parasites are enzootic into other estuaries via shellfish reef restoration projects could transport them to new regions. These parasites infect oyster tissue and not shell material, however it is likely that shells from wild and /or cultured molluscs that may be procured from processors or retailers for recycling would retain some residual host tissue in areas such as retractor muscle scars, mantle tissue and so on. Neither the infective stage nor the mode of transmission for paramyxids have been identified to date. Important information, such as the minimum infective dose required for an index case to occur is therefore not available, however being parasites theoretically infection by just one infective stage can result in successful transmission. Since there is little evidence that these parasites can be transmitted horizontally directly through the water or vertically between generations, their spread via contaminated recycled mollusc shells would occur only if sufficient viable infective stages were introduced into areas where susceptible alternate hosts and oyster final hosts were present under environmental conditions suitable for transmission.

At this time the lifecycles of these parasites are unknown, however for *Marteilia sydneyi* and *M. refringens*, some of their alternate hosts include planktonic crustaceans or benthic polychaetes (Audemard et al. 2002, Larsson and Koie 2005, Boyer et al. 2013, Ward et al. 2016). Both host groups are ubiquitous in the Australian environment (Rainer and Hutchings 1977, Bennett 1987), meaning that the presumed indirect lifecycle of these parasites may not be a barrier to their wider dissemination through movements of mollusc

shells. As there may be pathways to expose shellfish reef restoration projects to *Marteilia* spp. and *Marteilioides* spp. via recycled mollusc shells, and suitable hosts may occur in the wild in areas where shellfish reefs are being restored, the risk of exposure and establishment is non-negligible, and the overall likelihood of exposure and establishment of these parasites is considered to be **Low.**

4.12.7 Consequence assessment

Although various species of *Marteilia* spp. and *Marteilioides* spp. are already present in populations of wild molluscs in some regions of Australia, other regions may be free of infection at this time. On the other hand, they mostly occur at low prevalence and there is little evidence to date to suggest that these other paramyxid parasites can cause major disease outbreaks or significant impacts on populations of wild or cultured molluscs in Australia. They are not listed by the OIE or NACA, nor are they listed as reportable diseases in any State, however any new or unidentified species may be referred to the relevant competent authority for identification given that *Marteilia refringens* is an OIE listed disease agent that is a reportable disease in all states, as are *M. sydneyi* and *Marteilioides chungmuensis* (Table 3). Hence the spread of paramyxids into new areas could potentially result in intervention by government authorities and disruption to aquaculture, commercial and recreational mollusc fisheries and shellfish reef restoration if attempts were made to identify the infection and prevent its further spread into uninfected areas. Considering all of these factors, establishment of *Marteilia* spp. or *Marteilioides* spp. in new areas could potentially cause economic harm, but may not necessarily cause significant biological consequences and/or significant and irreversible environmental effects for wild oyster fisheries. Taking all of these factors into consideration, the consequences of establishment of *Marteilia* spp. or *Marteilioides* spp. via recycled mollusc shells is considered to be **Low.**

4.12.8 Risk estimation

The risk estimation for *Marteilia* spp. or *Marteilioides* spp. is determined by combining the likelihood of release and exposure (from Table 5) with the consequences of establishment (Table 6) to arrive at a risk estimation (Table 7). The risk estimate for infection with *Marteilia* spp. or *Marteilioides* spp. does not exceed the ALOP for any of the release pathways, suggesting that additional risk management is not required for these disease agents.

Risk estimate for infection with *Marteilia* spp. and *Marteilioides* spp.

	Unmitigated	Desiccation			Heat		Salinity	Other
Treatment	None	3 mo.	4 mo.	6 mo.	55°C >10 min	80°C >5 min	0 ppt for >24 hrs	4% acetic acid 30 min
Combined likelihood of release and exposure	Low	E low?	Neg	Neg	V low?	E low?	V low?	V low?
Consequences of establishment	Low	Low	Low	Low	Low	Low	Low	Low
Risk estimation	V low	Neg?	Neg	Neg	Neg?	Neg?	Neg?	Neg?

4.13 Infection with *Marteilioides chungmuensis*

4.13.1 Aetiologic agent: *Marteilioides chungmuensis* is a protozoan parasite classified in the Order Paramyxida within the Phylum Endomyxa (Adl et al. 2019). *Marteilioides chungmuensis* infects the ovary of *Crassostrea gigas* in Japan and Korea forming large grossly visible nodules in the gonad which make affected oysters unmarketable (Matsusato and Masumura 1981, Itoh et al. 2002, 2004a,b, Lester and Hine 2017).

4.13.2 OIE List: No **NACA List:** Yes **Zoonotic:** No

4.13.3 Australia's status: Other species of *Marteilioides* (including uncharacterized species) occur in various regions of Australia (see Section 4.12), however *Marteilioides chungmuensis* has not been recorded from Australia and is considered exotic.

4.13.4 Epizootiology

The paramyxid *Marteilioides chungmuensis* causes one of the most important diseases of Pacific oysters (*Crassostrea gigas*) in Asia. The problem was first reported in *C. gigas* cultured in the Hiroshima prefecture in Japan where oyster culturists had long been aware of the occurrence of oysters with grossly visible, abnormally enlarged nodular lesions in the ovary during the winter months (Seki 1934). Affected *C. gigas* were considered deformed and unmarketable, however other species of oysters (including *Crassostrea nippona*, *Saccostrea echinata*, *Ostrea denselamellosa* and *Ostrea denselamellosa futamiensis*) were not affected (Seki 1934, Matsusato and Masumura 1981). The cause of the condition was unknown, but over the years it was variously reported to correlate with environmental stresses such as rapid changes in salinity and water temperature, while post-war studies suggested the lesion was a type of tumour (Matsusato et al. 1977, Matsusato and Masumura 1981). By 1974 it appeared that the condition had spread throughout south-west Japan from the initial focus near Hiroshima to several bays to the west of Hiroshima Bay, as well as Sado Island on the north west coast at a prevalence of 6.7% in wild oysters and between 0 and 12% in cultured oysters (Matsusato and Masumura 1981). In the late 1970's a similar disease was first reported from *C. gigas* in Korea, and a few years later again in Chungmu Bay, South Korea in 1984, after which the new paramyxid parasite was described as *Marteilioides chungmuensis* by Comps et al. (1986).

Closer study of the parasite at Gokasho Bay in southern Japan using histology, found that infection prevalence was seasonal, with highest total prevalence over 50% in cultured oysters, (with a sex ratio of 1:1 male/female this suggests 100% prevalence in female oysters) and peak intensity (50-60% of oocytes infected) of infections occurring during the summer spawning season at water temperatures of 25-27°C (Imanaka et al. 2001). After spawning, prevalence decreased into autumn and winter being lowest (18-20% prevalence in cultured oysters) when water temperatures dropped to 13°C (Imanaka et al. 2001). Later studies confirmed that the development of the parasite was related to the gonad maturation cycle of the host, with infection prevalence and intensity being highest during the oysters summer spawning period (Ngo et al. 2003, Park et al. 2003, Tun et al. 2008b). Histology revealed that not all infected oysters display grossly visible nodular lesions, while studies using more sensitive molecular diagnostic methods (nested PCR) found that both male and female oysters can be infected (Itoh et al. 2004a) and a prepatent period of infection of at least 3 weeks in seawater at 24°C was required before infection could be detected in the gonad by histopathology (Tun et al. 2008b). The molecular

method was around twice as sensitive as histology, confirming that *M. chungmuensis* was present year-round in both male and female oysters, but disease occurred only in female oysters during summer and autumn when prevalence peaked at 80% (Tun et al. 2008b).

The parasite causes increased mortality (>50%) in heavily infected female oysters in late summer and autumn (Tun et al. 2008a), and infected oysters may spawn later in the year than normal (Tun et al. 2008b). The presence of the parasite also influences the condition of the oyster and its metabolic recovery after spawning, as post-spawn *C. gigas* infected with *M. chungmuensis* become watery from depleted glycogen reserves (Park et al. 2003). The parasite also influences the survival of the ova as infected oocytes are sterile (Tun et al. 2008b). Those oysters that survive into winter resorb the gonad and most change to males which greatly reduces the impact of the infection (and mortality rate), as sporulation does not occur in male oysters (Itoh et al. 2004a, Tun et al. 2008b).

It appears that water temperatures above 18°C are required to initiate *M. chungmuensis* infections (Tun et al. 2008b). The parasite enters the oyster via the epithelium of the gills, mantle and palps followed by extrasporogenic multiplication in the connective tissues (Itoh et al. 2004a). The parasites then migrate via the haemolymph into the gonad epithelium, where they invade immature oocytes (Itoh et al. 2004a) and commence sporulation at water temperatures above 20°C (Tun et al. 2008b). Most of the time two secondary cells of *M. chungmuensis* occur in each mature oyster oocyte, with each secondary cell containing one developing spore (Comps et al. 1986, Itoh et al. 2002). However, oocytes containing up to six secondary cells (each containing one developing spore) have been rarely observed (Imanaka et al. 2001). Mature spores occur inside eggs within the lumen of the genital tubule (Itoh et al. 2002), and spores are released with ova via the genital canal (Tun et al. 2008b). Little is known about the lifecycle once the spores exit the oyster and enter the environment. Direct horizontal oyster-to-oyster transmission does not appear to be possible, however, and it is assumed that as for *Marteilia sydneyi* and *M. refringens*, alternate hosts may be required to complete the lifecycle (Imanaka et al. 2001, Itoh et al. 2004a, Tun et al. 2008b). It appears that *M. chungmuensis* has high host specificity for *C. gigas*, however Itoh et al. (2004b) reported the same parasite infecting the gonad of the Iwagaki oyster (*Crassostrea nippona*) cultured in an area of Japan where *M. chungmuensis* was enzootic in cultured *C. gigas*.

4.13.5 Release assessment

Marteilioides chungmuensis has not been recorded from Australian molluscs and is considered exotic, however it is included in this risk analysis as it is listed as a notifiable disease agent that could cause serious harm if it were introduced at some point in the future. Furthermore, several uncharacterised *Marteilioides* spp. are already known to occur in Australian oysters. For example, Wolf (1977) reported a *Marteilioides*-like parasite at prevalence of 57% in healthy female *S. echinata* from the Northern Territory (43% overall prevalence). Hine and Thorne (2000) later confirmed the presence of a *Marteilioides* sp. in the same host in Darwin Harbour at a prevalence of 9.6%. Furthermore, the occasional detection of new paramyxids in Australian oysters at low prevalences demonstrates that the full range of paramyxid infections present in the various species of Australian molluscs remains to be determined. Because of this, there is a non-negligible risk that *M. chungmuensis* may occur in molluscs that are recycled for shellfish reef restoration in Australia. Neither the infective stage nor the mode of transmission for any of these parasites have been identified to date, however there is little evidence to suggest that other paramyxids can be transmitted horizontally and directly through the water or vertically between generations. The available evidence suggests that polychaetes,

copepods and amphipods that may be associated with mollusc shells must be considered potential alternative hosts for paramyxids (see Larsson and Koie 2005, Ward et al. 2016). Unfortunately, it appears that the resistance of infective or vegetative stages of these parasites to desiccation, freshwater, heat or acetic acid has not been determined. Taking into account information above, and given the likely propagule pressure experienced via the various anthropogenic introduction pathways, the likelihood estimations for the release of *M. chungmuensis* via the identified risk pathways are listed below.

Release assessment for *Marteilioides chungmuensis*

	Unmitigated	Desiccation			Heat		Salinity	Other
Treatment	None	3 mo.	4 mo.	6 mo.	55°C >10 min	80°C >5 min	0 ppt for >24 hrs	4% acetic acid 30 min
Likelihood of release	Moderate	E low?	Neg	Neg	Low?	E low?	V low?	V low?

4.13.6 Exposure assessment

While rock oysters around Australia are already at risk from natural exposure to *Marteilioides* spp., translocation of infected oyster shells from areas where these parasites are enzootic into other estuaries via shellfish reef restoration projects could transport them to new regions. These parasites infect oyster tissue and not shell material, however it is likely that shells from wild and /or cultured molluscs that may be procured from processors or retailers for recycling would retain some residual host tissue in areas such as retractor muscle scars, mantle tissue and so on. Neither the infective stage nor the mode of transmission for paramyxids have been identified to date, however it is known that *M. chungmuensis* can be transmitted when water temperatures exceed 18°C (Tun et al. 2008b). Important information, such as the minimum infective dose required for an index case to occur is therefore not available, however being parasites theoretically infection by just one infective stage can result in successful transmission. Since there is little evidence that these parasites can be transmitted horizontally directly through the water or vertically between generations, their spread via contaminated recycled mollusc shells would occur only if sufficient viable infective stages were introduced into areas where susceptible alternate hosts and oyster final hosts were present under environmental conditions suitable for transmission.

At this time the lifecycles of these parasites are unknown, however for *Marteilia sydneyi* and *M. refringens*, some of their alternate hosts include planktonic crustaceans or benthic polychaetes (Audemard et al. 2002, Larsson and Koie 2005, Boyer et al. 2013, Ward et al. 2016). Both host groups are ubiquitous in the Australian environment (Rainer and Hutchings 1977, Bennett 1987), meaning that the presumed indirect lifecycle of these parasites may not be a barrier to their wider dissemination through movements of mollusc shells. As there may be pathways to expose shellfish reef restoration projects to these parasites via recycled mollusc shells, and populations of wild or cultured *C. gigas* may occur at water temperatures above 18°C in some of the areas where shellfish reefs are being restored, the risk of exposure and establishment is non-negligible, and the overall likelihood of exposure and establishment of these parasites is considered to be **Low.**

4.13.7 Consequence assessment

Although various species of *Marteilioides* spp. are already present in populations of wild molluscs in some regions of Australia, the presence of grossly visible nodular lesions in the gonad typical of infection by *M. chungmuensis* has not been reported. The vast majority of *C. gigas* in Australia occur in populations cultured in Tasmania, southern NSW and South Australia, which are remote from the locations where *Marteilioides* spp. have been previously recorded either in northern NSW (Anderson and Lester 1992) or in northern Australia (Wolf 1977, Hine and Thorne 2000). It appears that *M. chungmuensis* and other *Marteilioides* spp. only cause problems where water temperatures exceed 20°C for many months, however this suggests they could be problematic if the culture of *C. gigas* was ever undertaken in northern Australia. *Marteilioides chungmuensis* is listed by NACA and is also a reportable disease in all States (Table 3). Hence the introduction of this parasite into Australia would potentially necessitate intervention by government authorities and disruption to aquaculture, commercial and recreational mollusc fisheries and shellfish reef restoration if attempts were made to contain the infection and prevent its further spread into uninfected areas. Considering all of these factors, establishment of *M. chungmuensis* in areas where *C. gigas* are cultured or potentially could be cultured in the future would cause significant economic harm through reduced marketability of affected oysters, have significant biological consequences and may also irreversibly affect wild and cultured populations of *C. gigas*. Taking all of these factors into consideration, the consequences of establishment of *M. chungmuensis* via recycled mollusc shells is considered to be **Moderate.**

4.13.8 Risk estimation

The risk estimation for *M. chungmuensis* is determined by combining the likelihood of release and exposure (from Table 5) with the consequences of establishment (Table 6) to arrive at a risk estimation (Table 7). The risk estimate for infection with *M. chungmuensis* exceeds the ALOP for some release pathways, suggesting that additional risk management is required for this disease agent.

Risk estimate for *Marteilioides chungmuensis*

	Unmitigated	Desiccation			Heat		Salinity	Other
Treatment	None	3 mo.	4 mo.	6 mo.	55°C >10 min	80°C >5 min	0 ppt for >24 hrs	4% acetic acid 30 min
Combined likelihood of release and exposure	Low	E low?	Neg	Neg	V low?	E low?	V low?	V low?
Consequences of establishment	Moderate	Mod	Mod	Mod	Mod	Mod	Mod	Mod
Risk estimation	Low	Neg?	Neg	Neg	V low?	Neg?	V low?	V low?

4.14 Infection with *Mikrocytos* spp. (including *M. mackini*)

4.14.1 Aetiologic agent: *Mikrocytos mackini* is a protozoan parasite classified in the Order Mikrocytida, a sister group to the Haplosporidia within the Phylum Endomyxa (Adl et al. 2019). Members of the Mikrocytida are microcell parasites of invertebrates, mainly oysters and clams, but also crustaceans (Hartikainen et al. 2014b). Members of the genus *Mikrocytos* infect oysters and clams and are characterised by their distinctive lack of mitochondria and haplosporosomes (Hine et al. 2001b) and their molecular sequences (Abbott and Meyer 2014, Abbott et al. 2014, Ramilo et al. 2014, Garcia et al. 2018).

4.14.2 OIE List: Yes **NACA List:** No **Zoonotic:** No

4.14.3 Australia's status: Not recorded. Members of the genus *Mikrocytos* are not known to occur in Australian molluscs, and are considered exotic. Microcell-like structures in abscess-like lesions in Sydney rock oysters *S. glomerata* from NSW with winter mortality disease (Roughley 1926) were described as *Mikrocytos roughleyi* by Farley et al. (1988). However, more recent studies suggest the microcell-like structures visually observed by Farley et al. (1988) are not a *Bonamia* or *Mikrocytos* species, hence *M. roughleyi* is *nomen dubium* (doubtful name) (Carnegie et al. 2014, Spiers et al. 2014). Microcell-like parasites visualized in *C. gigas* in South Australia (Diggles 2003) currently remain *incerte sedis* (uncertain placement).

4.14.4 Epizootiology

In 1960, a mass mortality event occurred in Pacific oysters (*Crassostrea gigas*) farmed in Henry Bay, Denman Island in British Columbia, Canada (Quayle 1961). Affected oysters exhibited numerous focal, greenish yellow pustules on the surface of the body/gonad, mantle, labial palps and adductor muscle, and brown (conchiolin) scarring of adjacent areas of the shell, but were otherwise in excellent "fat" condition (Quayle 1988, Farley et al. 1988). Mortalities started in early spring (May-June) when water temperatures were just beginning to rise to 9°C from the winter low of 7°C, and it was estimated that between 17 and 53% of the cultured oysters died, depending on their age and location on the beach (Quayle 1961, 1988). Overall mortalities were around 34% with highest mortality rates (53%) occurring in oysters at the lowest tide levels (0.3 metre tide level) and in individuals more than 2 years old (Quayle, 1961, 1988, Bower 1988). Between 1960 and 1994, the disease spread to nearby areas and mortalities at Henry Bay varied between 11% in 1967 (Quayle, 1988) to a high of 48% in 1988 (Bower 1988, Hervio et al. 1996). Although the cause of the disease was unknown, laboratory experiments demonstrated horizontal transmission by cohabitation (Quayle 1961), and in 1963, Dr J.G. Mackin identified the causative agent of what was by then called Denman Island disease as small, intracellular microcells 2-5 µm in diametre, which were intimately associated with the necrotic abscess-like lesions and these were subsequently described as *Mikrocytos mackini* by Farley et al. (1988). Similar microcells were also reported in *C. gigas* sampled from Hawaii, and *Ostrea lurida* from Yaquina Bay, Oregon, USA (Farley et al. 1988). Subsequent studies increased the geographic and host range when Bower et al. (1997) found that *M. mackini* can also cause disease in several other oyster species including eastern oysters *Crassostrea virginica*, European flat oysters *Ostrea edulis* and Olympia oyster *Ostrea lurida* (then called *O. conchaphila*), while Elston et al. (2012) found *M. mackini* also infected Kumamoto oysters *Crassostrea sikamea* in California during years where those oysters were exposed to low water temperatures.

Research has found that Pacific oysters infected with *M. mackini* require a prolonged period of low water temperature in order for Denman Island disease to develop. Hervio et al. (1996) found that *M. mackini* requires its host to encounter a seawater temperature below 10°C for at least 3 months before disease is expressed, while oysters previously exposed at low water temperatures then held at >15°C could retain *M. mackini* at subclinical levels for at least 6 months (Hervio et al. 1996). The effect of temperature on the disease process and prepatent period explains the seasonality of Denman Island disease in the field, with new hosts probably being infected in the previous spring during disease outbreaks when water temperatures are still low. Increasing water temperatures, which occur shortly after exposure, delay development of the disease over the summer months, then the disease develops over the 4-5 month winter period of cold water temperatures, resulting in the appearance of diseased oysters early in the spring (Hervio et al. 1996, Bower et al. 1997).

Farley et al. (1988) also detected large numbers of *Mikrocytos*-like microcells in Sydney rock oysters (*S. glomerata*) sampled during an outbreak of winter mortality in NSW (Roughley 1926). Winter mortality is a sporadic disease of SRO which occurs in winter after periods of high salinity (>29 ppt) following a sudden drop in water temperature below 14°C (Wolf 1967, Hand et al. 1998, see also Section 4.20). However, it is now considered that the microcells observed by Farley et al. (1988) are not a *Bonamia* or even a *Mikrocytos* species (Carnegie et al. 2014, Spiers et al. 2014), but instead are more likely to be an undescribed microcell. This is similar to the situation with other microcell-like parasites that have been visualized in *C. gigas* in South Australia during histopathological surveys (Diggles 2003), which also currently remain *incerte sedis* (uncertain placement). Since *C. gigas* is also known to carry *Bonamia* sp. (see Lynch et al. 2010), the parasite in South Australia could be a *Bonamia* sp., but other microcells could also be implicated including *Mikrocytos* and *Paramikrocytos* (see Farley et al. 1988, Bower et al. 1997, Abbott et al. 2014, Hartikainen et al. 2014b).

In the last decade molecular diagnostic tools have been used to discover several new species of *Mikrocytos*. Agents found in oysters include *Mikrocytos boweri*, which was described from Olympia oysters *Ostrea lurida* in British Columbia, Canada (Abbott et al. 2011, 2014). *M. boweri*-like parasites have also been detected from several other regions and hosts including European flat oysters (*Ostrea edulis*) from the east coast of Canada (Gagné et al. 2008), and *C. gigas* in China (Wang et al. 2010b), and *Mikrocytos mimicus* which was detected in cultured *C. gigas* during a disease outbreak (c. 20% mortality) in two year old oysters exposed to a protracted period of cold weather (estimated to be below 10°C) for up to three months near Norfolk, UK (Hartikainen et al. 2014b). Other species of *Mikrocytos* have also been detected infecting clams, including the *Mikrocytos*-like parasite found in Manila clams *Ruditapes philippinarum* from Galicia (NW Spain) (Ramilo et al. 2014), and two other species (*Mikrocytos veneroïdes* and *Mikrocytos donaxi*) discovered in diseased *Donax trunculus* sampled from wild and aquacultured beds, respectively, in France (Garcia et al. 2018). The apparent broad distribution of these parasites was one of the reasons why *Mikrocytos mackini* was delisted by the World Animal Health Organization (OIE) as an internationally reportable disease in 2007. However, these disease agents remain reportable in Australia (Table 3), and clearly the use of molecular diagnostic methods in the past decade has shown that *Mikrocytos* spp. are broadly distributed parasites of molluscs (Hartikainen et al. 2014b). It would seem prudent, therefore, to apply these same diagnostic methods to ascertain the identity of the *Mikrocytos*-like agents known to occur in Sydney rock oysters and Pacific oysters in Australia.

4.14.5 Release assessment

Mikrocytos mackini has not been recorded from Australian molluscs and is considered exotic, however it is included in this risk analysis as it is listed as a notifiable disease agent that could cause serious harm if it were

introduced at some point in the future. Furthermore, *Mikrocytos*-like agents are known to occur in Sydney rock oysters from NSW during winter mortality outbreaks (see Section 4.20) and also in apparently healthy Pacific oysters from South Australia (Diggles 2003). Unfortunately, the specific identity of these two microcells is unknown, and nothing is known about their survival or transmission characteristics. However, if these agents are considered novel Mikrocytids, there is evidence that not only *M. mackini*, but also *Mikrocytos mimicus* requires exposure of the infected oyster to protracted periods of cold weather (below 10°C) for several months prior to disease expression (Hartikainen et al. 2014b). Taken together, these findings suggest that low temperature dependence may occur across the genus *Mikrocytos* (see Abbott and Meyer 2014).

The occasional detection of microcell-like parasites in Australian oysters at low prevalences demonstrates that the full range of infections present in the various species of Australian molluscs remains to be determined. Because of this, there is a non-negligible risk that *Mikrocytos*-like disease agents may occur in molluscs that are recycled for shellfish reef restoration in Australia. Unfortunately, it appears that the resistance of infective stages of these parasites to desiccation, freshwater, heat or acetic acid has not been determined. Taking into account information above, and given the likely propagule pressure experienced via the various anthropogenic introduction pathways, the likelihood estimations for the release of *Mikrocytos* spp. (including *M. mackini*) via the identified risk pathways are listed below.

Release assessment for infection with *Mikrocytos* spp. (including *M. mackini*)

	Unmitigated	Desiccation			Heat		Salinity	Other
Treatment	None	3 mo.	4 mo.	6 mo.	55°C >10 min	80°C >5 min	0 ppt for >24 hrs	4% acetic acid 30 min
Likelihood of release	High	Neg?	Neg	Neg	Low?	E low?	Low?	V low?

4.14.6 Exposure assessment

While *Mikrocytos* spp. infect oyster tissue and not shell material, it is likely that shells from wild and /or cultured oysters that may be procured from oyster processors or retailers for recycling would retain some residual host tissue in areas such as retractor muscle scars, mantle tissue and so on. These parasites transmit directly from oyster-to-oyster, though various epidemiological details such as the minimum infective dose required for successful transmission are not presently known. However, it is known that successful transmission occurs during the cooler periods of the year where water temperatures are below 10°C (Abbott and Meyer 2014). Bivalves are efficient filter feeders and therefore are also efficient particle collectors, and can collect spores and infective stages of a wide range of bivalve disease agents (Barber and Ford 1992, Ford et al. 2009). Because of this, oysters are particularly susceptible to infection by protozoan infective stages delivered via the water, and this is one of the reasons why movements of infected oysters are highly likely to result in exposure and establishment of oyster pathogens in new areas, as has been demonstrated several times in other parts of the world (Elston et al. 1986, Friedman and Perkins 1994, Howard 1994, Bishop et al. 2006). As there may be pathways to expose shellfish reef restoration projects to *Mikrocytos* spp. via recycled mollusc shells, environmental conditions are likely to be suitable for disease transmission in many parts of southern Australia during the winter months, and suitable hosts may occur in the wild in areas where shellfish reefs are

being restored, the risk of exposure and establishment is non-negligible, and the overall likelihood of exposure and establishment of *Mikrocytos* spp. (including *M. mackini*) is considered to be **Moderate**.

4.14.7 Consequence assessment

Although some *Mikrocytos* -like microcells are already present in populations of wild and cultured molluscs in some regions of Australia, other regions may be free from infection at this time. The vast majority of *C. gigas* in Australia occur in populations cultured in Tasmania, southern NSW and South Australia, many of which are cultured in locations where water temperatures may decline below 10°C during the winter months. This suggests that *M. mackini* or other Mikrocytids could be problematic in the culture of *C. gigas* in southern Australia if it were introduced and established. *Mikrocytos mackini* is listed by the OIE and is also a reportable disease in all States (Table 3). Hence the introduction of this parasite into Australia may have significant trade implications, and would likely result in intervention by government authorities. Such interventions may cause disruption to aquaculture (particularly for *C. gigas*), as well as commercial and recreational mollusc fisheries and shellfish reef restoration if attempts were made to contain the infection and prevent its further spread into uninfected areas. Nevertheless, if *M. mackini* was detected in the wild, there would appear to be little chance of eradication. Taking all of these factors into consideration, the consequences of establishment of *Mikrocytos* spp. (including *M. mackini*) via recycled mollusc shells is considered to be **High.**

4.14.8 Risk estimation

The risk estimation for *M. mackini* and other species of *Mikrocytos* is determined by combining the likelihood of release and exposure (from Table 5) with the consequences of establishment (Table 6) to arrive at a risk estimation (Table 7). The risk estimate for infection with *M. mackini* and other species of *Mikrocytos* exceeds the ALOP for some release pathways, suggesting that additional risk management is required for these disease agents.

Risk estimate for infection with *Mikrocytos* spp. (including *M. mackini*)

	Unmitigated	Desiccation			Heat		Salinity	Other
Treatment	None	3 mo.	4 mo.	6 mo.	55°C >10 min	80°C >5 min	0 ppt for >24 hrs	4% acetic acid 30 min
Combined likelihood of release and exposure	Moderate	Neg?	Neg	Neg	Low?	E low?	Low?	V low?
Consequences of establishment	High	High	High	High	High	High	High	High
Risk estimation	High	Neg?	Neg	Neg	Mod?	V low?	Mod?	Low?

4.15 Infection with *Minchinia* spp. (including *M. occulta*)

4.15.1 Aetiologic agent: Parasites of the genus *Minchinia* are protozoans which are members of the Order Haplosporida in the Phylum Endomyxa (Adl et al. 2019). Members of the genus *Minchinia* infect mainly oysters or crabs and develop spores 2-12 µm in greatest dimension without ornamentation and the epispore cytoplasm is never attached to the spore wall (Azevedo and Hine 2016). Those that infect molluscs appear to have an indirect life cycle, requiring alternate (possibly non-molluscan) hosts (Haskin and Andrews 1988, Powell et al. 1999).

4.15.2 OIE List: No **NACA List:** No **Zoonotic:** No

4.15.3 Australia's status: The *Haplosporidium* sp. originally reported from rock oysters *Saccostrea cucullata* near Exmouth in WA by Hine and Thorne (2002) was reclassified as a species of *Minchinia* on the basis of a molecular study (Bearham et al. 2007), and subsequently described as *Minchinia occulta* Bearham et al. (2008b).

4.15.4 Epizootiology

Haplosporidian parasites of the genus *Minchinia* can cause disease outbreaks or significant impacts on populations of wild or cultured clams, oysters and mussels worldwide (Arzul and Carnegie 2015, Ward et al. 2016, 2019, Ramilo et al. 2018). In Australia, the haplosporidians *Haplosporidium hinei* and *Minchinia occulta* have been associated with sporadic but heavy mortalities in wild western or hooded rock oysters (*Saccostrea cucullata*) and hatchery reared pearl oysters (*Pinctada maxima*) in Western Australia (Hine and Thorne 1998, 2000, 2002, Jones and Creeper 2006, Bearham et al. 2008b, 2008c, 2009). In the early 1990s mortalities of up to 80% were recorded among wild *S. cucullata* by energy companies operating around Airlie Island in the Montebello Islands near Exmouth in North West WA (Hine and Thorne, 2002, Bearham et al. 2008c). The companies submitted samples for diagnosis, and a new haplosporidian species parasitizing moribund rock oysters was subsequently identified by Hine and Thorne (2002) as a *Haplosporidium* sp. The samples contained single, bi-nucleate and multinucleate plasmodia as well as spore stages (5.6–6.7 × 3.3–4.0 µm) throughout the connective tissues of *S. cucullata* (121 out of 791 oysters infected or 15.3% overall prevalence), but not in *S. glomerata* (0 out of 808 oysters infected), however *S. glomerata* were only sampled from areas where *S. cucullata* were uninfected (Hine and Thorne 2002). Subsequent investigations found the maximum site prevalence at Varanus Island where 90 of 322 *S. cucullata* (27.9%) were infected, with lower prevalence in the Montebello Islands (4/26 or 15.4%), Airlie Island (26 of 211 or 12.3%), East Lewis Island (3/44 or 16.8%) and Rosily Island (1/31 or 3.2%) (Hine and Thorne 2000, 2002). Few oysters were lightly infected, with the disease appearing progressive and fatal, but there was no evidence of movements of oysters in and out of the remote areas affected, suggesting the epizootic may have been a natural event (Hine and Thorne 2002).

A few years later, DNA sequence analysis of archived samples together with fresh samples obtained from the Montebello Islands suggested the identity of this parasite was aligned more closely with the genus *Minchinia* (see Bearham et al. 2007). Morphologically, the parasite described by Bearham et al. (2007) consisted of uni-nucleated naked cells that superficially resembled a *Bonamia* species and was eventually described as

Minchinia occulta (see Bearham et al. 2008a, 2008c). Mixed infections of *M. occulta* and *H. hinei* were also detected in hatchery reared *P. maxima* with *M. occulta* occurring at prevalences up to 26.7% during disease outbreaks (see Bearham et al. 2009). The uninucleate and multinucleate vegetative stages of *M. occulta* in *S. cucullata* occur in the connective tissues of the gills, mantle and around digestive diverticulae, multinucleate plasmodia with up to 25 nuclei occurred in connective tissue adjacent to the digestive tract, while sporulation was confined to the connective tissues around, but not within, the digestive tubules (Hine and Thorne 2002, Bearham et al. 2008c). Infected oysters were in poor condition due to the absence of gonad tissue overlying the digestive tissue, with some clinically diseased *S. cucullata* having meat/shell ratios around 1/3 normal size (Hine and Thorne 2002). Little else is known about *M. occulta* except that, like most other haplosporidians, infections are usually systemic and terminal (Hine and Thorne 2002, Diggles et al. 2002). Haplosporidian vegetative stages can proliferate within host connective tissues in the mantle, gills and leydig tissues forming masses of multi-nucleated plasmodia, while sporulation also occurs within connective tissues in the case of *M. occulta* (see Hine and Thorne 2002, Bearham et al. 2007). Eventual host death is thought to be due to overwhelming numbers of parasites interfering with normal organ functions.

The failure to transmit *M. occulta* and other haplosporidians directly by cohabitation or injection of spores suggests they have an indirect lifecycle requiring an alternate host (Burreson and Ford 2004, Bearham 2008), however nothing is known about the life cycle of *M. occulta* (see Bearham 2008). For other haplosporidians, the earliest vegetative stages of *Haplosporidium nelsoni* are found in the epithelia of the gills and palps, suggesting that for *H. nelsoni* the infective stage is waterborne and can be easily spread (Haskin and Andrews 1988). Despite this, neither the infective stage nor the mode of transmission for *H. nelsoni* has ever been identified (Powell et al. 1999, Sunila et al. 2000), although it is known that the infective stage for *H. nelsoni* can pass through a 1 mm filter (Sunila et al. 2000), and a 150 µm filter, but not a 1 µm filter followed by UV irradiation at a dose of 30 mJ/cm^2 (Ford et al. 2001). Hartikainen et al. (2014a) found evidence that *Minchinia tapetis* DNA was seasonally present in plankton samples (0.45-20µm and >20µm range) in April (springtime), suggesting *Minchinia* spp. infective stages may be planktonic, or may utilize planktonic metazoan hosts in their life cycles at certain times of year. However, Bearham (2008) could not transmit *M. occulta* infection in the laboratory using plankton samples.

4.15.5 Release assessment

Minchinia occulta has been detected in *S. cucullata* near Exmouth in WA at prevalences of up to 28% at Varanus Island using histology (Hine and Thorne 2002). However, histology appears to be only half as sensitive for detecting infections of *M. occulta* compared to molecular diagnostic techniques (see Bearham 2008, Bearham et al. 2008a), suggesting the prevalence of the parasite in wild populations of *S. cucullata* may exceed 50% at times, though at other times it is undetectable in oysters from the same locations where heavy mortalities occurred in the past (Bearham et al. 2008c). The difficulty in obtaining regular samples from such remote locations is the main reason why it is currently not known whether *Minchinia occulta* exhibits seasonal or longer-term temporal variation in infection prevalence or intensity. *Minchinia occulta* is known to be present in rock oysters in some parts of WA, but those infections have only been recorded a handful of times (Hine and Thorne 2002, Jones and Creeper 2006, Bearham et al. 2008b, 2009). However, at those times the prevalence of the parasite has been relatively high (up to 27.9%) at certain locations, and the infection has been associated with significant mortalities of wild oysters (Hine and Thorne 2002). Furthermore, *M. occulta* has also been detected in hatchery reared pearl oyster (*P. maxima*) spat farmed in several areas of WA,

suggesting that these parasites may be widely distributed in rock oysters that may be acting as alternate reservoir hosts (Bearham et al. 2009).

The emergence of haplosporidian diseases in species such as *S. cucullata* and *P. maxima* and occurrence of *Bonamia*-like parasites (which could also be uninucleate stages of haplosporidians such as *M. occulta*, see Bearham 2008, Bearham et al. 2007, 2008a, 2008b, 2009) in species such as *C. gigas* in SA (Diggles 2003) and molluscs in other regions of Australia (Heasman et al. 2004) demonstrate that the full range of haplosporidian infections that are present in the various species of molluscs in Australia remains to be determined. There is little evidence that *Minchinia* spp. can be transmitted horizontally through the water or vertically between generations without requiring an alternate host (Burreson and Ford 2004, Bearham 2008). While the lifecycle of these parasites is unknown, *Minchinia* sp. are known to be associated with plankton at certain times of year (Hartikainen et al. 2014a). Given the presence of these parasites in some rock oyster populations there is a non-negligible risk that *Minchinia* spp. disease agents may occur in molluscs that are recycled for shellfish reef restoration in Australia. Unfortunately, the resistance of infective stages of these parasites to desiccation, freshwater, heat or acetic acid has apparently not been determined. Taking into account information above, and given the likely propagule pressure experienced via the various anthropogenic introduction pathways, the likelihood estimations for the release of *Minchinia* spp. (including *M. occulta*) via the identified risk pathways are listed below.

Release assessment for infection with *Minchinia* spp. (including *M. occulta*)

	Unmitigated	Desiccation			Heat		Salinity	Other
Treatment	None	3 mo.	4 mo.	6 mo.	55°C >10 min	80°C >5 min	0 ppt for >24 hrs	4% acetic acid 30 min
Likelihood of release	Moderate	E low?	Neg	Neg	Low?	E low?	Low?	V low?

4.15.6 Exposure assessment

While *Minchinia* spp. infect oyster tissue and not shell material, it is likely that shells from wild and /or cultured molluscs that may be procured from processors or retailers for recycling would retain some residual host tissue in areas such as retractor muscle scars, mantle tissue and so on. There is little evidence that *Minchinia* spp. can be transmitted horizontally through the water or vertically between generations. Spread of haplosporidians via contaminated recycled mollusc shells would therefore occur only if sufficient viable infective stages were introduced into areas where susceptible alternate hosts and/or oyster final hosts were present under environmental conditions suitable for transmission. Because unknown alternate host(s) are presumably needed in order to complete the lifecycle of haplosporidians (Haskin and Andrews 1988, Ford et al. 2001, Diggles et al. 2002, Bearham 2008, Hartikainen et al. 2014a), the exposure pathway required for transmission remains unknown, as does important information such as the minimum infective dose required for an index case to occur, though being a parasite, theoretically infection by just one infective stage can result in successful transmission. If an index case did occur, these disease agents are highly pathogenic and it would be likely that the infected bivalve would become diseased, after which transmission and further spread from the index case may occur.

The sporadic detection and apparently restricted distribution of these parasites may be due to inadequate knowledge of the disease status of oysters in Australia, but also could be due to the fact that their alternate hosts may also be restricted in distribution and their populations may be influenced by environmental variables. The fact that *S. cucullata*, but not *S. glomerata* were infected with *M. occulta* (see Hine and Thorne 2002) suggests that these parasites may have high host specificity, which may reduce the chances of exposure and establishment. However, Hine and Thorne (2002) pointed out that *S. glomerata* was sampled only from areas where *S. cucullata* were uninfected, hence it remains possible that other species of oysters are also susceptible to *M. occulta*. Furthermore, the fact that some haplosporidians (e.g. *Haplosporidium nelsoni*) have been translocated and established infections in new regions (Friedman 1996, Burreson et al. 2000, Renault et al. 2000a), suggests that some of the presumptive alternate hosts may be widespread and/or ubiquitous (e.g. planktonic copepods, Hartikainen et al. 2014a), or that these parasites may have lower host specificity for the alternate host. As there may be pathways to expose shellfish reef restoration projects to *Minchinia* spp. via recycled mollusc shells, environmental conditions are likely to be suitable for disease transmission in many parts of Australia and suitable hosts may occur in the wild in areas where shellfish reefs are being restored, the risk of exposure and establishment is non-negligible, and the overall likelihood of exposure and establishment of haplosporidians is considered to be **Low.**

4.15.7 Consequence assessment

Although *Minchinia* spp. are already present in populations of wild molluscs in some regions of Australia, other regions appear free of infection at this time. There is evidence that haplosporidians like *M. occulta* can cause major disease outbreaks resulting in upwards of 80% mortality in wild oyster populations (Hine and Thorne, 2002, Bearham et al. 2008c). The introduction of these parasites into new areas could therefore have significant impacts on populations of both wild and cultured molluscs. These disease agents are no longer listed by the OIE or NACA, but *M. occulta* remains listed as a reportable disease in WA (Table 3). Hence the spread of these parasites to new areas could adversely impact trade and result in intervention by government authorities. Such interventions may cause disruption to aquaculture, commercial and recreational mollusc fisheries and shellfish reef restoration if attempts were made to contain the infection and prevent its further spread into uninfected areas. Nevertheless, if *Minchinia* spp. were detected in the wild, there would appear to be little chance of eradication. Considering all of these factors, establishment of *Minchinia* spp. into new areas would likely have significant biological consequences and could cause economic harm to oyster aquaculture together with significant irreversible environmental effects for wild oyster populations. Taking all of these factors into consideration, the consequences of establishment of *Minchinia* spp. (including *M. occulta*) via recycled mollusc shells is considered to be **High.**

4.15.8 Risk estimation

The risk estimation for *M. occulta* and other species of *Minchinia* is determined by combining the likelihood of release and exposure (from Table 5) with the consequences of establishment (Table 6) to arrive at a risk estimation (Table 7). The risk estimate for infection with *M. occulta* and other species of *Minchinia* exceeds the ALOP for some release pathways, suggesting that additional risk management is required for these disease agents.

Risk estimate for infection with *Minchinia* spp. (including *M. occulta*)

	Unmitigated	Desiccation			Heat		Salinity	Other
Treatment	None	3 mo.	4 mo.	6 mo.	55°C >10 min	80°C >5 min	0 ppt for >24 hrs	4% acetic acid 30 min
Combined likelihood of release and exposure	Low	E low?	Neg	Neg	V low?	E low?	V low?	V low?
Consequences of establishment	High	High	High	High	High	High	High	High
Risk estimation	Moderate	V low?	Neg	Neg	Low?	V low?	Low?	Low?

4.16 Infection with *Perkinsus* spp. (including *P. olseni*, *P. chesapeaki*)

4.16.1 Aetiologic agent: *Perkinsus* spp. are protozoa classified in the Family *Perkinsidae* within the Kingdom Alveolata (which also includes phyla containing dinoflagellates, apicomplexans, and ciliates, see Zhang et al. 2011, Adl et al. 2019). Members of the Perkinsidae are parasites of marine molluscs, particularly oysters, clams and gastropods. This section includes *Perkinsus olseni* and those *Perkinsus* spp. other than *Perkinsus marinus* that causes Dermo disease in American oysters (see Section 4.17).

4.16.2 OIE List: Yes **NACA List:** Yes **Zoonotic:** No

4.16.3 Australia's status: *Perkinsus olseni* infections have been reported in a variety of species of molluscs from all Australian states except Tasmania (Lester and Davis 1981, Goggin and Lester 1995, Hine and Thorne 2000, Liggins and Upston 2010, Dang et al. 2015, 2017).

4.16.4 Epizootiology

Members of the genus *Perkinsus* within the Family *Perkinsidae* are closely related to dinoflagellates (Reece et al. 1997, Zhang et al. 2011). These obligate protistan parasites are known to infect a wide range of marine molluscs in many regions of the world (Villalba et al. 2004). The life cycle of *Perkinsus* spp. is direct and involves infection of the filter feeding host mainly via the gills and palps (Wang et al. 2018) followed by vegetative proliferation within the host by trophozoites that undergo successive bipartitioning. Other stages that have been observed include hypnospores, zoosporangia and biflagellated zoospores, the latter which are probably natural dispersal and infective stages (Goggin et al. 1989). When the host dies or when host tissues infected by *Perkinsus* spp. are incubated in fluid thioglycollate medium (FTM), the trophozoites enlarge and develop a thick cell wall, becoming easy to visualise after staining with lugols iodine (Ray 1966). When these enlarged hypnospore stages are transferred into seawater, they form zoosporangia and production of hundreds to thousands of zoospores occurs within the original cell wall (Villalba et al. 2004). The biflagellated zoospores 3-5 µm in size leave the zoosporangium through discharge tubes and enter the water to reinfect new hosts via the gills, palps and digestive tract (Villalba et al. 2004, Wang et al. 2018). Infection of susceptible molluscs can occur horizontally through the water by cohabitation via contact with zoospores, but trophozoites and hypnospores have also been shown experimentally to cause infection (Goggin et al. 1989), and the disease can be transmitted via mechanical vectors such as ectoparasitic snails (White et al. 1987).

The first described species of *Perkinsus* was *Perkinsus marinus*, the agent responsible for "Dermo disease" that has been associated with significant mortality events in American oyster (*Crassostrea virginica*) populations along the southern and eastern coasts of the United States since the late 1940's (Mackin et al. 1950, Ray 1954, 1996, Andrews 1996, see Section 4.17). The second *Perkinsus* species described was *Perkinsus olseni*, which was originally identified from blacklip abalone *Haliotis rubra* with grossly visible yellowish pustular lesions in the foot muscle near Port Lincoln in Spencer Gulf, SA as early as 1972 (Lester and Davis 1981). Several foci of infection near Port Lincoln around the Eyre Peninsula were known to divers and the distribution of the parasite appeared to be relatively stable, until around a decade later *P. olseni* was associated with mortalities in greenlip abalone *Haliotis laevigata* around 140 km away on the western side of Gulf St Vincent in SA during a particularly hot period of weather (Lester 1986, O'Donoghue et al. 1991,

Goggin and Lester 1995). Since the early 1990's the same parasite has been associated with mass mortality events in blacklip abalone along the central and southern coast of NSW during the summer months (Lester and Hayward 2005, Liggins and Upston 2010).

In SA the presence of *P. olseni* in infected abalone of all sizes was characterized by the presence of macroscopic necrotic nodules (0.5-8.0 mm in diametre) in the foot muscle and mantle (O'Donoghue et al. 1991). The disease process is facilitated by high water temperatures >20°C and high salinities, but at temperatures under 15°C infected abalone may be able to combat the infection (Lester 1986, Lester and Hayward 2005). *Perkinsus olseni* has also been reported from 30 out of 84 species of molluscs examined from the Great Barrier Reef (Goggin and Lester 1987), from pearl oysters *Pinctada maxima* from Torres Strait (Norton et al. 1993b) as well as several species of molluscs from WA (Hine and Thorne 2000, Dang et al. 2017). Until recently the *Perkinsus* –like parasites from Australian molluscs have all been identified as *P. olseni* (see Murrell et al. 2002, Lester and Hayward 2005), that was until Dang et al. (2015) reported the presence of both *P. olseni* and *Perkinsus chesapeaki* in mud arc cockles (*Anadara trapezia*) collected from Moreton Bay, QLD. The identity of *P. chesapeaki* in this host was later confirmed (Reece et al. 2017). *Perkinsus olseni* has also been recorded in many other regions worldwide, including in cockles (*Austrovenus stutchburyi*) in the North Island of New Zealand, where its distribution is probably limited by temperature (Hine and Diggles 2002b), and in Manila clams *Tapes philippinarum* in South Korea (where it has been associated with mass mortalities) as well as Japan, China, Vietnam, Europe and Uruguay (Villalba et al. 2004, Park et al. 2005). Infections of *P. olseni* in clams, cockles and oysters in Asia is often associated with disease that causes intense tissue inflammation and haemocyte infiltration around the infected areas, reduced reproductive effort, retarded gonad maturation and death (Choi and Park 2010, Waki et al. 2018). At least 6 other species of *Perkinsus* are currently recognised, including *P. qugwadi*, *P. chesapeaki*, *P. andrewsi*, *P. mediterraneus*, *P. beihaiensis* and *P. honshuensis*, all of which are parasites of molluscs (Villalba et al. 2004, Dungan and Reece 2006, Moss et al. 2008, Kang et al. 2016). Research in Panama and Tokyo Japan suggests that various *Perkinsus* species have been spread globally through international shipping (Pagenkopp Lohan et al. 2018, Itoh et al. 2019).

Vegetative stages of *P. olseni* can survive in dead hosts at 4°C and 0°C for several days with high survival during development of prezoosporangia, while they also survive freezing (-60°C) in both dried abalone tissue (19% survival after 28 days) and in abalone tissue stored in seawater (37% survival after 197 days at -60°C) (Goggin et al. 1990). Zoospores can survive in seawater at temperatures of 20-25°C for up to 28 days (Chu and Greene 1989), while hypnospores are susceptible to millimolar concentrations of silver nanoparticles (Bravo-Guerra et al. 2020). Vegetative stages can survive in dead molluscs underwater at normal environmental temperatures (15-25°C) for over 3 weeks (Ray 1954), which is much longer than the 2-4 days it takes for hypnospores to form and complete zoosporogenesis (Chu and Greene 1989), as shown by natural transmission of *Perkinsus* spp. after the death of its host (Villalba et al. 2004).

4.16.5 Release assessment

In Australia *Perkinsus* spp. have been detected in two areas of SA from abalone *H. laevigata*, *H. rubra*, *H. cyclobates*, *H. scalaris*, cockles *Barbatia pistachia*, *Katelysia rhytiphora*, razor shells *Pinna bicolor*, and scallops *Chlamys bifrons*, in NSW from *H. rubra*, *H. laevigata* and *H. roei*, in QLD from pearl oysters *Pinctada maxima* and 23 other families of bivalves from the Great Barrier Reef, and in Victoria from *O.*

angasi, (Lester and Davis 1981, Goggin and Lester 1987, 1995, Norton et al. 1993b, Lester and Hayward 2005). *Perkinsus* spp. has also been detected from several areas of WA in numerous hosts including pearl oysters (*Pinctada albina*, *Pinctada fucata*, *Pinctada margaritifera*), rock oysters *S. glomerata* and *S. cucullata*, hammer shells (*Malleus meridianus*), razor shells (*Isognomon isognomon*, *Pinna bicolour* and *P. deltoides*), cockles (*Anadara* sp., *Costacallista impar*, *Circe plicatina*), scallops (*Amusium balloti*) and greenlip abalone (see Hine and Thorne 2000, Dang et al. 2017). Greenlip abalone (*H. laevigata*) appear to be particularly susceptible to *P. olseni* infection, while blacklip abalone (*H. rubra*) appear to be relatively resistant to infection (O'Donoghue et al. 1991, Lester and Hayward 2005), though may still suffer epizootics at high water temperatures (Liggins and Upston 2010). In contrast, in Australia to date *Perkinsus chesapeaki* has only been recorded from mud arc cockles (*Anadara trapezia*) in Moreton Bay (Dang et al. 2015)

It is already known that *Saccostrea glomerata* and *S. cucullata* can be infected with *P. olseni* (see Hine and Thorne 2000), and given the parasites low host specificity, it is reasonable to assume that other species of oysters will also be susceptible to infection. Lester and Davis (1981) exposed *S. glomerata* to *P. olseni* zoospores at lowered salinities (20-30 ppt) and did not detect any infections using RFTM three months later, however Goggin et al. (1989) exposed *S. glomerata* and hairy mussels (*Trichomya hirsuta*) in aquaria to motile zoospores of *P. olseni* and found both species to be "largely refractory" to infection, with prevalence and intensity in *S. glomerata* highest (prevalence 100%, intensity up to 0.8 hypnospores/mm^2) in oysters exposed to *P. olseni* zoospores obtained from *A. trapezia*. The vegetative stages of *P. olseni* are extremely resistant and can survive in dead host tissue in water at ambient temperatures for several days during development of prezoosporangia (Goggin and Lester 1995), and even remain viable after 197 days at -60°C (Goggin et al. 1990). They are, however, inactivated by 10 minutes exposure to 50°C in brine (120 ppt salinity) and after 6 hours in freshwater (Goggin et al. 1990). The resistance of vegetative and infective stages of *P. olseni* and other *Perkinsus* spp. to desiccation, or acetic acid has not been determined, however it is known that *P. marinus* does not survive 115 days desiccation in piles of oyster shells where average temperatures ranged between 6 and 38°C inside the pile and reached a peak of 47°C on the surface (Bushek et al. 2004). All *Perkinsus* spp. can be transmitted horizontally through the water, and are known to occur in mollusc populations in several parts of mainland Australia, hence there is a non-negligible risk that these disease agents may occur in molluscs that are recycled for shellfish reef restoration in Australia. Taking into account the information above, and given the likely propagule pressure experienced via the various anthropogenic introduction pathways, the likelihood estimations for the release of *Perkinsus* spp. (including *P. olseni* and *P. chesapeaki*) via the identified risk pathways are listed below.

Release assessment for infection with *Perkinsus* spp. (including *P. olseni, P. chesapeaki*)

	Unmitigated	Desiccation			Heat		Salinity	Other
Treatment	None	3 mo.	4 mo.	6 mo.	55°C >10 min	80°C >5 min	0 ppt for >24 hrs	4% acetic acid 30 min
Likelihood of release	High	V low	E Low	Neg	E low	E low?	Neg	V low?

4.16.6 Exposure assessment

While *Perkinsus* spp. (including *P. olseni* and *P. chesapeaki*) infect oyster tissue and not shell material, it is

likely that shells from wild and /or cultured molluscs that may be procured from processors or retailers for recycling would retain some residual host tissue in areas such as retractor muscle scars, mantle tissue and so on. Molluscs in several regions of mainland Australia are already at risk from natural exposure to *P. olseni*, however, it is not known whether there are any strain differences between *P. olseni* isolates from different parts of Australia (Hine and Thorne 2000), and it is not known whether *P. chesapeaki* occurs in locations other than Moreton Bay. *Perkinsus* spp. can be transmitted horizontally through the water (Goggin et al. 1989) and their vegetative and infective stages can survive in water for at least 28 days (Chu and Greene 1989) as well as for many days in dead host tissue during development of prezoosporangia. The infective stages of *Perkinsus* spp. can even survive passage through the gut of scavenger teleosts (Hoese 1964).

The number of *P. olseni* parasites in the tissue of infected hosts can exceed 2 x 10^6 parasites/gram of tissue (Choi and Park 2010, Waki et al. 2018, Wang et al. 2018). In contrast, the minimum infective dose required for transmission to new hosts varies depending on host species and other environmental factors, but for *P. marinus* may be as few as 100 prezoosporangia (Chu and Volety 1997), or as high as 1 x 10^5 infective stages/oyster (Andrews 1996). This means that very little residual infected tissue on the shell of recycled molluscs may be required to provide an infectious dose. This massive replication potential explains why *Perkinsus* spp. is easily transmitted and can cause acute mass mortalities when susceptible molluscs are held at high density (Goggin et al. 1989). Furthermore, all life stages of the parasite are potentially infective, thus infected dying molluscs can liberate large numbers of infective stages (Bushek et al. 2002), which can then be concentrated within the digestive tract of nearby filter feeding bivalves as well as many other species of filter feeding invertebrates which could act as mechanical vectors. Thus, if an index case occurred, transmission and further spread from the index case to new hosts would be highly likely. Environmental conditions as well as host immune status will play important roles in transmission and establishment of *P. olseni* and other *Perkinsus* spp. in an index case. Water temperatures above 20°C appear to be required to increase the chances of infection, and transmission does not appear to occur in water of low salinity (<10 ppt). As there may be pathways to expose shellfish reef restoration projects to *Perkinsus* spp. via recycled mollusc shells, environmental conditions are likely to be suitable for disease transmission in most parts of mainland Australia and suitable hosts may occur in the wild in areas where shellfish reefs are being restored, the risk of exposure and establishment is non-negligible, and the overall likelihood of exposure and establishment of *Perkinsus* spp. (including *P. olseni* and *P. chesapeaki*) is considered to be **High.**

4.16.7 Consequence assessment

Although *P. olseni* and *P. chesapeaki* are already present in populations of wild molluscs in many parts of Australia, the distribution of this parasite is often sporadic at smaller scales (Lester 1986, Lester and Hayward 2005), and some regions of Australia may remain free from infection at this time. *Perkinsus* spp. infections can cause epizootic disease in wild molluscs (Hine and Thorne 2002, Liggins and Upston 2010), and have been proven to be implicated in long term declines in mollusc populations (Waki et al. 2018). There is also evidence that *P. olseni* can also cause sub-lethal disease (reduction of fecundity, growth, and condition) which can have significant impacts at the population level in both wild and cultured molluscs. *Perkinsus olseni* is listed by the OIE and NACA as a reportable disease, and remains listed as a reportable disease in all States (Table 3). Hence the spread of *Perkinsus* spp. into new areas is likely to adversely impact trade and may result in intervention by government authorities. Such interventions may cause disruption to aquaculture, commercial and recreational mollusc fisheries and shellfish reef restoration if attempts were made to contain

the infection and prevent its further spread into uninfected areas. Considering all of these factors, establishment of *Perkinsus* spp. (including *P. olseni* and *P. chesapeaki*) into new areas would likely have significant biological consequences and could cause economic harm to mollusc aquaculture together with significant irreversible environmental effects for wild mollusc populations. Taking all of these factors into consideration, the consequences of establishment of these parasites via recycled mollusc shells is considered to be **High.**

4.16.8 Risk estimation

The risk estimation for *P. olseni*, *P. chesapeaki* and other species of *Perkinsus* is determined by combining the likelihood of release and exposure (from Table 5) with the consequences of establishment (Table 6) to arrive at a risk estimation (Table 7). The risk estimate for infection with *P. olseni*, *P. chesapeaki* and other species of *Perkinsus* exceeds the ALOP for some release pathways, suggesting that additional risk management is required for these disease agents.

Risk estimate for infection with *Perkinsus* spp. (including *P. olseni, P. chesapeaki*)

	Unmitigated	Desiccation			Heat		Salinity	Other
Treatment	None	3 mo.	4 mo.	6 mo.	55°C >10 min	80°C >5 min	0 ppt for >24 hrs	4% acetic acid 30 min
Combined likelihood of release and exposure	High	V low	E Low	Neg	E low	E low?	Neg	V low?
Consequences of establishment	High	High	High	High	High	High	High	High
Risk estimation	High	Low	V low	Neg	V low	V low?	Neg	Low?

4.17 Infection with *Perkinsus marinus*

4.17.1 Aetiologic agent: *Perkinsus marinus* (formerly known as *Dermocystidium marinum*, or *Labyrinthomyxa marina*) is a protozoan classified in the Family *Perkinsidae* within the Kingdom Alveolata (Zhang et al. 2011, Adl et al. 2019) that causes "Dermo disease" in American oysters.

4.17.2 OIE List: Yes **NACA List:** No **Zoonotic:** No

4.17.3 Australia's status: Other species of *Perkinsus* occur in various regions of Australia (see Section 4.16), however *Perkinsus marinus* has not been recorded from Australia and is considered exotic.

4.17.4 Epizootiology

As early as the 1930's, mortalities of up to 95% of American oysters (*Crassostrea virginica*) were reported during the summer and early fall in certain bays in Louisiana and throughout the wider Gulf Coast area of the Gulf of Mexico, USA (Ray 1954). The mortality events were associated with warm summer water temperatures (over 20°C and less than 35°C) and salinities over 15 ppt and were worst in larger oysters more than 1 or 2 years old during a drought period in the mid 1940's (Ray 1954). Affected oysters were thin, weak and in poor condition with gaping (inability to keep the shell valves closed) prior to death (Ray 1954, 1996, Villalba et al. 2004). Due to the fact that multi-million dollar lawsuits were filed against oil companies by oystermen in Louisiana in 1946 after they accused oil spills of causing oyster kills, funding was provided to four research groups to study the problem (Ray 1996). After two years study, oil contamination was ruled out, as was infection by gregarines (*Nematopsis* spp.), oyster drills (*Thais* spp.) or mudworm (*Polydora* spp.) (Ray 1954, 1996). Instead, two of the research groups independently found the oyster mortalities were correlated with heavy infections by a new a spherical organism that was present in the haemolymph and tissues of diseased oysters and absent in healthy ones (Ray 1954, 1996). The researchers saw that the new disease agent possessed characteristics reminiscent of fungi and collaborated to describe it as *Dermocystidium marinum* (Dermo) and the disease became known as "Dermo disease" (Mackin et al. 1950, Ray 1954, 1996, Ray and Chandler 1955, Villalba et al. 2004).

While originally thought to be a fungus, subsequent studies found that *D. marinum* had several developmental, morphological and ultrastructural features that indicated otherwise. Mackin and Ray (1966) suggested that the parasite may be a member of the Labyrinthulomycota due to the observation that cultured *D. marinum* cells were amoeboid and glide along "mucoid tracks", and proposed renaming it *Labyrinthomyxa marina*. Ultrastructural studies undertaken by Perkins (1969) found some resemblance to fungal structures, but he later found evidence that *L. marina* zoospores have an apical complex, suggesting they were protozoans which should instead be placed in the Subphylum Apicomplexa (Perkins 1976b). However, it was noted the zoospores were morphologically distinct from all other known apicomplexans, leading to the conclusion that *L. marina* was unique and deserving of the establishment of a new Class (Perkinsea), genus, and the new species name *Perkinsus marinus* (see Levine 1978). More recent molecular taxonomic evidence has supported the uniqueness of these parasites within the Family *Perkinsidae*, but found they were more closely related to dinoflagellates than apicomplexans (Goggin and Barker 1993, Murrell et al. 2002) and higher taxonomic

revisions have placed both these groups with the ciliates in the Kingdom Alveolata (Cavalier-Smith 1993, Zhang et al. 2011, Adl et al. 2019).

Research into the disease process advanced quickly once it was discovered that trophozoites of the parasite isolated from oyster tissues would survive, grow in size and sporulate *in-vitro* (Ray 1952, 1954, 1966). Ray (1952) observed that when oyster tissues infected by *P. marinus* were incubated in fluid thioglycollate medium (FTM), trophozoites enlarged up to 300 µm in diametre and developed a thick wall, thus becoming a new stage he called the hypnospore. Then he found that when hypnospores produced in FTM are transferred into seawater, sporulation began and progressed with successive vegetative proliferations resulting in development of hundreds of biflagellated zoospores within the original hypnospore wall (Ray 1952, 1954). The addition of antibiotic and antifungal reagents to the FTM enhanced its sensitivity as a new diagnostic tool that could be used to detect *P. marinus*, in what has since become known as Rays FTM technique (RFTM) (Ray 1952, 1966). For routine diagnosis, excised fragments of oyster heart, rectum, mantle and gill are incubated in RFTM at room temperature for up to 7 days, then stained with Lugols iodine (Ray 1952, 1966). The hypnospores enlarge during incubation then become visible under a dissecting microscope when stained blue-black with the iodine, allowing quantitation of the number of parasites present using a scale proposed by Mackin (Mackin Scale, in Ray 1954) in which a numerical value of 0.5, 1, 2, 3, 4 or 5 was given to describe the intensity of infection (very light to heavy).

Development of the RFTM method allowed researchers to delimit the geographic extent of *P. marinus* infections quickly and inexpensively (Ray 1954). They found that *P. marinus* was widespread in *C. virginica* throughout the Gulf of Mexico from Texas to Florida as well as the south east coast of the US as far north as Virginia and various high salinity areas of Chesapeake Bay (Ray 1954). Oysters three to four months old were found to be refractory to infection, however the parasite was often causing mortalities of greater than 80% in adult populations of American oysters during periods of high water temperatures (>25°C) and salinities (>15 ppt) (Ray 1996). The same parasite was also found at lower prevalence and intensities in other bivalves including *Crassostrea rhizophorae*, *C. ariakensis*, *Ostrea* spp., clams, and scallops (Ray 1954, Calvo et al. 1999, 2000, 2001). *Perkinsus marinus* can also infect *Crassostrea gigas*, but the infections do not cause disease (Barber and Mann 1994, Calvo et al. 1999). Higher filtration rates and longer exposure to infective particles could explain why *P. marinus* prevalence is higher in adult than in juvenile oysters living in the same environments (Villalba et al. 2004). Subsequent studies showed that distribution of *P. marinus* in oysters and other bivalves in these regions and also in Mexico is seasonally variable depending on environmental factors, with the parasite being favoured by high water temperatures and high salinity with peak infection intensity and mortality occurring in late summer (Chu and Greene 1989, Burreson et al. 1996). Natural infection appears absent in estuarine “sanctuaries” where salinity is less than 9-12 ppt (Burreson et al. 1994, 1996). It appears that transmission does not proceed below 9 ppt, however, once established in an oyster host, *P. marinus* can persist within the oyster tissues for at least 3 months at salinities around 5 ppt (Villalba et al. 2004).

The lifecycle of *P. marinus* was found to be direct and involves horizontal transmission involving infection of the filter feeding host mainly via the gills and palps followed by vegetative proliferation within the gut epithelium and other organs by trophozoites that undergo successive bipartitioning (Ray and Chandler 1955, Villalba et al. 2004). Other stages that have been observed inside oyster tissues include hypnospores, zoosporangia and biflagellated zoospores, the latter which are probably natural dispersal and infective stages although all types of developmental stages are infective for new oyster hosts (Goggin et al. 1989, Villalba et

al. 2004). When the host dies (likely due to starvation followed by organ dysfunction due to massive infections of parasites) or when host tissues infected by *Perkinsus* spp. are incubated in RFTM, the trophozoites enlarge and develop a thick cell walled hypnospore, which sporulates liberating large numbers of biflagellate zoospores into the environment via the faeces or upon autolytic breakdown of the host tissue (Villalba et al. 2004). Ray (1954) studied parasites cultured in thioglycolate medium and found that growth of *P. marinus* was greatest at 30°C and became retarded at 35°C. The vast majority of *P. marinus* trophozoites were inactivated when exposed to 35-37°C water temperature for 60 hours, and did not tolerate salinities below 1.5 ppt (Ray 1954, Ray and Chandler 1955, Burreson et al. 1994). Other treatments shown to inactivate *P. marinus* cells within host tissues included exposure to greater than 115 days desiccation, high temperatures (50°C >18 hours or 60°C for 1 hour) and freshwater for 30 minutes (Bushek et al. 1997, 2004, Bushek and Howell 2000).

4.17.5 Release assessment

Perkinsus marinus has not been recorded from Australian molluscs and is considered exotic, however it is included in this risk analysis as it is listed as a notifiable disease agent that could cause serious harm if it were introduced at some point in the future. In Australia the closely related *Perkinsus olseni* has been reported from a wide range of abalone and bivalve hosts in QLD, NSW, Vic, SA, and WA, while *P. chesapeaki* has only been recorded from mud arc cockles (*Anadara trapezia*) in Moreton Bay (see Section 4.16). *Perkinsus marinus* appears to infect and cause disease mainly in American oysters (*C. virginica*), however it is known to also be able to infect *C. gigas*, which tolerates the infection and does not experience disease (Barber and Mann 1994). A range of clam species are known to be susceptible to infection, suggesting that hosts susceptible to *P. marinus* probably do occur in the Australian environment. The vegetative stages of *P. marinus* can survive in dead host tissue at ambient temperatures for several days during development of prezoosporangia (Ray 1954). However, they are inactivated by exposure to high temperatures (50°C >18 hours or 60°C for 1 hour) and freshwater for 30 minutes (Bushek et al. 1997). The vegetative stages of *P. marinus* are relatively resistant to desiccation, with complete inactivation requiring around 115 days desiccation in piles of oyster shells where average temperatures ranged between 6 and 38°C inside the pile and reached a peak of 47°C on the surface (Bushek et al. 2004). The resistance of *P. marinus* stages to acetic acid are unknown. Taking into account the information above, and given the likely propagule pressure experienced via the various anthropogenic introduction pathways, the likelihood estimations for the release of *Perkinsus marinus* via the identified risk pathways are listed below.

Release assessment for infection with *Perkinsus marinus*

	Unmitigated	Desiccation			Heat		Salinity	Other
Treatment	None	3 mo.	4 mo.	6 mo.	55°C >10 min	80°C >5 min	0 ppt for >24 hrs	4% acetic acid 30 min
Likelihood of release	High	V low	E Low	Neg	Low	E low?	Neg	V low?

4.17.6 Exposure assessment

While *Perkinsus marinus* infects oyster tissue and not shell material, it is likely that shells from wild and /or cultured molluscs that may be procured from processors or retailers for recycling would retain some residual host tissue in areas such as retractor muscle scars, mantle tissue and so on. Molluscs in several regions of mainland Australia are already at risk from natural exposure to *P. olseni* and *P. chesapeaki*, however, they would be naïve to *P. marinus* and many native species are likely to be susceptible to infection. *Perkinsus marinus* transmits horizontally through the water (Goggin et al. 1989) and environmental conditions favourable for parasite survival are found throughout mainland Australia. The vegetative and infective stages of *P. marinus* can survive in water for at least 28 days (Chu and Greene 1989) as well as for many days in dead host tissue during development of prezoosporangia. The infective stages of *Perkinsus* spp. can even survive passage through the gut of scavenger teleosts (Hoese 1964).

The minimum infective dose required for transmission to new hosts for *P. marinus* varies depending on host species and other environmental factors, but may be as few as 100 prezoosporangia (Chu and Volety 1997), or as high as 1 x 10^5 infective stages/oyster (Andrews 1996). This means that very little residual infected tissue on the shell of recycled molluscs may be required to provide an infectious dose. All life stages of the parasite are potentially infective, thus infected dying molluscs can liberate large numbers of infective stages (Bushek et al. 2002), which can then be concentrated within the digestive tract of nearby filter feeding bivalves as well as many other species of filter feeding invertebrates which could act as mechanical vectors. Thus, if an index case occurred, transmission and further spread from the index case to new hosts would be highly likely. Environmental conditions as well as host immune status will play important roles in transmission and establishment of *P. marinus* in an index case. Water temperatures above 20°C are required to increase the chances of infection, and transmission does not appear to occur in water of low salinity (<9 ppt). As there may be pathways to expose shellfish reef restoration projects to *P. marinus* via recycled mollusc shells, environmental conditions are likely to be suitable for disease transmission in most parts of mainland Australia and suitable hosts may occur in the wild in areas where shellfish reefs are being restored, the risk of exposure and establishment is non-negligible, and the overall likelihood of exposure and establishment of *P. marinus* is considered to be **High.**

4.17.7 Consequence assessment

Although other *Perkinsus* spp. are already present in populations of wild molluscs in some regions of Australia, *P. marinus* has never been recorded here and is considered exotic. *Perkinsus marinus* infections can cause epizootic disease in wild molluscs, and can have significant impacts at the population level in both wild and cultured molluscs. *Perkinsus marinus* is listed by the OIE as a reportable disease, and remains listed as a reportable disease in all States (Table 3). Hence the spread of *P. marinus* into new areas is likely to adversely impact trade and may result in intervention by government authorities. Such interventions may cause disruption to aquaculture, commercial and recreational mollusc fisheries and shellfish reef restoration if attempts were made to contain the infection and prevent its further spread into uninfected areas. Considering all of these factors, establishment of *P. marinus* into new areas would likely have significant biological consequences and could cause economic harm to mollusc aquaculture together with significant irreversible environmental effects for wild mollusc populations. Taking all of these factors into consideration, the consequences of establishment of these parasites via recycled mollusc shells is considered to be **High.**

4.17.8 Risk estimation

The risk estimation for *P. marinus* is determined by combining the likelihood of release and exposure (from Table 5) with the consequences of establishment (Table 6) to arrive at a risk estimation (Table 7). The risk estimate for infection with *P. marinus* exceeds the ALOP for some release pathways, suggesting that additional risk management is required for this disease agent.

Risk estimate for infection with *Perkinsus marinus*

	Unmitigated	Desiccation			Heat		Salinity	Other
Treatment	None	3 mo.	4 mo.	6 mo.	55°C >10 min	80°C >5 min	0 ppt for >24 hrs	4% acetic acid 30 min
Combined likelihood of release and exposure	High	V low	E Low	Neg	Low	E low?	Neg	V low?
Consequences of establishment	High	High	High	High	High	High	High	High
Risk estimation	High	Low	V low	Neg	Mod	V low?	Neg	Low?

4.18 Akoya oyster disease

4.18.1 Aetiologic agent: Mass mortality of Akoya oysters (*Pinctada fucata martensii*) is caused by an infectious filterable disease agent (Morizane et al. 2002, Wada 2007). The disease agent displays tissue trophism for the adductor muscle, haemolymph and particularly the mantle (Nakayasu et al. 2004). Recent data suggest a spirochaete bacterium Candidatus *Maribrachyspira akoyae* is implicated in the disease syndrome (Matsuyama et al. 2017, 2018, 2019).

4.18.2 OIE List: No **NACA List:** No **Zoonotic:** ?

4.18.3 Australia's status: Not recorded. Akoya oyster disease is not known to occur in Australian molluscs, and the disease is considered exotic.

4.18.4 Epizootiology

Mass mortalities of juvenile, adult and seeded Japanese oysters (or Akoya oyster, *Pinctada fucata martensii*) due to a disease that turned the adductor muscle yellowish to red-brown in colour were first reported in Yusu Bay and Uchiumi Bay, in Ehime Prefecture, south western Japan during 1994 (Wada 1997, Miyazaki et al. 1999, Jones 2007a). Mortalities were associated with high water temperatures above 25°C, and in 1996 and 1997, overall losses of around 50 percent of production in western Japan were reported (Miyazaki et al. 1999), with losses as high as 80% in some localised areas (Tomaru et al. 2001). Since that time, mass mortalities of Akoya oysters have occurred annually during the summer and autumn months at these and other pearl farming bays in western Japan, resulting in significant economic losses and around 66% reduction in pearl farming employment (Yoshinaga and Kokonoe 2018).

Clinical signs in diseased oysters include reduced growth and sluggish closure of shell valves on stimulation of the mantle margin, atrophy and discolouration of the adductor muscle, mantle margin and body, and transparency of the mantle (Wada 2007). The adductor muscle appears yellowish or reddish brown, especially during periods of higher water temperature (Miyazaki et al. 1999) and affected oysters produce poor quality pearls (Kobayashi et al. 1999, Miyazaki et al. 1999). Various factors such as toxic algal blooms, and environmental factors related to water temperature anomalies and food limitation were initially investigated as potential contributing factors to the disease outbreak (Tomaru et al. 2001). However, the disease originally emerged after importation of oysters from China (Wada 1997, Miyazaki et al. 1999) and was subsequently apparently spread by movements of oysters between pearl oystering bays, suggesting an infectious agent was involved (Miyazaki et al. 1999). Initial studies found the disease appeared to be horizontally transmissible between oysters via co-habitation and transplantation of mantle tissue from affected oysters (Kurokawa et al. 1999), as well as injection of 0.45µm filtered haemolymph from diseased oysters (Morizane et al. 2002), with adductor dysfunction similar to that observed in natural cases of disease causing widely opened shell valves shortly before death after 2 to 3 months (Morizane et al. 2002).

Miyazaki et al. (1999) reported the presence of small (25-30 nm) virus-like particles in the muscle fibres of the adductor, foot, gill and cardiac muscle, and reported horizontal transmission of the disease via inoculation of virus isolated in culture from these organs resulting in high mortalities of up to 100% within 20 days.

However, Nakajima (1999) could not isolate any viral agents from affected oysters using 14 different cell lines (including cell lines used by Miyazaki et al. 1999), although he did confirm that the disease could be transmitted horizontally by cohabitation and inoculation. In contrast, Hirano et al. (2002) failed to transmit the disease horizontally when Akoya oysters were cohabited in an aquaculture setting, and more detailed epidemiological studies by the same group found "no viral, bacterial, mycotic, or parasitic causative organisms" in diseased oysters, suggesting that Akoya oyster disease was more likely to be due to environmental changes related to a combination of high water temperatures (average winter water temperature greater than 15°C and summer water temperatures over 25°C), and the existence of organic pollution originating from nearby fish farms (Hirano et al. 2005). A study by the same research group (Sugishita et al. 2005) found that similar pathological changes (blebbing and necrosis in the epithelial cells of the digestive organ) and mortalities of Akoya oysters could be initiated by exposure of oysters to oxidized oils similar to those found in fish feeds, suggesting that organic pollution caused by fish farming, exposed oysters to increased suspended solids containing lipid peroxides, resulting in disease (Sugishita et al. 2005). These results lead to some authors concluding that Akoya oyster disease was a result of environmental stress caused by nearby urbanisation and industrialisation (Kuchel et al. 2011).

Despite the results from Hirano et al. (2005) and Sugishita et al. (2005), other research groups continued to find the disease was transmissible, with Nakayasu et al. (2004) injecting the supernatant of tissue homogenates to find that the highest concentrations of the putative disease agent occurred in the mantle, followed by the adductor muscle, heart and haemolymph. Mortalities of the experimentally infected oysters began to occur after 3 months and continued until 5 months at water temperatures of 23-25°C (Nakayasu et al. 2004). This ongoing evidence of an infectious aetiology from several separate research groups spurred utilisation of 16S rRNA-based metagenomic technologies (next generation sequencing) to try to identify genes present in affected oysters and absent from healthy oysters (Matsuyama et al. 2017). This research found that genes isolated only in diseased oysters were mostly bacterial in origin, suggesting that the causative agent of Akoya oyster disease was a bacterial pathogen. The hypothesis was supported by controlled trials which showed the onset and progression of disease could be prevented by treating naïve oysters injected with the 0.45 µm filtered haemolymph of diseased oysters with penicillin via bath-administration (Matsuyama et al. 2017). Matsuyama et al. (2017, 2019) detected genes of bacteria from the Phylum Spirochaetes which were most abundant in the mantle and haemolymph of diseased oysters, thus aligning with the bioassay results of Nakayasu et al. (2004) more than a decade earlier. Furthermore, *in-situ* hybridization and immunostaining found a *Brachyspira*-like bacterium (Candidatus *Maribrachyspira akoyae*) in haemolymph smears from diseased oysters, but not from healthy oysters (Matsuyama et al. 2017).

Spirochaete bacteria are known pathogens of terrestrial animals and humans (e.g. see Hampson 2017, Edmondson et al. 2018), however it is not known if Candidatus *Maribrachyspira akoyae* is of zoonotic importance to humans at this time. These bacteria are slow growing, fastidious and notoriously difficult to culture (Hampson 2017, Edmondson et al. 2018, Matsuyama et al. 2019). Pathological investigations of oysters with Akoya oyster disease revealed at the early stages of the disease, marked necrosis, loss of cytoplasm and surface blebbing in the epithelial cells of the stomach and tubules of the digestive gland, spreading to other organ systems as disease progressed (Hirano et al. 2005). The sequential pathology of diseased oysters suggested that "*some cellular injury factors were ingested with food, were absorbed, and caused damage to cells in various organs*" (Hirano et al. 2005). The fact that spirochaete bacteria are known to attach to intestinal epithelia and cause enteric pathologies in animals and humans (Hampson 2017) thus

aligns with the pathology associated with Akoya oyster disease, and the role of the bacterium in the disease process is strongly suggested by the consistent detection of Candidatus *M. akoyae* in oysters from growing areas experiencing disease outbreaks associated with reddish-brown colouration of the adductor (Matsuyama et al. 2018). Furthermore, qPCR found that spirochete genes were most abundant in the mantle, adductor muscle, and haemolymph of affected oysters, and that spirochete genes were detected in the haemolymph 2 weeks after experimental infection, and their number continued to increase with time until mortality (Matsuyama et al. 2019). Together, these results are epidemiologically consistent with field observations and strongly suggest that a spirochete bacterium causes Akoya oyster disease (Matsuyama et al. 2019). Genetic data from Japan found that isolates of Candidatus *Maribrachyspira akoyae* sourced from 11 locations around Japan were similar (Matsuyama et al. 2018). The high degree of genetic uniformity between isolates from different geographic areas suggests the bacterium expanded from a recent bottleneck and was probably introduced into Japan from pearl oysters imported from China (Matsuyama et al. 2018).

4.18.5 Release assessment

For the purposes of this RA, it will be assumed that Akoya oyster disease is caused by a *Brachyspira*-like spirochete bacterium, and that the causative agent is exotic to Australia. However, it is included in this risk analysis as it is listed as a notifiable disease that could cause serious harm if it were introduced at some point in the future. It is known that spirochaete bacteria are commonly associated with marine bivalves (Husmann et al. 2010), however they can also survive free in the environment for extended periods as some are known facultative or obligate anaerobes found in large numbers in microbial mats in mud or biofilms on underwater surfaces (Shivani et al. 2015). *Brachyspira*-like spirochetes are robust and are also able to tolerate freezing, given that it is well known that spirochaete isolates can be successfully recovered after long term freezing (Edmondson et al. 2018). The full range of infections present in the various species of Australian molluscs remains to be determined, and it also appears possible that these disease agents could be introduced into Australia via ballast water, or with infected hosts as biofouling on the hulls or sea chests of commercial shipping, fishing vessels and pleasure craft (Howard 1994, Deveney et al. 2017), or in imported mollusc products used as bait or burley (Diggles 2020a). Because of this, there is a non-negligible risk that spirochaete bacteria may occur in molluscs that are recycled for shellfish reef restoration in Australia. Unfortunately, it appears the resistance of Candidatus *Maribrachyspira akoyae* to desiccation, freshwater, heat or acetic acid has not been determined. Taking into account the information above, and given the likely propagule pressure experienced via the various anthropogenic introduction pathways, the likelihood estimations for the release of Akoya oyster disease via the identified risk pathways are listed below.

Release assessment for Akoya oyster disease

	Unmitigated	Desiccation			Heat		Salinity	Other
Treatment	None	3 mo.	4 mo.	6 mo.	55°C >10 min	80°C >5 min	0 ppt for >24 hrs	4% acetic acid 30 min
Likelihood of release	Moderate	E low?	Neg	Neg	Low	E low?	Low?	V low?

4.18.6 Exposure assessment

While Candidatus *Maribrachyspira akoyae* infects oyster mantle and adductor tissue and not shell material, it is likely that shells from wild and /or cultured molluscs that may be procured from processors or retailers for recycling would retain some residual host tissue in areas such as retractor muscle scars, mantle tissue and so on. To date Candidatus *M. akoyae* is only known from diseased Akoya oysters, and it is not known whether it can infect other bivalve species, even though Akoya oyster disease was also reported to affect scallops and Pacific oysters (see Jones 2007a). Because of this lack of information, it is not clear whether this disease agent can infect bivalves that occur in Australia and/or whether bacteria released through various pathways could come in contact with susceptible hosts. On the other hand, the environmental conditions in many parts of mainland Australia would appear suitable for establishment of Akoya oyster disease, which requires water temperatures above 25°C for disease to be expressed. If susceptible molluscs occurred in Australia and were exposed to viable infective stages via one of the identified pathways, infection may occur via horizontal transmission if an infective dose was introduced into an area where susceptible hosts were present under conditions suitable for transmission. However, the minimum infective dose for these disease agents is not known. If an index case occurred, the disease agent may be able to persist and establish in the local oyster population, however to date there is no evidence that Akoya oyster disease has been spread via international shipping and become established in wild and cultured populations of molluscs in new locations. As there may be pathways to expose shellfish reef restoration projects to spirochaete bacteria via recycled mollusc shells, environmental conditions are likely to be suitable for disease transmission in many parts of mainland Australia and suitable hosts may occur in the wild in areas where shellfish reefs are being restored, the risk of exposure and establishment is non-negligible, and the overall likelihood of exposure and establishment of Akoya oyster disease is considered to be **Very low.**

4.18.7 Consequence assessment

Akoya oyster disease has never been recorded in Australia and is considered exotic. Its introduction into Australia would therefore likely have highly significant ramifications, both ecologically and financially, the latter due to the fact that the disease has caused significant economic impacts in Japan. Spirochaete infections can cause disease in humans (Hampson 2017, Edmondson et al. 2018), but it is not known whether Candidatus *Maribrachyspira akoyae* is of zoonotic importance at this time. Given the existing pearl oyster industry in northern Australia and interest in developing a TRO aquaculture industry, establishment of such a major pathogen would represent a significant obstacle to future investment in such industries, especially if Australian pearl oyster species were also susceptible to infection. However, as Akoya oyster disease is not listed by the OIE or NACA, its presence would not have significant international trade implications. However, its detection in Australia would likely require intervention by government authorities as the disease is under official control in QLD, WA and the NT (Table 3). This could cause disruption to normal trade in mollusc commodities by commercial fisheries and mollusc gathering by recreational fishers to try to limit potential spread into uninfected areas. Nevertheless, once these bacteria were detected, unless it was in an enclosed system which could be effectively sanitised there would appear to be little chance of eradication. Taking all of these factors into consideration, the consequences of establishment of these disease agents via recycled mollusc shells is considered to be **High.**

4.18.8 Risk estimation

The risk estimation for Akoya oyster disease is determined by combining the likelihood of release and exposure (from Table 5) with the consequences of establishment (Table 6) to arrive at a risk estimation (Table 7). The risk estimate for Akoya oyster disease exceeds the ALOP for some release pathways, suggesting that additional risk management is required for this disease agent.

Risk estimate for Akoya oyster disease

	Unmitigated	Desiccation			Heat		Salinity	Other
Treatment	None	3 mo.	4 mo.	6 mo.	55°C >10 min	80°C >5 min	0 ppt for >24 hrs	4% acetic acid 30 min
Combined likelihood of release and exposure	Very low	E low?	Neg	Neg	V low	E low?	V low?	E low?
Consequences of establishment	High	High	High	High	High	High	High	High
Risk estimation	Low	V low?	Neg	Neg	Low	V low?	Low?	V low?

4.19 Oyster oedema disease

4.19.1 Aetiologic agent: None identified to date.

4.19.2 OIE List: No **NACA List:** No **Zoonotic:** No

4.19.3 Australia's status: Oyster oedema disease (OOD) has been recorded from hatchery reared pearl oyster (*Pinctada maxima*) spat in Exmouth Gulf, Western Australia (WA) (Humphrey and Barton 2009, Jones et al. 2010, Goncalves et al. 2017).

4.19.4 Epizootiology

Mass mortalities occurred in farmed silver lip pearl oysters (*Pinctada maxima*) in Exmouth Gulf, Western Australia (WA) in October 2006 (Madin 2007, Humphrey and Barton 2009, Jones et al. 2010). Around 2.8 million *P. maxima* of all class sizes (up to 120 mm shell height) died, including approximately 60% of recently-seeded oysters, with mortalities up to 90% or higher in smaller oysters with an apparent spread of the disease to all lease sites in Exmouth Gulf (Madin 2007, Humphery and Barton 2009). Gross signs included severe mantle retraction, muscle weakness, mild oedema of the mantle tissue and palps, and mortality (Jones et al. 2010). Only *P. maxima* were affected, with other bivalves including blacklip pearl oysters *P. margaritifera* and Shark Bay pearl oysters (*P. albina*) or other marine organisms (fish, crustaceans) remaining apparently healthy (Jones et al. 2010). Histopathology revealed focal loss of epithelial cells along the mantle margins but otherwise no haemocyte inflammatory processes or known pathogens were present (Jones et al. 2010). Based on the oedematous tissue changes which occurred in affected oysters (Jones et al. 2010), the syndrome was called oyster oedema disease (OOD). Since 2006, OOD has continued to sporadically effect *P. maxima* farming in some locations in WA (Goncalves et al. 2017).

Water samples taken at the time of the initial outbreak showed no known toxic algal species were present. Instead, the initial disease outbreak appeared to follow movements of personnel (divers) and was consistent with a propagating infectious process (Jones et al. 2010). Water quality parametres were apparently normal, and the affected oysters had been recently feeding (Jones et al. 2010). However, unlike the situation with Akoya oyster disease (see Section 4.18), attempts to transmit OOD under experimental conditions exposing naïve oyster spat to tissue homogenates from oysters diagnosed with OOD were equivocal, and did not point to an infectious cause (Humphery and Barton 2009). The high susceptibility of juvenile pearl oysters to disease suggested the immunocompetence of the host may be an important factor in the condition (Bearham et al. 2009). The aetiology of the oedematous lesions associated with OOD is perplexing as oysters are osmoconformers (Jones et al. 2010). The OOD lesions could therefore be due to 1. a pathogenic agent affecting the epithelial cells directly, 2. a variety of insults (including pathogens) that provoke a generalized non-specific oedematous response either at the cellular level or through a neuroendocrine-mediated response, or 3. oedema could also be the outcome of a flaccid hydraulic system, perhaps indicating a loss of muscular control (Jones et al. 2010).

Electron microscopy did not detect any viruses or virus-like particles in tissues from OOD cases (Jones et al. 2010, Goncalves et al. 2017). However, subsequent studies using real time PCR (qPCR) showed there was

clear evidence for the presence of at least one *Chlamydia*-like organism (*maxima*-CLO), as well as *Simkania negevensis* and other intracellular bacteria at high prevalences in diseased oysters. However, both types of CLO were present in both diseased and healthy *P. maxima*, hence their presence did not appear to bear any consistent relationship with disease or OOD mortalities and a causative role in a disease process was not established (Crockford and Jones 2012, Goncalves et al. 2017).

Goncalves et al. (2017) used next generation nucleotide sequencing and high throughput qPCR to compare OOD-affected *P. maxima* with healthy control oysters to identify any nucleotide sequences in the OOD-affected oysters that might come from an infectious agent such as a virus, bacteria or parasite. They found clear differences between the cDNA sequences present in oysters affected by OOD when compared to healthy controls (Goncalves et al. 2017). A number of sequences (5) were strongly associated with OOD and the abundance of some of these sequences was correlated with increasing mortality, however unlike the situation with spirochaete bacteria in Akoya oyster disease, none of the differential sequences in *P. maxima* from WA were closely related to any known infectious agents (Goncalves et al. 2017). Instead, it is thought they may instead simply reflect differences in the pathobiome of dying oysters – it was not clear whether the associated sequences were undescribed microbes or undescribed oyster genes (Goncalves et al. 2017). Nevertheless, the strong relationship between these sequences, OOD and mortality meant that they may be useful predictors of mortality, although their lack of resemblance to known infectious agents leaves open the possibility that OOD is not an infectious disease and may have some other cause (Goncalves et al. 2017).

4.19.5 Release assessment

While OOD is known to be occur in pearl oysters (*Pinctada maxima*) from some areas of WA, it has not been reported in other parts of northern Australia. Furthermore, it appears that if OOD is caused by a disease agent, that agent is difficult to transmit horizontally (Humphery and Barton 2009) and appears highly host specific for *P. maxima*, as it does not appear to cause disease in even closely related pearl oysters such as *P. margaritifera* or *P. albina*. Thus, at this time there is no evidence that OOD could infect or be transmitted to other oyster species. Taking into account the information above, the risk of release of OOD via the various anthropogenic introduction pathways is likely to be negligible, and no further analysis is required for this disease.

Release assessment for oyster oedema disease

	Unmitigated	Desiccation			Heat		Salinity	Other
Treatment	None	3 mo.	4 mo.	6 mo.	55°C >10 min	80°C >5 min	0 ppt for >24 hrs	4% acetic acid 30 min
Likelihood of release	Negligible	Neg	Neg	Neg	Neg	Neg	Neg	Neg

4.20 Winter mortality (*Mikrocytos roughleyi*)

4.20.1 Aetiologic agent: Microcell-like structures occur in in abscess-like lesions in Sydney rock oysters *S. glomerata* from NSW with winter mortality disease (Roughley 1926). These were described as *Mikrocytos roughleyi* by Farley et al. (1988). However, more recent molecular studies suggest the microcell-like structures visually observed by Farley et al. (1988) are not a *Bonamia* or *Mikrocytos* species and the disease syndrome could be due to an undescribed microcell-like organism (the winter mortality disease agent) and/or adverse environmental factors (Carnegie et al. 2014, Spiers et al. 2014).

4.20.2 OIE List: No **NACA List:** No **Zoonotic:** No

4.20.3 Australia's status: Winter mortality disease has been reported to affect Sydney rock oysters (*S. glomerata*) in NSW from Port Stephens south to the NSW/Victoria border (Adlard and Lester 1995, Spiers et al. 2014).

4.20.4 Epizootiology

A disease syndrome called winter mortality was first reported in oysters from the Georges River, near Sydney in the 1920's (Roughley 1926). The disease was characterised by unusual mortalities of larger oysters in the downstream areas of affected estuaries during the late winter months (August -September) during periods of low water temperature (10-14°C) and high salinity (>29 ppt) (Roughley 1926, Hand et al. 1998, Nell 2001). Affected oysters gape and display occasional focal abscess-like lesions in the vesicular connective tissue of the gills, mantle, labial palps, gonad and adductor muscle (Roughly 1926, Wolf 1967, Farley et al. 1988). Histologically the affected oysters can have moderate to large numbers of small (1-3 µm) microcell-like bodies in affected tissues, which led Farley et al. (1988) to describe the microcells as a new species, *Mikrocytos roughleyi*, based on histological sections alone, because it was reminiscent of *Mikrocytos mackini* due to the fact that both parasites were associated with abscess-like lesions in a cupped oyster species during periods of low water temperature. The disease was originally detected in the Georges River in Sydney, however similar problems have been reported in *S. glomerata* as far north as Port Stephens and throughout estuaries in southern NSW to the Victorian border (Adlard and Lester 1995, Smith et al. 2000). Mortalities as high as 70% have been attributed to the disease (Adlard and Lester 1995), with mortality rates able to be reduced by increasing the intertidal growing height of oysters by 150 to 300 mm above their usual height or by transferring oysters to upstream locations before the winter months (Smith et al. 2000). Importantly, mortality rates can be increased by lowering oysters into deeper water to increase the time they are submerged (Smith et al. 2000).

Research into winter mortality progressed slowly for around 7 decades due to its sporadic occurrence and short window of disease expression, as well as diagnostic difficulties (Adlard and Lester 1995). The development of molecular diagnostic techniques promised improved diagnostic sensitivity and the earliest studies allowed presumptive identification of *M. roughleyi* using molecular probes not only in several estuaries in southern NSW, but also in three *S. glomerata* from Moreton Bay in QLD (Adlard and Lester 1995). However, the test used by Adlard and Lester (1995) amplified only a very small part of the genome and was not specific for *M. roughleyi* or even for the genus *Mikrocytos*, hence the relevance of their results are not clear (Carnegie et al.

2014). Then Cochennec-Laureau et al. (2003) utilised TEM and molecular techniques to investigate the taxonomic affinities of microcells in *S. glomerata* from southern NSW affected by winter mortality. They found that the parasite they isolated had molecular and ultrastructural (presence of haplosporosomes and mitochondria) characteristics that were more closely aligned with *Bonamia* sp., and they suggested reclassification of *M. roughleyi* as *Bonamia roughleyi* (Cochennec-Laureau et al. 2003). However, the most recent molecular diagnostic evidence indicates that some *S. glomerata* in southern NSW (Georges River, Pambula River) are infected with *Bonamia exitiosa* and *Haplosporidium* sp. at low prevalence (Carnegie et al. 2014), suggesting that the parasite genetically and morphologically identified in *S. glomerata* by Cochennec-Laureau et al. (2003) was a mis-identified *B. exitiosa*, and therefore the parasite named *B. roughleyi* is *nomen dubium* (doubtful name) (Carnegie et al. 2014).

While *Bonamia exitiosa* was unambiguously detected using molecular methods in *S. glomerata* from southern NSW at very low prevalences (mean 3% in the Georges River using cPCR), pathological evidence of the role of a *Bonamia* spp. causing disease in oysters dying from winter mortality remains elusive (Engelsma et al. 2014, Spiers et al. 2014, Carnegie et al. 2014). This was because the incidence of winter mortality disease in the populations of Georges River *S. glomerata* studied was substantially higher (up to 85% of oysters showed focal abscesses or other signs of disease in September) than the prevalence of *B. exitiosa* (maximum prevalence 10% in September using cPCR), and ISH showed that the numerous microcells that were visible in around 50% of the oysters showing signs of winter mortality in September were not a known species of *Bonamia* or *Mikrocytos* (see Spiers et al. 2014). The predominant microcell in *S. glomerata* affected by winter mortality is therefore not a *Bonamia* spp. or *Mikrocytos* spp. (see Spiers et al. 2014, Carnegie et al. 2014), and thus the taxonomic affinities of the microcell associated with winter mortality disease currently remains unknown.

Engelsma et al. (2014) summarized the current state of play by concluding the work done by Spiers et al. (2014) shows that *Bonamia exitiosa* in *S. glomerata* was not responsible for winter mortality, and that the parasite visually observed as *Mikrocytos roughleyi* by Farley et al. (1988) is not a *Bonamia* or *Mikrocytos* species (Carnegie et al. 2014, Spiers et al. 2014). This suggests that *M. roughleyi* is also *nomen dubium*. These microcell-like bodies associated with winter mortality may not even represent a member of the Haplosporidia, and could instead represent a protist from an as yet unidentified group (Spiers et al. 2014). The lack of correlation between the presence of the microcell-like bodies and disease may be explained by the fact that peaks in winter mortality disease coincide with low oyster condition, which are related to low levels of phytoplankton (a major food source) during dry winters (Hand et al. 1998), hence an important role for adverse water quality or other unfavourable environmental factors in the disease syndrome is plausible and cannot be ruled out (Spiers et al. 2014). Furthermore, a correlation of winter mortality disease with reduced water quality is possible given that winter mortality generally affects larger oysters older than 2 years of age (Roughley 1926), which filter greater volumes of water (Hand et al. 1998), and raising the growing height of the oysters reduces mortalities (Smith et al. 2000).

As has been found for infections with other microcell parasites such as *Bonamia ostreae*, selective breeding over many generations may be able to develop strains of cultured *S. glomerata* that are resistant to winter mortality (Dove et al. 2013). In contrast, wild oyster populations will likely be much slower to acquire natural resistance due to genetic dilution by cross breeding with susceptible oysters (Flannery et al. 2014, Morga et al. 2017).

4.20.5 Release assessment

The taxonomic affinities of the enigmatic, microcell-like bodies associated with winter mortality in Sydney rock oysters from southern NSW during the winter months remain unknown. Furthermore, nothing is known about their survival or transmission characteristics, except that reducing the time oysters spend in cold (10-14°C) and high saline (>29 ppt) waters reduces the mortality rate (Smith et al. 2000). However, if these agents are considered novel Mikrocytids, there is evidence from not only *M. mackini*, but also *Mikrocytos mimicus* that they require exposure of the infected oyster to protracted periods of cold weather (below 10°C) for several months prior to disease expression (Hartikainen et al. 2014b). The occasional detection of microcell-like parasites in Australian oysters at low prevalences demonstrates that the full range of infections present in the various species of Australian molluscs remains to be determined. Because of this, there is a non-negligible risk that uncharacterized disease agents including *Mikrocytos*-like disease agents may occur in molluscs that are recycled for shellfish reef restoration in Australia. Unfortunately, the resistance of infective stages of these disease agents to desiccation, freshwater, heat or acetic acid has not been determined. Taking into account information above, and given the likely propagule pressure experienced via the various anthropogenic introduction pathways, the likelihood estimations for the release of the winter mortality disease agent via the identified risk pathways are listed below.

Release assessment for winter mortality (*Mikrocytos roughleyi*)

	Unmitigated	Desiccation			Heat		Salinity	Other
Treatment	None	3 mo.	4 mo.	6 mo.	55°C >10 min	80°C >5 min	0 ppt for >24 hrs	4% acetic acid 30 min
Likelihood of release	High	Neg?	Neg	Neg	Low?	E low?	Low?	V low?

4.20.6 Exposure assessment

While the winter mortality disease agent infects oyster tissue and not shell material, it is likely that shells from wild and /or cultured molluscs that may be procured from processors or retailers for recycling would retain some residual host tissue in areas such as retractor muscle scars, mantle tissue and so on. The mode of transmission of these disease agents is unknown, however it is known that maintaining oysters under the water for longer periods increases the mortality rate (Smith et al. 2000), suggesting that transmission of the disease occurs via the water column. Whether the disease agent transmits directly from oyster-to-oyster via the water is not clear, nor are various epidemiological details such as the minimum infective dose required for successful transmission. Bivalves are efficient filter feeders and therefore are also efficient particle collectors, and can collect spores and infective stages of a wide range of bivalve disease agents (Barber and Ford 1992, Ford et al. 2009). Winter mortality disease mainly affects larger oysters older than 2 years, possibly because they filter larger volumes of water and thus may be exposed to larger quantities of infective stages. At this time the balance of evidence suggests that winter mortality disease is due to infection by the enigmatic, microcell-like bodies, rather than being an environmental problem. As there may be pathways to expose shellfish reef restoration projects to the winter mortality disease agent via recycled mollusc shells, environmental conditions are likely to be suitable for disease transmission in many parts of southern Australia during the winter months, and suitable hosts may occur in the wild in areas where shellfish reefs are being restored, the risk of exposure

and establishment is non-negligible, and the overall likelihood of exposure and establishment of the winter mortality disease agent is considered to be **Moderate**.

4.20.7 Consequence assessment

Although enigmatic *Mikrocytos* -like microcells are already present in populations of wild and cultured molluscs in some regions of Australia, winter mortality disease is presently restricted to southern NSW and it appears that other regions may be free from infection at this time. Sydney rock oysters in northern NSW and QLD are likely to occur in waters too warm for winter mortality to become problematic, however *S. glomerata* also occur in parts of southern WA where water temperatures may decline below 14°C during the winter months while salinities remain above 30 ppt. This suggests that the winter mortality disease agent could be problematic in other parts of southern Australia if it were introduced and established. The winter mortality disease agent is not listed by the OIE or NACA, but is listed as a reportable disease in NSW, Tasmania and WA (Table 3). Hence the introduction of this disease agent into new regions may have significant trade implications, and could result in intervention by government authorities. Such interventions may cause disruption to aquaculture, commercial and recreational mollusc fisheries and shellfish reef restoration if attempts were made to contain the infection and prevent its further spread into uninfected areas. While selective breeding over many generations may be able to develop strains of oysters resistant to winter mortality, it appears likely that this will take several decades to achieve meaningful increases in survival of cultured populations. In contrast, wild oyster populations will likely be even slower to acquire natural resistance due to genetic dilution by cross breeding with susceptible oysters. Taking all of these factors into consideration, the consequences of establishment of the winter mortality disease agent via recycled mollusc shells is considered to be **Moderate.**

4.20.8 Risk estimation

The risk estimation for the winter mortality disease agent is determined by combining the likelihood of release and exposure (from Table 5) with the consequences of establishment (Table 6) to arrive at a risk estimation (Table 7). The risk estimate for the winter mortality disease agent exceeds the ALOP for some release pathways, suggesting that additional risk management is required for these disease agents.

Risk estimate for winter mortality (*Mikrocytos roughleyi*)

	Unmitigated	Desiccation			Heat		Salinity	Other
Treatment	None	3 mo.	4 mo.	6 mo.	55°C >10 min	80°C >5 min	0 ppt for >24 hrs	4% acetic acid 30 min
Combined likelihood of release and exposure	Moderate	Neg?	Neg	Neg	Low?	E low?	Low?	V low?
Consequences of establishment	Moderate	Mod	Mod	Mod	Mod	Mod	Mod	Mod
Risk estimation	Moderate	Neg?	Neg	Neg	Low?	V low?	Low?	V low?

4.21 Boring mussels

4.21.1 Invasive agent: Boring mussels of the genus *Lithophaga*, that penetrate the shell of molluscs forming holes that sometimes breach the nacreous layer (Jones 2007a).

4.21.2 OIE List: No **State Pest Lists:** No **Zoonotic:** No

4.21.3 Australia's status: Boring molluscs *Lithophaga* spp. are common fouling organisms regularly encountered in pearl oyster farming in northern Australia (Humphrey et al. 1998, Jones 2007a).

4.21.4 Epizootiology

Biofouling can be problematic in the aquaculture of marine molluscs (Taylor et al. 1997, Bannister et al. 2019). For example, otherwise healthy populations of larger and older silver lip pearl oysters *Pinctada maxima* farmed in the NT often display severe shell damage due to epiphytic organisms, while younger oysters have less shell damage (Humphrey et al. 1998). Boring bivalve molluscs of the genus *Lithophaga* are common in larger older cultured pearl oysters throughout the Indo-West Pacific (Sims 1993, Doroudi 1996), including in northern Australia (Humphrey et al. 1998) where they have been reported to produce large holes of up to 1-2 cm diametre, sometimes disrupting or breaching the nacreous layer (Jones 2007a). In his study of the borer infestations of pearl oysters in the Persian Gulf, Doroudi (1996) implicated *Lithophaga hanleyana* and *L. malaccana* as the main problematic boring mussel species in that region.

Boring bivalves are a natural component of marine reef ecosystems, where they penetrate into limestone substrates by means of chemical secretions, forming burrows where they may live for up to 50 years or more (Brickner et al. 1993, Galinou-Mitsoudi and Sinis 1995, Kefi et al. 2014). Larvae of *Lithophaga* spp. settle on limestone substrates at a length of > 260 µm and some species can grow to at least 80 mm long (Galinou-Mitsoudi and Sinis 1995), however the majority of *Lithophaga* populations do not survive their first year and thus seldom exceed 4 mm in diametre (Galinou-Mitsoudi and Sinis 1997). Larval settlement can occur at high densities, but only in subtidal areas at certain times of the year (Galinou-Mitsoudi and Sinis 1997, Kefi et al. 2014). Once recruited to a mollusc shell, boring bivalves can penetrate the calcium carbonate layers, creating numerous holes and tracts within the shell matrix, subsequently weakening the shell (Humphrey and Norton 2005). Full thickness penetration of the shell valves by such organisms may result in irregular areas of dark colouration due to brown melanisation reactions from the host, which are often associated with secondary infection by microbial agents, especially bacteria (Diggles et al. 2007). A dynamic interaction exists between boring organisms and the the host mollusc, as damage to the shell matrix is subsequently covered by deposition of nacre, often with brown conchiolin deposits as melanin is excreted to try to wall off invasion by microorganisms during active infections (Humphrey and Norton 2005, Diggles et al. 2007).

Despite destruction of the shell by borers, studies have found that fouling did not affect the survival of l-year-old *Pinctada maxima* and regular removal of fouling at 2-4-week intervals improved growth and reduced shell deformity (Taylor et al. 1997). Their study found that those oysters handled most frequently (cleaned fortnightly) along with those cleaned every 4 weeks, were significantly larger than those handled every 8 weeks or after 16 weeks (Taylor et al. 1997). However, once oysters exceed around 3 years of age, shell borers can begin to damage the shell, and if left uncleaned the shells can be damaged to such an extent that shell

disarticulation can occur if a severe infestation occurs near the shell hinge (B.K. Diggles, personal observation).

4.21.5 Release assessment

Several species of boring mussels occur widely throughout northern Australia, mainly in coral reef areas, including *Lithophaga antillarum, L. divaricalx, L. hanleyana, L. laevigata, L. lessepsiana, L. lima, L. malaccana, L. nasuta, L. obesa*, and *L. teres*, amongst others (see Wilson 1979, Kleemann 1980, 1984, 1995). It is most likely some of these species, particularly those known to inhabit dead coral, also infest the shells of pearl oysters (Doroudi 1996, Humphrey et al. 1998, Jones 2007a). Boring mussels can probably infect virtually any sufficiently large bivalve mollusc that occurs in subtidal areas for prolonged periods, however larval boring mussels only settle subtidally at certain times of the year (Galinou-Mitsoudi and Sinis 1995, 1997, Kefi et al. 2014), and these larvae are unlikely to survive drying in air for several hours as would occur if oysters or mussels occur in intertidal areas. It is thus reasonable to assume that the calcareous shells of rock oysters and other molluscs growing subtidally in many parts of Australia will be exposed to infection by boring mussels and hence it is highly likely that these organisms would occur in shells recycled for shellfish reef restoration in Australia. While the larval stages of *Lithophaga* spp. cannot tolerate desiccation, it is likely that adult mussels will be able to tolerate emersion for longer periods, however the exact length of time they can survive out of the water is unknown. Juveniles of other mussel species (blue mussels *Mytilus* spp. and greenlip mussels *Perna canaliculus*) experienced 100% mortality after 7 days desiccation in direct sunlight and 10-11 days desiccation, respectively in temperature controlled (14.5-18.5°C) rooms (Hopkins et al. 2016).

Similarly, resistance of the life stages of *Lithophaga* spp. to freshwater, heat or acetic acid has also apparently not been determined. However, other mussel species, such as juvenile (<1 year old) blue mussels (*Mytilus edulis*) experienced 33% mortality after exposure to a 90 second dip in 5% acetic acid (vinegar) to treat them for infestations by tunicates (*Styela* spp.) (Swan 2006), and Atalah et al. (2016) recorded 97.5% mortality of juvenile (3 month old) Australian blue mussels (*Mytilus galloprovincialis*) exposed to 4% acetic acid for 1 hour, and 100% mortality after 24 hours exposure. Furthermore, Denny (2008) found that greenlip mussels dipped in 4% acetic acid for only 2 minutes then left out of the water for 24 hours without rinsing, suffered 57-75% mortality. Exposure to seawater at 50°C for 30 seconds and at 60°C 10 seconds also resulted in 100% mortality of *M. galloprovincialis* (see Sievers et al. 2019), however juvenile (16-36 mm shell length) greenlip mussels survived over 48 hours in freshwater at ambient (10-20°C) water temperatures (Forrest and Blakemore 2006). From these data it seems likely that boring mussels (*Lithophaga* spp.) will be susceptible to desiccation, heat and acetic acid exposure. Taking into account the information above, and given the likely propagule pressure experienced via the various anthropogenic introduction pathways, the likelihood estimations for the release of boring mussels via the identified risk pathways are listed below.

Release assessment for boring mussels

	Unmitigated	Desiccation			Heat		Salinity	Other
Treatment	None	3 mo.	4 mo.	6 mo.	55°C >10 min	80°C >5 min	0 ppt for >24 hrs	4% acetic acid 30 min
Likelihood of release	High	Neg	Neg	Neg	Neg	Neg	Mod	E low

4.21.6 Exposure assessment

Molluscs in many parts of Australia are already at risk from natural exposure to boring mussels, however shellfish reef restoration efforts could transport these agents into new regions where their survival and establishment will depend on the availability of suitable hosts and favourable environmental conditions. Infection and establishment of boring mussels in new hosts would occur only if mature *Lithophaga* spp. survived transport and successfully reproduced. *Lithophaga* spp. mature at around 2 years of age and 0.9-1 cm in length, hence if reproductively active *Lithophaga* spp. were to be translocated, the recycled mollusc shells would need to be infested with relatively large, macroscopically visible mussels. Sexes of *Lithophaga* spp. are mostly separate (Galinou-Mitsoudi and Sinis 1994), though a small percentage (c. 1.5%) of hermaphroditic specimens may occur in any given population (Kefi et al. 2014), suggesting that few individual mussels would need to be translocated to establish breeding populations in new areas. Gametogenesis occurs once water temperatures exceed 18-20°C and larvae of boring mussels are released on a seasonal basis in the summer months (Galinou-Mitsoudi and Sinis 1994, Kefi et al. 2014), with the appearance of newly settled individuals occurring around two months after the liberation of larvae into the water column (Galinou-Mitsoudi and Sinis 1997). The *Lithophaga* spp. populations in the Mediterranean and Adriatic Seas persist in highly saline (20-37 ppt) waters throughout a wide range of water temperatures (11.5-35°C) (Kefi et al. 2014), suggesting boring mussels can tolerate a wide range of environmental conditions.

Under natural circumstances, susceptible molluscs would not need to be in close proximity to shell material infected with boring mussels for horizontal transmission to occur, and a single viable planktonic larvae is sufficient to initiate colonization of the host mollusc. As these borers are ectocommensals, the immune system of the host mollusc is likely to play little or no role in modulation of the infection process, hence the main factor controlling boring mussel establishment in new regions will be environmental conditions. Proliferation of boring molluscs will be encouraged in areas near natural coral reefs and whenever shellfish reefs are restored subtidally, however given their relatively slow growth, it may take several years for newly established populations to become reproductively self-sufficient (Galinou-Mitsoudi and Sinis 1995, Kefi et al. 2014). Taking these various factors into consideration, given that boring mussels may be present in recycled mollusc shells, and environmental conditions are likely to be suitable for their transmission in many parts of Australia, the risk of exposure and establishment is non-negligible, and the overall likelihood of exposure and establishment of boring mussels is considered to be **Low**.

4.21.7 Consequence assessment

Boring mussels are already present in many regions of Australia, however the distribution of some species may be restricted to certain areas at this time. There is evidence that boring mussels have been associated with damage to the shells of older pearl oysters cultured subtidally in northern Australia, however they are opportunistic commensals and not primary pathogens in wild and cultured oysters. Boring mussels are not listed by the OIE or NACA as a reportable disease, and they are not listed as a reportable disease in any Australian State (Table 3). Furthermore, control of these agents in cultured molluscs is likely to be relatively straightforward through employment of intertidal culture, and they do not cause problems in oysters that are less than 3 years old, hence they are unlikely to be problematic in rock oyster or mussel culture as the commercial products are likely to reach marketable size within this time period. Their presence would, however, potentially cause marketability issues for cultured molluscs due to shell damage, and the potential

for economic loss due to this must be considered. Considering all of these factors, establishment of boring mussels in new areas would potentially have mild biological consequences, and would likely have insignificant environmental effects for ecosystems and wild mollusc fisheries. Taking all of these factors into consideration, the consequences of introduction of boring mussels via recycled mollusc shells is likely to be **Very low**.

4.21.8 Risk estimation

The risk estimation for boring mussels is determined by combining the likelihood of release and exposure (from Table 5) with the consequences of establishment (Table 6) to arrive at a risk estimation (Table 7). The risk estimate for boring mussels does not exceed the ALOP for any of the release pathways, suggesting that additional risk management is not required for these marine pests.

Risk estimate for infection with boring mussels

	Unmitigated	Desiccation			Heat		Salinity	Other
Treatment	None	3 mo.	4 mo.	6 mo.	55°C >10 min	80°C >5 min	0 ppt for >24 hrs	4% acetic acid 30 min
Combined likelihood of release and exposure	Low	Neg	Neg	Neg	Neg	Neg	Low	E low
Consequences of establishment	Very low	V low	V low	V low	V low	V low	V low	V low
Risk estimation	Negligible	Neg	Neg	Neg	Neg	Neg	Neg	Neg

4.22 Boring sponges

4.22.1 Invasive agent: Boring sponges from the Family *Clionaidae* (Class Demospongiae), including members of the genus *Cliona* and *Pione*, which infect and erode the shell matrix of bivalves.

4.22.2 OIE List: No **State Pest Lists:** Yes (WA) **Zoonotic:** No

4.22.3 Australia's status: Boring sponges of the genus *Cliona* and *Pione* are common biofouling organisms sometimes encountered in culture of Sydney rock oysters (Wesche et al. 1997) along Australia's east coast, and regularly encountered during pearl oyster farming activities throughout northern Australia (Humphrey et al. 1998, Fromont et al. 2005, Jones 2007a).

4.22.4 Epizootiology

Boring sponges of the Family *Clionaidae* are a group of endolithic sponges that colonise limestone surfaces such as live and dead corals, but also the shells of bivalves, including rock oysters (Wesche et al. 1997, Carver et al. 2010, Carroll et al. 2015, Stubler et al. 2017). They are interchangeably referred to as boring, bio eroding, or excavating sponges due to their ability to chemically bioerode limestone and other calcium carbonate substrates (Pomponi 1980). These sponges cause major biofouling problems in bivalve aquaculture (Fitridge et al. 2012, Bannister et al. 2019), especially for the pearl oyster industry in northern Australia, where their presence leads to severe erosion of the shell matrix and premature removal of shells from pearl production (Taylor et al. 1997, de Nys and Ison 2004, Fromont et al. 2005, Jones 2007a, Daume et al. 2010). In the culture of silver-lip pearl oyster *Pinctada maxima*, clionaid boring sponges cause a condition known as "red bum" or "red arse" that may render a shell so fragile that it may collapse during handling and cleaning resulting in shell disarticulation (Fromont et al. 2005, Humphrey and Norton 2005, Southgate and Lucas 2008). These problems due to bioerosion by sponges are chronic, and mainly affect older pearl oysters greater than 3 years of age (Guenther et al. 2006, Daume et al. 2010, Carroll et al. 2015), in which full thickness penetration of the shell valves may result in secondary infection by microbial agents, especially bacteria (Humphrey and Norton 2005). A dynamic interaction exists between boring organisms and the deposition of nacre by the oyster, as damage to the shell matrix is subsequently covered by deposition of nacre, often with brown conchiolin deposits as melanin is deposited to try to wall off invasion by microorganisms during active infections (Humphrey and Norton 2005, Diggles et al. 2007). This increase in shell deposition has a metabolic cost, as the sponge causes the oysters to divert energy into shell maintenance and repair at the expense of somatic growth, which tends to result in reduced growth rate of heavily affected oysters (Fromont et al. 2005, Carroll et al. 2015).

Fromont et al. (2005) surveyed the sponges that excavated shells of *Pinctada maxima* in northern Australia and found the most common species was *Cliona dissimilis*, a khaki-brown coloured tunicate which occurred in 62% of shells examined from pearl oyster farms in WA, with the red coloured *Pione velans* and the yellow-brown *C. orientalis* also present at lower prevalences of 18% and 5%, respectively. *Cliona orientalis* had previously been reported from Australia in living and dead coral on the Great Barrier Reef, but it was subsequently found in *P. maxima* from the NT and WA (Fromont et al. 2005). The presence of clionaid sponges in pearl oyster culture is problematic because these oysters are held for over 3 years subtidally for

pearl production, which provides sufficient time for chronic bioerosion by sponges to adversely affect shell structure and nacre production, resulting in the need for regular labour intensive shell cleaning in order to prevent loss of the oldest oysters that carry the largest, most valuable pearls (deNys and Ison 2004, Guenther et al. 2006, Daume et al. 2010). Indeed, control of biofouling is estimated to cost between 25- 30% of the operating costs of a pearl farm in northern Australia (deNys and Ison 2004).

Wesche et al. (1997) was the first to record clionaid sponges from the shells of rock oysters in Australia when they described *Cliona vastifica* and *C. celata* in 12-18 month old cultured Sydney rock oysters *Saccostrea glomerata* from the Pimpama River, Moreton Bay. These clionaids occurred at prevalences of around 6% in live oysters, but prevalence increased to 43.6% in dead shells, with infection by the sponges usually greatest in the left valve of the oyster (cup) and correlating with prolonged immersion periods, with oysters grown at mid-intertidal levels being free from infection (Wesche et al. 1997). The effect of clionaid boring sponges on the health of *S. glomerata* and other oyster species is otherwise unknown, but is likely to be similar to that recorded in pearl oysters, as suggested by data from overseas which found that eastern oysters (*Crassostrea virginica*) in the USA colonized by *Cliona celata* exhibited significantly slower growth rates relative to uncolonized oysters, but with otherwise equivocal impacts on oyster condition and survival (Carroll et al. 2015). Furthermore, the adverse effects on rock oyster growth were again only observed in larger, older *C. virginica* greater than 70-80 mm shell length (Carroll et al. 2015).

Treatment and prevention of clionaid sponge infestations involves either moving shells to trays in mid/upper intertidal locations (Wesche et al. 1997), or in the case of subtidal pearl oyster culture, regular cleaning/defouling of the shell is required either manually by mechanical scrubbing or by using a high-pressure hose, while there has also been some success with freshwater baths for 30–60 min, and application of antifouling paints such as "PearlSafe®" (de Nys and Ison 2004, Jones 2007a). PearlSafe® is a wax-based emulsion that creates an impervious layer, effectively smothering and killing the boring sponge within 4 weeks of application, and preventing its regrowth (Southgate and Lucas 2008). Other successful treatments have included hypersaline dips in 90% brine for 6 minutes, which have been proven to reduce prevalence of clionaid infestations by an order of magnitude and improve oyster survival and growth (Carver et al. 2010), while acetic acid dips were effective, but resulted in oyster mortality (Carver et al. 2010).

4.22.5 Release assessment

Clionaid sponges occur widely throughout northern Australia, and indeed the cosmopolitan distributions of some species of *Cliona* are possibly a consequence of artificial transport of molluscs as biofouling on shipping and/or in ballast water (Wesche et al. 1997). Boring sponges release their gametes during the late summer months (Carver et al. 2010) and the calcareous shell of any sufficiently large mollusc that occurs in subtidal areas for prolonged periods must be considered potentially susceptible to infection. It is thus reasonable to assume that the shells of molluscs growing subtidally in many parts of Australia will be naturally exposed to infection by boring sponges, and hence it is highly likely that these organisms would occur in shells recycled for shellfish reef restoration in Australia.

It is known that boring sponge propagules (which are ciliated planktonic larvae) do not survive drying in air for several hours, as occurs if molluscs are cultured in intertidal areas (Wesche et al. 1997). While the planktonic propagules of *Cliona* cannot tolerate desiccation, mature sponges within the shell matrix are likely

to be able to tolerate desiccation for longer periods, however the exact length of time they can survive out of the water is unknown. Carver et al. (2010) examined the effectiveness of acetic acid against the boring sponge *Cliona celata* on the shell of adult eastern oysters (*Crassostrea virginica*) and found that while >90% of the boring sponges could be eliminated by exposure to 10% acetic acid for 10 min, even the most dilute treatment (a 30 second dip in 5% acetic acid) resulted in 56% mortality of treated oysters. Miller et al. (2010) found that *C. celata* had a high tolerance to salinity and heat stress between the ranges of 18-33°C and 22-42 ppt salinity, however it appears that the resistance of various life stages of *Cliona* spp. to higher temperatures or freshwater have not been determined. Taking into account the information above, and given the likely propagule pressure experienced via the various anthropogenic introduction pathways, the likelihood estimations for the release of boring sponges via the identified risk pathways are listed below.

Release assessment for boring sponges

	Unmitigated	Desiccation			Heat		Salinity	Other
Treatment	None	3 mo.	4 mo.	6 mo.	55°C >10 min	80°C >5 min	0 ppt for >24 hrs	4% acetic acid 30 min
Likelihood of release	High	E low?	Neg	Neg	Low?	E low?	V low?	E low

4.22.6 Exposure assessment

Molluscs in many parts of Australia are already at risk from natural exposure to boring sponges, however, shellfish reef restoration efforts could transport these agents into new regions where their survival and establishment will depend on the availability of suitable hosts and favourable environmental conditions. Boring sponges can reproduce sexually or asexually by fragmentation (Carver et al. 2010), suggesting that few infected mussels would need to be translocated to establish breeding populations in new areas. It is known that oyster-to-oyster contact may result in horizontal transfer of boring sponges (Warburton 1958). It is also possible that infection and establishment of boring sponges in new hosts can occur via the water column when sponges reproduce sexually, which can occur during mass spawning events during the summer months (Mariani et al. 2000). Once *Cliona* spp. release their gametes, the larval stages are weak swimmers (Mariani et al. 2000) but may remain planktonic for 2 days prior to settlement (Warburton 1966), after which juvenile sponges develop 10 -15 days later (Mariani et al. 2000). This means susceptible molluscs do not need to be in close proximity to oyster shells infected with boring sponges for horizontal transmission to occur, and a single viable gamete is likely to be sufficient to initiate colonization of the host mollusc (Warburton 1966). As these borers are ectocommensals, the immune system of the host is likely to play little or no role in modulation of the infection process, hence the main factors controlling infection intensity (and therefore whether disease occurs at all), will be environmental conditions and the age of the oysters. Proliferation of boring sponges will be encouraged in any areas near natural rocky or coral reefs and whenever oysters or other molluscs occur subtidally for long time periods, but transmission during mass spawning events only occurs at specific periods during summer. Taking these various factors into consideration, given that boring sponges may be present in recycled mollusc shells, and environmental conditions are likely to be suitable for their transmission in many parts of Australia, the risk of exposure and establishment is non-negligible, and the overall likelihood of exposure and establishment of boring mussels is considered to be **Low**.

4.22.7 Consequence assessment

Boring sponges are already present in many regions of Australia, however the distribution of some species may be restricted to certain areas at this time. There is evidence that boring sponges have been associated with damage to the shells of pearl oysters and older Sydney rock oysters cultured subtidally in northern Australia, however they are opportunistic commensals and not primary pathogens in wild and cultured oysters. Boring sponges are not listed by the OIE or NACA as reportable diseases, however they are a reportable pest in WA (Table 3). Because of this, the spread of boring sponges into new areas in WA may result in intervention by government authorities and cause disruption to aquaculture, commercial and recreational mollusc fisheries and shellfish reef restoration if attempts were made to contain the infection and prevent its further spread into uninfected areas. Fortunately, control of these agents in cultured molluscs is relatively straightforward through employment of intertidal culture, and given they only cause problems in molluscs greater than 3 years old, they are unlikely to be problematic in rock oyster or mussel culture as the commercial products generally reach a marketable size within that time period. Their presence could, however, cause issues in pearl oyster culture and could also potentially cause marketability issues for other cultured molluscs due to shell damage. Considering all of these factors, establishment of boring sponges in new areas would potentially have relatively mild biological consequences, and would likely have insignificant environmental effects for ecosystems and wild mollusc fisheries. Taking all of these factors into consideration, the consequences of introduction of boring sponges via recycled mollusc shells is likely to be **Very low**.

4.22.8 Risk estimation

The risk estimation for boring sponges is determined by combining the likelihood of release and exposure (from Table 5) with the consequences of establishment (Table 6) to arrive at a risk estimation (Table 7). The risk estimate for boring sponges does not exceed the ALOP for any release pathways, suggesting that additional risk management is not required for these marine pests.

Risk estimate for boring sponges

	Unmitigated	Desiccation			Heat		Salinity	Other
Treatment	None	3 mo.	4 mo.	6 mo.	55°C >10 min	80°C >5 min	0 ppt for >24 hrs	4% acetic acid 30 min
Combined likelihood of release and exposure	Low	E low?	Neg	Neg	Low?	E low?	V low?	E low
Consequences of establishment	Very Low	V low	V low	V low	V low	V low	V low	V low
Risk estimation	Negligible	Neg?	Neg	Neg	Neg?	Neg?	Neg?	Neg

4.23 Invasive barnacles

4.23.1 Invasive agent: Barnacles are crustaceans classified within the Subclass Cirripedia, adults of which are usually sessile attached to hard substrates or other organisms. Species of barnacles that are regarded as problematic and invasive such that they are listed as unwanted pests in Australia include *Balanus* (*Amphibalanus*) *improvisus* (bay barnacle), *B. eburneus* (ivory barnacle), *B. glandula* (common acorn barnacle), and *Chthamalus proteus* (Atlantic barnacle).

4.23.2 OIE List: No **State Pest Lists:** Yes (QLD, NSW, SA, WA, NT) **Zoonotic:** No

4.23.3 Australia's status: *Balanus improvisus* is considered to be established in southern WA since the 1950s, however the other species listed above are considered exotic to Australia (DAWR 2019).

4.23.4 Epizootiology

Barnacles are crustaceans that, as adults, are usually sessile attached to hard substrata or to other organisms inside a tough calcareous shell. They are prolific biofouling organisms, producing large numbers of planktonic larvae which are capable of settling on and adhering to most types of hard substrate in subtidal areas (Allen 1953). Because of their hardiness and ability to tolerate a wide range of environmental conditions, barnacles have become one of the most common and well known groups of invasive organisms that have been spread to many regions worldwide as biofouling or in ballast water via international and domestic shipping (Allen 1953, Murphy and Paini 2010, Carlton et al. 2011, Kauano et al. 2017). For example, the bay barnacle (*Balanus improvisus*), originally from the north Atlantic, is known to be amongst the first exotic pest introductions into North America, becoming established in Ecuador before the 1850's (Darwin 1854) and being detected along the Pacific coast of north America only a few years after the start (1849) of the "gold rush" that brought hundreds of ships from the Atlantic Ocean (and elsewhere) to California (Carlton et al. 2011). Bay barnacles were also one of the first invasive pests known to be translocated with oysters, when *A. improvisus* was introduced to the Pacific coast of North America with commercial oyster shipments from the Atlantic seaboard sometime after 1869 (Carlton et al. 2011). Other species of barnacles known to be translocated with oysters include *Fistulobalanus albicostatus* which was introduced into California on a regular basis since 1930 in shipments of Pacific oysters (*Crassostrea gigas*) (Carlton et al. 2011).

In Australia, exotic barnacles from the northern hemisphere are commonly recorded on the hulls of ships that have returned from operations in the northern Pacific and Atlantic Oceans (Allen 1953). However, due to the difficulty in identifying and distinguishing some of the exotic species from native barnacles, incursions are often well established before they are detected (Hayes et al. 2005, Murphy and Paini 2010, DAWR 2019). At least nine species of exotic barnacles are now considered established in Australia, including *Balanus amphitrite*, *B. albicostatus*, *B. trigonus*, *B. reticulatus*, *Megabalanus rosa*, *M. tintinnabulum*, *Notomegabalanus algicola* and *Lepas* (*Anatifa*) *anserifera* (see Hayes et al. 2005, DAWR 2019). The listed *B. improvisus* is native to the north Atlantic, but has been spread worldwide, dominating and outcompeting native invertebrate species for food and space throughout Europe, North and South America, the west coast of Africa and New Zealand (Murphy and Paini 2010), the latter country where it was initially observed on an oil

platform which had been transported from Japan (Forster and Willan 1979). This species was reported in southern WA during the 1940s (Bishop 1951), and its presence in Australia was uncertain (Allen 1953), but it is now considered established (Naser et al. 2015, DAWR 2019).

While there are several species of invasive barnacle of concern (see 4.23.1), *Balanus improvisus* will be used as a model example of an invasive barnacle because it is extremely hardy and known to occur on oyster shells. This species can tolerate a wide range of temperatures (-2 to 35°C), water depths (up to 160 metres), oxygen levels (down to 1 mg/L) and salinities (0-44 ppt), although 6-30 ppt is optimal and it can survive (but not reproduce) in freshwater (Murphy and Paini 2010, Carlton et al. 2011). Adult *B. improvisus* can settle at high densities (up to 4370 individuals/m^2) and are notorious for fouling most hard surfaces including clogging intake pipes and heat exchangers, imposing significant costs to shipping and fish farming, as well as industries such as power plants for which cooling water from the sea is used (Murphy and Paini 2010 and references cited therein). The duration of the pelagic larval stages prior to settlement is up to 48 days at 10ºC, but less than 9 days at 22°C, hence *B. improvisus* is likely to be able to be translocated via ballast water (Murphy and Paini 2010). Adult *B. improvisus* can live for up to 4 years and is a common epibiont on oyster shells (Murphy and Paini 2010) and has also been recorded on the carapace of invasive crabs in the Persian Gulf (Naser et al. 2015).

4.23.5 Release assessment

Various species of native barnacles occur throughout Australia, and the calcareous shells of molluscs grown in intertidal and subtidal areas will be exposed to colonization by these natural epibionts. However, several exotic species of barnacles are also now established in Australia, including *B. improvisus* that occurs in southern WA and which is listed as an unwanted marine pest in some states (Hayes et al. 2005, DAWR 2019). Barnacles release their larvae during the warmer months and the calcareous shell of any sufficiently large mollusc that occurs in intertidal or subtidal areas for prolonged periods must be considered potentially susceptible to colonisation. It is thus reasonable to assume that the shells of molluscs growing subtidally in many parts of Australia will be exposed to barnacles, and it is certain that these organisms occur in shells recycled for shellfish reef restoration in Australia (B.K. Diggles, personal observations).

While some barnacle species, including *B. improvisus*, and *Balanus glandula/crenatus* can tolerate freshwater for periods of several days to more than a week (Brock et al. 1999), it is known that barnacles are susceptible to heat, and desiccation. For example, Sasikumar et al. (1992) found that adult handbell barnacles (*Megabalanus tintinnabulum*) 5-30 mm diametre experienced 100% mortality after being exposed to hot water (37°C for 200 minutes, 40°C for 156 minutes, or 47°C for 10 minutes). Barnacles are, however, more robust compared to most other biofouling species, with Piola and Hopkins (2012) finding that barnacles native to New Zealand and Australia (*Elminius modestus*, *E. plicata*) exhibited 100% survival after 1 hour at 37.5°C, a temperature that killed nearly all other species of biofouling examined. Instead, exposure to a temperature of 42.5°C for 20 minutes was required to kill 100% of *E. modestus* and *E. plicata* (see Piola and Hopkins 2012). Barnacles are also known to be very tolerant of desiccation due to the ability of their outer calcareous plates to retain their internal moisture for long periods (Fitridge et al. 2012). Kauano et al. (2017) found that around 22% and 50%, respectively, of *Balanus improvisus* and *Balanus amphitrite* survived after 96 hours emersion, while Foster (1971) found that 8% of a population of the common rock barnacle *Balanus balanoides* survived 258 hours desiccation (10.75 days) at 10°C. The limit of survival for barnacles exposed to desiccation is likely

to depend on their size as well as ambient temperature and humidity (Foster 1971, Hummel et al. 1988, Hopkins et al. 2016), but when held at ambient temperatures (10-20°C) it appears to be unlikely to be much longer than the 13 days reported by Carlton et al. (2011) regarding observations that acorn barnacles (*Balanus glandula*) which survived transport from an oyster harvest in Puget Sound until re-submergence in seawater in a laboratory in Connecticut nearly 2 weeks later.

There appears to be little information available on the susceptibility of barnacles to acetic acid. Growcott et al. (2016) noted that the most resilient biofouling groups to in-water chemical treatments are shelled organisms (e.g. mussels and barnacles), which can close their shell and stop feeding for long periods (up to several weeks, see Miossec et al. 2009) to prevent uptake of the chemical and therefore limit soft tissue damage. This suggests that barnacles may be resistant to short term dips in acetic acid. Taking into account the likely propagule pressure experienced via the various anthropogenic introduction pathways, the likelihood estimations for the release of invasive barnacles via the identified risk pathways are listed below.

Release assessment for invasive barnacles

	Unmitigated	Desiccation			Heat		Salinity	Other
Treatment	None	3 mo.	4 mo.	6 mo.	55°C >10 min	80°C >5 min	0 ppt for >24 hrs	4% acetic acid 30 min
Likelihood of release	High	Neg	Neg	Neg	V low	Neg	High	V low

4.23.6 Exposure assessment

Molluscs in all areas of Australia are already at risk from natural exposure to native and introduced barnacles, however, shellfish reef restoration efforts could transport these agents into new regions where their survival and establishment will depend on the availability of suitable hosts and favourable environmental conditions. Most barnacles are cross fertilizing hermaphrodites (Darwin 1854), suggesting that few individuals need to be translocated to establish breeding populations in new areas. Once fertilised by themselves or adjacent barnacles, the fertilized eggs are brooded in the mantle cavity before nauplius larvae hatch out and are released into the water column where they swim, feed and undergo a series of molts over a period of days or weeks (duration depending on water temperature) before metamorphosing into cyprid larvae that settle onto suitable substrates, using its first antennae and various chemical cues to find a suitable place to attach (Anderson 1994). The cyprid larvae of most species are especially attracted by the presence of attached adults of their own kind, and once settled they attach using cement glands then metamorphose to become juvenile barnacles (Anderson 1994). The effective larval dispersal period means susceptible molluscs do not need to be in close proximity to oyster shells infected with barnacles for horizontal transmission to occur, and a single viable cyprid larvae is likely to be sufficient to initiate colonization of the host mollusc. As barnacles are ectocommensals, the immune system of the host is likely to play little or no role in modulation of the colonisation process, hence the main factors determining colonization will be environmental conditions. Proliferation of barnacles will be encouraged in any areas where hard settlement substrates are available, including whenever oysters or other molluscs occur subtidally. Taking these various factors into consideration, given that barnacles may be present in recycled mollusc shells, and environmental conditions

are likely to be suitable for their transmission throughout Australia, the risk of exposure and establishment is non-negligible, and the overall likelihood of exposure and establishment of barnacles is considered to be **High**.

4.24.7 Consequence assessment

Both native and introduced barnacles are already present in many regions of Australia, however the distribution of some invasive species, such as *Balanus* (*Amphibalanus*) *improvisus* is currently restricted to certain areas at this time. Invasive barnacles such as *B. improvisus* can have a range of detrimental effects on the ecology of receiving ecosystems, including outcompeting native benthos for space and food, fouling of the hulls of watercraft or other underwater equipment, clogging intake pipes and heat exchangers of power plants, and imposing significant costs to shipping and fish farming (Murphy and Paini 2010 and references cited therein). A variety of barnacle species are listed as unwanted pests in QLD, NSW, SA, WA and the NT (Table 3). However, because they require specialist expertise to identify, once they are introduced into a new area, barnacle incursions are often well established before they are detected (Hayes et al. 2005, Murphy and Paini 2010, DAWR 2019), meaning there is usually little chance of eradication. Considering all of these factors, establishment of invasive barnacles in new areas would potentially have relatively mild to moderate biological consequences for ecosystems, but may cause considerable nuisance to marine infrastructure, fisheries and aquaculture industries. Taking all of these factors into consideration, the consequences of introduction of invasive barnacles via recycled mollusc shells is likely to be **Low**.

4.23.8 Risk estimation

The risk estimation for invasive barnacles is determined by combining the likelihood of release and exposure (from Table 5) with the consequences of establishment (Table 6) to arrive at a risk estimation (Table 7). The risk estimate for invasive barnacles exceeds the ALOP for some release pathways, suggesting that additional risk management is required for these marine pests.

Risk estimate for invasive barnacles

	Unmitigated	Desiccation			Heat		Salinity	Other
Treatment	None	3 mo.	4 mo.	6 mo.	55°C >10 min	80°C >5 min	0 ppt for >24 hrs	4% acetic acid 30 min
Combined likelihood of release and exposure	High	Neg	Neg	Neg	V low	Neg	High	V low
Consequences of establishment	Low	Low	Low	Low	Low	Low	Low	Low
Risk estimation	Low	Neg	Neg	Neg	Neg	Neg	Low	Neg

4.24 Invasive ctenophores (comb jellies)

4.24.1 Invasive agent: Comb jellies (also known as ctenophores) are a group of gelatinous invertebrates classified within the Phylum Ctenophora, which differ from jellyfish and hydroids (Phylum Cnidaria) in that they do not possess stinging cells called nematocysts. Species of comb jellies that are problematic and invasive such that they are listed as unwanted pests in Australia include *Beroe ovata* and *Mnemiopsis leidyi*.

4.24.2 OIE List: No **State Pest Lists:** Yes (QLD, NSW, SA, WA, NT) **Zoonotic:** No

4.24.3 Australia's status: Not recorded. *Beroe ovata* and *Mnemiopsis leidyi* are not known to occur in Australian waters, and are considered exotic (DAWR 2019).

4.24.4 Epizootiology

In the late 1980's and early 1990's the pelagic component of the Black Sea ecosystem experienced a massive bloom of the ctenophore *Mnemiopsis leidyi*, a few years after it was accidentally introduced (probably via ballast water) in the early 1980's (Neirmann 2004). Unlike most other gelatinous invertebrates, *Mnemiopsis leidyi* is a macrophagic medusae, which grows to around 90 mm in diametre and is capable of predating on objects up to 1 cm long (Kideys et al. 2005). All forms of zooplankton are natural food items including copepods, cladocerans, larvae of crustaceans and bivalves and ichthyoplankton (fish eggs and larvae) (Purcell et al. 2001, Kideys et al. 2005). Like other ctenophores, *M. leidyi* feeds superfluously - even when it is full it continues to capture prey, regurgitating large quantities of undigested plankton in a bolus of mucus (GESAMP 1997). Thus, its deleterious impact on zooplankton and micronekton greatly exceeds its ability to assimilate captured prey (GESAMP 1997).

This species inhabits surface waters from 0-25 metres depth above seasonal thermoclines in waters ranging between 1 and 32ºC and 3.4 to 70 ppt salinity (Purcell et al. 2001, Murphy and Paini 2010). The natural range of *M. leidyi* extends throughout the estuaries and inshore waters of the western Atlantic along the coasts of North and South America, where it is known to impart a significant trophodynamic effect on zooplankton (Purcell et al. 2001) and carbon transfer within coastal ecosystems (Condon and Steinberg 2008). For example, in parts of its native range like Chesapeake Bay, during the summer months a *M. leidyi* density of 12 adult ctenophores per cubic metre can remove 23-32% of the zooplankton stock per day, including 30–100% of fish eggs per day, but < 5% of the fish larvae (Purcell et al. 2001). In parts of its invasive range, *M. leidyi* populations have been found to filter copepods from 5% to 80% of the water column daily, greatly reducing the food available for larval and juvenile fishes (Riisgård et al. (2007).

After its introduction and spread throughout the Black Sea, *M. leidyi* invaded the Azov, Marmara, and eastern Mediterranean Seas, and in 1999 it appeared for the first time in the Caspian Sea (Purcell et al. 2001). Its invasion into these systems in the absence of its natural predators (which include other ctenophores and jellyfish) coincided with drastic ecological changes in the zooplankton community and severe declines in fish populations, particularly Black Sea anchovy (*Engraulis encrasicolus*), European sprat (*Sprattus sprattus*), Mediterranean horse mackerel (*Trachurus mediterraneus*) and other small pelagic fisheries (Neirmann 2004, Kideys et al. 2005). These fisheries declines have most often been attributed to the invasion of *Mnemiopsis leidyi*, which competes directly with zooplanktivorous fishes for food, and which also eats fish eggs and larvae

(Purcell et al. 2001). However, as pointed out by Neirmann (2004), the bloom of *M. leidyi* in the Black Sea may have been facilitated by heavy fishing that reduced the anchovy biomass to the point where the zooplankton biomass normally consumed by the small pelagic fishes became available for exploitation by *Mnemiopsis leidyi*. This, combined with pollution and eutrophication of the water body and the hermaphroditic reproduction mode of *M. leidyi* (which allowed it to outcompete endemic jellyfish such as *Aurelia aurita*) is probably what led to the enormous bloom of the ctenophore during 1988-89 (Neirmann 2004, Kideys et al. 2005). Once established, *M. leidyi* outcompeted fishes and endemic jellyfish for zooplankton resources until 1992, reaching peak biomasses of up to 2000 grams wet weight (c. >3000 individuals) per cubic metre in 1989 in the Black Sea (Kideys et al. 2005) . At its zenith, the invasive *M. leidyi* blooms in the Black Sea and elsewhere reached a total biomass of c. 900 million tons (comparable to 10 times the world's annual fish harvest, see Murphy and Paini 2010). Populations of small pelagic fishes eventually increased again, probably because of reduced fishing pressure in subsequent years, allowing some control over the *M. leidyi* population because fish are more successful in competing for the same zooplankton food sources, due to their speed and activity (Neirmann 2004).

The *M. leidyi* bloom in the Black Sea was eventually bought under control by the unintentional introduction and establishment around October 1997 of one of its natural predators, another larger ctenophore *Beroe ovata* (see Purcell et al. 2001, Kideys et al. 2005). *Beroe ovata* is also native to the western Atlantic, where it feeds almost exclusively on other ctenophores and imparts control over *M. leidyi* populations wherever they co-occur (GESAMP 1997, Kideys et al. 2005, Bat et al. 2007). Shiganova et al. (2001) found that the newly invasive population of *B. ovata* in the Black Sea ingested up to 10% of the *M. leidyi* population each day, resulting in a 5-fold increase in abundance of zooplankton and 20-fold increase in ichthyoplankton populations. Mutlu (2009) noted that the biomasses and abundances of not only *M. leidyi*, but also native jellyfish *Aurelia aurita* and *Pleurobrachia pileus*, were all substantially reduced following introduction of *Beroe ovata* in the Black Sea. The introduction of *Mnemiopsis leidyi* into regions other than the Black Sea has also resulted in substantial ecological changes. For example, Roohi et al. (2008) found negative impacts of *M. leidyi* on zooplankton communities in the Southern Caspian Sea. Zooplankton abundance decreased 2-5 fold, while zooplankton species diversity was halved following invasion compared to pre-invasion baseline samples. Cladocerans were most adversely affected, with only 1 of 24 species previously reported subsequently present. Riisgård et al. (2007) monitored predation effects of *M. leidyi* after its introduction into the coastal waters of the Netherlands and Denmark during the summer of 2006. They found *M. leidyi* populations at some locations were high (up to 300 grams wet weight (c. 800 individuals 5-15 mm diametre) per cubic metre), representing a very high ctenophore biomass that caused a collapse in zooplankton populations (Riisgård et al. 2007). When *M. leidyi* invaded the Baltic Sea in 2006, widespread predation of copepods and larvae of barnacles and jellyfish (*Aurelia aurita*) was noted (Javidpour et al. 2009a), together with rapid increases in its population size, sometimes exceeding an order of magnitude growth in less than a week during late summer (Javidpour et al. 2009b).

Ctenophores including the genera *Mnemiopsis* and *Beroe* are simultaneous hermaphrodites with direct development (GESAMP 1997). In its natural range, spawning in *M. leidyi* occurs at night between water temperatures of 11-29°C and salinities 6-30 ppt, usually peaking during the summer months at temperatures between 19-23°C after which the spherical larvae hatch in 20–24 hours (Purcell et al. 2001). Growth is rapid and when food availability is not limited, *M. leidyi* population biomass doubles daily, approaches the doubling time of phytoplankton (Purcell et al. 2001, Kideys et al. 2005), while *B. ovata* populations can double in less than 10 days (Kideys et al. 2004). Maturity in *M. leidyi* occurs in around 3 weeks after which many thousands

of eggs (up to 14,000 per day) may be produced (Purcell et al. 2001). This combination of high feeding, growth and reproduction rates enables *M. leidyi* populations to bloom under favourable conditions during the summer months (Purcell et al. 2001). The *M. leidyi* population biomass tends to increase more quickly after warmer winters when the average size of overwintering medusae is larger, and conversely, spring ctenophore population size is low after cold winters (Purcell et al. 2001).

4.24.5 Release assessment

Both *Mnemiopsis leidyi* and *Beroe ovata* have not been recorded from Australia and are considered exotic, however, they are included in this risk analysis as they are listed as unwanted marine pests in some states (Hayes et al. 2005, DAWR 2019, Table 3). Various species of native ctenophores occur throughout Australia, and whilst they are not normally associated with molluscs it is possible that live molluscs collected from the wild and from aquaculture leases could come in contact with larval or adult ctenophores at various stages of their life history in the water column, and thus they could be gathered together during mollusc harvesting. It appears that *M. leidyi* can naturally tolerate salinities less than 2 ppt (Purcell et al. 2001), and hence could potentially survive in freshwater for short periods of time. In contrast, *B. ovata* appears slightly less tolerant of low salinities, disintegrating when salinity was reduced to 3-4 ppt (Shiganova et al. 2001). Ctenophores and other gelatinous invertebrates do not tolerate desiccation, are unlikely to tolerate heat (though exact upper heat tolerances have apparently not been published), and are sensitive to acetic acid as shown by the quick disintegration of *M. leidyi* in 1% acidic Lugols solution containing around 0.1% acetic acid (Engell-Sorensen et al. 2009). Taking into account the likely propagule pressure experienced via the various anthropogenic introduction pathways, the likelihood estimations for the release of invasive ctenophores via the identified risk pathways are listed below.

Release assessment for invasive ctenophores (comb jellies)

	Unmitigated	Desiccation			Heat		Salinity	Other
Treatment	None	3 mo.	4 mo.	6 mo.	55°C >10 min	80°C >5 min	0 ppt for >24 hrs	4% acetic acid 30 min
Likelihood of release	Very low	Neg	Neg	Neg	E low?	Neg	E low	E low?

4.24.6 Exposure assessment

All areas of Australia are already at risk from natural exposure to native ctenophores, however, shellfish reef restoration efforts could transport ctenophores into new regions where their survival and establishment will depend on the availability of favourable environmental conditions. Ctenophores like *Mnemiopsis* and *Beroe* are simultaneous hermaphrodites, which means that in theory, a single animal could successfully invade and establish a population in a new area (GESAMP 1997). It is this attribute, together with their ability to reproduce over a wide range of environmental conditions, that makes these organisms well-suited for translocation via ballast water (GESAMP 1997). However, as ctenophores are free living within the water column, they are not usually associated with oysters or other benthic molluscs, though they could be gathered together during mollusc harvesting. Furthermore, they are unlikely to survive long periods out of the water during handling and transport of mollusc shells. Taking these various factors into consideration, given that ctenophores could be present with recycled mollusc shells, and environmental conditions are likely to be

suitable for their transmission throughout Australia, the risk of exposure and establishment is non-negligible, and the overall likelihood of exposure and establishment of ctenophores is considered to be **Very low**.

4.23.7 Consequence assessment

Introduction of *Mnemiopsis leidyi* into coastal ecosystems outside its natural range where it is released from its natural predators has resulted in cascading trophic affects that have altered entire ecosystems, including significant reductions in zooplankton, ichthyoplankton and zooplanktivorous fish (Murphy and Paini 2010). This ctenophore also outcompetes native jellyfish species and can result in local extinction of some native zooplankton species such as copepods which are important food source for fish larvae, resulting in collapse of fisheries. Introduction of *Beroe ovata* into ecosystems affected by *M. leidyi* has been proposed as the only plausible solution for areas where the latter has become established (Kideys et al. 2005). However, such biocontrols are likely to have unwanted side effects due to increased predation pressure on native ctenophores and jellyfish (Mutlu 2009), as well as other impacts as they act as biological pollutants which restructure the lower trophic levels of affected ecosystems (Shiganova et al. 2019). Furthermore, high numbers of gelatinous invertebrates can clog fishing nets, contaminate fish catches, damage fish in sea cage aquaculture, and block the pumps and filters of coastal power and desalination plants (Purcell et al. 2007, Gibbons et al. 2016). Both *M. leidyi* and *B. ovata* are listed as unwanted pests in QLD, NSW, SA, WA and the NT (Table 3), however once they are introduced into a new area, there is virtually no chance of eradication (DAWR 2019). Considering all of these factors, establishment of invasive ctenophores into new areas would potentially have major irreversible biological consequences for ecosystems, and would cause considerable economic damage to marine infrastructure, fisheries and aquaculture industries. Taking all of these factors into consideration, the consequences of introduction of invasive ctenophores via recycled mollusc shells is likely to be **High**.

4.24.8 Risk estimation

The risk estimation for invasive ctenophores is determined by combining the likelihood of release and exposure (from Table 5) with the consequences of establishment (Table 6) to arrive at a risk estimation (Table 7). The risk estimate for invasive ctenophores does not exceed the ALOP for any of the release pathways, suggesting that additional risk management is not required for these marine pests.

Risk estimate for invasive ctenophores (comb jellies)

	Unmitigated	Desiccation			Heat		Salinity	Other
Treatment	None	3 mo.	4 mo.	6 mo.	55°C >10 min	80°C >5 min	0 ppt for >24 hrs	4% acetic acid 30 min
Combined likelihood of release and exposure	Extremely Low	Neg	Neg	Neg	E low?	Neg	E low	E low?
Consequences of establishment	High	High	High	High	High	High	High	High
Risk estimation	Very low	Neg	Neg	Neg	V low?	Neg	V low	V low?

4.25 Invasive crabs

4.25.1 Invasive agent: Crabs are invertebrates classified in the Phylum Arthropoda, Subphylum Crustacea within the Order Decapoda. Species of crabs that are problematic and invasive such that they are listed as unwanted pests in Australia include *Callinectes sapidus*, *Carcinus maenas*, *Charybdis japonica*, *Eriocheir sinensis*, *Hemigrapsus penicillatus*, *H. sanguineus*, *H. takanoi*, *Pachygrapsus fakaravensis*, *Petrolisthes elongatus*, and *Rhithropanopeus harrisii*. Also listed is a horseshoe crab (*Carcinoscorpius rotundicauda*) which is another type of marine arthropod classified within the Order Xiphosura, Family *Limulidae*.

4.25.2 OIE List: No **State Pest Lists:** Yes (all states) **Zoonotic:** No

4.25.3 Australia's status: *Carcinus maenas* (European shore crab) is considered an established marine pest of national significance to Australia. It was introduced before 1900 and is now established in the temperate waters of New South Wales, South Australia, Tasmania and Victoria, and was also reported once from WA (Thresher et al. 2003). *Petrolisthes elongatus* (New Zealand half crab) is established in Tasmania, where it was introduced from New Zealand with live flat oysters (*Ostrea chilensis*) or dry ballast rocks in the late 1800s (King 1997, Gregory et al. 2012).

4.25.4 Epizootiology

Crabs within the Order Decapoda include many highly adaptable and robust species which, with the advent of international shipping and the globalisation of trade, have proven to be some of the worlds most effective invasive marine organisms (Roche and Torchin 2007, Murphy and Paini 2010, Handfling et al. 2011, Forsstrom et al. 2015). International translocations of several of the most highly invasive crab species are likely to have occurred by the 18th and early 19th centuries, initially via hull fouling and dry ballast in shipping (Ojaveer et al. 2018). Crab invasions are also likely to have been facilitated by movements of commodities including oysters (Roche and Torchin 2007, Gregory et al. 2012), marine debris, and more recently since the late 19th century, as larvae in ship ballast water (Handfling et al. 2011, Briski et al. 2012, Ojaveer et al. 2018). In many cases, subsequent studies have shown that these invasive crab species are usually generalists which easily adapt to new environments, where they can be an important restructuring force in marine communities by outcompeting native species and altering ecosystem functioning (Handfling et al. 2011, Gregory et al. 2012, Kotta and Ojaveer 2012, Ojaveer et al. 2018, Young and Elliott 2020).

One of the earliest recorded invasions was by the European shore crab (*Carcinus maenas*), a relatively large (up to 100 mm carapace width), robust and highly adaptable crab species that is ranked as one of the world's top 100 worst invaders (Murphy and Paini 2010). *Carcinus maenas* is native to the northeast Atlantic from northern Africa to Norway and Iceland, and is the most common intertidal decapod in Europe (Young and Elliott 2020). It is a generalist which is known to feed on a wide range of sessile and mobile epifauna and infauna, consuming prey items from at least 104 families and 158 genera of plants, animals and protists. Adults generally feed in the top few centimetres of sediment, although they have been observed digging pits up to 15 cm deep to extract large clams (Murphy and Paini 2010). In its native range in Europe, *C. maenas* population densities have been estimated at 0.1-20 adult crabs per square metre with up to 2000 juveniles per square metre in suitable habitats during summer. However, in 1817 *C. maenas* was found for the first time in

North America near New York and New Jersey, presumably carried from Europe in solid ballast or on the outside of wooden ship hulls (Young and Elliott 2020). In the 200 years since, *C. maenas* has expanded its range north to Canada and Newfoundland, often reaching massive abundances (in Nova Scotia, Canada, 15000 *C. maenas* were taken in 2 traps in 24 hours, see Murphy and Paini 2010), dramatically effecting intertidal ecology as well as commercially important mollusc fisheries for soft-shell clams (*Mya arenaria*) (see Young and Elliott 2020). This species has also been introduced into South Africa (c. 1983), the west coast of the USA (c. 1989), Argentina (1999), and of course, Australia where it was introduced before 1900 and is now established in New South Wales (south of Sydney), South Australia, Tasmania and Victoria (Thresher et al. 2003, Murphy and Paini 2010, Young and Elliott 2020).

Another highly invasive crab is the Chinese mitten crab (*Eriocheir sinensis*). This is also a relatively large (up to 80 mm carapace width) and adaptable omnivorous species which feeds on a wide variety of algae, detritus, and benthic macroinvertebrates from intertidal areas to about 10 metres depth (Murphy and Paini 2010). *Eriocheir sinensis* is native to temperate and tropical inshore areas of east Asia between Vladivostok (Russia) and southern China, including Japan and Taiwan (Murphy and Paini 2010). However, it has also been introduced via shipping throughout Europe where it now occurs between Finland in the north, throughout the northeast Atlantic seaboard, Baltic and Mediterranean Seas, as well as via Europe into North America including the Mississippi Delta, San Francisco Bay, the Great Lakes and the St Lawrence River in Canada (Murphy and Paini 2010, Handfling et al. 2011). *Eriocheir sinensis* has a catadromous life history, with reproduction and larval development occurring in the marine environment, followed by migration of juveniles up to freshwater regions of rivers and creeks where they can also migrate overland (Murphy and Paini 2010). Because of this life history, *E. sinensis* can be a vector for several notifiable disease agents including white spot syndrome virus (WSSV) which causes white spot disease (WSD) (Ding et al. 2015) and in freshwater areas, crayfish plague (infection by *Aphanomyces astaci*) (see Svoboda et al. 2014, Tilmans et al. 2014).

While larger crab species are easier to detect (and therefore usually the first to be recorded outside their natural ranges), there are many species of smaller crabs that are also hardy, adaptable and easily translocated, but are less easily identified and tend to remain cryptic in their new habitats until they become well established (Roche and Torchin 2007). One of these smaller species is Harris' mud crab (*Rhithropanopeus harrisii*) which grows to around 25 mm carapace width. This species is native to the Atlantic and Caribbean coasts of North America, but was introduced into San Francisco Bay on the west coast in the late 1800's probably via translocations of American oysters (*Crassostrea virginica*) from the east coast (Roche and Torchin 2007). It is a highly invasive crab that is cryptic, eurythermal and euryhaline, able to adapt to a wide range of temperatures and salinities which allows it to outcompete native crab species causing significant alterations to estuarine food webs (Murphy and Paini 2010). In its native range, adult *R. harrisii* are mainly found subtidally under structures in brackish waters, reaching high population densities in salinities below 10 ppt, and occurring down to 200 metres depth. However, in its introduced range it can extend its distribution well inland into freshwater lakes via fish restocking efforts or bait bucket transfers (Patton et al. 2010).

Because of its high environmental tolerances and adaptability, *R. harrisii* has been spread by anthropogenic activities to over 21 countries (Roche and Torchin 2007), including Japan, Europe, and the Middle East, becoming established in the Black Sea by the late 1930's, the Mediterranean soon after (Zalota et al. 2016) and more recently the Baltic Sea (Fowler et al. 2013, Forsström et al. 2015). The main dispersal vector for this species is thought to be via ballast water, however other vectors are also important, including oyster

translocations, and fouling of vessel hulls and sea chests, the latter being a particularly effective method of international translocation of decapod crustaceans (Coutts and Dodgshun 2007). A strong association with translocation by shipping is shown by the establishment of *R. harrisii* in the Panama Canal (Roche and Torchin 2007). As for other invasive crab species, introduced *R. harrisii* may alter food webs, with the potential to displace native crustaceans as well as benthophagous fish species (Roche and Torchin 2007). In high densities, *R. harrisii* is responsible for fouling water intake pipes in the Caspian Sea and Texas, and can also cause economic loss to fishermen by spoiling fish in gill nets (Roche and Torchin 2007). Finally, Roche and Torchin (2007) point out this species (as well as all other decapods) can host WSSV, making it a potential vector for WSD, and possibly other internationally important diseases of crustaceans.

The New Zealand half crab (*Petrolisthes elongatus*) is another small crab species (up to 18 mm carapace width) that may be associated with oyster movements, as this was probably how this species was introduced from New Zealand into Tasmania sometime in the late 1800s (King 1997, Gregory et al. 2012). Other smaller crab species (up to 50 mm carapace width) that may be associated with shellfish reefs or mollusc aquaculture are listed as unwanted pests in Australia include shore crabs within the Family *Grapsidae* (the Asian shore crab *Hemigrapsus sanguineus*, brush-clawed shore crabs (*Hemigrapsus takanoi*, *H. penicillatus*), and Polynesian grapsid crab *Pachygrapsus fakaravensis*). Adults of larger swimming crabs (Family *Portunidae*) are active swimmers which can live on sandy or muddy substrates, but their juveniles may still be cryptogenic and associated with shellfish reefs. Two portunids are listed as unwanted pests including the blue crab *Callinectes sapidus* (native to the east coast of North America where it grows to over 220 mm carapace width, but also introduced into many parts of Asia and Europe), and the Asian paddle crab *Charybdis japonica*. The latter species grows to around 110 mm carapace width and is native to China, Japan, Russia, and Malaysia, but has recently been introduced into northern New Zealand (Fowler and McLay 2013).

The final crab listed as an unwanted pest in Western Australia is not a decapod but instead is the mangrove horseshoe crab (*Carcinoscorpius rotundicauda*), a chelicerate in the Family *Limulidae* that is native to tropical marine and brackish waters throughout Asia and common in waters surrounding ports in that region (McDonald et al. 2015a). There is only one documented international introduction of this species (into New Zealand in 1910), however, there is no clear evidence in the scientific literature that horseshoe crabs pose a pest translocation risk (McDonald et al. 2015a).

4.25.5 Release assessment

Many of the invasive crab species of concern are currently exotic to Australia at this time, however *Carcinus maenas* is established in the temperate waters of New South Wales, South Australia, Tasmania and Victoria, while *Petrolisthes elongatus* occurs in subtidal and intertidal areas of Tasmania. All of these species can be cryptogenic while juveniles and most have been reported to be associated with oyster translocations or shellfish reefs (Roche and Torchin 2007, McKindsey et al. 2007, Murphy and Paini 2010, Gregory et al. 2012). These invasive crab species of concern are highly adaptable and can tolerate a wide range of temperatures and salinities as well as desiccation for long periods. For example, Cohen and Zabin (2009) reported studies that found *Carcinus maenas* can survive over 60 days out of water when sheltered under seaweed, and over 100 days when held in bottles with damp gravel, and could resume normal feeding after 94 days without food. However, Darbyson et al. (2009) found that *C. maenas* isolated without any cover in dry conditions at a mean air temperature of 29°C all died after 7 days, but that 60% of crabs survived 7 days when

seawater or a damp rope was present. Other crab species may be less resistant to desiccation, as shown by Jones and Greenwood (1982) who found that small (0.1 gram) *Petrolisthes elongatus* began to die after 6 hours desiccation at 16°C and 70-80% relative humidity, with 100% mortality within 11 hours. However, in the same experiments they showed that larger (0.76 gram) *P. elongatus* exhibited 100% survival under the same conditions, with death likely to occur only after 24 hours emersion, and that ultimate tolerance to desiccation increased with both body size and ambient humidity (Jones and Greenwood 1982).

Adults of several of the crab species of concern may be able to tolerate freshwater for periods exceeding 24 hours, including *Eriocheir sinensis* and *Rhithropanopeus harrisii* both of which can live in freshwater for indefinite periods. Other species such as *Carcinus maenas* and *Hemigrapsus sanguineus* cannot tolerate freshwater but can tolerate salinities as low as 3-5 ppt for over 24 hours in the case of *H. sanguineus* (see Murphy and Paini 2010) or over a month in the case of *C. maenas* (see Young and Elliot 2020). Invasive crabs are sensitive to heat, with Best et al. (2014) finding that exposing juvenile (<16 mm carapace width) *C. maenas* to salt water heated to 45°C for 1 minute or 55°C for 5 seconds resulted in 100% mortality. Tolerance of crabs to higher water temperatures is not widely reported, however Adams et al. (2019) showed that the heartbeat of crayfish (*Procambarus clarkii*) stops within 10 seconds when they are exposed to water temperatures of 80°C, probably due to coagulation/cooking of muscle proteins. Invasive crabs are also likely to be sensitive to acetic acid (DAWR 2015b), however it appears the tolerance of key invasive crab species to immersion in 4% acetic acid has not been determined. Taking into account the likely propagule pressure experienced via the various anthropogenic introduction pathways, the likelihood estimations for the release of invasive crab species via the identified risk pathways are listed below.

Release assessment for invasive crabs

	Unmitigated	Desiccation			Heat		Salinity	Other
Treatment	None	3 mo.	4 mo.	6 mo.	55°C >10 min	80°C >5 min	0 ppt for >24 hrs	4% acetic acid 30 min
Likelihood of release	High	Low	Neg	Neg	Neg	Neg	Mod	Low?

4.25.6 Exposure assessment

Invasive crabs have already become established in several parts of temperate Australia, however other areas may remain free at this time. Invasive crabs are commonly found associated with shellfish reefs and are often cryptic in mollusc shell materials (Cohen and Zabin 2009), which means that shellfish reef restoration efforts could transport invasive crabs into new regions where their survival and establishment will depend on the availability of favourable environmental conditions. With the exception of the mangrove horseshoe crab (*Carcinoscorpius rotundicauda*), invasive crabs in general are highly adaptable species that can tolerate a broad range of temperatures and salinities (Handfling et al. 2011). For example, the optimal temperature range for *R. harrisii* where successful reproduction can occur is 15-25ºC, but adults can survive and persist between 0.9-35ºC (Murphy and Paini 2010), while *C. maenas* can survive between temperatures of 0-35°C (Young and Elliot 2020). Species such as *C. maenas* and *R. harrisii* are also extremely tolerant of low oxygen levels, and it is these attributes, together with their ability to outcompete native species for food and reproduce over a wide range of environmental conditions, that allows these organisms to establish populations in new areas after translocation (Handfling et al. 2011). Taking these various factors into consideration, given that invasive

crab species are commonly associated with shellfish reefs and aquacultured molluscs and thus could be present with recycled mollusc shells, and environmental conditions are likely to be suitable for their transmission throughout Australia, the risk of exposure and establishment is non-negligible, and the overall likelihood of exposure and establishment of invasive crab species is considered to be **High**.

4.25.7 Consequence assessment

Introduction and establishment of invasive crab species into new areas of Australia would likely have significant ramifications, both ecologically and financially. Because various species of invasive crabs are listed as unwanted pests in all States (Table 3), their detection in new areas may necessitate intervention by government authorities and disruption to shipping and other coastal activities if attempts were made to try to eradicate the invasive population and prevent its further spread. However, once they are introduced into a new area, for the cryptic species of invasive crabs often by the time they are detected and identified there is virtually no chance of eradication (DAWR 2019). Invasive crabs can outcompete native species, resulting in significant ecological damage to freshwater, estuarine and inshore aquatic ecosystems, including irreversible changes to food webs and potential loss of biodiversity through displacement of native crustacean species (Murphy and Paini 2010, Handfling et al. 2011). The introduction of invasive decapods can also facilitate entry or spread of important crustacean pathogens, particularly WSSV which has recently established in southeast Queensland (Diggles 2020b), but also *Aphanomyces astaci*, which would have significant ramifications for domestic and international trade of crustacean products produced by Australia's fisheries and aquaculture industries if they were introduced or spread into new regions. Taking all of these factors into consideration, the consequences of introduction of invasive crab species into new areas via the identified risk pathways are likely to be at least **Moderate** based on ecological damage alone, and would be even be higher if notifiable diseases such as WSD or crayfish plague were introduced with them.

4.25.8 Risk estimation

The risk estimation for invasive crab species is determined by combining the likelihood of release and exposure (from Table 5) with the consequences of establishment (Table 6) to arrive at a risk estimation (Table 7). The risk estimate for invasive crab species exceeds the ALOP for some release pathways, suggesting that additional risk management is required for these marine pests.

Risk estimate for invasive crabs

	Unmitigated	Desiccation			Heat		Salinity	Other
Treatment	None	3 mo.	4 mo.	6 mo.	55°C >10 min	80°C >5 min	0 ppt for >24 hrs	4% acetic acid 30 min
Combined likelihood of release and exposure	High	Low	Neg	Neg	Neg	Neg	Mod	Low?
Consequences of establishment	Moderate	Mod	Mod	Mod	Mod	Mod	Mod	Mod
Risk estimation	Moderate	Low	Neg	Neg	Neg	Neg	Mod	Low?

4.26 Invasive clams

4.26.1 Invasive agent: Clams are invertebrates classified within the Class Bivalvia in the Phylum Mollusca. Species of clams that are problematic and invasive such that they are listed as unwanted pests in Australia include *Anadara transversa*, *Corbula amurensis*, *Ensis directus*, *Mya japonica*, *M. arenaria*, *Theora lubrica*, and *Varicorbula gibba*.

4.26.2 OIE List: No **State Pest Lists:** Yes (all states) **Zoonotic:** No

4.26.3 Australia's status: The Asian semelid bivalve (*Theora lubrica*) was found established in Port Phillip Bay in 1958 (Hewitt et al. 2004), the European clam (*Varicorbula gibba*) has established populations in Tasmania and Victoria (the latter since 1987) (Hayes et al. 2005), while the Japanese softshell clam (*Mya japonica*) was detected in the Prosser River on Tasmania's east coast in 2018 (Grove et al. 2018).

4.26.4 Epizootiology

Bivalve molluscs (including clams, oysters, mussels) are amongst the most common groups of biofouling organisms found in fouling communities on boat hulls and aquaculture equipment (Brock et al. 1999, McKindsey et al. 2007, Murphy and Paini 2010, Cahill et al. 2019) . With the advent of international shipping and the globalisation of trade, bivalve molluscs have proven to be some of the worlds most effective invasive marine organisms (Murphy and Paini 2010, Ovajeer et al. 2018). Threats posed by invasive clams (Families *Arcidae*, *Corbulidae*, *Myidae*, *Pharidae*, and *Semelidae*) will be examined in this Section, whilst threats due to other invasive bivalves such as mussels (Families *Mytilidae*, *Dressinidae*) and oysters (Families *Ostreidae* and *Anomiidae*) will be examined in Sections 4.27 and 4.28.

Anthropogenic movements of invasive clam species comprise perhaps the earliest known international translocations of marine organisms. There is evidence that the soft shelled clam (*Mya arenaria*) was translocated from its native range along the east coast of North America to Europe, possibly as early as the 13th century by the Vikings as a food item or via hull fouling or rock ballast (Petersen et al. 1992, Strasser 1999, Cross et al. 2016, Ovajeer et al. 2018). *Mya arenaria* lives in soft muddy sediments from the upper intertidal to subtidal zones where it filters phytoplankton and organic detritus from the water column (Murphy and Paini 2010). It is a large (up to 15 cm in shell length and 6 cm in height), deep burrowing, commercially important mollusc widely used for food and bait, and it commonly dominates benthic infauna in estuaries throughout its native range (Murphy and Paini 2010). After its introduction into Europe, *M. arenaria* spread into many areas including the Baltic, Mediterranean, Black and Adriatic seas where it appears to have outcompeted many native bivalve species for space and food and dominates many benthic soft sediment ecosystems (Strasser 1999, Murphy and Paini 2010). The closely related Japanese softshell clam (*Mya japonica*) which is native to temperate areas of the north west Pacific Ocean has similar invasive characteristics, and in 2018 specimens of this species up to 15 cm shell length were reported for the first time in the southern hemisphere in the Prosser River at Orford on Tasmania's east coast (Grove et al. 2018, Dann et al. 2020). Investigations revealed high population densities of adult and juvenile clams, indicating that *M. japonica* had likely been present at Orford for at least 10 years (certainly since July 2013), possibly arriving as veliger larvae in ballast water from an Asian-origin ship docked at a nearby international woodchip export facility (Grove et al. 2018).

When *M. arenaria* was introduced into San Francisco Bay on the west coast of the United States (probably on oyster spat) sometime in the mid-late 19th century (Strasser 1998, Wasson et al. 2001, Grove et al. 2018, Dann et al. 2020), it eventually replaced native clams (*Macoma nasuta*) and underpinned successful fisheries for many decades (Murphy and Paini 2010). However, *M. arenaria* was then itself outcompeted after introduction of the exotic Asian basket clam (*Corbula amurensis*), which arrived from its native range (South East Asia) in ballast water sometime in the mid 1980's (Carlton et al. 1990). Within two years, *C. amurensis* almost completely displaced *M. arenaria* and the remaining native bivalves within San Francisco Bay, virtually eliminating biodiversity and completely altering the structure of the benthic ecosystem as it reached densities of over 10,000 per square metre at some sites (Carlton et al. 1990). The Asian basket clam is a relatively small (2-3 cm shell length), fast growing species which partially buries into soft muddy or sandy sediments in intertidal and subtidal areas, leaving 1/2 to 2/3 of its shell above the sediment in order to feed (Murphy and Paini 2010). *Corbula amurensis* is a very resilient species able to tolerate low oxygen as well as a wide range of salinities from freshwater to 35 ppt and water temperatures between 8 and 23°C (Murphy and Paini 2010). Dense populations of *C. amurensis* filter large quantities of phytoplankton from the water column, and are able to reproduce within a few months after settlement from the plankton, meaning they are able to rapidly outcompete slower growing native species for space and food, resulting in their displacement (Murphy and Paini 2010).

The European clam *Varicorbula gibba* is another small clam species (maximum size 2 cm) native to Europe where it inhabits sandy-mud sediments in subtidal areas with the ability to attach to gravel and stones by a single byssal thread (Murphy and Paini 2010). It is regarded as a pest due to its high growth rate and tolerance of a broad range of environmental conditions, being able to tolerate severe hypoxia for several weeks, and is considered an indicator of environmental degradation caused by pollution, low oxygen content, or increased turbidity (Aquenal 2008a, Albano et al. 2018). Like the Asian basket clam, the European clam can also achieve very high population densities (> 50000 per square metre) and therefore has the potential to compete with native species for food and space (Curry and Parry 1999, Aquenal 2008a, Murphy and Paini 2010). Experiments done in Port Phillip Bay demonstrated a negative impact of *V. gibba* on the growth rates of commercial scallops (*Pecten fumatus*), probably due to competition for food (Talman and Keough 2001). The European clam was not present in Port Phillip Bay in the 1970's (Curry and Parry 1999), but was identified in samples collected as early as 1987 (Hewitt et al. 2004, Hayes et al. 2005), and was most likely introduced to Australia as larvae in ballast water or adults in the sea chests of shipping (Hewitt et al. 2004, Aquenal 2008a).

The Asian semelid bivalve *Theora lubrica* is another small (maximum size 1.5 cm) clam with an almost transparent shell. This species is native to the northwest Pacific including Russia, Japan and Hong Kong, but has established invasive populations in many parts of the world, including North America, Europe, New Zealand (Hayes et al. 2005) and Port Phillip Bay where it has been recorded since the 1950's (Hewitt et al. 2004). This species lives in muddy sediments, from the low tide mark to depths of around 100 metres, and is considered to be an indicator species for pollution due to its preference for hypoxic sediments rich in organic matter. *Theora lubrica* populations exhibit rapid growth rates, high densities and near continuous recruitment, which increases the probability of uptake of their larvae in ballast water (Murphy and Paini 2010). These life history characteristics also mean *T. lubrica* can outcompete slower growing native species for space and food (Hayes et al. 2005, Murphy and Paini 2010).

The transverse arc clam *Anadara transversa* is a small (maximum 3.5 cm) species native to sandy and muddy subtidal sediments along the east coast of North America, but it has been translocated to many regions outside its natural range including the Mediterranean, Aegean and Adriatic Seas (Albano et al. 2018). Like the other invasive clams described above, *A. transversa* is fast growing (Nerlovic et al. 2018), and tolerant of eutrophic conditions, with evidence from the Mediterranean that increasing hypoxia and pollution during the 1970's were the necessary prerequisites driving the success of its invasion process (Albano et al. 2018). The jack-knife clam (*Ensis directus*) is another clam native to sandy intertidal and subtidal sediments along the east coast of North America. These long but thin razor clams grow to a large size (16-17 cm long and 2.5-2.8 cm wide), and were introduced into Europe along the German coast of the North Sea in the late 1970's, probably via larvae in ballast water (Murphy and Paini 2010). Since then *E. directus* has spread rapidly (up to 75 km/year) to Denmark, Sweden, Norway, the Netherlands, Belgium, France, and Britain. This species may compete against native bivalves and alter benthic community structure, but it is most problematic due to the loss of public amenity as its sharp shells can deter tourists with possible cuts and bacterial infection of the resulting wounds, whilst piles of shells along the foreshore affect visual amenity (Murphy and Paini 2010).

4.26.5 Release assessment

Populations of some invasive clam species already occur in Victoria (*Varicorbula gibba*, *Theora lubrica*) and Tasmania (*Mya japonica*, *V. gibba*), however several other invasive clam species of concern are currently exotic to Australia at this time. Being sediment burrowers, clams have limited potential for spread through biofouling and are more usually spread by planktonic larvae in ballast water discharged from shipping (Carlton et al. 1990, Dann et al. 2020). Nevertheless, several invasive clam species have been translocated with oyster spat in the past (e.g. *M. arenaria*, see Strasser 1998), and some (e.g. *A. transversa*, *V. gibba*) have one or more byssus threads which allow them to attach to hard surfaces (which could include mollusc shells collected from subtidal or intertidal areas) as biofouling (Murphy and Paini 2010, Nerlovic et al. 2018). All invasive clam species of concern are highly adaptable and can tolerate a wide range of environmental temperatures and salinities, whilst several species (e.g. *A. transversa*, *C. amurensis*, *T. lubrica*, *V. gibba*) are advantaged by pollution, low oxygen levels and other anthropogenic impacts (Albano et al. 2018).

Several of the clam species of concern may be able to tolerate freshwater for periods exceeding 24 hours by shutting their shell valves, including *Corbula amurensis* which can tolerate freshwater for at least 30 days (Carlton et al. 1990). It is known that bivalves, including invasive clam species, generally do not tolerate desiccation for long periods (Cohen and Zabin 2009, Hopkins et al. 2016), and that subtidal species tend to be less tolerant of emersion than intertidal species (Hummel et al. 1988). Thus, tolerance of invasive clams to desiccation is likely to be less than that of the intertidal Pacific oyster (*Crassostrea gigas*), which required up to 34 days desiccation before 100% mortality was recorded by Hopkins et al. (2016). Tolerance of invasive clams to higher water temperatures is not widely reported, however Leach (2011) exposed other (presumably more tolerant) intertidal species of bivalves (mussels *M. edulis* and *Trichomya hirsuta*) to thermal treatments within a replica sea chest and recorded 100 % mortality after 10 minutes at 60°C.

Invasive clams are also likely to be sensitive to acetic acid, however it appears the tolerance of key invasive clam species to immersion in 4% acetic acid has not been determined. Other intertidal bivalves, such as juvenile (<1 year old) blue mussels (*Mytilus edulis*) experienced 33% mortality after exposure to a 90 second dip in 5% acetic acid (Swan 2006), while Carman et al. (2016) recorded 100% mortality of juvenile (15-25

mm shell length) *Mytilus edulis* after 5 min exposure to 5% acetic acid. Furthermore, Atalah et al. (2016) recorded 97.5% mortality of juvenile (3 month old) Australian blue mussels (*Mytilus galloprovincialis*) exposed to 4% acetic acid for 1 hour, and 100% mortality after 24 hours exposure, whilst Denny (2008) exposed greenlip mussels (*Perna canaliculus*) to 4% acetic acid for only 2 minutes then left them out of the water for 24 hours without rinsing, resulting in 57-75% mortality. Therefore, taking into account the likely propagule pressure experienced via the various anthropogenic introduction pathways, the likelihood estimations for the release of invasive clam species via the identified risk pathways are listed below.

Release assessment for invasive clams

	Unmitigated	Desiccation			Heat		Salinity	Other
Treatment	None	3 mo.	4 mo.	6 mo.	55°C >10 min	80°C >5 min	0 ppt for >24 hrs	4% acetic acid 30 min
Likelihood of release	High	Neg	Neg	Neg	V. low	Neg	High	V. low

4.26.6 Exposure assessment

Invasive clams have already become established in several parts of temperate Australia, probably after their introduction via ballast water, however other areas may remain free at this time. Juveniles of invasive clams may be associated with shellfish reefs and they may be collected and translocated with mollusc shell materials (Strasser 1998, Wasson et al. 2001, Grove et al. 2018), which means that shellfish reef restoration efforts could transport invasive clams into new regions where their survival and establishment will depend on the availability of favourable environmental conditions. As detailed above, invasive clams are highly adaptable species that can tolerate a broad range of temperatures and salinities and adverse environmental conditions such as low oxygen levels and eutrophication (Albano et al. 2018), and it is these attributes, together with their ability to outcompete native species for food and reproduce over a wide range of environmental conditions, that allows these organisms to successfully establish populations in new areas after translocation (Strasser 1999). On the other hand, it appears that invasive clam species are less likely to be able to establish in areas where environmental conditions remain relatively undisturbed (Albano et al. 2018), while under some circumstances alien clams may establish populations for several years, then disappear (e.g. alien *Mya arenaria* reported in Elkhorn Slough, south of San Francisco Bay on the west coast of the United States in 1916 are apparently no longer present, see Wasson et al. 2001). Taking these various factors into consideration, given that invasive clam species may be associated with shellfish reefs and aquacultured molluscs and thus could be present with recycled mollusc shells, and environmental conditions are likely to be suitable for their transmission throughout many parts of Australia, the risk of exposure and establishment is non-negligible, and the overall likelihood of exposure and establishment of invasive clam species is considered to be **High**.

4.26.7 Consequence assessment

Introduction and establishment of invasive clam species into new areas of Australia may have important ramifications for local ecosystems, but the magnitude and extent of these effects would depend on the species introduced and the location of the introduction. For example, introductions of the smaller, faster growing species such as *Corbula amurensis* into locations that have suffered anthropogenic disturbance has resulted in losses of important mollusc fisheries and dramatic cascading trophic affects and biodiversity losses that have

altered entire ecosystems (Carlton et al., 1990, Murphy and Paini 2010). However, on the other hand, the introduction of *Varicorbula gibba* into Port Phillip Bay and other areas of Tasmania was considered to have had relatively low impacts (Murphy and Paini 2010), although some effects of competition against native species remained apparent (Talman and Keough 2001). Indeed, the introduction of some of the larger edible species, such as soft shell clams (*Mya arenaria*, *M. japonica*) has resulted in development of economically important fisheries in some parts of their introduced ranges (Strasser 1999, Wasson et al. 2001). However, these fisheries benefits have come in some circumstances at the expense of restructuring of benthic soft sediment ecosystems, and the effects (detrimental or otherwise) of the recent incursion of *M. japonica* into the east coast of Tasmania remains to be determined (Grove et al. 2018). The potential of various species of clams to vector and transmit several important disease agents, including *Perkinsus olseni*, *Perkinsus marinus*, *Marteilia refringens*, OsHV-1 µVar and other malacoherpesviruses, also needs to be taken into account (Tables 9, 10).

Because various species of invasive clams are listed as unwanted pests in all States (Table 3), their detection in new areas may necessitate intervention by government authorities and disruption to shipping and other coastal activities if attempts were made to try to eradicate the invasive population and prevent its further spread. However, once they are introduced into a new area, often by the time they are detected and identified they are well established and there is virtually no chance of eradication (Grove et al. 2018, DAWR 2019). Taking all of these factors into consideration, the consequences of introduction of invasive clam species into new areas via the identified risk pathways are likely to be **Low** based on ecological damage alone, but would be higher if notifiable diseases such as *Perkinsus* spp., *Marteilia* spp., OsHV-1 µVar or other malacoherpesviruses were introduced with them, as described in Sections 4.3, 4.4, 4.10, 4.12, 4.16, and 4.17.

4.26.8 Risk estimation

The risk estimation for invasive clams is determined by combining the likelihood of release and exposure (from Table 5) with the consequences of establishment (Table 6) to arrive at a risk estimation (Table 7). The risk estimate for invasive clams exceeds the ALOP for some release pathways, suggesting that additional risk management is required for these marine pests.

Risk estimate for invasive clams

	Unmitigated	Desiccation			Heat		Salinity	Other
Treatment	None	3 mo.	4 mo.	6 mo.	55°C >10 min	80°C >5 min	0 ppt for >24 hrs	4% acetic acid 30 min
Combined likelihood of release and exposure	High	Neg	Neg	Neg	V. low	Neg	High	V. low
Consequences of establishment	Low	Low	Low	Low	Low	Low	Low	Low
Risk estimation	Low	Neg	Neg	Neg	Neg	Neg	Low	Neg

4.27 Invasive mussels

4.27.1 Invasive agent: Mussels are bivalves classified within the Families *Mytilidae* and *Dressenidae* in the Phylum Mollusca. Species of mussels that are problematic and invasive such that they are listed as unwanted pests in Australia include *Arcuatula senhousia*, *Brachidontes pharaonis*, *Mytilopsis sallei*, *Perna canaliculus*, *Perna perna*, and *Perna viridis*.

4.27.2 OIE List: No **State Pest Lists:** Yes (all states) **Zoonotic:** No

4.27.3 Australia's status: The Asian bag mussel (*Arcuatula senhousia*) has been introduced and has established in several parts of Australia, including Victoria (Portland and Port Phillip Bay), northern and eastern Tasmania, south west South Australia, and Cockburn Sound, Lower Swan River and Fremantle in Western Australia. The Asian green mussel (*Perna viridis*) is exotic to Australia, but is known to have established and recruited in small numbers in Trinity Inlet, Cairns between 2001 and 2003 prior to eradication (Stafford et al. 2007). It is regularly detected in foreign vessel biofouling in Australian waters (Heersink et al. 2014). The black striped mussel (*Mytilopsis sallei*) is exotic to Australia, but is known to have established in Cullen Bay Marina in Darwin in 1999, but was successfully eradicated (Ferguson 2000, Willan et al. 2000, Bax et al. 2002, Neil et al. 2005). It is occasionally detected in foreign vessel biofouling in Australian waters.

4.27.4 Epizootiology

Bivalve molluscs (including clams, oysters, mussels) are amongst the most common groups of biofouling organisms found in fouling communities on boat hulls and aquaculture equipment (Brock et al. 1999, McKindsey et al. 2007, Murphy and Paini 2010, Cahill et al. 2019) . With the advent of international shipping and the globalisation of trade, bivalve molluscs have proven to be some of the worlds most effective invasive marine organisms (Murphy and Paini 2010, Ovajeer et al. 2018). Threats posed by invasive mussels (Families *Mytilidae*, *Dressinidae*) will be examined in this Section, whilst threats due to invasive clams (Families *Arcidae*, *Corbulidae*, *Myidae*, *Pharidae*, and *Semelidae*) were examined in Section 4.26, whilst invasive oysters (Families *Ostreidae* and *Anomiidae*) will be examined in Section 4.28.

The Asian bag mussel (*Arcuatula senhousia*, syn. *Musculista senhousia*) is a relatively small (up to 3 cm long) species of mussel classified within the Family *Mytilidae* that is native to Japan, China, Vietnam and Singapore and other coastal areas of the north west Pacific Ocean (Hayes et al. 2005). This species has proven to be a particularly invasive and troublesome fouling organism that forms dense aggregations on soft or hard substrata in intertidal to subtidal habitats along coastal and estuarine shorelines to depths of around 20 metres. It has undergone substantial human-mediated range extensions in the 20th century via biofouling, ballast water and accidental introductions (Neil et al. 2005, Murphy and Paini 2010). Its present day distribution now extends throughout the northern Pacific Ocean from southern Siberia to Singapore and the north and central Philippines, the Mediterranean, north-west America, New Zealand (introduced prior to 1980), and Australia, being first recorded here in 1982 (Willan 1987, Neil et al. 2005). *Arcuatula senhousia* was probably introduced to Washington and California with Pacific oysters (*Crassostrea gigas*), but was also thought to be introduced by ballast water and/or ship fouling into the Mediterranean and the north-east Pacific (Hayes et al. 2005, Murphy and Paini 2010). This species has a "remarkable" thermal tolerance (Neil et al. 2005), high

tolerance to low oxygen levels as well as high fecundity and rapid growth to maturity (9 months or less) that allows it to dominate benthic communities by outcompeting and displacing native species (Willan 1987). It can burrow into soft sediments and also settles in dense aggregations known as byssal mats (or bags) which may smother native bivalves (Willan 1987) and restrict the growth of some species of seagrass, but may also increase infaunal density and species richness because they provide additional habitat for many species (Hayes et al. 2005). *Arcuatula senhousia* prefers to settle in groups on soft substrata, but is a highly adaptive species capable of fouling wooden wharf pilings and other man-made structures (Hayes et al. 2005, Murphy and Paini 2010). Since its introduction and establishment in Australia in the early 1980's in the Swan River (see Willan 1987), populations of *A. senhousia* have also become established in Port Phillip Bay and Portland (Victoria), northern and eastern Tasmania, southwest South Australia, as well as several other locations in south west Western Australia (Cockburn Sound, Fremantle) (DAWR 2019).

The Asian green mussel (*Perna viridis*) is an edible shellfish classified within the Family *Mytilidae* which colonises both hard and soft substrates in intertidal and subtidal areas of coastal, estuarine and tidal creek shores. This species was originally native to the north-western Pacific Ocean, but has undergone substantial human-mediated range extensions since the 19th century via biofouling and deliberate transfers for both subsistence and commercial use (Neil et al. 2005). Its present day distribution now extends throughout the tropical Indo-Pacific from the Middle East to southern Japan, islands in Micronesia and Papua New Guinea, where it is a dominant fouling organism and a major fouling pest for power plant cooling systems (Murphy and Paini 2010). As it grows to a quite large size (adults can reach up to 16 cm shell length) *P. viridis* is used for aquaculture in countries such as India, Malaysia, Singapore, Indonesia, the Philippines, southern China (including Hong Kong) and French Polynesia (Neil et al. 2005). *Perna viridis* can tolerate wide environmental extremes in water temperature and salinity and has a high tolerance to pollution, hence it readily establishes populations in new geographical locations, with known introductions in the USA (Gulf of Mexico, Florida, Georgia), the Caribbean (Trinidad, Jamaica), South America (Venezuela), and also in Cairns, Australia (Murphy and Paini 2010). Because of this, Hayes et al. (2005) ranked *P. viridis* as number 1 high priority in their list of international marine invaders.

This species successfully established in Trinity Inlet in Cairns in August 2001 after being introduced as fouling of the hull of a foreign fishing vessel that was seized by the Australian Customs Service (Neil et al. 2005). The foreign fishing vessel was moored in Trinity Inlet for 1 year before being defouled and scuttled at a deepwater location. During defouling *P. viridis* was identified at high densities (>25/m^2) on the hull and in water intakes, and subsequent surveys between late 2001 and July 2003 detected mussels on other floating structures throughout the inlet (n=40, shell length range 30.1-161 mm, mean 95 mm), indicating at least one spawning and successful recruitment event had taken place (Neil et al. 2005). At least four additional recruits from this Trinity Inlet population are known to have been translocated to Innisfail 90 km south of Cairns on the hull of a yacht during this period (Stafford et al. 2007). These were removed and subsequent surveillance has suggested this species either has not established, or remains present in very low densities in Trinity Inlet (Neil et al. 2005, Murphy and Paini 2010). Heersink et al. (2014) found that the propagule pressure for *Perna viridis* entering Australia is very high, with an estimated 10s to 100s of *P. viridis* fouled vessels entering Australian ports each year, however relatively few establishments have been recorded, leading to suggestions the risk of invasion by *P. viridis* may not be as high as originally estimated (Wells 2017, Heersink et al. 2020). A significant body of research and analysis has been done to assess the likelihood of *P. viridis* being released into Australian waters (Hayes et al. 2005, Neil et al. 2005, Summerson et al. 2007, Richmond et al. 2010,

Heersink et al. 2014). These analyses concluded that environmental conditions are likely to be suitable for establishment of *P. viridis* around a large proportion of the northern Australian coastline (Summerson et al. 2007, Richmond et al. 2010, Heersink et al. 2020). The optimal annual temperature range for *P. viridis* is 26-32ºC, but it can survive 7-32 ºC (Murphy and Paini 2010). It is primarily found at salinities of 18-33 ppt but can survive 0-80 ppt (Murphy and Paini 2010).

The black striped false mussel (*Mytilopsis sallei*) is small (maximum 3 cm shell length) shellfish classified within the Family *Dressenidae* which readily colonises all littoral habitats in intertidal and shallow subtidal areas of marine, coastal, estuarine and tidal creek shores. This species was originally native to tropical and sub-tropical waters of the western Atlantic from Colombia to the Gulf of Mexico, but has undergone substantial human-mediated range extensions in the 20th century via biofouling and ballast water introductions (Neil et al. 2005, Murphy and Paini 2010). Its present day distribution now extends throughout the Atlantic coastline of North and South America including Florida, the Caribbean, West Indies, and by the late 20th century *M. sallei* had spread to Asia and the Middle East being recorded from India (1967), Japan (1974), Taiwan (1977), Indonesia, Singapore and Hong Kong by 1980 (Murphy and Paini 2010) and the Philippines soon after (Neil et al. 2005). In areas where it has been introduced, *M. sallei* becomes a dominant fouling organism and a major fouling pest for power plant cooling systems, wharves, marinas, seawalls and other intertidal and subtidal structures (Murphy and Paini 2010). Unlike *P. viridis*, due to its small size *M. sallei* is of limited use as a human foodstuff and indeed due to its massive biofouling potential its presence is considered a threat to aquaculture expansion (Murphy and Paini 2010). *Mytilopsis sallei* can tolerate wide environmental extremes in water temperature and salinity and has a high tolerance to pollution, hence it readily establishes populations in new geographical locations, especially in artificial or disturbed environments.

In Australia, *Mytilopsis sallei* successfully established and was found in large numbers in Cullen Bay Marina in Darwin in 1999, after it was thought to be introduced via fouling of the hull of yachts or fishing vessels that had frequented ports in Indonesia (Neil et al. 2005). Baseline surveys 6 months earlier did not detect *M. sallei* in the same locations, suggesting it was introduced sometime in late 1998. At the same time, a small population of *M. sallei* was discovered in a separate marina and a few individuals were found in a third marina (Bax et al. 2002, Neil et al. 2005). Rapid responses by authorities and the fortuitous location of the major infestation within a lock-gate protected marina allowed an effective response including use of chemicals such as chlorine and copper sulphate, resulting in effective eradication (Ferguson 2000, Willan et al. 2000, Neil et al. 2005).

The brown mussel (*Perna perna*) is an edible mussel that grows to a relatively large size (up to 17 cm shell length) and which colonises both hard and soft substrates in intertidal and particularly subtidal areas of coastal, estuarine and tidal creek shores (Murphy and Paini 2010). It was originally native to the central and south-western Atlantic, the south-eastern Atlantic and the southwestern Indian Oceans including the southern Caribbean, Brazil, Uruguay, south east and west coasts of Africa, India, and Sri Lanka, but has undergone human-mediated range extensions via biofouling and ballast water introductions into Venezuela, the Mediterranean, and the Gulf of Mexico (Texas) (Murphy and Paini 2010, GISD 2015). As it grows to large sizes, the brown mussel is used for human consumption in many countries, but has been associated with outbreaks of paralytic shellfish poisoning in Venezuela and elsewhere (GISD 2015). *Perna perna* can tolerate reasonably wide environmental extremes in water temperature and salinity, but mainly occurs in subtropical

regions and is relatively less invasive compared to *P. viridis* or *M. sallei*. Indeed, a near extinction of *P. perna* populations in the Gulf of Mexico occurred in 1997 when surface water temperatures neared 30ºC (Murphy and Paini 2010).

Like the other invasive mussel species discussed above, environmental damage associated with invasions by *P. perna* is mainly due to its ability to outcompete native bivalves for food and space. In areas where it has been introduced, *P. perna* becomes a dominant fouling organism and a major fouling pest for power plant cooling systems (where it can co-exist with *P. viridis*), wharves, marinas, seawalls, and other intertidal and subtidal structures (Murphy and Paini 2010). In its native range in Brazil, fouling by *P. perna* on navigation buoys requires their removal every six months to avoid sinking the buoys, which affects shipping safety, while on artificial substrata in south Texas, beds of *P. perna* can be composed of several layers and attain thicknesses in excess of 20 cm which causes significant nuisance fouling as well as exclusion of native species (Murphy and Paini 2010).

The New Zealand green lip mussel *Perna canaliculus* is an edible mussel that grows to a very large size (maximum 24 cm) and forms the basis of an important aquaculture industry in New Zealand. This species is listed on the Australian Priority Marine Pest species list[13] and is also listed as an unwanted organism in Western Australia (Table 3). It is thought to have similar invasive potential as other *Perna* species, and thus may be able to outcompete Australian blue mussels (*Mytilus galloprovincialis*) (see Menge et al. 2007) if it were to be introduced into Australia. This species is also a known host for *Perkinsus olseni*[14] in New Zealand (Castinel et al. 2019), is imported into Australia in large quantities for retail sale and its shells are commonly found together with oyster shells during shell recycling activities undertaken from Australian restaurants (B.K. Diggles, personal observations).

4.27.5 Release assessment

Populations of the Asian bag mussel (*Arcuatula senhousia*) have already established in several parts of Australia, however other areas remain free, and several other invasive mussel species of concern are currently exotic to Australia at this time. Mussels are common biofouling organisms which attach by byssus threads to a wide range of surfaces, including aquacultured oysters and their furniture, as well as wild oysters collected from subtidal or intertidal areas, and some invasive mussel species have been translocated with oyster spat in the past (e.g. *Arcuatula senhousia* with *C. gigas*, see Murphy and Paini 2010). All of the invasive mussel species of concern are highly adaptable and can tolerate a wide range of environmental temperatures and salinities, whilst some species (e.g. *A. senhousia*, *M. sallei*, *P. viridis*) are advantaged by pollution, low oxygen levels and other anthropogenic impacts (Murphy and Paini 2010).

All of the mussel species of concern are likely to be able to tolerate freshwater for periods exceeding 24 hours by shutting their shell valves, including juvenile (16-36 mm shell length) *Perna canaliculus* which can tolerate freshwater for at least 2 days and up to 5 days at 10°C (Forrest and Blakemore 2006). Other species, such as *Mytilopsis sallei*, can persist in freshwater environments for up to 9 months (Summerson et al. 2007, Murphy and Paini 2010). On the other hand, it is known that invasive mussel species do not tolerate desiccation for

[13] https://www.marinepests.gov.au/pests/identify/nz-green-mussel

[14] https://www.oie.int/wahis_2/public/wahid.php/Reviewreport/Review?page_refer=MapFullEventReport&reportid=15953

long periods (Cohen and Zabin 2009). For example, the median emersion tolerance time in *Perna perna* at 25ºC was <100 hours (c. 4 days) regardless of relative humidity, and increased to around 11 days at 15ºC depending on relative humidity (Murphy and Paini 2010). In another study, juvenile blue mussels (*Mytilus* spp.) and greenlip mussels (*Perna canaliculus*) experienced 100% mortality after 7 days desiccation in direct sunlight and 10-11 days desiccation, respectively in temperature controlled (14.5-18.5°C) rooms (Hopkins et al. 2016).

Tolerance of mussels to higher water temperatures have been examined and their thermal tolerance zones and ultimate lethal temperature depend on mussel size as well as water temperature and time of immersion (e.g. Wallis 1977). Rajagopal et al. (1995a) found that exposure of juvenile (9 mm) *Perna indica* to 38°C water for 2 hours resulted in 100% mortality, whilst Rajagopal et al. (1995b) found that exposure of juvenile (2 mm) *Perna viridis* to 39°C water for 73 minutes resulted in 100% mortality. Exposure to seawater at 50°C or higher for 30 seconds also resulted in 100% mortality of *M. galloprovincialis* up to 60 mm shell length (Sievers et al. 2019). At even higher water temperatures, Leach (2011) recorded 100% mortality in mussels (*M. edulis* and *Trichomya hirsuta*) exposed to 60°C for 10 minutes within a replica sea chest. Invasive mussels are also known to be sensitive to acetic acid, including juvenile (<1 year old) blue mussels (*Mytilus edulis*) which experienced 33% mortality after exposure to a 90 second dip in 5% acetic acid (Swan 2006), while Carman et al. (2016) recorded 100% mortality of juvenile (15-25 mm shell length) *Mytilus edulis* after 5 min exposure to 5% acetic acid. Furthermore, Atalah et al. (2016) recorded 97.5% mortality of juvenile (3 month old) Australian blue mussels (*Mytilus galloprovincialis*) exposed to 4% acetic acid for 1 hour, and 100% mortality after 24 hours exposure, whilst Denny (2008) exposed greenlip mussels (*Perna canaliculus*) to 4% acetic acid for only 2 minutes then left them out of the water for 24 hours without rinsing, resulting in 57-75% mortality. Taking into account the information above, and given the likely propagule pressure experienced via the various anthropogenic introduction pathways, the likelihood estimations for the release of invasive mussels via the identified risk pathways are listed below.

Release assessment for invasive mussels

	Unmitigated	Desiccation			Heat		Salinity	Other
Treatment	None	3 mo.	4 mo.	6 mo.	55°C >10 min	80°C >5 min	0 ppt for >24 hrs	4% acetic acid 30 min
Likelihood of release	High	Neg	Neg	Neg	Neg	Neg	High	V low

4.27.6 Exposure assessment

Invasive mussels such as *A. senhousia* have already become established in several parts of temperate Australia, probably after their introduction via ballast water (Willan 1987), however areas around the subtropical and tropical coastlines of Australia remain free of *A. senhousia* at this time (Richmond et al. 2010), while several exotic invasive mussel species continue to cause incursions from time to time (Bax et al. 2002, Neil et al. 2005, Heersink et al. 2014, 2020). Juvenile mussels are commonly associated with shellfish reefs and they may be collected and translocated with mollusc shell materials (B.K. Diggles, personal observations), which means that shellfish reef restoration efforts could transport invasive mussels into new regions where their survival and establishment will depend on the availability of favourable environmental conditions. A significant amount of research and analysis has been done to assess the likelihood of establishment of invasive

mussels in Australian waters (Hayes et al. 2005, Neil et al. 2005, Summerson et al. 2007, Richmond et al. 2010, Heersink et al. 2014). These analyses concluded that environmental conditions are likely to be suitable for establishment of *P. viridis* and *M. sallei* around the entire northern Australian coastline (Summerson et al. 2007, Richmond et al. 2010). In contrast, *P. perna* may be restricted to more brackish harbours and estuaries in regions south of the Tropic of Capricorn (Richmond et al. 2010), while as it originates from New Zealand, *P. canaliculus* would likely be restricted to temperate regions. As detailed above, invasive mussels are highly adaptable species that can tolerate a broad range of temperatures and salinities and adverse environmental conditions such as low oxygen levels and eutrophication (Murphy and Paini 2010), and it is these attributes, together with their ability to outcompete native species for food and reproduce over a wide range of environmental conditions, that allows these organisms to successfully establish populations in new areas after translocation. Taking these various factors into consideration, given that invasive mussel species may be associated with shellfish reefs and aquacultured molluscs and thus could be present with recycled mollusc shells, and environmental conditions are likely to be suitable for their transmission throughout many parts of Australia, the risk of exposure and establishment is non-negligible, and the overall likelihood of exposure and establishment of invasive mussel species is considered to be **High.**

4.27.7 Consequence assessment

Introduction and establishment of invasive mussels into new areas of Australia would likely have highly significant ramifications, both ecologically and financially, the latter due to their being a major cause of unwanted fouling of important marine infrastructure (e.g. power plant cooling water systems, bridges, vessels, shipping buoys, docks, seawalls) (Murphy and Paini 2010 and references cited therein). Because various species of invasive mussels are listed as unwanted pests in all States (Table 3), their establishment in new regions of Australia would necessitate significant intervention by government authorities and disruption to normal shipping and other coastal activities if attempts were made to try to prevent their further spread into uninfected areas. Even so, once they are introduced into a new area, only in exceptional circumstances of early detection and through significant expense may there be a chance of eradication (Bax et al. 2002, Neil et al. 2005). Invasive mussels can outcompete native species due to their faster growth, reproduction and higher pollution tolerance, resulting in significant ecological damage to estuaries and inshore areas that would likely be irreversible, including loss of biodiversity through displacement of native shellfish and seagrass habitats (Murphy and Paini 2010 and references cited therein). Some invasive mussels (e.g. *Perna viridis*) are also known to bioaccumulate heterotrophic bacteria, enteric viruses, toxins and heavy metals to very high levels, thus posing a risk to human health if they are eaten, while heavy fouling of visible surfaces can result in losses of public/tourist amenity (Murphy and Paini 2010). Their introduction may also facilitate entry of new mollusc pathogens and parasites, including *Perkinsus olseni*, *Perkinsus marinus*, *Marteilia refringens*, OsHV-1 µVar and other malacoherpesviruses which may be harmful to native species, as has been documented for other shellfish introductions (Howard 1994, Bishop et al. 2006, Lynch et al. 2010, Tables 9, 10). Taking all of these factors into consideration, the consequences of introduction of invasive mussel species into new areas via the identified risk pathways are likely to be **High** based on ecological damage alone, but could be even higher if notifiable diseases such as *Perkinsus* spp., *Marteilia* spp., OsHV-1 µVar or other malacoherpesviruses were introduced with them, as described in Sections 4.3, 4.4, 4.10, 4.12, 4.16, and 4.17 .

4.27.8 Risk estimation

The risk estimation for invasive mussels is determined by combining the likelihood of release and exposure (from Table 5) with the consequences of establishment (Table 6) to arrive at a risk estimation (Table 7). The risk estimate for invasive mussels exceeds the ALOP for some release pathways, suggesting that additional risk management is required for these marine pests.

Risk estimate for invasive mussels

	Unmitigated	Desiccation			Heat		Salinity	Other
Treatment	None	3 mo.	4 mo.	6 mo.	55°C >10 min	80°C >5 min	0 ppt for >24 hrs	4% acetic acid 30 min
Combined likelihood of release and exposure	High	Neg	Neg	Neg	Neg	Neg	High	V low
Consequences of establishment	High	High	High	High	High	High	High	High
Risk estimation	High	Neg	Neg	Neg	Neg	Neg	High	Low

4.28 Invasive oysters

4.28.1 Invasive agent: Oysters are bivalves classified within the Families *Ostreidae* and *Anomiidae* in the Phylum Mollusca. Species of oysters that are listed as unwanted pests in Australia include *Crassostrea ariakensis*, *C. gigas*, *C. virginica*, and *Monia noblis*.

4.28.2 OIE List: No **State Pest Lists:** Yes (QLD, WA) **Zoonotic:** No

4.28.3 Australia's status: The Pacific oyster (*Crassostrea gigas*) was introduced unsuccessfully into Western Australia for aquaculture purposes in 1947, but was successfully introduced into Tasmania in 1948 (Hayes et al. 2005). Feral populations of *C. gigas* have since been spread (probably by shipping) to other areas including several rivers and bays in NSW including Botany Bay, Port Stephens and the Hawkesbury River, as well as the Port River in South Australia, after which it has formed the basis of economically important aquaculture industries in these regions. This species was also deliberately introduced into locations in Victoria and Spencer Gulf and Coffin Bay/west coast region of South Australia for the purposes of aquaculture development (Murphy and Paini 2010).

4.28.4 Epizootiology

Bivalve molluscs (including clams, oysters, mussels) are amongst the most common groups of biofouling organisms found in fouling communities on boat hulls and aquaculture equipment (Brock et al. 1999, McKindsey et al. 2007, Murphy and Paini 2010, Cahill et al. 2019). With the advent of international shipping and the globalisation of trade, bivalve molluscs have proven to be some of the worlds most effective invasive marine organisms (Murphy and Paini 2010, Ovajeer et al. 2018). Threats posed by invasive oysters (Families *Ostreidae* and *Anomiidae*) will be examined in this Section, whilst threats due to other invasive bivalves such as clams (Families *Arcidae*, *Corbulidae*, *Myidae*, *Pharidae*, and *Semelidae*) and mussels (Families *Mytilidae*, *Dressinidae*) were examined in Sections 4.26 and 4.27, respectively.

As mentioned throughout this document, it is well known that transfers of live oysters as well as oyster shell materials are highly effective as primary and secondary vectors for introduction of a wide range of pest and disease agents (AFFA 2002, Bushek et al. 2004, Forrest and Blakemore 2006, Mineur et al. 2007, Cohen and Zabin 2009, Ojaveer et al. 2018). However, during the process of oyster shell recycling for shellfish reef restoration, it is also possible that inadvertent transfer of live oysters (both juveniles and adults) may also occur, especially if oyster shell materials are obtained directly from oyster leases or wholesale oyster chucking enterprises (B.K. Diggles, personal observations). In such circumstances, without appropriate mitigation it is possible that viable oysters could be translocated into new areas during shellfish reef restoration (Ruesink et al. 2005).

The Suminoe oyster (*Crassostrea ariakensis*), also known as the Chinese River Oyster, is a large (maximum size 24 cm) edible rock oyster native to China and possibly an early introduction into Japan (Calvo et al. 2000, Fofonoff et al. 2018). Its occurrence outside China and Japan is uncertain because of taxonomic issues relating to its synonymy with *Crassostrea rivularis* (which has been reported throughout SE Asia including India, Pakistan, Malaysia and Borneo) and *C. hongkongensis* in some studies (Calvo et al. 2000, Wang et al. 2004).

It is known that *C. ariakensis* was inadvertently introduced to the west coast of North America in the 1970's with shipments of *C. gigas* from Japan (Breese and Malouf 1977). Intentional introductions of *C. ariakensis* for aquaculture development were considered on the east coast of North America and France, due to the higher resistance of this species to pathogens including *Perkinsus marinus* and *Haplosporidium nelsoni* (see Calvo et al. 2000), but after further study these introductions did not go ahead due to the high susceptibility of *C. ariakensis* to *Bonamia exitiosa* (see Cochennec et al. 1998, Burreson et al. 2004, Bishop et al. 2006, Audemard et al. 2008, 2014). This oyster naturally occurs in muddy intertidal zones in areas where salinities are between 10 and 30 ppt, however it can tolerate exposures to salinities > 35 ppt and as low as 2 ppt, and appears to be able to grow faster than (and thus has the potential to outcompete) native oyster species (Calvo et al. 2000, Fofonoff et al. 2018).

The Pacific oyster (*Crassostrea gigas*) is a large (maximum shell length over 30 cm) fast growing edible rock oyster originally native to coastlines of Asia in the north west Pacific Ocean including Russia, China, Korea and Japan (Fofonoff et al. 2018). This species has been deliberately introduced into many regions of the world for aquaculture development throughout the 20th century (Shatkin et al. 1997), including both east and west coasts of North America, Europe, and Australia (Ruesink et al. 2005, Murphy and Paini 2010, Fofonoff et al. 2018). It has also been accidentally introduced into many locations, including New Zealand in 1971 where it was presumably introduced via ballast water or as hull fouling on ships (Dinamani 1971, Smith et al. 1986, Shatkin et al. 1997). In many locations outside its natural range *C. gigas* has become invasive and exhibited significant range expansions, often facilitated by global warming (Diederich et al. 2005) and spread by biofouling (Deveney et al. 2017), ballast water or larval settlement, with native oyster species often being outcompeted by feral *C. gigas* populations due to their high growth rates, fecundity (Ruesink et al. 2005, Anglès d'Auriac et al. 2017) and ability to cope with high turbidity (Bayne 1999, 2002).

In Australia, Pacific oysters were deliberately introduced into Western Australia in 1947, but they did not establish, however they were established in Tasmania in 1948 and were also deliberately introduced into Victoria and South Australia for aquaculture development (Ayers 1991, Murphy and Paini 2010). The Pacific oyster was accidentally introduced into Port Stephens in New South Wales sometime prior to 1985, and was declared a noxious species because it outgrows and displaces the native Sydney rock oyster (*Saccostrea glomerata*), however after several years of trying to eradicate *C. gigas*, the NSW government ended eradication attempts and allowed its cultivation (Ayers 1991, Murphy and Paini 2010). In areas of NSW where both species now occur, Pacific oysters grow faster and can potentially outcompete Sydney rock oysters (Bayne 1999, Honkoop and Bayne 2002, Krassoi et al. 2008, Bishop et al. 2010, Scanes et al. 2016). Bayne (1999, 2002) found that part of the competitive advantage enjoyed by *C. gigas* over *S. glomerata* is due to its higher ventilation rate and greater metabolic efficiencies which make it a faster feeder, particularly at higher food concentrations. It is also known that significant inhibition of filtration occurs in *S. glomerata* at a comparatively low concentration of particulate matter, between 10 and 15 mg/l, compared to > 100 mg/l for *C. gigas* (see Bayne 2002). These differences in filtration capacity (>50% lower filtration rate than *C. gigas* in laboratory studies (Wilkie et al. 2013) and in the field when turbidity is >15 mg/L) disadvantage *S. glomerata* in anthropogenically modified estuaries with increased turbidity, which is one reason why *C. gigas* is an invasive threat in estuaries where *S. glomerata* occurs.

Pacific oysters recruit to hard surfaces in intertidal and subtidal areas in estuaries and sheltered inshore areas, settling in dense aggregations which limit the food and space available for native rock oyster species (Murphy and Paini 2010). They spawn during the summer months when water temperatures are between 18.5 and

25°C, with maximum growth attained at 30°C, but adult oysters are robust and can tolerate water temperatures between -1.5 and 35°C and salinities between 5 and 41 ppt (Murphy and Paini 2010, Fofonoff et al. 2018). Pacific oysters are known to harbour many invasive pests, including mudworms, barnacles, Clionaid sponges, tunicates and invasive macroalgae (Mineur et al. 2007), and many disease agents including viruses (iridoviruses, OsHV-1 µVar), parasites (*Bonamia exitiosa*, *Bonamia ostreae*, *Haplosporidium nelsoni*, *Marteilia refringens*, *Marteilioides chungmuensis*, *Perkinsus marinus*), as well as commensals (e.g. the copepod *Mytilicola orientalis*) - see above Sections for more details for each of these disease agents.

The American eastern oyster (*Crassostrea virginica*) is a large (up to 25 cm shell length) edible rock oyster native to shallow estuarine waters along the east coast of North and Central America from the Gulf of St. Lawrence to Venezuela (Fofonoff et al. 2018). The eastern oyster was deliberately translocated to many locations during the 19th century including the west coast of North America (from British Columbia to southern California), Hawaii, and Europe. The eastern oyster is currently established in Pearl Harbour in Hawaii, and a remnant population survives in British Columbia, but the vast majority of these introductions were unsuccessful (Fofonoff et al. 2018), hence it appears that *C. virginica* is less invasive than some other oyster species like *C. gigas*. Nevertheless, *C. virginica* is a robust species which can tolerate salinities between 2 and 42 ppt, and water temperatures between -1.8 to 36ºC (Fofonoff et al. 2018), and is known to harbour several listed disease agents including viruses (malacoherpesviruses), and parasites (*Bonamia exitiosa*, *Bonamia ostreae*, *Haplosporidium nelsoni*, *Marteilia refringens*, *Mikrocytos mackini*, and *Perkinsus marinus* - see above Sections for more details for each of these disease agents.

Jingle shells or saddle oysters (*Anomia nobilis*) are small (up to 10 cm) flat bivalves that superficially resemble true oysters (Family *Ostreidae*), but they are classified in the Family *Anomiidae* due to various morphological differences including their mode of attachment onto substrates, which is via a byssal plug passing through an opening in the right (lower) shell valve (Bishop Museum 2002). This species is native to the tropical Indo-West Pacific region but was probably introduced into the Hawaiian Islands in the early 19th century via ship-fouling as it is a very common fouling organism, typically found subtidally or intertidally on pier pilings and floating docks in characteristic stacks one on top of the other. The ecological impact of this species is unstudied, but observations suggest it may compete for space with native invertebrates (Bishop Museum 2002).

4.28.5 Release assessment

Populations of Pacific oysters (*Crassostrea gigas*) have already established in several parts of Australia where they underpin economically important aquaculture industries, such that around 80% of mollusc shells recycled from processors and retailers in south east Queensland for shellfish restoration between 2017 and 2020 have been *C. gigas* shells (B.K. Diggles, personal observations). However, *C. gigas* and several other invasive oyster species are listed as pests of concern, and some of these (*C. ariakensis*, *C. virginica*, *A. nobilis*) are exotic to Australia at this time. Oysters are common biofouling organisms which attach to a wide range of surfaces, but particularly other oyster shells due to chemical signalling which attracts planktonic spat to conspecifics (Tamburri et al. 2006). Most of the invasive oyster species of concern can tolerate a wide range of environmental temperatures and salinities, whilst species like *C. gigas* are also advantaged by anthropogenic impacts (Scanes et al. 2016).

All of the oyster species of concern are likely to be able to tolerate freshwater for periods exceeding 24 hours by shutting their shell valves, including *C. gigas* juveniles and adults which can survive freshwater dips (Rolheiser et al. 2012) in this manner as well as immersion for several weeks at or below 2 ppt (Miossec et al. 2009). It is known that invasive oysters cannot tolerate desiccation for long periods (Cohen and Zabin 2009), however they do tend to be more desiccation-resistant compared to most other taxa. For example, Hopkins et al. (2016) found that small adult (mean 39 gram wet weight) *C. gigas* exposed to direct sunlight outdoors (mean air temperature 20.3°C) began dying after 3 days with 100% mortality after 16 days, however when held indoors in a temperature controlled (14.5-18.5°C) room, 100% mortality took 34 days. This was around three times longer than tolerated by mussels (*Mytilus* and *Perna* spp.) which experienced 100% mortality after 10-11 days desiccation in the same temperature controlled room (Hopkins et al. 2016).

Tolerance of oysters to higher water temperatures have been examined and, like mussels, their thermal tolerance zones and ultimate lethal temperature depend on oyster size as well as water temperature and time of immersion. However, it appears that oysters can tolerate significantly higher temperatures than other taxa (Mountfort et al. 1999, Rajagopal et al. 2005, Piola and Hopkins 2012). For example, Rajagopal et al. (2005) found that *C. gigas* had upper temperature tolerance limits much greater than similar sized *Mytilus* spp., with a significant effect of size observed (greater thermal tolerance with increasing size). At 40°C water temperature, 100% mortality of 11 mm spat and 54 mm *C. gigas* took 96 and 167 min, respectively, while at 43°C, 100% mortality occurred in *C. gigas* of all sizes within 1 hour (Rajagopal et al. 2005). In contrast, Piola and Hopkins (2012) found 50.6% of adult (>50 mm) *C. gigas* had survived 60 minutes exposure to 55°C, and 100% mortality of adult *C. gigas* was achieved only following exposure to 57.5°C for 60 min, or 60°C for 30 min. Limited data are available for other oyster species, however Sievers et al. (2019) found that 100% of both small (1.5 cm shell length) and large (5 cm shell length) Australian flat oysters (*Ostrea angasi*) were killed after exposure to 50°C or higher water temperature for longer than 30 seconds

Invasive oysters are also likely to be sensitive to acetic acid, including adult (mean 8.5 cm, 89 gram wet weight) *C. gigas* which experienced 100% mortality after 5 minutes or more immersion in 5% acetic acid (Rolheiser et al. 2012). There are apparently no data for tolerances of *Anomia nobilis* to any of these treatments, however given it appears that *C. gigas* is larger and relatively more robust compared to most (if not all) other invasive bivalves (Hopkins et al. 2016), it will be assumed that any treatments that kill *C. gigas* will also kill equivalent sized *C. ariakensis*, *C. virginica* and *A. noblis*. Taking into account the information above, and given the likely propagule pressure experienced via the various anthropogenic introduction pathways, the likelihood estimations for the release of invasive oysters via the identified risk pathways are listed below.

Release assessment for invasive oysters

	Unmitigated	Desiccation			Heat		Salinity	Other
Treatment	None	3 mo.	4 mo.	6 mo.	55°C >10 min	80°C >5 min	0 ppt for >24 hrs	4% acetic acid 30 min
Likelihood of release	High	Neg	Neg	Neg	Mod	Neg	High	V low

4.28.6 Exposure assessment

Invasive oysters such as *C. gigas* have already become established in several parts of temperate and subtropical Australia, however some parts of NSW as well as WA, QLD and the NT remain free of *C. gigas* at this time, whilst the other listed invasive oyster species of concern have never been recorded from Australia. "Overcatch" of viable juvenile oysters is translocated in virtually every shipment of oyster shell material collected during mollusc shell recycling in south east Queensland (B.K. Diggles, personal observations), which means that without mitigation shellfish reef restoration efforts would transport invasive oysters into new regions where their survival and establishment will depend on the availability of favourable environmental conditions. As detailed above, invasive oysters are generally highly adaptable species that can tolerate a broad range of temperatures and salinities and thus the environment is likely to be suitable for their establishment around much of the Australian coastline. Taking these various factors into consideration, given that at least one invasive oyster species (*C. gigas*) is commonly collected during mollusc shell recycling in Australia, and environmental conditions are likely to be suitable for its survival and establishment in many parts of Australia, the risk of exposure and establishment is non-negligible, and the overall likelihood of exposure and establishment of invasive oyster species is considered to be **High.**

4.28.7 Consequence assessment

Introduction and establishment of invasive oysters into new areas of Australia would likely have significant ecological ramifications through displacement of native oyster species. Because various species of invasive oysters are listed as unwanted pests in QLD and WA (Table 3), their establishment in these jurisdictions could necessitate significant intervention by government authorities and disruption to normal shipping and other coastal activities if attempts were made to try to prevent their further spread into uninfected areas. Even so, once they are introduced into a new area, only in exceptional circumstances of early detection and through significant expense may there be a chance of eradication (Bax et al. 2002, Neil et al. 2005). Invasive oysters can outcompete native species due to their faster growth, reproduction and higher pollution tolerance, resulting in ecological changes to estuaries and inshore areas that would likely be irreversible. Invasive oysters including *C. gigas* may also compete directly with endemic oysters through competitive feeding including predation of their larval stages (Ezgeta-Balic et al. 2020). However, in places where *C. gigas* has co-occurred with native *S. glomerata* in NSW for over 50 years, populations of the latter have coexisted (Krassoi et al. 2008) and often remained stable in some areas (Bishop et al. 2010), though *C. gigas* makes up to 85% of oyster populations in some parts of invaded systems like Port Jackson (Scanes et al. 2016). The introduction of invasive oysters may also facilitate entry or spread of mollusc pathogens and parasites, including *Bonamia exitiosa*, *Bonamia ostreae*, *Haplosporidium nelsoni*, *Marteilia refringens*, *Marteilioides chungmuensis*, *Mikrocytos mackini*, *Perkinsus olseni*, *Perkinsus marinus*, iridoviruses, OsHV-1 µVar and other malacoherpesviruses which may be harmful to native species, as has been documented for other shellfish introductions (Howard 1994, Bishop et al. 2006, Lynch et al. 2010, Tables 9, 10). Taking all of these factors into consideration, the consequences of introduction of invasive oyster species into new areas via the identified risk pathways are likely to be **Moderate** based on ecological damage alone, but could be even higher if notifiable diseases were introduced with them, as described in Sections 4.2, 4.3, 4.4, 4.6, 4.7, 4.8, 4.9, 4.10, 4.13, 4.14, 4.16, and 4.17.

4.28.8 Risk estimation

The risk estimation for invasive oyster species is determined by combining the likelihood of release and exposure (from Table 5) with the consequences of establishment (Table 6) to arrive at a risk estimation (Table 7). The risk estimate for invasive oyster species exceeds the ALOP for some release pathways, suggesting that additional risk management is required for these marine pests.

Risk estimate for invasive oysters

	Unmitigated	Desiccation			Heat		Salinity	Other
Treatment	None	3 mo.	4 mo.	6 mo.	55°C >10 min	80°C >5 min	0 ppt for >24 hrs	4% acetic acid 30 min
Combined likelihood of release and exposure	High	Neg	Neg	Neg	Mod	Neg	High	V low
Consequences of establishment	Moderate	Mod	Mod	Mod	Mod	Mod	Mod	Mod
Risk estimation	High	Neg	Neg	Neg	Mod	Neg	High	V low

4.29 Invasive polychaetes (including mudworms)

4.29.1 Invasive agent: Polychaete worms are classified in the Phylum Annelida, Class Polychaeta within Families *Sabellidae*, *Serpulidae* and *Spionidae*. Species of invasive polychaete worms that are listed as unwanted pests in Australia include serpulids (*Hydroides dianthus*), sabellids (*Euchone limnicola*, *Sabella spallanzanii*) and spionids such as *Marenzelleria* spp. and mudworms (*Boccardia* spp., *Polydora* spp.).

4.29.2 OIE List: No **State Pest Lists:** Yes (all states) **Zoonotic:** No

4.29.3 Australia's status: The deposit feeding sabellid polychaete *Euchone limnicola* has been introduced into NSW (Botany Bay), Victoria (Port Phillip Bay) and SA (Port River in Adelaide), while the European fan worm *Sabella spallanzanii* has been introduced and become established in various ports and harbours in NSW, Victoria, Tasmania, SA and WA (Murphy and Paini 2010). Mudworm infections have been reported in the shells of oysters and other molluscs from all Australian states (Walker 2011), and certain species of these agents (*Polydora* spp., *Boccardia* spp.) are reportable pests in some jurisdictions (Victoria, SA, the NT).

4.29.4 Epizootiology

Various species of polychaete worms are common biofouling organisms, several are ectocommensal on bivalve molluscs, and all are easily spread through anthropogenic activities including oyster transfers and international shipping (Link et al. 2009, Murphy and Paini 2010, Ojaveer et al. 2018). Calcareous tube dwelling polychaetes (Family *Serpulidae*) are notorious biofoulers that are easily transported and introduced into new areas, with the native habitats of many species of tube worms of the genus *Hydroides* uncertain due to their wide dissemination in the 18th and 19th centuries by shipping (Link et al. 2009). For example, the tube worm *Hydroides elegans* is thought to have been introduced into Port Jackson prior to its original description there in 1883 (Murphy and Paini 2010). *Hydroides dianthus* develops tubes around 20 mm long and appears to be native to the east coast of North America, but has been introduced into many regions, including as recently as 2006 into Tokyo Bay, Japan (Link et al. 2009), although it still thought to be exotic to Australia (Link et al. 2009, Murphy and Paini 2010). *Hydroides dianthus* is a prominent fouling organism commonly found in high numbers on the shells of mussels (*Mytilus galloprovincialis*) in the Mediterranean Sea (Cinar et al. 2008), while *H. elegans* has been reported to overgrow juvenile oysters causing mortalities in Japan as well as generating problematic biofouling that interferes with mariculture equipment (Link et al. 2009).

Sabellid fan worms (Family *Sabellidae*) are a group of deposit dwelling polychaetes that are well known invasive organisms. The sabellid polychaete *Euchone limnicola* grows to around 12 mm long and is native to the north east Pacific Ocean along the west coast of North America (Murphy and Paini 2010). Since it was first recorded in Victoria (Port Phillip Bay) in 1984, this introduced species has been found in other high traffic port areas in NSW (Botany Bay), and SA (Port River in Adelaide) where it inhabits soft muddy sediments down to around 10 metres water depth (Hayes et al. 2005, Murphy and Paini 2010). This species establishes dense populations within the sediments, possibly competing with native species for food and space (Hayes et al. 2005). The European fan worm *Sabella spallanzanii* is a large (up to 40 cm long) tube dwelling worm native to inshore waters of the Mediterranean Sea and the European Atlantic coast (Read et al. 2011). This species is generally found in shallow subtidal areas in harbours and sheltered embayments down to

around 30 metres water depth (Murphy and Paini 2010). The European fan worm was probably introduced into Australia in the early 1980's in Port Phillip Bay via hull fouling or ballast water, and is now often found in the soft sediments in many ports and harbours throughout southern Australia (Ahyong et al. 2017), where it is generally found in clumps attached to dead bivalve shells, live ascidians or sea grass (Murphy and Paini 2010). It also can be found in extensive beds, for example in parts of Cockburn Sound (WA) and Port Phillip Bay (Victoria), where it attains densities greater than 100 individuals per square metre, and readily colonises man-made structures such as wharf piles, channel markers, pontoons, and submerged wrecks (Murphy and Paini 2010). The European fan worm has also invaded New Zealand, where it was first detected in 2008 in Port Lyttelton in the South Island, then subsequently near Auckland in the North Island (Read et al. 2011). Eradication was attempted, but this was abandoned within 2 years of its original detection when it became clear that the invasion had spread widely in many areas throughout both islands, largely due to the massive reproductive ability of *S. spallanzanii* which can also regenerate adult worms by architomy (regeneration of fragments of the body into new individuals) following trauma-related damage (Read et al. 2011). The introduction of this species into New Zealand probably originated from shipping from Australia via ballast water or hull fouling (Ahyong et al. 2017). Fan worms filter feed using a crown of feeding tentacles that capture suspended matter including phytoplankton and zooplankton, but their main detrimental effect is probably via competition for space as they outcompete and displace native species in soft sediments (O'Brien et al. 2006, Read et al. 2011).

Spionid polychaetes (Family *Spionidae*) are predominantly free living worms that are found in muddy estuarine sediments worldwide, however anthropogenic activities have increased the distribution of some spionid species, including red gilled mudworms of the genus *Marenzelleria*, which have been translocated via ballast water from international shipping from the east coast of North America to many new regions in Europe (Bastrop et al. 1997) where they can attain densities of several thousand individuals per square metre (Murphy and Paini 2010). *Marenzelleria* spp. tend to be larger (up to 11 cm long) than most native burrowing polychaetes and can tolerate low oxygen and high sulphide concentrations, and in places such as the Baltic Sea there were concerns they would outcompete native polychaetes through competition for food and space (Murphy and Paini 2010, Fofonoff et al. 2018). However, closer study of the situation in the Baltic Sea suggests that these invasions may have been beneficial, as the larger *Marenzelleria* spp. tend to burrow deeper into the substrate than do native polychaetes, reducing competition (Urban-Malinga et al. 2013), whilst the increased bioturbation allows deeper oxygen penetration into the sediment (Bonaglia et al. 2013). This increased oxidation of the sediments in turn increases the area available for aerobic meiobenthos, thus improving sediment biodiversity as well as nutrient cycling which may help mitigate eutrophication and harmful cyanobacteria blooms (Maximov et al. 2015).

Other species of spionid polychaetes commonly use the shells of bivalve molluscs and abalone as settlement substrates (Read 2010, Walker 2011) as they feed on suspended or resuspended particles or plankton (Dauer et al. 1981). Most spionids infesting mollusc shells are small (up to 2.5 cm long) ectocommensals with the planktonic larvae settling on the outer shell, however some larvae can settle inside the mantle cavity and/or on the edge of the shell lip, with the growing worm establishing a cover of mucus and debris while enlarging a burrow on the inner surface of the shell valve in the extrapallial space, accumulating sediment and detritus inside their burrow as the mollusc covers it with nacre, resulting in shell blistering (thus the name "mudworm") (Read 2010). At least 37 species of spionids have been recorded in Australia to date, with at least 12 species of *Polydora* and 10 species of *Dipolydora* occurring on the east coast alone (see Walker 2009, 2011). The main species that are usually reported to be problematic in mollusc aquaculture include *Polydora*

websteri, *P. haswelli* and *P. hoplura*, *Boccardia knoxi* and *B. chilensis*) (see Nell 2001, Lleonart et al. 2003a, 2003b, Sato-Okoshi et al. 2017). Low intensity infections are innocuous and usually confined to the outer shell, however some species may cause unsightly mud blisters in the inner shell and abscesses in the adductor muscle if the blister contacts the tissue (Whitelegge 1890). It is notable that many of the early museum specimens originally identified as *P. websteri* from Australian oysters were, upon re-examination, other species such as various *Dipolydora* spp., while some specimens identified as *P. polybranchia* by Haswell (1885) and Whitelegge (1890) from *S. glomerata* were re-identified as *Boccardia polybranchia* and *P. wellingtonensis* (see Walker 2009, 2011).

Prevalence and intensity of mudworm infestations vary considerably with local water quality and growing height of oysters. Light mudworm infections rarely cause mortalities and infected oysters can usually be marketed, however mud blisters may interfere with shucking and reduce the commercial value of oysters to be served on the half-shell (Nell 2001). Prevalence and intensity of infection increases in the vicinity of muddy substrates (Whitelegge 1890), resulting in reduced oyster growth (Wargo and Ford 1993), while infections can be reduced by off bottom bivalve culture techniques preferably at heights that dry out the mollusc for at minimum 2 hours in each tidal cycle (Nell 2001). While trans-Tasman exports of live oysters from New Zealand were commonplace during the late nineteenth century, there is no evidence that mudworms were problematic in New Zealand at that time (Read 2010). The earliest reports of mudworm in New Zealand only date from the early 1970s and only from northern New Zealand, whereas a century earlier at least one of these pest worms had already become widespread along eastern Australian coasts (Nell 2001, Ogburn et al. 2007, Read 2010). This suggests that "mudworm disease" of wild and cultured sub tidal oysters on Australia's east coast from around 1870 onwards (Roughley 1939) was probably not "*due to introduction of exotic mudworms from New Zealand*", as hypothesized by some authors (see Ogburn et al. 2007). Instead, proliferation of native mudworm species probably occurred due to increased eutrophication and organic enrichment of Australian estuaries (Saville-Kent 1891), a process that began to be noticeable after floods from 1870 onwards due to catchment clearing and development (McCulloch et al. 2003, Diggles 2013). Mudworms are very abundant in muddy tidal flats compared to clean sandy areas, as mudworm settlement is stimulated by high microbial counts associated with muddy sediments (Sebesvari et al. 2006). This suggests that organic enrichment/eutrophication and sedimentation (the first anthropogenic changes that tend to occur in estuaries following any extensive clearing or development in the catchment, see Paterson et al. 2003) will promote increased abundance of mudworms (Nell 2001, Diggles 2013). In effect, Haswell (1885) noted this by stating "*some local circumstances, such as muddiness of the water produced by increasing traffic, tend to decrease the vital powers of the oysters and thus favour the inroads of the parasites*", while Saville-Kent (1891) stated "*this so called worm disease is essentially a dirt disease*" and "*the worm disease, or, as it may be more correctly termed, the mud disease*". The subsequent disappearance of sub tidal oyster beds throughout much of the east coast (Ogburn et al. 2007) is more likely due to spatfall failure (spat set does not occur on dirty surfaces covered in sediment trapped by the algae generated by organic enrichment), as well as QX disease in recent years (Diggles 2013).

Mortalities of *S. glomerata* reported in Moreton Bay after floods in the mid 1890's were attributed to "mudworm disease" (Brisbane Courier 1898), however today, while heavy mudworm infections reduce the growth rate of cupped oysters, they are seldom fatal (Wargo and Ford 1993, Read 2010, B.K. Diggles, personal observations), although hyperinfections of *P. hoplura* and *B. knoxi* have caused mortalities of up to 50% in aquacultured abalone (Lleonart 2002, Lleonart et al. 2003a, 2003b). Whitelegge (1890) also described

mudworm hyperinfections in subtidal oysters resulting in mortality, hence it remains plausible that the "mudworm epizootics" of the late 19th and early 20th centuries were instead indicators of structural shifts in the biota of the affected estuaries due to increasing sediment loads bought down from newly modified catchments after floods (Diggles 2013). Given the existence of many species of endemic spionid polychaetes (Walker 2011, Sato-Okoshi 2017), increased sedimentation and organic enrichment in estuaries resulting in proliferation of endemic polychaetes (in the form of both mudworms and the polychaete intermediate hosts of *M. sydneyi*, see Adlard and Nolan 2015 and Section 4.11) appears the most parsimonious explanation for the emergence of both mudworm and QX disease, with QX becoming more prominent since the 1970s due to further declines in water quality causing more frequent immunosuppression of the oysters (Peters and Raftos 2003, Green and Barnes 2010).

4.29.5 Release assessment

Several species of invasive polychaetes occur in various jurisdictions throughout southern Australia, including species of mudworm of concern to mollusc farmers (Walker 2009, 2011, Sato-Okoshi et al. 2008, 2017). Some of these species may have restricted distributions, such as *Boccardia knoxi*, which may prefer cooler waters as it has been recorded in Australia only from south Western Australia and Tasmania (Walker 2011). However, it is reasonable to assume that mudworms can infect the shells of virtually any mollusc species that occurs near muddy substrates. Sabellids and serpulids are also commonly associated with bivalve shells (Cinar et al. 2008, Link et al. 2009, Murphy and Paini 2010), hence there is a non-negligible risk that these unwanted pests may occur in mollusc shells that are recycled for shellfish reef restoration in Australia.

It is known that some invasive polychaetes are susceptible to freshwater. For example, Nel et al. (1996) found that exposing mudworms (*Polydora hoplura*) on the shells of Pacific oysters (*C. gigas*) to freshwater for 12 hours significantly reduced (but did not eliminate) the infections, while Nell (2007) recommended 48 hours immersion in freshwater to kill *P. websteri* on *S. glomerata*. Dunphy et al. (2005) recorded 98% mortality of *Boccardia* spp. on the shells of bluff oysters (*Ostrea chilensis*) after 5 hours immersion in freshwater, and Lleonart (2002) found immersion of *Boccardia* spp. in freshwater for 10 minutes *in-vitro* resulted in 100% mortality for spionids removed from their feeding tubes, but only 30-40% mortality after 2 hours when the worms were exposed to freshwater while remaining inside their feeding tubes in the shells of abalone (which experienced 80% mortality during that time). Sabellid tubeworms do not tolerate freshwater (Brock et al. 1999), and it is known that *Sabella spallanzanii* is susceptible to freshwater baths and experiences 100% mortality after 1.5-2 hours immersion in freshwater while remaining within its feeding tube (Jute and Dunphy 2017). However, adults of *Marenzelleria* spp. can tolerate freshwater and their larvae can also develop at low salinities of >5 ppt (Fofonoff et al. 2018).

It is known that polychaetes are susceptible to desiccation and that their mortality rates during emersion increase with ambient temperature (Hummel et al. 1988). For example, in a study by Kauano et al. (2017) only around 6% and 8% of sabellid and serpulid worms survived after 6 and 30 hours emersion, respectively. *Sabella spallanzanii* is susceptible to desiccation and King (2017) recorded 100% mortality in *S. spallanzanii* after 2 days (48 hours) emersion in a temperature controlled (18°C) room. Desiccation is also effective against spionids, with minimal survival of larval mudworms (*Boccardia* spp.) after drying in air for 4 hours (Lleonart 2003a), while adult mudworms (*Polydora* spp.) also cannot survive drying for more than 7 to 10 days out of the water in ambient conditions typical of temperate NSW (Nell 2007). Invasive polychaetes are also

susceptible to hot water, with Nel et al. (1996) recording a significant reduction in the survival of *Polydora hoplura* (40% mortality) after Pacific oysters (*C. gigas*) were dipped in 70°C seawater for 40 seconds. There is also evidence that invasive polychaetes are susceptible to acetic acid. For example, King (2017) found that immersion of *Sabella spallanzanii* in a 5% solution of acetic acid for 1 minute resulted in 75% mortality, while 100% mortality was observed in all S. *spallanzanii* exposed to 5% acetic acid for longer than 4 minutes. However, Forrest et al. (2007) found that the serpulid *Hydroides elegans* survived a 4 minute dip in 4% acetic acid. Taking into account the information above, and given the likely propagule pressure experienced via the various anthropogenic introduction pathways, the likelihood estimations for the release of invasive polychaetes via the identified risk pathways are listed below.

Release assessment for invasive polychaetes (including mudworms)

	Unmitigated	Desiccation			Heat		Salinity	Other
Treatment	None	3 mo.	4 mo.	6 mo.	55°C >10 min	80°C >5 min	0 ppt for >24 hrs	4% acetic acid 30 min
Likelihood of release	High	Neg	Neg	Neg	V low	Neg	Mod	V low

4.29.6 Exposure assessment

Molluscs in all parts of Australia are already at risk from natural exposure to spionid mudworms, and invasive polychaetes such as *S. spallanzanii* and *E. limnicola* have already become established in several parts of temperate Australia, however some areas around the subtropical coastlines of Australia remain free of *S. spallanzanii* at this time. Mudworms and invasive polychaetes are commonly associated with shellfish reefs and mudworms in particular are regularly collected and translocated with mollusc shell materials (B.K. Diggles, personal observations), which means that shellfish reef restoration efforts could transport invasive polychaetes into new regions where their survival and establishment will depend on the availability of favourable environmental conditions. Polychaetes can multiply via asexual reproduction via either architomy or paratomy (division of the parent body into two halves with reconstitution of the missing halves by regeneration, see Walker 2011) as well as sexually via external fertilization in the water column when populations have male and female individuals (Walker 2011). Female spionids are able to sexually reproduce at an age of 3 months, producing multiple broods of eggs each year which hatch into planktonic larvae which settle on a seasonal basis (Handley 2000, Lleonart et al. 2003a, 2003b) when appropriate settlement cues are present (Sebesvari et al. 2006). Thus, under favourable conditions a single larvae or adult worm may be sufficient to initiate colonization and establishment (Whitelegge 1890). Invasive polychaetes can tolerate a broad range of environmental temperatures and salinities and adverse conditions such as low oxygen levels and eutrophication (Murphy and Paini 2010), and it is these attributes, together with their ability to outcompete native species for food and reproduce over a wide range of environmental conditions, that allows these organisms to successfully establish populations in new areas after translocation. Taking these various factors into consideration, given that invasive polychaete species may be associated with shellfish reefs and aquacultured molluscs and thus could be present with recycled mollusc shells, and environmental conditions are likely to be suitable for their transmission throughout many parts of Australia, the risk of exposure and establishment is non-negligible, and the overall likelihood of exposure and establishment of invasive polychaetes is considered to be **High.**

4.29.7 Consequence assessment

Spionid mudworms are already present in most (if not all) regions of Australia, and *S. spallanzanii* and *E. limnicola* are also widely distributed in several harbours in the southern States, however the distribution of some of these species may be restricted to certain areas at this time, while others (e.g. *Hydroides dianthus*) remain exotic. There is evidence that heavy spionid mudworm infections have been associated with reduced growth of rock oysters, while disease outbreaks due to mudworms have been rarely reported in abalone cultured at high densities in enclosed systems. Heavy fouling by serpulid tubeworms has also been linked to mortalities of juvenile oysters in Japan as well as generating problematic biofouling that interferes with mariculture equipment (Link et al. 2009), while *S. spallanzanii* is highly invasive and changes the ecology of benthic systems by outcompeting native species. In contrast, spionid mudworms infecting mollusc shells (*Polydora* spp., *Boccardia* spp.) are more accurately described as opportunistic commensals of molluscs reared under suboptimal conditions rather than primary pathogens, and their control in cultured molluscs is relatively straightforward. Polychaetes associated with mollusc shells must also be considered potential alternative hosts for paramyxids including *Marteilia* spp. and *Marteilioides* spp. (see Section 4.12, Tables 9, 10). Shell infecting spionid mudworms are not listed by the OIE or NACA as reportable diseases, but they are listed as important pests in Victoria, SA and the NT. The free living spionids *Marenzelleria* spp. are listed as pests in QLD, SA, WA and the NT, however there is some evidence that their introduction may be beneficial for anthropogenically disturbed areas which are affected by eutrophication. The invasive sabellid *S. spallanzanii* is listed as an unwanted organism in all states, but this species as already become widely distributed throughout Australia's southern States, while *H. dianthus* is unwanted in WA and *E. limnicola* is unwanted in the NT. Taking all of these factors into consideration, the consequences of introduction of invasive polychaete species into new areas via the identified risk pathways are likely to be **Low**.

4.29.8 Risk estimation

The risk estimation for invasive polychaetes is determined by combining the likelihood of release and exposure (from Table 5) with the consequences of establishment (Table 6) to arrive at a risk estimation (Table 7). The risk estimate for invasive polychaetes exceeds the ALOP for some release pathways, suggesting that additional risk management is required for these marine pests.

Risk estimate for invasive polychaetes (including mudworms)

	Unmitigated	Desiccation			Heat		Salinity	Other
Treatment	None	3 mo.	4 mo.	6 mo.	55°C >10 min	80°C >5 min	0 ppt for >24 hrs	4% acetic acid 30 min
Combined likelihood of release and exposure	High	Neg	Neg	Neg	V low	Neg	Mod	V low
Consequences of establishment	Low	Low	Low	Low	Low	Low	Low	Low
Risk estimation	Low	Neg	Neg	Neg	Neg	Neg	Low	Neg

4.30 Invasive seaweeds

4.30.1 Invasive agent: Seaweeds are macroalgae classified within the Phyla Chlorophyta, Ochrophyta, Phaeophyceae and Rhodophyta. Species of seaweed that are listed as unwanted pests in Australia include *Caulerpa taxifolia*, *Codium fragile*, *Fucus evanescens*, *Grateloupia turuturu*, *Polysiphonia setacea*, *Sargassum muticum*, and *Undaria pinnatifida*.

4.30.2 OIE List: No **State Pest Lists:** Yes (all states) **Zoonotic:** No

4.30.3 Australia's status: Several invasive seaweeds have already been recorded in Australia, including *Caulerpa taxifolia* which is indigenous to northern Australia (NT, WA and QLD as far south as Moreton Bay), but which has been introduced into many estuaries, harbours and ports in NSW, SA and Tasmania (Murphy and Paini 2010, Wiltshire and Deveney 2017). *Codium fragile* was first detected in Tasmania in 1890 and has spread to several locations in Victoria, NSW, SA and WA (Schaffelke and Deane 2005, Provan et al. 2008, McDonald et al. 2015b), whilst *Grateloupia turuturu* was first detected in Tasmania in 2004 (Saunders and Withall 2006). *Undaria pinnatifida* (Japanese kelp) occurs in Victoria and Tasmania, and is considered an established marine pest of national significance (DAWR 2019).

4.30.4 Epizootiology

A range of species of macroalgae (also known as seaweeds) have been reported to be invasive and problematic in many parts of the world after their introduction via biofouling or ballast water of domestic or international shipping, mollusc translocations or via the aquarium trade (Lewis 1999, Mineur et al. 2007, 2008, Schaffelke and Hewitt 2007, Murphy and Paini 2010). Indeed, many arrivals of exotic seaweeds have been recorded in the immediate surroundings of mollusc farming areas (Mineur et al. 2007). The most common effect of alien macroalgae in their new receiving environments are usually changed competitive relationships resulting from overgrowth of the alien species resulting in displacement and reduced abundances/biomass of native macroalgae or seagrasses and reductions in biodiversity (Schaffelke and Hewitt 2007).

The Aquarium *Caulerpa* or "killer algae" *Caulerpa taxifolia* is a green algae (Phylum Chlorophyta) native to northern Australia (NT, WA and QLD as far south as Moreton Bay) that has proven to be highly invasive following introductions into estuaries, harbours and ports in NSW, SA and Tasmania, as well as overseas where it has invaded parts of California and large areas of the Mediterranean Sea following its introduction off the coast of Monaco in 1981 (Creese et al. 2004, Murphy and Paini 2010, Wiltshire and Deveney 2017). The most probable method of introduction of *C. taxifolia* into the Mediterranean was likely via the aquarium trade where this species is used as a decorative plant, however once introduced its relatively rapid spread in the Mediterranean was probably facilitated by short-distance transport of thallus fragments entangled in boating gear and fishing nets (Creese et al. 2004). The highest abundances of this species tend to occur in areas with highest anthropogenic activity as propagules are generated by damage (e.g. boat propellers, anchors, trampling) (Creese et al. 2004). In areas overseas where it has been introduced *C. taxifolia* tends to outcompete seagrasses and homogenise benthic ecosystems, resulting in losses of both seagrass and fisheries production (Creese et al. 2004, Murphy and Paini 2010). However, in Australia the impacts of *C. taxifolia* on seagrasses are not as severe, with research in NSW indicating *C. taxifolia* tends to grow primarily on the edges

of seagrass beds (Glasby 2013), and in areas where seagrasses are not present it may create habitat similar to native seagrasses in some regards (York et al. 2006), but it can differ from native seagrass habitats in relation to invertebrates living in the sediments (Gallucci et al. 2012, Gribben et al. 2013).

"Dead mans fingers", or *Codium fragile* is another green macroalgae (Phylum Chlorophyta) which was originally described as various, of which the subspecies *C. fragile* ssp. *tomentosoides*, originally thought to be native to Japan, is extremely invasive (Trowbridge 1998). Translocation of *C. fragile* ssp. *tomentosoides* is usually attributed to the fouling of ship hulls or on shipments of shellfish, including oysters, scallops and clams (Lewis 1999, Hewitt et al. 1999, Lyons and Scheibling 2009, McDonald et al. 2015b). Molecular analysis of sub species of *C. fragile* previously identified as native to various regions showed that the invasive *tomentosoides* strain has been colonizing new habitats across the world via hull fouling on shipping for centuries, sometimes nearly 100 years before it was noticed (Provan et al. 2008). These data suggest *C. fragile* ssp. *tomentosoides* (taxonomically more correctly called *C. fragile* ssp. *fragile*, see Provan et al. 2008), was spread into the Australasian region in the early 19th century, being first observed in New Zealand in 1841 and in Australia (Tasmania) by 1890 (Fofonoff et al. 2018). The alga has since spread rapidly around south eastern Australia and is currently known at several locations in eastern Tasmania, Victoria, NSW, SA and WA (Schaffelke and Deane 2005, McDonald et al. 2015b). Some of the negative impacts of *C. fragile* introductions include nuisance fouling of natural and artificial surfaces (particularly shipping-related infrastructure such as wharves, jetties, rip rap, and moorings), disruption of shellfish culture and clogging of fishing nets (Schaffelke and Deane 2005). The tendency of this species to overgrow and smother oyster beds has earned it the nickname "oyster thief" (McDonald et al. 2015b).

The brown algae *Fucus evanescens* (Phylum Ochrophyta) is native to coastlines near the Arctic circle including Iceland, the Faeroes Islands and northern Norway, but became established in European waters in the late 19th century and invaded the Baltic Sea in the 1950's (Schueller and Peters 1994, Wikström et al. 2002). The species rapidly spread outwards from the first known introduction, colonising harbours along European coastlines as it was translocated by hull fouling on shipping, but after the original range expansion further spread became limited and the species is still largely confined to harbour areas in the Baltic Sea (Schueller and Peters 1994). Studies in Sweden suggested that invasion of *F. evanescens* interacted with other brown algae communities (including other species of *Fucus*), however the direct effect on biodiversity is probably low (Wikström and Kautsky 2004).

The Asian seaweed or "devils tongue weed" *Grateloupia turuturu* is one of the largest known red algae (Phylum Rhodophyta) which is native to shorelines of the northwest Pacific Ocean from Vladivostok, Russia to South Korea, but has been introduced to both the west and east coasts of North America, Europe, the Mediterranean Sea and in localized regions of Australia, New Zealand and Brazil (Fofonoff et al. 2018). This species was first recorded in Australia in January 2004 near Bicheno along the east coast of Tasmania (Saunders and Withall 2006), and was found in New Zealand soon after (D'Archino et al. 2007). As *G. turuturu* has wide temperature and salinity tolerances, rapid growth and reproduction, it is considered to be a nuisance organism with a high capacity for invasiveness and ability to outcompete native macroalgae (Fofonoff et al. 2018).

Polysiphonia setacea (syn. *Womersleyella setacea*) is a red algae (Phylum Rhodophyta) that was originally described from the Hawaiian Islands, but has been introduced into many other regions including the Mediterranean Sea in the late 1980s where it formed dense, almost monospecific turfs (Rindi et al. 1999). This

species has subsequently spread widely into adjacent areas including the Adriatic Sea (Battelli and Rindi 2008), whilst closely related species such as the invasive *Polysiphonia morrowii* are known to colonise Pacific oyster (*C. gigas*) reefs (Emilia Croce and Parodi 2014). Similarly, the strangle weed or wireweed (*Sargassum muticum*) is a large brown algae (Phylum Phaeophyceae) originally native to the western Pacific Ocean from southern Russia, China Korea and Japan that was introduced into the west coast of North America and also Europe in shipments of Pacific oysters *C. gigas* from Asia (Lewis 1999, Fofonoff et al. 2018). It is another fast growing and highly fecund species with broad temperature and salinity tolerances which allow it to outcompete and displace native macroalgae (Murphy and Paini 2010). *Sargassum muticum* was first recorded from the west coast of North America in 1944 on Vancouver Island in British Columbia, a few years after translocations of *C. gigas* from Japan. By the early 2000s it had colonized most of the Pacific coast of Baja California (Fofonoff et al. 2018). In Europe, it was first collected in Southeast England in 1971 and rapidly spread north and south, reaching Norway by 1988, Spain by 1985, Venice by 1992 and Morocco by 2012 where it colonises hard surfaces in sheltered locations to depths of around 4 metres (Fofonoff et al. 2018).

The Japanese seaweed or wakame (*Undaria pinnatifida*) is another large brown macroalgae (Phylum Phaeophyceae) native to the Northwest Pacific Ocean, from Russia to the coasts of Japan, Korea, China and Hong Kong where it is cultivated for food. This species has been translocated broadly via hull fouling or ballast water and become established throughout cold-temperate and subtropical waters worldwide including the west coast of North America, Europe, the Mediterranean Sea, Argentina, New Zealand and Australia (Lyons and Scheibling 2009, South et al. 2017, Fofonoff et al. 2018). In its introduced range it fouls aquaculture equipment and boating structures and competes with native algal species from the intertidal zone down to a depth of 25 metres, reducing biodiversity in some places due to its fast growth and high reproductive output which outcompetes native species, but increasing habitat structure and diversity in others (Murphy and Paini 2010, Fofonoff et al. 2018). Japanese seaweed was first recorded in Australia in 1988 on the east coast of Tasmania then was subsequently found in western Port Phillip Bay in Victoria in 1996 (Hewitt et al. 1999, Lewis 1999). Studies suggest that *U. pinnatifida* was likely delivered to Tasmania and Port Phillip Bay in two different introduction events from international shipping via spores in ballast water or via hull-fouling (Lyons and Scheibling 2009, South et al. 2017). In Tasmania and New Zealand, *U. pinnatifida* has been observed mainly at boat ramps or boat moorings, and its domestic spread has been attributed to accidental transfer from recreational and commercial boat fleets (Lyons and Scheibling 2009, Bollen et al. 2017, South et al. 2017).

4.30.5 Release assessment

Several species of invasive seaweeds already occur in various jurisdictions throughout Australia, including *C. taxifolia* in northern Australia, NSW, SA and Tasmania, *Codium fragile* and *Undaria pinnatifida* in Tasmania and Victoria (*C. fragile* also occurs in southern NSW, SA and WA), and *Grateloupia turuturu* in Tasmania. All of these species of invasive seaweeds have been found associated with shellfish reefs or mollusc shells and several have also been translocated internationally on the shells of *C. gigas* and/or other oyster species (Mineur et al. 2007). For these reasons, there is a non-negligible risk that these unwanted pests may occur on mollusc shells that are recycled for shellfish reef restoration in Australia.

It is known that invasive seaweeds are susceptible to freshwater. For example, most invasive seaweeds of concern will not tolerate salinities below 15 ppt (O'Laughlin et al. 2006), with *Sargassum muticum* being most

tolerant remaining viable at 10 ppt (Fofonoff et al. 2018), whilst *Caulerpa taxifolia* does not tolerate 10 ppt for longer than 3 hours prior to total mortality (Theil et al. 2007). However, in contrast Yarish et al. (1979) reported that *Polysiphonia subtilissima* can survive in 5 ppt, and died only after exposure to freshwater for 3.5 days, while *Undaria pinnatifida* gametocytes survived 2 days in freshwater at 10°C and 1 day at 20°C (Forrest and Blakemore 2006).

It is known that invasive seaweeds are susceptible to desiccation. For example, 100% mortality of both plantlets and gametocytes of *Undaria pinnatifida* were recorded after 3 days at 10°C and 55-85% relative humidity, however at high relative humidity (>95%) plantlets and gametocytes of *U. pinnatifida* survived over 2 months at 10°C, and 3 weeks (plantlets) to 6 weeks (gametocytes) at 20°C (Forrest and Blakemore 2006). Bollen et al. (2017) also found that *U. pinnatifida* spores survived up to 5 days desiccation at 15°C and 59% relative humidity. *Codium fragile* can also survive periods of emersion of up to 90 days in a plastic ziplock bag in water vapour saturated air at 17.5°C and 90% relative humidity (Schaffelke and Deane 2005). However, it appears *U. pinnatifida* and *C. fragile* are relatively tolerant of desiccation compared to other species of macroalgae. For example, Sant et al. (1996) found that *C. taxifolia* survived up to 10 days emersion when kept in the dark at 18°C and high (90%) humidity, however Creese et al. (2004) found that medium and large clumps of *C. taxifolia* were all dead after 3 days desiccation in the shade with wet rope in a simulated anchor box.

Invasive seaweeds are also susceptible to hot water, as shown by the gametophytes of *Undaria pinnatifida* which are killed when exposed to water temperatures of 45°C for 45 seconds, or 55-60°C for 5 seconds (Wotton et al. 2004, Forrest and Blakemore 2006). There is also evidence that invasive seaweeds are susceptible to acetic acid. For example, Forrest et al. (2007) found that *U. pinnatifida* was highly susceptible to dips in both 2% and 4% acetic acid, with 100% mortality of all life stages after 4 minutes exposure to 2% acetic acid and 1 minute exposure to 1% acetic acid. Taking into account the information above, and given the likely propagule pressure experienced via the various anthropogenic introduction pathways, the likelihood estimations for the release of invasive seaweeds via the identified risk pathways are listed below.

Release assessment for invasive seaweeds

	Unmitigated	Desiccation			Heat		Salinity	Other
Treatment	None	3 mo.	4 mo.	6 mo.	55°C >10 min	80°C >5 min	0 ppt for >24 hrs	4% acetic acid 30 min
Likelihood of release	High	V low	Neg	Neg	Neg	Neg	Mod	Neg

4.30.6 Exposure assessment

Invasive seaweeds including *C. taxifolia*, *Codium fragile*, *Undaria pinnatifida* and *Grateloupia turuturu* have already become established in several areas of Australia, however some areas remain free of these species at this time. It is known that invasive seaweeds commonly grow on shellfish reefs and can be translocated with mollusc shell materials which means that shellfish reef restoration efforts could transport invasive seaweeds into new regions where their survival and establishment will depend on the availability of favourable environmental conditions. Once introduced into a new area, invasive seaweeds can multiply rapidly and the various species of concern exhibit a range of reproductive strategies, reproducing not only sexually but also

parthenogenetically and vegetatively via asexual reproduction (Hewitt et al. 1999, Creese et al. 2004, Murphy and Paini 2010, McDonald et al. 2015b). Furthermore, invasive seaweeds can tolerate a broad range of environmental temperatures and salinities, and often thrive in anthropogenically modified environments and it is these attributes that allow them to outcompete native species for space and successfully establish populations in new areas after translocation (Hewitt et al. 1999, Creese et al. 2004, Schaffelke and Hewitt 2007, Lyons and Scheibling 2009, Murphy and Paini 2010, Fofonoff et al. 2018). Taking these various factors into consideration, given that invasive seaweeds may be associated with shellfish reefs and aquacultured molluscs and thus could be present with recycled mollusc shells, and environmental conditions are likely to be suitable for their transmission throughout many parts of Australia, the risk of exposure and establishment is non-negligible, and the overall likelihood of exposure and establishment of invasive seaweeds is considered to be **High.**

4.30.7 Consequence assessment

Invasive seaweeds have already impacted several estuaries, bays and harbours in Australia's southern States, where there is evidence that they have displaced native species and altered ecosystem structure and function (Hewitt et al. 1999, Creese et al. 2004, Schaffelke and Hewitt 2007, Murphy and Paini 2010). Their establishment in new areas can also cause significant nuisance fouling which has adverse effects on marine infrastructure (Murphy and Paini 2010) and aquaculture (Fitridge et al. 2012, Bannister et al. 2019) and requires considerable economic investment to control (Schaffelke and Hewitt 2007). In some circumstances eradication of invasive seaweeds has been possible. For example, Glasby et al. (2005) reported how localised invasions of *Caulerpa taxifolia* in estuaries in NSW were controlled using application of sea salt at a concentration of 50 kg per square metre, which rapidly killed the seaweed and allowed native species to recolonise the affected area within 6 months. An incursion of *C. taxifolia* into the West Lakes system near Adelaide in South Australia was also effectively eradicated by pumping freshwater into the closed system until salinities were around 10 ppt, at which the seaweed could no longer survive (Schaffelke and Hewitt 2007). Wotton et al. (2004) described how *U. pinnatifida* was eradicated from a sunken trawler in the Chatham Islands in New Zealand. In this case, the vessel was treated by divers using hot (70°C) water for a minimum of 10 minutes using two methods: plywood boxes attached to the hull and with the water within them heated by elements, or an underwater flame torch (Wotton et al. 2004). It took four weeks to treat the vessel, at a cost of over $2 million AUD that was covered by vessel insurance (Wotton et al. 2004, Schaffelke and Hewitt 2007).

The rare examples where invasive seaweeds have been successfully eradicated are, however, vastly outnumbered by instances where they have become permanently established, as eradication is simply not logistically or economically feasible for the majority of invasions (Schaffelke and Hewitt 2007, Wiltshire and Deveney 2017). Nevertheless, because various species of invasive seaweed are listed as pests in all States (Table 3), their establishment in new areas within these jurisdictions could necessitate significant intervention by government authorities and disruption to normal shipping and other coastal activities if attempts were made to try to prevent their further spread into uninfected areas. Invasive seaweeds can outcompete native species due to their faster growth, reproduction and higher pollution tolerance, resulting in ecological changes to estuaries and inshore areas that in most circumstances are irreversible. Taking all of these factors into consideration, the consequences of introduction of invasive seaweed species into new areas via the identified risk pathways are likely to be **High**.

4.30.8 Risk estimation

The risk estimation for invasive seaweeds is determined by combining the likelihood of release and exposure (from Table 5) with the consequences of establishment (Table 6) to arrive at a risk estimation (Table 7). The risk estimate for invasive seaweeds exceeds the ALOP for some release pathways, suggesting that additional risk management is required for these marine pests.

Risk estimate for invasive seaweeds

	Unmitigated	Desiccation			Heat		Salinity	Other
Treatment	None	3 mo.	4 mo.	6 mo.	55°C >10 min	80°C >5 min	0 ppt for >24 hrs	4% acetic acid 30 min
Combined likelihood of release and exposure	High	V low	Neg	Neg	Neg	Neg	Mod	Neg
Consequences of establishment	High	High	High	High	High	High	High	High
Risk estimation	High	Low	Neg	Neg	Neg	Neg	High	Neg

4.31 Invasive seastars

4.31.1 Invasive agent: Seastars are invertebrates classified in the Family *Asteriidae*, Class Asteroidea within the Phylum Echinodermata. The Northern Pacific seastar (*Asterias amurensis*) is listed as an unwanted pest in all Australian states.

4.31.2 OIE List: No **State Pest Lists:** Yes (all states) **Zoonotic:** No

4.31.3 Australia's status: *Asterias amurensis* has been introduced and is now established in parts of the southern and eastern coast of Tasmania and in Port Phillip Bay Victoria, and is considered an established marine pest of national significance to Australia (DAWR 2019).

4.31.4 Epizootiology

Echinoderms occur in marine waters worldwide, and several species within this group have been translocated internationally by human activities (O'Hara 1999). Their benthic lifestyle tends not to favour the distribution of echinoderms via hull fouling on modern shipping, however the wooden shipping in previous centuries was highly likely to distribute live adult echinoderms (particularly seastars), as do sea chests on modern shipping today (O'Hara 1999). Furthermore, planktonic echinoderm larvae are sometimes found in ballast water, and translocations of live oysters or other bivalves for fisheries or aquaculture may also transport cryptogenic echinoderm juveniles (O'Hara 1999, Dommisse and Hough 2004).

One very well studied invasive echinoderm is the Japanese seastar (*Asterias amurensis*), a large seastar (up to 50 cm diameter) that is native to the north west Pacific Ocean along the temperate coastlines of Russia, China, Korea and Japan (Murphy and Paini 2010). In its native range it is usually found in intertidal and subtidal zones that are protected from wave action, but has also been recorded offshore at a depth of 200 metres. A voracious predator of other invertebrates (including bivalves, gastropods, crabs, barnacles, ascidians, bryozoans, other echinoderms and dead fishes), *Asterias amurensis* is considered a major pest for the shellfish farming industries within its native range (Hatanaka and Kosaka 1959), where it is in turn predated upon by other seastar and Alaskan king crabs (Murphy and Paini 2010). When translocated into new areas where it is released from its natural predators, *A. amurensis* has proven to be highly invasive and has outcompeted and displaced native echinoderms, significantly impacted bivalve populations and adversely affected ecological processes, fisheries and aquaculture industries (Ross et al. 2004, 2006, Aquenal 2008b).

The Japanese seastar was probably introduced into Australia sometime in the 1980's as it was first observed in the Derwent estuary in south east Tasmania in 1986, but was mis-identified as a native seastar species (*Uniophora granifera*) until 1992 when concerns arose about buildup of its numbers near wharves in Hobart (Goggin 1998, Ross et al. 2006, Murphy and Paini 2010). The vector for introduction of *A. amurensis* into Australian waters is thought to be either via planktonic larvae introduced with ballast water, or juveniles or adults introduced in the sea chests of international shipping from Japan (Goggin 1998, O'Hara 1999). Ballast water introduction is favoured by the prolonged duration (up to 120 days at 10-12°C) of the various planktotrophic developmental stages including coeloblast, gastrula, bipinnaria and brachiolaria larvae (Bruce et al. 1995, Goggin 1998). Populations of *A. amurensis* increased rapidly in the first 15 to 20 years after its introduction, with population densities approaching 10 juveniles and adults per square metre at some locations

(Bruce et al. 1995). Attempts to manually remove adult seastar from around Hobart wharves in 1993 resulted in removal of many tens of thousands of seastar, with no apparent effect on the population (Goggin 1998, Aquenal 2008b). Larval densities in the plankton peaked during the late winter months during a prolonged winter/spring spawning period and exceeded 1100 larvae per cubic metre, the highest ever recorded for seastar larvae (Bruce et al. 1995). The distribution of *A. amurensis* spread into adjacent areas along the coast of south east Tasmania, probably by larval dispersal but also potentially through translocation of cryptogenic juveniles in fishing gear or during aquaculture translocations (e.g. scallop spat bags, mussel ropes or oyster movements) (Bruce et al. 1995, Goggin 1998, Dommisse and Hough 2004). A mature *A. amurensis* was then found in Port Phillip Bay, Victoria by scallop dredgers in August 1995 (Goggin 1998, O'Hara 1999), whilst the first juvenile *A. amurensis* were observed in Port Phillip Bay on grow out ropes in a mussel farm in January 1998 (Goggin 1998). The species is now well established in Port Phillip Bay with populations of nearly 100 million recorded by the year 2000 (Parry and Cohen 2001), and several million individuals still present today (DAWR 2019). Genetic studies suggested that the invasion of Port Phillip Bay originated from *A. amurensis* populations in Tasmania, possibly through ballast water or translocation of live adults in the sea chests of domestic shipping (O'Hara 1999).

The introduction of *A. amurensis* affected the benthic ecology of affected areas by becoming the dominant invertebrate predator in the Derwent River ecosystem, depleting native bivalve populations and also causing significant nuisance fouling and predation of commercially important bivalve shellfish in southeast Tasmania (Goggin 1998, Ross et al. 2004, 2006). Predation by *A. amurensis* has resulted in significant stock losses in Tasmanian scallop farms (Dommisse and Hough 2004). Furthermore, in this part of Tasmania, the combined effect of predation by European shore crab *C. maenas* and *A. amurensis* on wild and commercially reared bivalves is thought to be more detrimental to their populations than the impacts of either predator alone (Ross et al. 2004).

4.31.5 Release assessment

The Japanese seastar is now established in parts of the southern and eastern coast of Tasmania and in Port Phillip Bay Victoria (DAWR 2019). Analysis based on the reported reproductive temperature tolerances of *A. amurensis* larvae (ranging from 5-10ºC to 17-23ºC) together with estimates of the maximum temperatures survivable by juveniles and adult seastars (23-25ºC) suggested that *A. amurensis* represents an invasive threat along the entire southern coastline of Australia (Summerson et al. 2007, DAWR 2019). It is known that *A. amurensis* has been found associated with aquacultured bivalves and this species could potentially be translocated during movements of oysters (Bruce et al. 1995, Goggin 1998, Dommisse and Hough 2004). For these reasons, there is a non-negligible risk that these unwanted pests may occur in molluscs that are recycled for shellfish reef restoration in Australia.

It is known that *A. amurensis* does not tolerate freshwater, with adult seastars experiencing mortality when they are exposed to freshwater for over 2 hours (Marine Pest Sectoral Committee 2015), while larval stages do not tolerate water below 9 ppt with 100% mortality occurring in less than 2 minutes (Goggin 1998). Adult *A. amurensis* are moderately tolerant of desiccation, with survival rates dropping to around 40% after 48 hours emersion (Marine Pest Sectoral Committee 2015). It is known that *A. amurensis* larvae do not tolerate water temperatures above 26°C (Goggin 1998), which suggests adult seastars are likely to be susceptible to hot water and unlikely to survive at water temperatures above 50°C for long periods, however the exact details of the

susceptibility of *A. amurensis* to elevated water temperatures have apparently not been determined. Similarly, it is likely that *A. amurensis* is susceptible to acetic acid (Marine Pest Sectoral Committee 2015), however unfortunately it appears the resistance of *A. amurensis* to acetic acid applied externally has not been determined, although it is known that seastars are susceptible to vinegar administered internally via injection (Moutardier et al. 2015). Taking into account the information above, and given the likely propagule pressure experienced via the various anthropogenic introduction pathways, the likelihood estimations for the release of invasive seastars such as *A. amurensis* via the identified risk pathways are listed below.

Release assessment for invasive seastars

	Unmitigated	Desiccation			Heat		Salinity	Other
Treatment	None	3 mo.	4 mo.	6 mo.	55°C >10 min	80°C >5 min	0 ppt for >24 hrs	4% acetic acid 30 min
Likelihood of release	High	Neg	Neg	Neg	E low?	Neg	Neg	E low?

4.31.6 Exposure assessment

The Japanese seastar has already become established in several areas of Tasmania and Port Phillip Bay, however many areas of southern Australia including South Australia, southern WA and southern NSW remain free of this species at this time despite conditions being suitable for its establishment (Summerson et al. 2007, DAWR 2019). It is known that *A. amurensis* is often found associated with aquacultured bivalves and its settled larvae and juveniles can be translocated with mollusc shell materials, which means that shellfish reef restoration efforts could transport *A. amurensis* into new regions where its survival and establishment will depend on the availability of favourable environmental conditions. *Asterias amurensis* is capable of both sexual and asexual reproduction and the seastar is also capable of regeneration from damaged body parts (Goggin 1998, Murphy and Paini 2010). Female seastars are capable of producing 10-25 million eggs per year, and the various larval stages can remain in the water column for about 120 days (Bruce et al. 1995), meaning that newly introduced populations of *A. amurensis* can quickly increase in size, outcompete native species for space and successfully establish populations in new areas after translocation (Goggin 1998, Ross et al. 2004, 2006). Taking these various factors into consideration, given that *A. amurensis* may be associated with shellfish reefs and aquacultured molluscs and thus could be present with recycled mollusc shells, and environmental conditions are likely to be suitable for its establishment throughout many parts of southern Australia, the risk of exposure and establishment is non-negligible, and the overall likelihood of exposure and establishment of *A. amurensis* is considered to be **High.**

4.31.7 Consequence assessment

The Japanese seastar has already impacted several estuaries, bays and harbours in Tasmania and Port Phillip Bay where there is evidence that it has displaced native species and altered ecosystem structure and function. The *A. amurensis* populations in the Derwent Estuary in Tasmania and Port Phillip Bay in Victoria have fluctuated from their initial peaks, but large populations have remained, and the invasive aggregations have maintained a large larval pool (DAWR 2019). By consuming a large quantity of bivalve species, which are functionally important on native assemblages, their presence in these locations has altered food web dynamics and reduced abundance and recruitment of native species (DAWR 2019). Given their high fecundity and

flexible reproductive strategies, eradication of *A. amurensis* populations requires their complete removal from the infested area (DAWR 2019). Eradication was not possible in Tasmania or Port Phillip Bay, and experience has shown attempts at eradication are unlikely to be successful if initial investigations determine that the species is widespread, cannot be contained, and is present in open coastal environments (Dommisse and Hough 2004, Marine Pest Sectoral Committee 2015). Nevertheless, because *A. amurensis* is listed as a notifiable pest in all States (Table 3), the establishment of this species in new areas within these jurisdictions could necessitate significant intervention by government authorities and disruption to normal shipping and other coastal activities if attempts were made to try to prevent its further spread into uninfected areas. *Asterias amurensis* can outcompete native species due to its fast growth and high reproductive output, resulting in ecological changes to estuaries and inshore areas that in most circumstances are irreversible. Taking all of these factors into consideration, the consequences of introduction of invasive seastars such as *A. amurensis* into new areas via the identified risk pathways are likely to be **High**.

4.31.8 Risk estimation

The risk estimation for invasive seastars is determined by combining the likelihood of release and exposure (from Table 5) with the consequences of establishment (Table 6) to arrive at a risk estimation (Table 7). The risk estimate for invasive seastars exceeds the ALOP for some release pathways, suggesting that additional risk management is required for these marine pests.

Risk estimate for invasive seastars

	Unmitigated	Desiccation			Heat		Salinity	Other
Treatment	None	3 mo.	4 mo.	6 mo.	55°C >10 min	80°C >5 min	0 ppt for >24 hrs	4% acetic acid 30 min
Combined likelihood of release and exposure	High	Neg	Neg	Neg	E low?	Neg	Neg	E low?
Consequences of establishment	High	High	High	High	High	High	High	High
Risk estimation	High	Neg	Neg	Neg	V low?	Neg	Neg	V low?

4.32 Invasive tunicates/ ascidians/ hydroids

4.32.1 Invasive agent: Tunicates and ascidians are invertebrates classified in the Class Ascidiacea Orders Aplousobranchia, Enterogona, Stolidobranchia, within the Phylum Chordata, whilst hydroids are classified within the Phylum Cnidaria, Class Hydrozoa. Species of tunicates that are invasive such that they are listed as unwanted pests in various Australian states include *Ciona intestinalis*, *Didemnum* spp. (exotic species, including *D. vexillum*), and *Styela clava*, while the hydroid *Blackfordia virginica* is listed as an unwanted pest in Western Australia.

4.32.2 OIE List: No **State Pest Lists:** Yes (QLD, NSW, Tas, SA, WA, NT) **Zoonotic:** No

4.32.3 Australia's status: The European seasquirt *Ciona intestinalis* is a common fouling organism in virtually all Australian ports, however this species may be declining in several of the locations where it was once common (Kott 1990, McDonald 2004). The clubbed tunicate *Styela clava* was first recorded in Australia in Hobsons Bay, Port Phillip Bay, Victoria in 1972, and has also been recorded in Sydney Harbour (Hewitt et al. 1999, DAWR 2019). Exotic species of *Didemnum* (e.g. *D. perlucidum*) have also been reported in WA (Munoz and McDonald 2014, Wells 2018).

4.32.4 Epizootiology

Tunicates are a group of sac-like sessile marine invertebrates classified in the Class Ascidiacea which are commonly known as 'sea squirts". Adult sea squirts possess by a tough leathery body wall or "tunic" made of cellulose materials and are common fouling organisms that settle (often at high densities) on hard underwater surfaces such as rocks, floats, pylons, or the hulls and sea chests of shipping as they feed by filter feeding on phytoplankton, zooplankton and organic materials (Murphy and Paini 2010). Sea squirts naturally occur on coastlines with low to moderate wave energies and have a relatively short lived (1-2 weeks) planktonic larval dispersal stage which tends to limit their natural dispersal (Locke 2009, Murphy and Paini 2010). However, some species have wide environmental tolerances, are highly fecund and are notorious for heavily fouling vessels, bivalves, aquaculture and fishing equipment and other artificial structures, and because of these attributes these species have been found to be highly invasive (Lambert 2009, Locke 2009).

Colonial sea squirts of the genus *Didemnum* (including *Didemnum vexillum*) are ascidians classified within the Order Aplousobranchia which are well known to be highly invasive (Locke 2009, Murphy and Paini 2010). A large number of species of *Didemnum* spp. are native to Australia which makes identification of exotic *Didemnum* spp. somewhat problematic to non-specialists (DAWR 2019), however some exotic *Didemnum* spp. (e.g. *Didemnum perlucidum* in the Swan River, and Pilbara, WA) have been detected when specialists examine the sea squirt fauna of ports and harbours where international shipping is concentrated (e.g. Munoz and McDonald 2014, Wells 2018). The "sea vomit" or "sea cheese" *Didemnum vexillum* is a colonial seasquirt probably native to Japan that has been introduced widely around the world including Europe, the Mediterranean Sea, both coasts of North America and New Zealand, where it was first recorded in 2001 and described in 2002 (Lambert 2009, Locke 2009). Lambert (2009) ruled out translocation of *D. vexillum* from Japan into many countries with Pacific oysters (*C. gigas*) during the 1950's and 1960's because there were no reports of sudden didemnid ascidian appearances prior to the 1970s. Instead, Lambert (2009) suggested

international introductions of *D. vexillum* (including to the type locality in New Zealand) are more likely from international shipping (either via hull or sea chest fouling), with subsequent local spreading by domestic vessels, drifting and reattachment of dislodged fragments, and movements of fouled aquaculture stock and gear. *Didemnum vexillum* develops carpet-like colonies over all types of hard surfaces, particularly artificial structures, as well as seagrass and bivalves down to depths of around 80 metres (Murphy and Paini 2010). It has proven to be highly invasive and outcompetes native benthic invertebrates for space, covering 50-90% of over 230 square km of bottom on offshore banks off the east coast of North America since establishing there in the early 1980's, where it has also become highly problematic overgrowing and smothering aquacultured oysters and mussels (Lambert 2009, Rolheiser et al. 2012, Ferguson et al. 2017). In New Zealand during the summer months *D. vexillum* requires less than 14 days to smother and kill greenlip mussels (*Perna canaliculus*) cultured on longlines (Murphy and Paini 2010). *Didemnum vexillum* can tolerate a wide range of water temperatures (-2°C to over 24°C) and salinities (20-36 ppt indefinitely), and survives daily 2 hour exposures to 10 ppt salinity for at least 2 weeks (Gröner et al. 2011). It can also reproduce sexually whenever water temperatures exceed around 13°C or asexually with new colonies produced through asexual budding and fragmentation (Fletcher et al. 2013). Because *D. vexillum* broods its embryos and the lecithotrophic tadpole larvae are free-swimming for only a few hours (maximum 36 hours), natural planktonic dispersal of *D. vexillum* is limited to less than 1 km (Fletcher et al. 2013). However, once larvae settle onto the substrate new colonies grow quickly by asexual reproduction via budding and wherever this species has been introduced it grows extremely rapidly, quickly covering large areas (hundreds of square metres) and overgrowing almost every other sessile species (Lambert 2009).

The European sea squirt *Ciona intestinalis* is a large (up to 15 cm) cylindrically shaped solitary ascidian classified within the Order Enterogona that is native to the shorelines of Europe, but has been spread worldwide by biofouling on shipping (Kott 1990). This species was first recorded in Australia in the 1870's from Sydney Harbour then in Port Phillip Bay in 1958 (Hewitt et al. 1999). *Ciona intestinalis* has since been recorded from virtually all Australian ports, including upstream estuarine and brackish water areas at depths of less than 10 metres (Hewitt et al. 1999), however there is evidence that this species may be declining in several of the locations where it was once common (Kott 1990, McDonald 2004). *Ciona intestinalis* is a known problematic biofouling organism of cultured shellfish in areas of Europe and North America (Carver et al. 2003), however it has been found that *D. vexillum* will outcompete and smother *Ciona intestinalis* in areas where both occur (see Murphy and Paini 2010). The clubbed tunicate (*Styela clava*) is a relatively large (16 cm) club shaped solitary ascidian classified within the Order Stolidobranchia with a short (<7 cm) stalk that is native to coastlines of the north west Pacific Ocean including Russia, China, Korea and Japan (Hewitt et al. 1999, Murphy and Paini 2010, DAWR 2019). As a fouling species, it is common on artificial surfaces and mussel lines in low wave energy environments and sheltered embayments up to 25 metres depth where it can grow to densities of up to 1500 individuals per square metre, overgrow aquacultured bivalves and outcompete native benthic invertebrates for space (Swan 2006, Murphy and Paini 2010). *Styela clava* was first recorded in Australia in 1972 in Port Phillip Bay, and later spread to Sydney Harbour (Hewitt et al. 1999, DAWR 2019). It is a hardy species, capable of withstanding a wide range of water temperatures (2-23°C) and salinities between 20 and >36 ppt and is a problematic fouling organism on vessels, aquaculture and fishing equipment and other artificial structures (Murphy and Paini 2010).

Hydroids are classified within the Phylum Cnidaria, Class Hydrozoa, and the Black Sea jellyfish *Blackfordia virginica* is a colonial hydroid with a conspicuous (1.5-2.2 cm diametre) dome shaped planktonic medusa stage and a small (0.5 mm polyps on stalks around 2 mm long) cryptic hydroid stage (Fofonoff et al. 2018).

The native range of *B. virginica* is unclear, but is thought to be the Black Sea, however it was first described from Virginia on the east coast of North America in 1904 (Fofonoff et al. 2018). This species has been introduced into estuarine environments in many parts of the world including Europe, the Caspian Sea, South Africa, Mexico, South America, China, India and the east and west coasts of the United States (Fofonoff et al. 2018). The short stalked polyps of *B. virginica* grow on aquatic vegetation and the undersides of hard substrates, can tolerate a wide range of water temperatures (3-30°C) but have only been reported from estuarine (2-22 ppt) habitats (Fofonoff et al. 2018), however the planktonic medusae can tolerate full strength seawater (36 ppt) and have been found in coastal waters where their feeding can be associated with a reduction in density of all zooplankton species (Murphy and Paini 2010). The predominate mode of translocation for *B. virginica* is uncertain but is likely to be associated with shipping, due to fact that most invaded areas are near shipping ports (Fofonoff et al. 2018).

4.32.5 Release assessment

Several species of invasive tunicates and ascidians occur in parts of southern Australia where they are common biofouling agents associated with aquacultured bivalves, however species such as *D. vexillum*, other non-native *Didemnum* spp. and *B. virginica* remain exotic to Australia at this time. There are also many examples of the spread of invasive tunicates and ascidians with movements of oysters and mussels (Denny 2008, Lambert 2009, Rolheiser et al. 2012, Ferguson et al. 2017). For these reasons, there is a non-negligible risk that these unwanted pests may occur in mollusc shells that are recycled for shellfish reef restoration in Australia.

There is evidence that some tunicates and ascidians do not tolerate freshwater. For example, Denny (2008) found that 10 minute dips in freshwater were insufficient to kill *Didemnum vexillum*, however Katayama and Ikeda (1987) demonstrated that *Didemnum moseleyi* (later confirmed as *D. vexillum* by Lambert 2009) exposed to freshwater survived for up to 2 hours in winter, but began to die after 15 minutes in summer. Juvenile and adult *Styela clava* are more resistant, but are unable to survive in freshwater for longer than 3 hours (Ramsay 2015a), which is similar to the case with juvenile and adult *Ciona intestinalis* which also experienced 100% mortality after 3 hours in freshwater (Ramsay 2015b). However, in contrast the colonial hydroid *Blackfordia virginica* can tolerate very low salinities (2 ppt) for long periods (Fofonoff et al. 2018). It is known that invasive tunicates, ascidians and cnidarians are all susceptible to desiccation. For example, Katayama and Ikeda (1987) noted mortality in *D. vexillum* after 5 hours or more of air drying in direct sunlight, although the magnitude of the effect was dependent on ambient temperature. Hopkins et al. (2016) found that adult ascidians (*Ciona* spp.) experienced 100% mortality after 24 hours desiccation in the shade at an average air temperature of 20.3°C, or in as little as 8 hours when placed in direct sunlight. Hillock and Costello (2013) found that exposure of *Styela clava* adults to direct sunlight resulted in mortality within 24 hours, but that survival was longer in the shade when the air temperature was cooler, whilst larger individuals of *S. clava* generally survived for longer out of seawater than smaller individuals, such that 2 weeks air exposure at 10°C was recommended to provide sufficient safety margin for complete eradication of *S. clava*.

Resistance of invasive tunicates and ascidians to heated seawater has been examined. Carver et al. (2003) found a 1 minute exposure of adult *Ciona intestinalis* to seawater heated to 40°C resulted in 100% mortality. Piola and Hopkins (2012) exposed *Ciona* spp. and *D. vexillum* to heated seawater in a simulated sea chest and found exposures to 37.5°C for 60 minutes, 40°C for 30 minutes and 42.5°C for 20 minutes all resulted in 100% mortality of both species. Sievers et al. (2019) exposed *Styela clava* and *C. intestinalis* to hot water and

found *C. intestinalis* was more susceptible, experiencing 100% mortality at 40°C for 1 minute, or 50°C for 10 seconds, whilst 100% mortality of *S. clava* required 60°C for at least 30 seconds.

It is also known that invasive tunicates and ascidians are sensitive to acetic acid. Denny (2008) exposed *D. vexillum* to short (3 seconds to 10 minute) dips in 0.1 to 10% acetic acid and found that while mortalities up to 80% were observed, no treatment consistently resulted in 100% mortality (except for dipping *D. vexillum* into 0.5% bleach for 20 seconds). However, Rolheiser et al. (2012) found that *D. vexillum* was controlled by 10 minute dips in 5% acetic acid. Seivers et al. (2019) exposed *Styela clava* and *C. intestinalis* to 2% and 5% acetic acid solutions and recorded 100% mortality in *C. intestinalis* exposed to 2% acetic acid for 1 minute or 5% acetic acid for 10 seconds at ambient temperature (20°C), whilst *S. clava* was more tolerant and 100% mortality was observed only when *S. clava* were exposed to 2% or 5% acetic acid for 1 minute at water temperatures 40°C or higher. There is limited information available on the ability of *B. virginica* to tolerate desiccation, hot water or acetic acid, however given the relatively small size of the polyps of this species, it is likely to be even more susceptible to these treatments than are *Stylea*, *Ciona* and *Didemnum*. Taking into account the information above, and given the likely propagule pressure experienced via the various anthropogenic introduction pathways, the likelihood estimations for the release of invasive tunicates, ascidians and hydroids via the identified risk pathways are listed below.

Release assessment for invasive tunicates / ascidians / hydroids

	Unmitigated	Desiccation			Heat		Salinity	Other
Treatment	None	3 mo.	4 mo.	6 mo.	55°C >10 min	80°C >5 min	0 ppt for >24 hrs	4% acetic acid 30 min
Likelihood of release	High	Neg	Neg	Neg	E low	Neg	V low	E low

4.32.6 Exposure assessment

Invasive tunicates and ascidians have already become established in several areas of Australia, however some areas remain free of these species at this time and other species of ascidians and hydroids remain exotic. It is known that invasive hydroids commonly overgrow shellfish and aquaculture equipment (Denny 2008, Ferguson et al. 2017) and thus can be translocated with mollusc shell materials, which means that shellfish reef restoration efforts could transport them into new regions where their survival and establishment will depend on the availability of favourable environmental conditions. All of the invasive tunicates, ascidians and hydroids of concern can tolerate a wide range of environmental conditions and can multiply rapidly using a range of sexual and asexual reproductive strategies (Lambert 2009, Locke 2009, Gröner et al. 2011, Fletcher et al. 2013), and it is these attributes that allow them to outcompete native species for space and successfully establish populations in new areas after translocation (Hewitt et al. 1999, Lambert 2009, Murphy and Paini 2010, Fofonoff et al. 2018). Taking these various factors into consideration, given that invasive tunicates, ascidians and hydroids may be associated with shellfish reefs and aquacultured molluscs and thus could be present with recycled mollusc shells, and environmental conditions are likely to be suitable for their transmission throughout many parts of Australia, the risk of exposure and establishment is non-negligible, and the overall likelihood of exposure and establishment of invasive tunicates, ascidians and hydroids is considered to be **High.**

4.32.7 Consequence assessment

Invasive tunicates and ascidians have already impacted several estuaries, bays and harbours in Australia's southern States, where there is evidence that they have displaced native species and altered ecosystem structure and function (Hewitt et al. 1999, Murphy and Paini 2010), although in the case of the long established European seasquirt (*Ciona intestinalis*), there is evidence that it may be declining in several locations (Kott 1990, McDonald 2004). On the other hand, one of the worst ascidian invaders, namely *Didemnum vexillum*, remains exotic to Australia at this time. Once *D. vexillum* was introduced into New Zealand it spread quickly and is now considered a significant threat to benthic ecological processes and mussel and salmon aquaculture (Forrest and Hopkins 2013). The extremely effective reproductive strategies of *D. vexillum* has meant that its eradication is generally not economically or logistically feasible unless the introduction is detected very early in a new location and immediate action is undertaken (Lambert 2009, Forrest and Hopkins 2013, Fletcher et al. 2013). Nevertheless, because various species of invasive tunicates, ascidians and hydroids are listed as pests in various States (Table 3), their establishment in new areas within these jurisdictions could necessitate significant intervention by government authorities and disruption to normal shipping and other coastal activities if attempts were made to try to prevent their further spread into uninfected areas. Species such as *Didemnum* spp. and *Stylea* spp. can also act as mechanical vectors for listed disease agents such as *Haplosporidium nelsoni* (see Messerman and Bowden 2016, Tables 9, 10). The establishment of exotic tunicates, ascidians and hydroids into new areas can thus cause significant economic damage, but they can also outcompete native species for space due to their faster growth, resulting in ecological changes to ecosystems that in most circumstances are irreversible. Taking all of these factors into consideration, the consequences of introduction of invasive tunicates, ascidians and hydroids into new areas via the identified risk pathways are likely to be **High** based on ecological damage alone, but could be even higher if notifiable diseases were introduced with them, as described in Section 4.9.

4.32.8 Risk estimation

The risk estimation for invasive tunicates, ascidians and hydroids is determined by combining the likelihood of release and exposure (from Table 5) with the consequences of establishment (Table 6) to arrive at a risk estimation (Table 7). The risk estimate for invasive tunicates, ascidians and hydroids exceeds the ALOP for some release pathways, suggesting that additional risk management is required for these marine pests.

Risk estimate for invasive tunicates / ascidians / hydroids

	Unmitigated	Desiccation			Heat		Salinity	Other
Treatment	None	3 mo.	4 mo.	6 mo.	55°C >10 min	80°C >5 min	0 ppt for >24 hrs	4% acetic acid 30 min
Combined likelihood of release and exposure	High	Neg	Neg	Neg	E low	Neg	V low	E low
Consequences of establishment	High	High	High	High	High	High	High	High
Risk estimation	High	Neg	Neg	Neg	V low	Neg	Low	V low

4.33 Invasive whelks/ gastropods/ limpets/ chitons

4.33.1 Invasive agent: Whelks, gastropods, limpets and chitons are invertebrates classified in the Classes Gastropoda and Polyplacophora in the Phylum Mollusca. Species of whelks, gastropods, limpets and chitons that are invasive such that they are listed as unwanted pests in various Australian states include *Chiton glaucus*, *Crepidula fornicata*, *Maoricolpus roseus*, and *Rapana venosa*.

4.33.2 OIE List: No **State Pest Lists:** Yes (all states) **Zoonotic:** No

4.33.3 Australia's status: The New Zealand screw shell (*Maoricolpus roseus*) has been introduced into Tasmania, Victoria, NSW, SA and QLD (Probst and Crawford 2008), with the original introductions into Tasmania probably occurring in the 1920's or 1930s following translocations of oysters from New Zealand (Greenhill 1965, Bax et al. 2003). The New Zealand chiton (*Chiton glaucus*) also occurs in south eastern Tasmania, and was possibly introduced in the same manner (Kershaw 1955).

4.33.4 Epizootiology

Marine gastropods and chitons (Phylum Mollusca) are common inhabitants of biofouling assemblages and because of this, several species have been reported to be invasive and problematic in many parts of the world after their introduction via biofouling or through translocations of oysters (Murphy and Paini 2010). One invasive gastropod is the American slipper limpet (*Crepidula fornicata*) which is a large (up to 5 cm) limpet-like snail native to the east coast of North America that was introduced into Britain in the late 19th century with translocations of American oysters (*Crassostrea virginica*) (see Blanchard 1997). In the early 20th century *C. fornicata* was moved together with oysters into Puget Sound on the west coast of North America, and also into several countries in Europe (Blanchard 1997). In France before World War II, only a few *C. fornicata* were observed in the oyster beds of South Brittany and Charentes, however in 1949 many more were collected on mussel and scallop beds in Normandy and elsewhere, suggesting more introductions via hull fouling from Britain or directly from North America during Allied shipping operations (Blanchard 1997). A second wave of translocations of *C. fornicata* into Europe occurred during the 1970's with the introduction of Pacific oyster (*Crassostrea gigas*) spat from Japan and the west coast of the USA (Blanchard 1997, Moulin et al. 2007). The American slipper limpet has also been translocated to Japan and South America, possibly in ships ballast water (Murphy and Paini 2010). This species is considered a serious pest of oyster and mussel aquaculture in Europe, the Mediterranean and North America, with dense aggregations of *C. fornicata* reducing growth and survival of mussels, as the gastropods form dense semi-permanent stacks in which male slipper limpets attach to the shells of larger females (Murphy and Paini 2010). These piles of limpets (which can be stacked up to 12 individuals high, Fofonoff et al. 2018) extend into the water column modifying benthic habitat and hydrodynamics, often totally colonising the sediment surface (Moulin et al. 2007). *Crepidula fornicata* reproduces prolifically and large numbers of slipper limpets (sometimes comprising more than 80% of total benthic community in terms of biomass) can make the substrate unsuitable for bivalve spat settlement, alter sediment characteristics by trapping suspended silt, faeces and pseudofaeces which alters benthic biodiversity and bio-geochemical cycles and transforms soft substrate to hard-shelled substrate (Murphy and Paini 2010). It is tolerant of a wide range of water temperatures (0-29°C) and salinities (15-40 ppt) (O'Loughlin et al. 2006, Fofonoff et al. 2018).

The Asian rapa whelk (*Rapana venosa*) is a relatively large (up to 17 cm) gastropod native to shorelines of the north west Pacific Ocean including Russia, China, Taiwan, and Japan (ICES 2004). It has been introduced into Europe including the Black Sea (by the 1940s), Adriatic and Mediterranean Seas (by the 1980's), the Atlantic coast of North America (by 1998), and South America (by the late 1990's), probably as part of the biofouling fauna on shipping, ballast water or as a cryptic passenger during oyster translocations (Mann and Harding 2000, ICES 2004, Fofonoff et al. 2018). In its introduced range it has proven to be a voracious predator responsible for declines in populations of shellfish of commercial value as it feeds on oysters, mussels, clams and scallops (Savini and Occhipinti-Ambrogi 2006, Murphy and Paini 2010, Fofonoff et al. 2018). *Rapana venosa* outcompetes native species due to its high rate of reproduction, fast growth and tolerance to low salinity, water pollution, oxygen deficiency and habitat disturbance (Zolotarev 1996, Munari and Mistri 2011, Harding and Mann 2016). Dispersal of *R. venosa* larvae via ballast water is also likely as it has a prolonged planktonic larval phase (14-80 days) (Murphy and Paini 2010). In its native range *R. venosa* demonstrates tolerance to a wide range of water temperatures (4-27°C), while in other parts of the world where it has been introduced its tolerance of a narrower range of salinities (12-39 ppt) restricts its potential invasive distribution (Mann and Harding 2003, ICES 2004). Predation of juvenile *R. venosa* by larger species of native crabs, such as blue crabs (*Callinectes sapidus*), may impart some degree of control over invasive populations (Harding 2003), as may female reproductive deformities (imposex) caused by the pollutant tributyltin (TBT) (Harding et al. 2017).

The New Zealand screw shell (*Maoricolpus roseus*) is a large (9 cm long, 2.5 cm wide) gastropod with a conical shell that is native to New Zealand where it is found burrowing in and on sediments on sandy or muddy bottoms from the intertidal zone to depths of up to 150 metres (Bax et al. 2003, Murphy and Paini 2010). In its natural range this species can occur at very high densities with over 2300 individuals per square metre being recorded in Otago Harbour (Rainer 1981). In the 1920's *M. roseus* was not found during extensive dredging of the D'Entecastreaux channel south of Hobart in Tasmania, but by 1964 it was very common in the same area (Greenhill 1965) where today it can reach densities of up to 1500 individuals per square metre (Probst and Crawford 2008). Greenhill (1965) provided evidence that *M. roseus* was probably introduced into Tasmania in the 1930s as specimens had been collected in the late 1930's and in the 1940's by scallop fishermen, whilst other authors have suggested the introduction occurred in dry ballast or during shipments of bluff oysters (*Ostrea chilensis*) and rock oysters (*Saccostrea glomerata*) from New Zealand which were held for sale at the Hobart Fish Market during the 1920's to the late 1930's, where they were kept alive in crates in the water (Bax et al. 2003). The New Zealand screw shell has since been recorded in a wide area throughout eastern Tasmania, Bass Strait, Victoria, SA, NSW and northwards to southern Queensland (Bax et al. 2003, Probst and Crawford 2008). It is not clear whether its spread into Australia's mainland states was due to natural larval dispersal (in some areas against prevailing currents) (Probst and Crawford 2008), or whether its spread was facilitated by other vectors such as fishing vessels and dredges (Bax et al. 2003). In Australia *M. roseus* lives on similar substrates as it does in its native range, at water temperatures between 8-20°C, feeding on suspended particulate matter which it filters from the water column (Murphy and Paini 2010). Studies conducted in Tasmania found that *M. roseus* competes directly with commercial scallops (*Pecten fumatus*) for space at low scallop densities, and for both food and space at high scallop densities (Reid et al. 2018).

The New Zealand chiton (*Chiton glaucus*) is a moderately sized (5 cm) chiton that is abundant throughout most of its natural range in New Zealand where it is found under intertidal rocks in both the North and South Islands and Stewart Island (Murphy and Paini 2010). During the day *C. glaucus* remains hidden under boulders, but at night large numbers emerge and move about along the tops of rocky reefs, where they can

withstand emersion periods of around 50% of the time due to their low rate of oxygen consumption (Murphy and Paini 2010). Chitons are common inhabitants of oyster reefs and the introduction of *C. glaucus* into south eastern Tasmania probably occurred in the 1920's via oyster translocations (Liversage and Kotta 2019) in the same manner as the New Zealand screw shell was introduced (Kershaw 1955, Bax et al. 2003). In south eastern Tasmania *C. glaucus* is now one of the most conspicuous chiton species found in the low intertidal zones (Kershaw 1955), however there are no studies of the impacts of *C. glaucus* in its introduced range (Murphy and Paini 2010), and there are indications that the impacts of non-native herbivorous chitons on native benthic fauna may be limited (Liversage and Kotta 2019).

4.33.5 Release assessment

Some species of invasive gastropods (*Maoricolpus roseus*) and chitons (*Chiton glaucus*) occur in parts of southern Australia where they are commonly associated with shellfish reefs or aquacultured molluscs, however other species such as *Crepidula fornicata* and *Rapana venosa* remain exotic to Australia at this time. However, there are several examples of spread of invasive gastropods and chitons with movements of oysters (Blanchard 1997, Bax et al. 2003, Fofonoff et al. 2018, Liversage and Kotta 2019). For these reasons, there is a non-negligible risk that these unwanted pests may occur with mollusc shells that are recycled for shellfish reef restoration in Australia.

There is evidence that some marine gastropods and chitons can tolerate short term exposure to low salinities. For example, Mann and Harding (2000, 2003) found that 2.3% of larvae of *Rapana venosa* survived exposure to 7 ppt for 15 days. There appears to be limited information available on the tolerance of *C. fornicata*, *M. roseus* and *C. glaucus* to freshwater, however another chiton from New Zealand (*Sypharochiton pelliserpentis*) experienced 100% mortality within 10 hours exposure to freshwater at higher temperatures (30°C), but only around 80-85% mortality after 24 hours at 10 or 20°C (Boyle 1969, 1970).

It is known that marine gastropods and chitons are susceptible to desiccation. Diederich et al. (2015) reported 100% mortality of juvenile *Crepidula fornicata* after 6 hours air exposure at 20°C and 75% relative humidity due to loss of >75% of tissue water, however adults (2.5-4.5 cm shell length) survived 10 hours desiccation at 26°C and 75% relative humidity. There appears to be limited information on the desiccation tolerance of *R. venosa*, *M. roseus* and *C. glaucus*, however it is known that smaller chitons are less tolerant of emersion than larger ones (Horn 1982), while very few of the largest *Sypharochiton pelliserpentis* survived after 48 hours desiccation at room temperature (16-22°C) under dry (<10% relative humidity) conditions (Boyle 1970). On the other hand, all *Sypharochiton pelliserpentis* survived 48 hours desiccation at the same temperature when the humidity was increased to 90% (Boyle 1970).

Resistance of some gastropods to heated seawater has been examined. Piola and Hopkins (2012) exposed gastropods (*Melagraphia aethiops*) to water temps of 37.5°C for 60 minutes in a simulated sea chest and recorded 100% mortality, however *Crepidula fornicata* from intertidal areas were able to survive exposure to body temperatures up to 42°C for 3 hours in air (Diederich and Pechenik 2013). There appears to be limited information on the heat tolerance of *R. venosa*, *M. roseus* and *C. glaucus*, however another chiton from New Zealand (*Sypharochiton pelliserpentis*) was killed by immersion in boiling water for a few seconds (Horn 1982). There also appears to be little information available on the resistance of invasive gastropods and chitons to acetic acid. Taking into account the information above, and given the likely propagule pressure experienced

via the various anthropogenic introduction pathways, the likelihood estimations for the release of invasive whelks, gastropods, limpets and chitons via the identified risk pathways are listed below.

Release assessment for invasive whelks/ gastropods/ limpets/ chitons

	Unmitigated	Desiccation			Heat		Salinity	Other
Treatment	None	3 mo.	4 mo.	6 mo.	55°C >10 min	80°C >5 min	0 ppt for >24 hrs	4% acetic acid 30 min
Likelihood of release	High	Neg	Neg	Neg	Low?	E low?	Mod ?	Mod ?

4.33.6 Exposure assessment

Some species of invasive gastropods and chitons have already become established in several areas of Australia, however other areas remain free of these species at this time and other species of whelks and limpets remain exotic. It is known that invasive whelks, gastropods, limpets and chitons commonly overgrow aquacultured shellfish and aquaculture equipment (Blanchard 1997, Bax et al. 2003, Liversage and Kotta 2019) and thus can be translocated with mollusc shell materials, which means that shellfish reef restoration efforts could transport them into new regions where their survival and establishment will depend on the availability of favourable environmental conditions.

Once introduced into a new region of Australia, all of the invasive whelks, gastropods, limpets and chitons of concern can tolerate a wide range of temperatures and salinities, can multiply rapidly and be translocated domestically via natural larval planktonic dispersal, biofouling or ballast water (Murphy and Paini 2010, DAWR 2019). However, for some of the species of concern the regional extent of their potential invasions will be limited by environmental factors. For example, analyses suggest that while *C. fornicata* could become established in Australia, it would be unlikely to survive and reproduce in estuaries north of Sydney when water temperatures are likely to be too warm (DAWR 2019). Nevertheless, the invasive biological characteristics of these species provide them with attributes that allow them to outcompete native species for space and successfully establish populations in new areas after translocation (Murphy and Paini 2010, Fofonoff et al. 2018). Taking these various factors into consideration, given that invasive whelks, gastropods, limpets and chitons can be associated with shellfish reefs and aquacultured molluscs and thus could be present with recycled mollusc shells, and environmental conditions are likely to be suitable for their transmission and establishment throughout many parts of Australia, the risk of exposure and establishment is non-negligible, and the overall likelihood of exposure and establishment of invasive whelks, gastropods, limpets and chitons is considered to be **High.**

4.33.7 Consequence assessment

Invasive gastropods and chitons have already been introduced into several estuaries, bays and harbours in Australia's southern States, where there is some evidence that their presence has impacted ecosystem structure and function. For example, the introduced *Maoricolpus roseus* competes directly with commercial scallops (*Pecten fumatus*) in Tasmania for space at low scallop densities, and for both food and space at high scallop densities (Reid et al. 2018). However, two of the worst whelk and limpet invaders, namely *Crepidula fornicata* and *Rapana venosa*, both remain exotic to Australia at this time. Once these latter two species were introduced outside their natural ranges they became highly problematic, resulting in ecological damage to

ecosystems and imparting significant financial impacts on bivalve fisheries and the commercial culture of bivalve shellfish (Blanchard 1997, ICES 2004, Fofonoff et al. 2018). Because of these reasons, various species of invasive whelks, gastropods, limpets and chitons are listed as pests in various States (Table 3), and their establishment in new areas within these jurisdictions could therefore necessitate intervention by government authorities and disruption to normal shipping and other coastal activities if attempts were made to try to prevent their further spread into uninfected areas. However, because they are often cryptic and difficult to discriminate from native species of similar appearance, incursions tend to be detected only after they are well established and there appears to be few instances of successful eradication of invasive whelks, gastropods, limpets and chitons (DAWR 2019). The establishment of exotic whelks, gastropods, limpets and chitons into new areas can thus potentially cause significant economic damage, but they may also compete with native species for food and space, resulting in ecological changes to ecosystems that in most circumstances are irreversible. Gastropods can also harbour a range of disease agents and their introduction can thus facilitate entry or spread of important mollusc pathogens, including apicomplexans, *Xenohaliotis californiensis*, *Haplosporidium nelsoni* and other haplosporidians, and *Perkinsus olseni* (see Diggles et al. 2002, Di Giorgio et al. 2014, Ituarte et al. 2014, Crosson et al. 2014, Messerman and Bowden 2016, Tables 9, 10). Taking all of these factors into consideration, the consequences of introduction of invasive whelks, gastropods, limpets and chitons into new areas via the identified risk pathways are likely to be **Moderate** based on ecological damage alone, but could be even higher if notifiable diseases were introduced with them, as described in Sections 2.1.3, 4.5, 4.8, 4.9 and 4.16.

4.33.8 Risk estimation

The risk estimation for invasive gastropods, whelks, limpets and chitons is determined by combining the likelihood of release and exposure (from Table 5) with the consequences of establishment (Table 6) to arrive at a risk estimation (Table 7). The risk estimate for invasive gastropods, whelks, limpets and chitons exceeds the ALOP for some release pathways, suggesting that additional risk management is required for these marine pests.

Risk estimate for invasive whelks/ gastropods/ limpets/ chitons

	Unmitigated	Desiccation			Heat		Salinity	Other
Treatment	None	3 mo.	4 mo.	6 mo.	55°C >10 min	80°C >5 min	0 ppt for >24 hrs	4% acetic acid 30 min
Combined likelihood of release and exposure	High	Neg	Neg	Neg	Low?	E low?	Mod ?	Mod ?
Consequences of establishment	Moderate	Mod	Mod	Mod	Mod	Mod	Mod	Mod
Risk estimation	Moderate	Neg	Neg	Neg	Low?	Neg?	Mod ?	Mod ?

5.0 Risk Mitigation

5.1. Risk Evaluation

The results from the risk assessment stage are summarised in Table 8 below.

Table 8. Summary table for risk estimate outcomes from the risk assessment.

	Unmitigated	Treatment						
Disease agents	No treatment	Desiccation at >20°C			Heat		Salinity	Other
		3 months	4 months	6 months	55°C >10 min	80°C >5 min	0 ppt >24 hrs	4% acetic acid 30 min
Viruses								
AbHV-1 (Abalone viral ganglioneuritis)	H	VL?	N	N	VL?	N	H?	M?
Iridoviroses of molluscs	L	VL	VL	N	VL	N	L	VL
Infection with OsHV1-µVar (POMS)	H	VL	N	N	N	N	H	L
Infection with malacoherpesviruses	M	N	N	N	N	N	M	VL
Bacteria								
Infection with *Xenohaliotis californiensis*	L	VL	N	N	VL?	VL?	L	L?
Protozoa								
Bonamia exitiosa and *Bonamia* spp.	H	N	N	N	M?	VL?	M	L?
Infection with *Bonamia ostreae*	H	N	N	N	M?	VL?	M	L?
Haplosporidosis	L	N?	N	N	VL?	N?	VL?	VL?
Infection with *Haplosporidium nelsoni* (MSX disease)	L	N?	N	N	VL?	N?	VL	VL?
Infection with *Marteilia refringens*	M	VL?	N	N	L?	VL?	L?	L?
Marteilia sydneyi (QX disease)	L	N?	N	N	VL?	N?	VL?	VL?
Marteilia spp. and *Marteilioides* spp.	VL	N?	N	N	N?	N?	N?	N?
Infection with *Marteilioides chungmuensis*	L	N?	N	N	VL?	N?	VL?	VL?
Infection with *Mikrocytos* spp. (including *M. mackini*)	H	N?	N	N	M?	VL?	M?	L?
Minchinia occulta, Minchinia spp.	M	VL?	N	N	L?	VL?	L?	L?
Infection with *Perkinsus olseni, P. chesapeaki, Perkinsus* spp.	H	L	VL	N	VL	VL?	N	L?
Infection with *Perkinsus marinus*	H	L	VL	N	M	VL?	N	L?
Unknown aetiology								
Akoya oyster disease	L	VL?	N	N	L	VL?	L?	VL?
Oyster oedema disease	N	N	N	N	N	N	N	N
Winter mortality (*M. roughleyi*)	M	N?	N	N	L?	VL?	L?	VL?
Invasive marine pests								
Boring mussels	N	N	N	N	N	N	N	N
Boring sponges	N	N?	N	N	N?	N?	N?	N
Invasive barnacles	L	N	N	N	N	N	L	N
Invasive cnidarians/comb jellies	VL	N	N	N	VL?	N	VL	VL?
Invasive crabs	M	L	N	N	N	N	M	L?
Invasive clams	L	N	N	N	N	N	L	N
Invasive mussels	H	N	N	N	N	N	H	L
Invasive oysters	H	N	N	N	M	N	H	VL
Invasive polychaetes	L	N	N	N	N	N	L	N
Invasive seaweeds	H	L	N	N	N	N	H	N
Invasive seastars	H	N	N	N	VL?	N	N	VL?
Invasive tunicates/ ascidians/ hydroids	H	N	N	N	VL	N	L	VL
Invasive whelks/ gastropods/ limpets	M	N	N	N	L?	N?	M?	M?

N = Negligible risk, VL = very low risk (= ALOP), L = low risk, M = moderate risk, H = high risk, E = extreme risk, ? = uncertainty due to lack of information

It is clear from Table 8 that the unmitigated risk posed by recycling mollusc shells exceeds the ALOP for nearly all of the pests and disease agents of concern. Furthermore, for several of the pest groups of concern, the consequences of establishment for their respective risk assessments were analysed based only on ecological risk, which was then used to calculate the final risk estimates shown in Table 8. However, as noted in the relevant risk assessments, the establishment of several of the pests examined would also represent an additional source of risk of introduction and establishment of one or more significant disease agents of concern. Table 9 outlines how consideration of additional disease vector risks would affect the consequences of establishment and unmitigated risk assessments for those groups of pests which may also act as vectors of important diseases.

Table 9. Unmitigated risk estimates for pest species of concern which can also act as vectors of disease.

Invasive marine pests	Vectors for the following diseases:	Risk estimate based on ecological risk alone	Risk estimate based on combined ecological and disease risks
Invasive crabs	WSSV *Aphanomyces astaci*	Medium	High
Invasive clams	OsHV-1 µVar (Sect. 4.3) Other malacoherpesviruses (Sect. 4.4) *Marteilia refringens* (Sect. 4.10) *Marteilia* spp., *Marteilioides* spp. (Sect. 4.12) *Perkinsus* spp., *P. olseni* (Sect. 4.16) *Perkinsus marinus* (Sect. 4.17)	Low	High
Invasive mussels	OsHV-1 µVar (Sect. 4.3) Other malacoherpesviruses (Sect. 4.4) *Marteilia refringens* (Sect. 4.10) *Marteilia* spp., *Marteilioides* spp. (Sect. 4.12) *Perkinsus* spp., *P. olseni* (Sect. 4.16) *Perkinsus marinus* (Sect. 4.17)	High	Extreme
Invasive oysters	Iridoviroses (Sect. 4.2) OsHV-1 µVar (Sect. 4.3) Other malacoherpesviruses (Sect. 4.4) *Bonamia exitiosa*, *Bonamia* spp. (Sect. 4.6) *Bonamia ostreae* (Sect. 4.7) Haplosporidosis (Sect. 4.8) *Haplosporidium nelsoni* (Sect. 4.9) *Marteilia refringens* (Sect. 4.10) *Marteilia* spp., *Marteilioides* spp. (Sect. 4.12) *Marteilioides chungmuensis* (Sect. 4.13) *Mikrocytos* spp., *M. mackini* (Sect. 4.14) *Perkinsus* spp., *P. olseni* (Sect. 4.16) *Perkinsus marinus* (Sect. 4.17)	High	Extreme
Invasive polychaetes	*Marteilia* spp., *Marteilioides* spp. (Sect. 4.12)	Low	Low
Invasive tunicates/ ascidians/ hydroids	*Haplosporidium nelsoni* (Sect. 4.9)	High	Extreme
Invasive whelks/ gastropods/ limpets	Apicomplexa (Sect. 2.1.3) *Xenohaliotis californiensis* (Sect. 4.5) Haplosporidosis (Sect. 4.8) *Haplosporidium nelsoni* (Sect. 4.9)	Medium	High

The revised risk estimates shown in the far right-hand column of Table 9 for the combined ecological and disease risks reflected the highest risk estimates for each disease of concern. However, for those invasive species where both the unmitigated ecological risks and unmitigated disease risks were both already considered to be high (i.e. invasive mussels, oysters, tunicates, ascidians and hydroids), the combined risk estimate was considered to be extreme (Table 9). As risk estimations (Table 7) were determined by combining

the likelihood of release and exposure (from Table 5) with the consequences of establishment (Table 6), when the revised consequences were applied based on establishment of both the invasive pest and one (or more) diseases of concern, the revised risk estimations for the various treatments for these key species groups are shown in Table 10.

Table 10. Revised risk estimates for pest species of concern which can also act as vectors of disease.

	Unmitigated	Treatment						
Disease agents	No treatment	Desiccation at >20°C			Heat		Salinity	Other
		3 months	4 months	6 months	55°C >10 min	80°C >5 min	0 ppt >24 hrs	4% acetic acid 30 min
Invasive marine pests								
Invasive crabs	H	M	N	N	N	N	H	M?
Invasive clams	H	N	N	N	L	N	H	L
Invasive mussels	E	N	N	N	N	N	E	M
Invasive oysters	E	N	N	N	E	N	E	M
Invasive polychaetes	L	N	N	N	N	N	L	N
Invasive tunicates/ ascidians/ hydroids	E	N	N	N	L	N	M	L
Invasive whelks/ gastropods/ limpets	H	N	N	N	M?	N?	H?	H?

N = Negligible risk, VL = very low risk (= ALOP), L = low risk, M = moderate risk, H = high risk, E = extreme risk, ? = uncertainty due to lack of information

5.2 Treatment options for risk mitigation

This section compares the various risk mitigation measures which were identified as being potentially able to reduce the risk estimate for each pest and disease back to within the ALOP (i.e. to reduce the risk to a probability of occurrence less frequent than 1 in 100 years). One or more of these risk mitigation methods could then form the basis of biosecurity protocols and standard operating procedures for mollusc shell recycling programs throughout the various Australian jurisdictions.

The four main risk mitigation methods examined in this risk analysis included:

1. Desiccation in sunlight at >20°C for 3, 4 or 6 months
2. Heat treatment in water to 55°C for >10 minutes or 80°C for >5 minutes
3. Exposure to freshwater for >24 hours
4. Exposure to 4% acetic acid (vinegar) for >30 minutes

The risk mitigation processes discussed in this section of the RA relate only to option evaluation, together with an appraisal of the utility of each option for reducing risks to within the ALOP. These options could then form the basis of a consultation process that engages Government and stakeholders to evaluate the biosecurity risks involved with unrestricted pathways/mechanisms/risk factors for entry of important pests and diseases with a view towards identifying practical mitigation options that would reduce the risks identified with mollusc shell recycling to an acceptable level within each jurisdiction. The final risk management methods chosen by governments within each jurisdiction would need to take into account a wide variety of pest, disease, industry and region-related factors (i.e. local weather conditions if methods such as desiccation are used).

5.2.1 Desiccation

Desiccation of mollusc shells in sunlight at >20°C for periods of 3, 4 or 6 months were considered. The relative merits of each time period were as follows.

3 months desiccation

Desiccation of mollusc shells in air for a minimum of 3 months was generally effective against a wide range of marine pests and disease agents (Table 8). However, there was some uncertainty with regard to the ability of several mollusc parasites (haplosporidians (excluding *Bonamia* spp.), *Minchinia* spp., *Marteilia* spp., and *Mikrocytos* spp.) to survive in host tissues for that time period, while some of the more resistant viruses (e.g. iridoviruses) may also be able to survive for over 100 days in host tissues depending on the ambient temperature (Langdon 1989). Furthermore, it is known that the vegetative stages of *P. marinus* are relatively resistant to desiccation, with complete inactivation requiring around 115 days desiccation in piles of oyster shells where average temperatures ranged between 6 and 38°C inside the pile and reached a peak of 47°C on the surface (Bushek et al. 2004). Exceptions for marine pests included the invasive crab *Carcinus maenas*, as well as the seaweed *Codium fragile* (Table 8). Cohen and Zabin (2009) reported studies that found *Carcinus maenas* can survive over 60 days out of water when sheltered under seaweed, and over 100 days when held in bottles with damp gravel, and could resume normal feeding after 94 days without food. Similarly, studies by Schaffelke and Deane (2005) found that *Codium fragile* can survive periods of emersion of up to 90 days in a plastic ziplock bag in water vapour saturated air at 17.5°C and 90% relative humidity. These data suggest that invasive crabs, seaweeds, *Perkinsus marinus* and possibly some iridoviruses may be able to potentially survive in the shade inside piles of recycled mollusc shells for over 3 months/90 days if shells are not regularly turned over, ambient humidities within the shell pile remain high (>75%), or whenever ambient temperatures are low (below 20°C).

4 months desiccation

Desiccation of mollusc shells in air for a minimum of 4 months at temperatures of >20°C appeared to be universally effective against all the known pests and disease agents of concern examined in the analysis (Table 8), with the caveat that the shells should be turned over at least once and remain relatively dry so that ambient humidity is lower than around 75%. Under such conditions where weather conditions allow the shell pile to become relatively dry and at a temperature above 20°C for long periods of time (e.g. tropical and subtropical parts of Australia), a desiccation period of 4 months/120 days should be sufficient to completely inactivate even the most resilient pests and disease agents, namely *C. maenas*, *C. fragile*, *P. marinus* and iridoviruses (see Langdon 1989, Bushek et al. 2004, Schaffelke and Deane 2005, Cohen and Zabin 2009), to a level within the ALOP (Tables 8, 10).

6 months desiccation

However, if ambient weather conditions at the recycling facility do not allow the shell pile to dry out for long periods, and/or air temperatures at the recycling facility do not exceed 20°C for several months during storage of the shells, extending the desiccation period to 6 months with at least one shell turnover event is recommended to provide sufficient safety margin to ensure the ALOP is met. Under such conditions (e.g. in

temperate Australia), desiccation of recycled mollusc shells for a minimum of 6 months should reduce the risk of introducing pests and diseases to negligible (i.e., a probability of occurrence less frequent than 1 in 100 years, Tables 8, 10).

5.2.2 Heat treatment

Two different levels of heat treatment of mollusc shells were considered, namely immersion in water at 55°C for >10 minutes, or immersion in water at 80°C for >5 minutes. The merits of each method were as follows.

55°C for 10 minutes

Treatment of recycled mollusc shells in water heated to 55°C for 10 minutes was generally effective against most pest species of concern, but not invasive oysters such as the Pacific oyster (*C. gigas*) which by virtue of their thick shells can survive 60 minutes exposure to 55°C, with 100% mortality of adult *C. gigas* being achieved only following exposure to 57.5°C for 60 min, or 60°C for 30 min (Piola and Hopkins 2012). There was also much uncertainty as to whether heating the water to 55°C for 10 minutes would be sufficient to inactivate a wide range of disease agents, particularly some of the microcells (*Bonamia* spp., *Mikrocytos* spp.) and parasites *Marteilia* spp., and *Minchinia* spp. (Table 8), whilst it is known that *Perkinsus marinus* requires exposure to 50°C for over 18 hours or 60°C for 1 hour before it is completely inactivated (Bushek et al. 1997). This suggests that exposing recycled mollusc shells to water heated to 55°C for 10 minutes would not meet the ALOP.

80°C for 5 minutes

Although there was some uncertainty surrounding the effectiveness of this method against several disease agents (Tables 8, 10), heating recycled mollusc shells in water at 80°C for 5 minutes or more was considered likely to be effective against all taxa of concern, including *P. marinus* (see Bushek et al. 1997), and thus the use of this method for sanitising recycled mollusc shells would be within the ALOP.

5.2.3 Freshwater (minimum 24 hours)

Exposure of recycled mollusc shells to freshwater for 24 hours was generally ineffective for inactivating most of the disease agents of concern (with a level of uncertainty for some diseases), and also was ineffective for inactivating some of the most important pest species of concern (Tables 8, 10). This suggests that freshwater baths of 24 hours duration would not meet the ALOP.

5.2.4 Acetic acid (4% for 30 minutes)

Exposure of recycled mollusc shells to 4% acetic acid (vinegar) for 30 minutes was shown to be effective against many important invasive marine pests, particularly seaweeds and tunicates but also clams, barnacles, boring sponges, and boring mussels (Table 8). However, there is limited information and much uncertainty surrounding the efficacy of this control method against some other pest species as well as most disease agents of concern. This suggests that exposure to 4% acetic acid for 30 minutes would not meet the ALOP.

5.3 Summary of suggested risk mitigation methods

The discussions in Section 5.2 reveal that several risk mitigation methods do not meet the ALOP requirements for a range of marine pests and/or disease agents of concern. For example, exposure to 4% acetic acid (vinegar) for 30 minutes is effective against a range of invasive marine pests, particularly seaweeds and tunicates, however there is limited information and much uncertainty surrounding the efficacy of this control method against some other pest species as well as most disease agents of concern. This rules out safe use of vinegar on recycled mollusc shells at this time. Similarly, while exposure to freshwater for 24 hours is likely to meet the ALOP for some pests and disease agents, it would be ineffective against many invasive pests, and its effectiveness against many disease agents is uncertain. Heating the water to 55°C for 10 minutes is useful against most pest species of concern, but not when disease agents are also considered (Tables 8, 10).

In contrast, although there is some uncertainty surrounding its effectiveness against several disease agents, heating the water to 80°C for 5 minutes or more was considered to be effective against all taxa, and thus its use for sanitizing recycled mollusc shells would be within the ALOP. Using heated water in this manner would appear to be acceptable for treating small volumes of mollusc shells for experimental reef restoration projects, however, treatment of the large quantities (i.e. 10's to 1000's of tonnes) of mollusc shells required for shellfish reef restoration at environmentally meaningful scales via the heated water method would be not only logistically difficult, but also likely to be cost prohibitive.

In contrast, desiccation of mollusc shells by drying them in the sun at a dedicated shell recycling facility for several months can be undertaken at vast scales (e.g. 1000's of tonnes of shell per annum, as is done in Maryland, USA, see Table 1), at minimal cost. Desiccation of mollusc shells in air for a minimum of 3 months was generally effective except against invasive crabs (e.g. *Carcinus maenas*), and seaweeds (e.g. *Codium fragile*) as well as *Perkinsus* spp. and possibly some malacoherpesviruses (e.g. AbHV-1) whenever ambient humidities within the shell pile are high (>75%) or ambient temperatures are low (below 20°C). Only desiccation at >20°C for 4 months or more was found to be universally effective against all known pests and disease agents of concern. Hence to reduce the risk of introducing pests and diseases via mollusc shell recycling to within the ALOP, desiccation for a minimum of 4 months is considered appropriate under weather conditions where the shell pile is relatively dry and at a temperature above 20°C for long periods (i.e. in tropical and subtropical parts of Australia) and turned over at least once. However, if ambient weather conditions at the recycling facility do not allow the shell pile to dry out or exceed 20°C for many months (e.g. as in temperate parts of Australia during the winter months), extending the desiccation period to 6 months (including at least one shell turnover during this time) is recommended to provide a safety margin which would ensure negligible risk (i.e. a probability of occurrence less frequent than 1 in 100 years).

It is notable that several jurisdictions in the USA which are situated in cool temperate climates (e.g. Maryland, New York City) with ambient temperatures well below 20°C for many months of the year, extend their shell drying periods from 6 to 12 months and/or must include the summer months during the shell drying period (Table 1). This approach would ensure that their shell recycling sanitation processes are likely to be effective against all of the pest and disease agents considered in this document, with the possible exception of Washington State on the north west coast of the USA, which is a jurisdiction in a cool temperate climate which requires only a 3 month drying period (Table 1).

6.0 Acknowledgements

The author thanks Chris Gilles, Simon Branigan, Simon Reeves, Anita Nedosyko, Charlotte Boesch, Ben Dyar, William Helt, Thomas C. Price, Murray Barton, Kitman Dyrting, Shane Roberts, Marty Deveney, Ben Rampano, Kevin deWitte, Steve Wesche, Simon Rowe, Kylie Russell and Katie Webb for their input into the technical report and/or their comments on early drafts of the manuscript. This study was supported by the Fisheries Research and Development Corporation (FRDC) under project 2019-005, with funding on behalf of the Australian Government, together with funding and in-kind support from The Nature Conservancy Australia.

7.0 References

Abbott CL, Meyer GR (2014). Review of *Mikrocytos* microcell parasites at the dawn of a new age of scientific discovery. *Diseases of Aquatic Organisms* 110: 25-32.

Abbott CL, Gilmore SR, Lowe G, Meyer G, Bower S (2011). Sequence homogeneity of internal transcribed spacer rDNA in *Mikrocytos mackini* and detection of *Mikrocytos* sp. in a new location. *Diseases of Aquatic Organisms* 93: 243−250.

Abbott CL, Meyer GR, Lowe G, Kim E, Johnson SC (2014). Molecular taxonomy of *Mikrocytos boweri* sp. nov. from Olympia oysters *Ostrea lurida* in British Columbia, Canada. *Diseases of Aquatic Organisms* 110: 65-70.

Abollo E, Ramilo A, Casas SM, Comesaña P, Cao A, Carballal MJ, Villalba A (2008). First detection of the protozoan parasite *Bonamia exitiosa* (Haplosporidia) infecting flat oyster *Ostrea edulis* grown in European waters. *Aquaculture* 274: 201–207.

Adams MJ, King AMQ, Carstens EB (2013). Ratification vote on taxonomic proposals to the International Committee on Taxonomy of Viruses (2013). *Archives of Virology* 158: 2023−2030.

Adams R, Stanley CE, Piana E, Cooper RL (2019). Physiological and behavioral indicators to measure crustacean welfare. *Animals* 9: 914. doi.org/10.3390/ani9110914 .

Adl SM, and 47 other co authors (2019). Revisions to the classification, nomenclature, and diversity of eukaryotes. *Journal of Eukaryotic Microbiology* 66: 4-119.

Adlard R (2000). *Bonamia* spp. disease of flat oysters. In: Heasman M, Lyall I (eds). Proceedings of the Workshop held on 3 March 2000 at the Sydney Fsh markets: Problems of producing and marketing the flat oyster *Ostrea angasi* in NSW. Fisheres Research Report Series: 6. June 2000, p. 24-27.

Adlard RD, Ernst I (1995). Extended range of the oyster pathogen *Marteilia sydneyi*. *Bulletin of the European Association of Fish Pathologists* 15: 119-121.

Adlard RD, Lester RJG (1995). Development of a diagnostic test for *Mikrocytos roughleyi*, the aetiological agent of Australian winter mortality of the commercial rock oyster, *Saccostrea commercialis* (Iredale and Roughley). *Journal of Fish Diseases* 18: 609-614.

Adlard RD, Nolan MJ (2008). Aquatic animal health subprogram: Identification of host interactions in the life cycle of QX disease. Report prepared by QLD Museum for FRDC project 2006/062. 60 pgs.

Adlard RD, Nolan MJ (2015). Elucidating the life cycle of *Marteilia sydneyi*, the aetiological agent of QX disease in the Sydney rock oyster (*Saccostrea glomerata*). *International Journal for Parasitology* 45: 419–426.

AFFA (2002). Import Risk Analysis (IRA) of non-viable bivalve molluscs. Technical Issues Paper. September 2002. Biosecurity Australia – Agriculture, Fisheries and Forestry Australia. 67 pgs.

Ahyong ST, Kupriyanova E, Burghardt I, Sun Y, Hutchings PA, Capa M, Cox SL (2017). Phylogeography of the invasive Mediterranean fan worm, *Sabella spallanzanii* (Gmelin, 1791), in Australia and New Zealand. *Journal of the Marine Biological Association of the United Kingdom* 97: 985-991.

Albano PG, Gallmetzer I, Haselmair A, Tomasovych A, Stachowitsch M, Zuschin M (2018). Historical ecology of a biological invasion: the interplay of eutrophication and pollution determines time lags in establishment and detection. *Biological Invasions* 20: 1417–1430.

Alderman DJ (1979). Epizootiology of *Marteilia refringens* in Europe. *Marine Fisheries Review* 41: 67-69.

Allen FE (1953). Distribution of marine invertebrates by ships. *Australian Journal of Marine and Freshwater Research* 4: 307 – 316.

Alleway HK, Connell SD (2015). Loss of an ecological baseline through the eradication of oyster reefs from coastal ecosystems and human memory. *Conservation Biology* 29: 795-804.

Anderson DT (1994). Barnacles. Structure, function, development and evolution. Chapman and Hall, London.

Anderson TJ, Lester RJG (1992). Sporulation of *Marteilioides branchialis* n. sp. (Paramyxea) in the Sydney rock oyster, *Saccostrea commercialis*: an electron microscope study. *Journal of Protozoology* 39: 502-508.

Anderson TJ, Hine PM, Lester RJG (1995). A *Steinhausia*-like infection in the ovocytes of Sydney rock oysters *Saccostrea commercialis*. *Diseases of Aquatic Organisms* 22: 143-146.

Andree K, Friedman CS, Moore JD, Hedrick RP (2000). A polymerase chain reaction assay for the detection of genomic DNA of a Rickettsiales-like prokaryote associated with withering syndrome in California abalone. *Journal of Shellfish Research* 19: 213–218.

Andrews JD (1966). Oyster mortality studies in Virginia. V. Epizootiology of MSX, a protistan pathogen of oysters. *Ecology* 47: 19-31.

Andrews JD (1968). Oyster mortality studies in Virginica. VII Review of epizootiology and origin of *Minchinia nelsoni*. *Proceedings of the National Shellfisheries Association* 58: 23-36.

Andrews JD (1982). Epizootiology of late summer and fall infections of oysters by *Haplosporidium nelsoni*, and comparison to annual life cycle of *Haplosporidium costalis*, a typical haplosporidian. *Journal of Shellfish Research* 2: 15-23.

Andrews JD (1996). History of *Perkinsus marinus*, a pathogen of oysters in Chesapeake Bay 1950-1984. *Journal of Shellfish Research* 15: 13-16.

Anglès d'Auriac MB, Rinde E, Norling P, Lapègue S, Staalstrøm A, Hjermann DØ, Thaulow J (2017). Rapid expansion of the invasive oyster *Crassostrea gigas* at its northern distribution limit in Europe: Naturally dispersed or introduced? *PLoS ONE* 12: e0177481.

Antonio DB, Andree KB, Moore JD, Friedman CS, Hedrick RP. (2000). Detection of Rickettsiales-like prokaryotes by in situ hybridization in black abalone, *Haliotis cracherodii*, with withering syndrome. *Journal of Invertebrate Pathology* 75: 180–182.

Aquenal (2008a). National Control Plan for the European or basket shell clam *Varicorbula gibba*. Report prepared for the Australian Government. 40 pgs.

Aquenal (2008b). National Control Plan for the Northern Pacific seastar *Asterias amurensis*. Report prepared for the Australian Government. 46 pgs.

Arzul I, Carnegie RB (2015). New perspectives on the haplosporidian parasites of molluscs. *Journal of Invertebrate Pathology* 131: 32-42.

Arzul I, Renault T, Lipart C, Davison AJ (2001a). Evidence for interspecies transmission of oyster herpesvirus in marine bivalves. *Journal of General Virology* 82: 865-870.

Arzul I, Renault T, Lipart C (2001b). Experimental herpes-like viral infections in marine bivalves: Demonstration of interspecies transmission. *Diseases of Aquatic Organisms* 46: 1-6.

Arzul I, Nicolas JL, Davison AJ, Renault T (2001c). French scallops: a new host for ostreid herpesvirus-1. *Virology* 290: 342-349.

Arzul I, Renault T, Thebault A, Gerard A (2002). Detection of oyster herpesvirus DNA and proteins in asymptomatic *Crassostrea gigas* adults. *Virus Research* 84: 151-160.

Arzul I, Gagnaire B, Bond C, Chollet B, Morga B, Ferrand S, Robert M, Renault T (2009). Effects of temperature and salinity on the survival of *Bonamia ostreae*, a parasite infecting flat oysters *Ostrea edulis*. *Diseases of Aquatic Organisms* 85: 151−164.

Arzul I, Langlade A, Chollet B, Robert M, Ferrand S, Omnes E, Lerond S, Couraleau Y, Joly J, François C Garcia C (2011). Can the protozoan parasite *Bonamia ostreae* infect larvae of flat oysters *Ostrea edulis* ? *Veterinary Parasitology* 179: 69-76.

Arzul I, Corbeil S, Morga B, Renault T (2017). Viruses infecting marine molluscs. *Journal of Invertebrate Pathology* 147: 118-135.

Atalah J, Brook R, Cahill P, Fletcher LM, Hopkins GA (2016). It's a wrap: encapsulation as a management tool for marine biofouling. *Biofouling* 32: 277-286.

Audemard C, Barnaud A, Collins CM, Le Roux F, Sauriau PG, Coustau C, Blachier P, Berthe FCJ (2001). Claire ponds as an experimental model for *Marteilia refringens* life-cycle studies: new perspectives. *Journal of Experimental Marine Biology and Ecology* 257: 87-108.

Audemard C, Le Roux F, Barnaud A, Collins C, Sautour B, Sauriau PG, De Montaudouin X, Coustau C, Combes C, Berthe FCJ (2002). Needle in a haystack: involvement of the copepod *Paracartia grani* in the life cycle of the oyster pathogen *Marteilia refringens. Parasitology* 124: 315–323.

Audemard C, Sajus M, Barnaud A, Sautour B, Sauriau P, Berthe F (2004). Infection dynamics of *Marteilia refringens* in flat oyster *Ostrea edulis* and copepod *Paracartia grani* in a claire pond of Marennes-Oleron Bay. *Diseases of Aquatic Organisms* 61: 103–111.

Audemard C, Carnegie RB, Bishop MJ, Peterson CH, Burreson EM (2008). Interacting effects of temperature and salinity on *Bonamia* sp. parasitism in the Asian oyster *Crassostrea ariakensis. Journal of Invertebrate Pathology* 98: 344−350.

Audemard C, Carnegie RB, Hill KM, Peterson CH, Burreson EM (2014). *Bonamia exitiosa* transmission among and incidence in, Asian oyster *Crassostrea ariakensis* under warm euhaline conditions. *Diseases of Aquatic Organisms* 110: 143–150.

Austin B, Austin DA (2007). Bacterial fish pathogens: disease in farmed and wild fish. 4th edition. Springer. 552 pgs.

AusVet (2011). OsHV-1 µVar International Workshop, Cairns, QLD, Australia, 9-12 August 2011. FRDC report , 101 pgs.

Ayres P (1991). Introduced Pacific oysters in Australia. In: Sutherland J, Osman R (eds) The ecology of *Crassostrea gigas* in Australia, New Zealand, France and Washington State. Maryland Sea Grant College, College Park, pgs 3–7. https://www.mdseagrant.org/store/pdfs/UM-SG-TS-92-07_ecology%20gigas%20australia.pdf

Azevedo C, Hine PM (2016). Haplosporidia. In: Archibald JM et al. (eds), Handbook of the Protists. Springer International Publishing, 29 pgs.

Bai CM, Wang CM, Xia J, Sun H, Zhang S, Huang J (2015). Emerging and endemic types of Ostreid herpesvirus 1 were detected in bivalves in China. *Journal of Invertebrate Pathology* 124: 98–106.

Bai CM, Gao W, Wang CM, Yu T, Zhang T, Qiu Z, Wang QC, Huang J (2016). Identification and characterization of ostreid herpesvirus 1 associated with massive mortalities of *Scapharca broughtonii* broodstocks in China. *Diseases of Aquatic Organisms* 118: 65–75.

Bai CM, Wang QC, Morga B, Shi J, Wang CM (2017). Experimental infection of adult *Scapharca broughtonii* with Ostreid herpesvirus SB strain. *Journal of Invertebrate Pathology* 143: 79–82.

Bai CM, Morga B, Rosani U, Shi J, Li C, Xin LS, Wang CM (2019). Long-range PCR and high-throughput sequencing of Ostreid herpesvirus 1 indicate high genetic diversity and complex evolution process. *Virology* 526: 81–90.

Balouet G (1979). *Marteilia refringens* - Considerations of the life cycle and development of Aber disease in *Ostrea edulis*. *Marine Fisheries Review* 41: 64-66.

Balouet G, Cahour A, Chastel C, (1979). Epidémiologie de la maladie de la glande digestive de l'huître plate: hypothèse sur le cycle de *Marteilia refringens*. *Haliotis* 8: 323-326.

Balseiro P, Aranguren R, Gestal C, Novoa B, Figueras A (2006). *Candidatus* Xenohaliotis californiensis and *Haplosporidium montforti* associated with mortalities of abalone *Haliotis tuberculata* cultured in Europe. *Aquaculture* 258: 63-72.

Bannister J, Sievers M, Bush F, Bloecher N (2019). Biofouling in marine aquaculture: a review of recent research and developments. *Biofouling*, 35: 631-648.

Barber RD, Ford SE (1992). Occurrence and significance of ingested haplosporidian spores in the eastern oyster, *Crassostrea virginica* (Gmelin, 1791). *Journal of Shellfish Research* 11: 371-375.

Barber BJ, Mann R (1994). Growth and mortality of eastern oysters *Crassostrea virginica* (Gmelin 1791), and Pacific oysters *Crassostrea gigas* (Thunberg, 1793) under challenge from the parasite *Perkinsus marinus*. *Journal of Shellfish Research* 13: 109-114.

Barber RD, Kanaley SA, Ford SE (1991a). Evidence for regular sporulation by *Haplosporidium nelsoni* (MSX; Ascetospora: Haplosporidiidae) in spat of the American oyster, *Crassostrea virginica*. *Journal of Protozoology* 38: 305–306.

Barber BJ, Ford SE, Littlewood DTJ (1991b). A physiological comparison of resistant and susceptible oysters *Crassostrea virginica* (Gmelin) exposed to the endoparasite *Haplosporidium nelsoni* (Haskin, Stauber & Mackin). *Journal of Experimental Marine Biology and Ecology* 146: 101-112.

Barbieri ES, Medina CD, Vazquez N, Fiorito C, Martelli A, Wigdorovitz A, Schwindt E, Morga B, Renault T, Parrenoe V, Baron PJ (2019). First detection of Ostreid herpesvirus 1 in wild *Crassostrea gigas* in Argentina. *Journal of Invertebrate Pathology* 166: 107222.

Barbosa-Solomieu V, Dégremont L, Vazquez-Juarez R, Ascencio-Valle F, Boudry P, Renault T (2005). Ostreid Herpesvirus 1 (OsHV-1) detection among three successive generations of Pacific oysters (*Crassostrea gigas*). *Virus Research* 107: 47-56.

Bastrop R, Rohner M, Sturmbauer C, Jurss K (1997). Where did *Marenzelleria* spp. (Polychaeta: Spionidae) in Europe come from? *Aquatic Ecology* 31: 119–136.

Bat L, Sahin F, Satilmis HH, Ustun F, Birinci-Ozdemir Z, Kideys AE, Shulman GE (2007). The changed ecosystem of the black sea and its impact on anchovy fisheries. *Journal of Fisheries Sciences* 1: 191-227.

Batista FM, Arzul I, Pepin JF, Ruano F, Friedman CS, Boudry P, Renault T (2007). Detection of ostreid herpesvirus 1 DNA by PCR in bivalve molluscs: a critical review. *Journal of Virological Methods* 139: 1–11.

Battelli C, Rindi F (2008). The extensive development of the turf-forming red alga *Womersleyella setacea* (Hollenberg) R. E. Norris (Rhodophyta, Ceramiales) in the Bay of Boka Kotorska, Montenegro (southern Adriatic Sea). *Plant Biosystems* 142: 120-125.

Baulch T, Ellard K (2011). Pathogen removal and monitoring in abalone live holding and processing effluent with specific reference to abalone herpesvirus. Advice document for Tasmanian abalone processors. Department of Primary Industries, Parks, Water and Environment. 20 pgs.

Baulch T, Ellard K, Bradshaw M (2013). Tasmanian abalone biosecurity project: Implementation Phase 1: Biosecurity Strategies for abalone processors. *Journal of Shellfish Research* 32: 33-35.

Bax, N, Hayes, K, Marshall, A, Parry, DL and Thresher, R (2002). Man-made marinas as sheltered islands for alien marine organisms: Establishment and eradication of an alien invasive marine species. In: Veitch, CR and Clout, MN International Conference on Eradication of Island Invasives, Auckland, New Zealand, 19-23 February 2001.

Bax NJ, McEnnulty FR, Gowlett-Holmes KL (2003). Distribution and biology of the introduced gastropod, *Maoricolpus roseus* (Quoy and Gaimard, 1834) (Caenogastropoda: Turritellidae) in Australia. Centre for Research on Introduced Marine Pests. Technical Report No. 25. CSIRO Marine Research, Hobart. 40 pgs.

Bayne BL (1999). Physiological components of growth differences between individual oysters (*Crassostrea gigas)* and a comparison with *Saccostrea commercialis*. *Physiological and Biochemical Zoology* 72: 705-713.

Bayne BL (2002). A physiological comparison between Pacific oysters (*Crassostrea gigas*) and Sydney rock oyster (*Saccostrea glomerata*): food, feeding and growth in a shared estuarine habitat. *Marine Ecology Progress Series* 232: 163-178.

Bearham D (2008). Identification and characterisation of two haplosporidian parasites of oysters in north Western Australia. Dissertation submitted to Murdoch University for the degree of Doctor of Philosophy. 211 pgs.

Bearham D, Spiers Z, Raidal S, Jones JB, Nicholls PK (2007). Molecular characterisation of a haplosporidian parasite infecting rock oysters *Saccostrea cucullata* in northern Western Australia. *Journal of Invertebrate Pathology* 95: 33–40.

Bearham D, Spiers Z, Raidal S, Jones JB, Nicholls PK (2008a). Detection of *Minchinia* sp. in rock oysters *Saccostrea cucullata* (Born 1778) using DNA probes. *Journal of Invertebrate Pathology* 97: 50-60.

Bearham D, Spiers Z, Raidal S, Jones JB, Burreson EM, Nicholls PK (2008b). Spore ornamentation of *Haplosporidium hinei* n. sp. (Haplosporidia) in pearl oysters *Pinctada maxima* (Jameson, 1901). *Parasitology* 135: 521–527.

Bearham D, Spiers Z, Raidal S, Jones JB, Nicholls PK (2008c). Spore ornamentation of *Minchinia occulta* n. sp. (Haplosporidia) in rock oysters *Saccostrea cucullata* (Born, 1778). *Parasitology* 135: 1271–1280.

Bearham D, Spiers Z, Raidal S, Jones JB, Nicholls PK (2009). Detection of *Minchinia occulta* in samples of pearl oysters *Pinctada maxima* infected by *Haplosporidium hinei. Australian Veterinary Journal* 87: 430-437.

Beck MW, Brumaugh RD, Airoldi L, Carranza A, Coen LD, Crawford C, Defeo O, Edgar GJ, Hancock B, Kay MC, Lenihan HS, Luckenbach MW, Toropova CL, Zhang G, Guo X (2011). Oyster reefs at risk and recommendations for conservation, restoration, and management. *Bioscience* 61: 107-116.

Bennett I (1987). W.J. Dakin's classic study Australian Seashores. A guide to the temperate shores for the beach-lover, the naturalist, the shore fisherman and the student. Angus and Robertson, 411 pgs.

Berthe FCJ, Hine PM (2003). *Bonamia exitiosa* Hine et al. 2001 is proposed instead of *B. exitiosus* as the valid name of *Bonamia* sp. infecting flat oysters *Ostrea chilensis* in New Zealand. *Diseases of Aquatic Organisms* 57: 181.

Berthe FCJ, Pernas M, Zerabib M, Haffner P, Thébault A, Figueras AJ (1998). Experimental transmission of *Marteilia refringens* with special considerations for its life cycle. *Diseases of Aquatic Organisms* 34: 135-144.

Berthe FCJ, Le Roux F, Adlard RD, Figueras A (2004). Marteiliosis in molluscs: A review. *Aquatic Living Resources* 17: 433-448.

Best K, McKenzie CH, Couturier C (2014). Investigating mitigation of juvenile European green crab *Carcinus maenas* from seed mussels to prevent transfer during Newfoundland mussel aquaculture operations. *Management of Biological Invasions* 5: 255–262.

Biosecurity Australia (2009). *Generic Import Risk Analysis Report for Prawns and Prawn Products*. Final Report. Biosecurity Australia, Canberra, Australia. 7 October 2009, 292 pgs.

Bishop MJ, Krassoi FR, McPherson RG, Brown KR, Summerhayes SA, Wilkie EM, O'Connor WA. (2010). Change in wild-oyster assemblages of Port Stephens, NSW, Australia, since commencement of non-native Pacific oyster (*Crassostrea gigas*) aquaculture. *Marine and Freshwater Research* 61: 714-723.

Bishop ML, Carnegie RB, Stokes NA, Peterson CH, Burreson EM (2006). Complications of a non-native oyster introduction: facilitation of a local parasite. *Marine Ecology Progress Series* 325: 145–152.

Bishop MWH (1951). Distribution of barnacles by ships. *Nature* 167: 531.

Bishop Museum (2002). Introduced marine species of Hawaii. *Anomia nobilis* Reeve, 1859. http://www2.bishopmuseum.org/HBS/invertguide/species/anomia_nobilis.htm, accessed 1 August 2020.

Blanchard M (1997). Spread of the slipper limpet, *Crepidula fornicata* (L. 1758) in Europe. Current state and consequences. *Scientia Marina* 61: 109-118.

Bollen M, Battershill CN, Pilditch CA, Bischof K (2017). Desiccation tolerance of different life stages of the invasive marine kelp *Undaria pinnatifida*: Potential for overland transport as invasion vector. *Journal of Experimental Marine Biology and Ecology* 496: 1-8.

Bonaglia S, Bartoli M, Gunnarsson JS, Rahm L, Raymond C, Svensson O, Shakeri Yekta S, Brüchert V (2013). Effect of reoxygenation and *Marenzelleria* spp. bioturbation on Baltic Sea sediment metabolism. *Marine Ecology Progress Series* 482: 43-55.

Bougrier S, Tige G, Bachere E, Grizel H (1986). *Ostrea angasi* acclimatization to French coasts. *Aquaculture* 58: 151-154.

Bower SM (1988). Circumvention of mortalities caused by Denman Island oyster disease during mariculture of Pacific oysters. *American Fisheries Society Special Publication* 18: 246–248.

Bower SM (2010). Synopsis of Infectious Diseases and Parasites of Commercially Exploited Shellfish. https://www.dfo-mpo.gc.ca/science/aah-saa/diseases-maladies/index-eng.html

Bower SM, McGladdery SE, Price IM (1994). Synopsis of infectious diseases and parasites of commercially exploited shellfish. *Annual Review of Fish Diseases* 4: 1-199.

Bower SM, Hervio D, Meyer GR (1997). Infectivity of *Mikrocytos mackini*, the causative agent of Denman Island disease in Pacific oysters *Crassostrea gigas*, to various species of oysters. *Diseases of Aquatic Organisms* 29: 110-116.

Boyer S, Chollet B, Bonnet D, Arzul I (2013). New evidence for the involvement of *Paracartia grani* (Copepoda, Calanoida) in the life cycle of *Marteilia refringens* (Paramyxea). *International Journal for Parasitology* 43: 1089–1099.

Boyle PR (1969). The survival of osmotic stress by *Sypharochiton pelliserpentis* (Mollusca: Polyplacophora). *Biological Bulletin* 136: 154-166.

Boyle PR (1970). Aspects of the ecology of a littoral chiton, *Sypharochiton pellisekpentis* (Mollusca: Polyplacophora). *New Zealand Journal of Marine and Freshwater Research* 4: 364-384.

Bradley T, Moody N, Crane M (2017). Description of an outbreak of *Bonamia exitiosa* in Victorian native oysters in 2015. In: Fourth FRDC Australasian Scientific Conference on Aquatic Animal Health & Biosecurity, Pullman Reef Hotel Cairns 10-14 July 2017. p 26.

Braid BA, Moore JD, Robbins TT, Hedrick RP, Tjeerdema RS, Friedman CS (2005). Health and survival of red abalone, *Haliotis rufescens*, under varying temperature, food supply and exposure to the agent of withering syndrome. *Journal of Invertebrate Pathology* 89: 219–231.

Branigan S, Fitzsimons J, Gillies CL (2020). Modern middens: Shell recycling for restoring an endangered marine ecosystem in Victoria, Australia. *Ecological Management and Restoration* doi: 10.1111/emr.12425

Bravo-Guerra C, Cáceres-Martínez J, Vásquez-Yeomans R, Pestryakov A, Bogdanchikova N (2020). Lethal effects of silver nanoparticles on *Perkinsus marinus*, a protozoan oyster parasite. *Journal of Invertebrate Pathology* 169: 107304.

Breese WP, Malouf RE (1977). Hatchery rearing techniques for the oyster *Crassostrea rivularis* (Gould). *Aquaculture* 12: 123-126.

Brickner I, Kramarsky-Winter E, Mokady O, Loya Y (1993). Speciation in the coral-boring bivalve *Lithophaga purpurea*: evidence from ecological, biochemical and SEM analysis. *Marine Ecology Progress Series* 101: 139-145.

Brisbane Courier (1898). The Oyster Industry. Ravages of the Worm Disease. Certain areas seriously affected. *Brisbane Courier*, Tuesday 1 February 1898, page 5. . http://trove.nla.gov.au/ndp/del/article/3665726

Briski E, Ghabooli S, Bailey SA, MacIsaac HJ (2012). Invasion risk posed by macroinvertebrates transported in ships' ballast tanks. *Biological Invasions* 14: 1843–1850.

Brock R, Bailey-Brock JH; Goody J (1999). A case study of efficacy of freshwater immersion in controlling introduction of alien marine fouling communities: the USS Missouri. *Pacific Science* 53: 223-231.

Bruce BD, Sutton CA, Lyne V (1995). Laboratory and field studies of the larval distribution and duration of the introduced seastar *Asterias amurensis* with updated amd improved prediction of the species spread based on a larval dispersal model. Final report to the Fisheries Research and Development Corporation, March 1995 – CSIRO Fisheries, Hobart. 66 pgs. https://www.frdc.com.au/Archived-Reports/FRDC%20Projects/1993-235-DLD.pdf

Burge CA (2010). Report of the 2010 Annual meeting of the National Reference Laboratories for Mollusc Diseases, Nantes, 23-24 March 2010. IFREMER, La Tremblade, France.

Burge CA, Griffin FJ, Friedman CS (2006). Mortality and herpesvirus infections of the Pacific oyster *Crassostrea gigas* in Tomales Bay, California, USA. *Diseases of Aquatic Organisms* 72: 31-43.

Burge CA, Strenge RE, Friedman CS (2011). Detection of oyster herpesvirus in commercial bivalves in northern California, USA: Conventional and quantitative PCR. *Diseases of Aquatic Organisms* 94: 107-116.

Burge CA, Reece KS, Dhar AK, Kirkland P, Morga B, Dégremont L, Faury N, Wippel BJ, MacIntyre A, Friedman CS (2020). First comparison of French and Australian OsHV-1 µvars by bath exposure. *Diseases of Aquatic Organisms* 138: 137-144.

Burreson EM (1994). Further evidence of regular sporulation by *Haplosporidium nelsoni* in small oysters, *Crassostrea virginica*. *Journal of Parasitology* 80: 1036-1038.

Burreson EM, Ford SE (2004). A review of recent information on the Haplosporidia, with special reference to *Haplosporidium nelsoni* (MSX disease). *Aquatic Living Resources* 17: 499–517.

Burreson EM, Ragone-Calvo LM, La Peyre JF, Counts F, Paynter Jr KT (1994). Acute osmotic tolerance of cultured cells of the oyster pathogen *Perkinsus marinus* (Apicomplexa: Perkinsida). *Comparative Biochemistry and Physiology A* 109: 575–582.

Burreson EM, Ragone-Calvo LM (1996). Epizootiology of *Perkinsus marinus* disease of oysters in Chesapeake Bay, with emphasis on data since 1985. *Journal of Shellfish Research* 15: 17–34.

Burreson EM, Stokes NA (2006). Chapter 5.2.2. Haplosporidiosis of oysters, In: Fish Health Section Blue Book, 2014 Edition, Suggested Procedures for the Detection and Identification of Certain Finfish and Shellfish Pathogens. Section 1, Diagnostic Procedures for Finfish and Shellfish Pathogens. Chapter 5 Diseases of Molluscan Shellfish. American Fisheries Society's Fish Health Section. http://afs-fhs.org/perch/resources/14069252585.2.2haplosporid2014.pdf).

Burreson EM, Stokes NA, Friedman CS (2000). Increased virulence in an introduced pathogen: *Haplosporidian nelsoni* (MSX) in the Eastern oyster *Crassostrea virginica*. *Journal of Aquatic Animal Health* 12: 1-8.

Burreson EM, Stokes NA, Carnegie RB (2004). *Bonamia* sp. (Haplosporidia) found in nonnative oysters *Crassostrea ariakensis* in Bogue Sound, North Carolina. *Journal of Aquatic Animal Health* 16: 1-9.

Bushek D, Howell TL (2000). The effect of UV irradiation on *Perkinsus marinus* and its potential use to reduce transmission via shellfish effluents. Northeastern Regional Aquaculture Center (NRAC) Publication No. 00-008., North Dartmouth, Massachusetts, USA, 4 pgs.

Bushek D, Holley R, Kelly M (1997). Treatment of *Perkinsus marinus*-contaminated materials. *Journal of Shellfish Research* 16: 330.

Bushek D, Ford SE, Chintala MM (2002). Comparison of *in vitro* cultured and wild-type *Perkinsus marinus*. III. Fecal elimination and its role in transmission. *Diseases of Aquatic Organisms* 51: 217-225.

Bushek D, Richardson D, Bobo MY, Coen L (2004). Quarantine of oyster shell cultch reduces the abundance of *Perkinsus marinus*. *Journal of Shellfish Research* 23: 369-373.

Buss JJ, Wiltshire K, Prowse T, Harris JO, Deveney MR (2017). Survey of current prevalence and intensity of *Bonamia* sp. on South Australian *Ostrea angasi* hatchery and farm sites. In: Fourth FRDC Australasian Scientific Conference on Aquatic Animal Health & Biosecurity, Pullman Reef Hotel Cairns 10-14 July 2017. p 28.

Buss JJ, Wiltshire KH, Prowse TA, Harris JO, Deveney MR (2019). *Bonamia* in *Ostrea angasi*: Diagnostic performance, field prevalence and intensity. *Journal of Fish Diseases* 42: 63-74.

Buss JJ, Harris JO, Tanner JE, Wiltshire KH, Deveney MR (2020a). Rapid transmission of *Bonamia exitiosa* by cohabitation causes mortality in *Ostrea angasi*. *Journal of Fish Diseases* 43: 227-237.

Buss JJ, Wiltshire K, Harris JO, Tanner JE, Deveney MR (2020b). Infection dynamics of *Bonamia exitiosa* on intertidal *Ostrea angasi* farms. *Journal of Fish Diseases* 43: DOI: 10.1111/jfd.13134

Buss JJ, Wiltshire K, Harris JO, Deveney MR (2020c). Decontamination of *Bonamia exitiosa*. *Aquaculture* 523: 735210.

Butt D, Raftos DA (2007). Immunosuppression in Sydney rock oysters (*Saccostrea glomerata*) and QX disease in the Hawkesbury River, Sydney. *Marine and Freshwater Research* 58: 213–221.

Butt D, Shaddick K, Raftos D (2006). The effect of low salinity on phenoloxidase activity in the Sydney rock oyster, *Saccostrea glomerata*. *Aquaculture* 251: 159-166.

Cahill P, Hickey C, Lewis P, Tait L, Floerl O (2019). Treatment agents for biofouling in internal pipework of recreational vessels. A review of pipework configurations, biofouling risk, and operational considerations. MPI Technical Paper No: 2019/03. March 2019. 62 pgs.

Cali A, Becnel JJ, Takvorian PM (2016). Microsporidia. In: J.M. Archibald et al. (eds.), *Handbook of the Protists*, Springer International Publishing AG 2016, DOI 10.1007/978-3-319-32669-6_27-1.

Calvo GW, Luckenbach MW, Allen SK, Burreson EM (1999). A comparative field study of *Crassostrea gigas* (Thunberg 1793) and *Crassostrea virginica* (Gmelin 1791) in relation to salinity in Virginia. *Journal of Shellfish Research* 18: 465–474.

Calvo GW, Luckenbach MW, Allen SK, Burreson EM (2000). Comparative field study of *Crassostrea ariakensis* and *Crassostrea virginica* in relation to salinity in Virginia. Special Report in Applied Marine Science and Ocean Engineering No. 360, March 2000. 25 pgs. https://www.vims.edu/_docs/oysters/calvo_ariakensis.pdf

Calvo GW, Luckenbach MW, Allen SK, Burreson EM (2001). A comparative field study of *Crassostrea ariakensis* (Fujita 1913) and *Crassostrea virginica* (Gmelin 1791) in relation to salinity in Virginia. *Journal of Shellfish Research* 20: 221–229.

Caraguel CGB, Ellard K, Moody NJG, Corbeil S, Williams LM, Mohr PG, Cummins DM, Hoad J, Slater J, Crane MStJ (2019). Diagnostic test accuracy when screening for Haliotid herpesvirus 1 (AbHV) in apparently healthy populations of Australian abalone *Haliotis* spp. *Diseases of Aquatic Organisms* 136: 199–207.

Carlton JT, Thompson JK, Scheme1 LE, Nichols FH (1990). Remarkable invasion of San Francisco Bay (California, USA) by the Asian clam *Potamocorbula amurensis*. I. Introduction and dispersal. *Marine Ecology Progress Series* 66: 81-94.

Carlton JT, Newman WA, Pitombo FB (2011). Barnacle invasions: Introduced, cryptogenic, and range expanding Cirripedia of North and South America. In: Galil BS et al. (eds.), In the Wrong Place - Alien Marine Crustaceans: Distribution, Biology and Impacts, Invading Nature - Springer Series in Invasion Ecology 6. Pgs 159-214.

Carman MR, Lindell S, Green-Beach E, Starczak VR (2016). Treatments to eradicate invasive tunicate fouling from blue mussel seed and aquaculture socks. *Management of Biological Invasions* 7: 101–110.

Carnegie RB, Burreson EM (2011). Declining impact of an introduced pathogen: *Haplosporidium nelsoni* in the oyster *Crassostrea virginica* in Chesapeake Bay. *Marine Ecology Progress Series* 432: 1–15.

Carnegie RB, Burreson EM, Hine PM, Stokes NA, Audemard C, Bishop MJ, Peterson CH (2006). *Bonamia perspora* n. sp. (Haplosporidia), a parasite of the oyster *Ostreola equestris*, is the first *Bonamia* species known to produce spores. *Journal of Eukaryotic Microbiology* 53: 232-245.

Carnegie RB, Stokes NA, Audemard C, Bishop MJ, Wilbur AE, Alphin TD, Posey MH, Peterson CH, Burreson EM (2008). Strong seasonality of *Bonamia* sp. infection and induced *Crassostrea ariakensis* mortality in Bogue and Masonboro Sounds, North Carolina, USA. *Journal of Invertebrate Pathology* 98: 335−343.

Carnegie RB, Hill KM, Stokes NA, Burreson EM (2014). The haplosporidian *Bonamia exitiosa* is present in Australia, but the identity of the parasite described as *Bonamia* (formerly *Mikrocytos*) *roughleyi* is uncertain. *Journal of Invertebrate Pathology* 115: 33–40.

Carrasco N, Arzul I, Chollet B, Robert M, Joly JP, Furones MD, Berthe FCJ (2008). Comparative experimental infection of the copepod *Paracartia grani* with *Marteilia refringens* and *Marteilia maurini*. *Journal of Fish Diseases* 31: 497-504.

Carrasco N, Hine PM, Durfort M, Andree KB, Malchus N, Lacuesta B, González M, Roque A, Rodgers C, Furones D (2013). *Marteilia cochillia* sp. nov., a new *Marteilia* species affecting the edible cockle *Cerastoderma edule* in European waters. *Aquaculture* 412–413: 223–230.

Carrasco N, Green T, Itoh N (2015). *Marteilia* spp. parasites in bivalves: a revision of recent studies. *Journal of Invertebrate Pathology* 131: 43-57.

Carroll JM, O'Shaughnessy KA, Diedrich GA, Finelli CM (2015). Are oysters being bored to death? Influence of *Cliona celata* on *Crassostrea virginica* condition, growth and survival. *Diseases of Aquatic Organisms* 117: 31-44.

Carver CE, Chisholm A, Mallet AL (2003). Strategies to mitigate the impact of *Ciona intestinalis* biofouling on shellfish production. *Journal of Shellfish Research* 22: 621-631.

Carver CE, Thériault I, Mallet AL (2010). Infection of cultured eastern oysters *Crassostrea virginica* by the boring sponge *Cliona celata*, with emphasis on sponge life history and mitigation strategies. *Journal of Shellfish Research* 29: 905-915.

Castinel A, Webb SC, Jones JB, Peeler EJ, Forrest BM (2019). Disease threats to farmed green-lipped mussels *Perna canaliculus* in New Zealand: review of challenges in risk assessment and pathway analysis. *Aquaculture Environment Interactions* 11: 291-304.

Catanese G, Grau A, Valencia JM, Garcia-March JR, Alvarez E, Vazquez-Luis M, Deudero S, Darriba S, Carballal MJ, Villalba A (2018). *Haplosporidium pinnae* sp. nov., a haplosporidan parasite associated with mass mortalities of the fan mussel, *Pinna nobilis*, in the Western Mediterranean Sea. *Journal of Invertebrate Pathology* 157: 9−24.

Cavalier-Smith T (1993). Kingdom protozoa and its 18 phyla. *Microbiological Reviews* 57: 953-994.

Chagot D, Boulo V, Hervio D, Mialhe E, Bachère E, Mourton C, Grizel H (1992). Interactions between *Bonamia ostreae* (Protozoa: Ascetospora) and haemocytes of *Ostrea edulis* and *Crassostrea gigas* (Mollusca: Bivalvia): Entry Mechanisms. *Journal of Invertebrate Pathology* 59: 241−249.

Chang PH, Kuo ST, Lai SH, Yang HS, Ting YY, Hsu CL, Chen HC (2005). Herpes-like virus infection causing mortality of cultured abalone *Haliotis diversicolor supertexta* in Taiwan. *Diseases of Aquatic Organisms* 65: 23-27.

Chen MH, Kuo ST, Renault T, Friedman CS, Chang PH (2012). Development of a polymerase chain reaction for the detection of abalone herpesvirus infection based on the DNA polymerase gene. *Journal of Virological Methods* 185: 1–6.

Cheslett D, McKiernan F, Hickey C, Collins E (2009). Viral gametocytic hypertrophy of the Pacific oyster *Crassostrea gigas* in Ireland. *Diseases of Aquatic Organisms* 83: 181-185.

Choi KS, Park KI (2010). Review on the protozoan parasite *Perkinsus olseni* (Lester and Davis 1981) infection in Asian waters. In: Coastal Environmental and Ecosystem Issues of the East China Sea. A. Ishimatsu and H.-J. Lie (Eds). Hiroshima University Press. pgs. 269–281.

Choi DL, Lee NS, Choi HJ, Park MA, McGladdery SE, Park MS (2004). Viral gametocytic hypertrophy caused by a papova-like virus infection in the Pacific oyster *Crassostrea gigas* in Korea. *Diseases of Aquatic Organisms* 59: 205-209.

Chu FL, Greene KH (1989). Effect of temperature and salinity on the *in-vitro* culture of the oyster pathogen *Perkinsus marinus* (Apicomplexa: Perkinsea). *Journal of Invertebrate Pathology* 53: 260-268.

Chu FL, Volety AK (1997). Disease processes of the parasite *Perkinsus marinus* in eastern oyster *Crassostrea virginica*: minimum dose for infection initiation, and interaction of temperature, salinity and infective cell dose. *Diseases of Aquatic Organisms* 28: 61-68.

Cicala F, Moore JD, Caceres-Martínez J, Del Río-Portilla MA, Hernandez-Rodríguez M, Vasquez-Yeomans R, Rocha-Olivares A (2017). Multigenetic characterization of *Candidatus* Xenohaliotis californiensis. *International Journal of Systematic and Evolutionary Microbiology* 67: 42–49.

Cinar ME, Katagan T, Kocak F, Ozturka B, Ergen E, Kocatas A, Onen M, Kirkim F, Bakir K, Kurt G, Daglia E, Acika S, Dogana A, Ozcan T (2008). Faunal assemblages of the mussel *Mytilus galloprovincialis* in and around Alsancak Harbour (Izmir Bay, eastern Mediterranean) with special emphasis on alien species. *Journal of Marine Systems* 71: 1-17.

Clark B (2020). History: Streets of Bribie. Banksia Beach. 19 June 2020. *The Bribie Islander* 116: 30-31. http://www.digsfish.com/Clark2020BanksiaBeachhistory.pdf

Cochennec N, Renault T, Boudry P, Chollet B, Gerard A (1998). *Bonamia*-like parasite found in the Suminoe oyster *Crassostrea rivularis* reared in France. *Diseases of Aquatic Organisms* 34:193–197.

Cochennec-Laureau N, Reece KS, Berthe FCJ, Hine PM (2003). *Mikrocytos roughleyi* taxonomic affiliation leads to the genus *Bonamia* (Haplosporidia). *Diseases of Aquatic Organisms* 54: 209–217.

Cohen AN, Zabin CJ (2009). Oyster shells as vectors for exotic organisms. *Journal of Shellfish Research* 28: 163-167.

Comps M (1969). Observations relatives à l'affection branchiale des huítres portugaises (*Crassostrea angulata* Lmk). *Revue des Travaux de I'Institut des Pêches Maritimes* 33: 151-160.

Comps M (1970). Observations sur les causes dune mortalite´anormal des huıˆtres plates dans le bassin de Marennes. *Revue des Travaux de I'Institut des Pêches Maritimes* 34: 317–326.

Comps M (1976). *Marteilia lengehi* n. sp. parasite de l'huitre *Crassostrea cucullata*. *Rev. Trav. Inst. Pêches Marit.* 40: 347–349.

Comps, M (1988). Epizootic disease of oysters associated with viral infections. In Disease Processes in Marine Bivalve Molluscs, ed Fisher, W.S. pp. 23-37. Bethesda, Maryland: American Fisheries Society Special Publication 18.

Comps M, Bonami JR (1977). Infection virale associe a des mortalites chex l'huitre *Crassostrea gigas* Th. *Comptes Rendus Hebdomadaire des seances de l'Academie des Sciences, Serie D, Sciences Naturelles* 285: 1139-1140.

Comps M, Tige G, Grizel H (1980). Etude ultrastructural d'un protiste parasite de l'huitre plate *Ostrea edulis* L. *Compte Rendu de l'Academie Science*, Paris 290, 383-384.

Comps M, Park MS, Desportes I (1986). Etude ultrastructurale de *Marteilioides chungmuensis* n.g., n. sp. parasite des ovocytes de l'huitre *Crassostrea gigas* Th. *Protistologica* 22: 279–285.

Comps M, Herbaut C, Fougerouse A (1999). Virus-like particles in pearl oyster *Pinctada margaritifera*. *Bulletin of the European Association of Fish Pathologists* 19: 85-88.

Comps M, Herbaut C, Fougerouse A, Laporte F (2001). Progress in pathological characterisation of Syndrome 85 in the black-lip pearl oyster *Pinctada margaritifera*. *Aquatic Living Resources* 14: 195-202.

Comtet T, Garcia C, LeCoguic Y, Joly JP (2004). First record of the microsporidian parasite *Steinhausia mytilovum* in *Mytilus* sp. (Bivalvia: Mytilidae) from France. *Diseases of Aquatic Organisms* 58: 261-264.

Condon RH, Steinberg DK (2008). Development, biological regulation, and fate of ctenophore blooms in the York River estuary, Chesapeake Bay. *Marine Ecology Progress Series* 369: 153-168.

Conrad JM (2015). Bioeconomics of a marine disease. *Marine Resource Economics* 30: 393-46.

Corbeil S, Arzul I, Robert M, Berthe FCJ, Besnard-Cochennec N, Crane MS (2006). Molecular characterisation of an Australian isolate of *Bonamia exitiosa*. *Diseases of Aquatic Organisms* 71: 81−85.

Corbeil S, Handlinger J, Crane MSJ (2009). Bonamiosis in *Ostrea angasi*. Australian and New Zealand Standard Diagnostic Procedure. http://www.scahls.org.au/__data/assets/pdf_file/0003/1516512/Bonamia.pdf

Corbeil S and 16 other co-authors (2010). Development and validation of a TaqMan® PCR assay for the Australian abalone herpes-like virus. *Diseases of Aquatic Organisms* 92: 1-10.

Corbeil S, McColl KA, Williams LM, Mohammad I, Hyatt AD, Crameri SG, Fegan M, Crane MJ (2012a). Abalone viral ganglioneuritis: Establishment and use of an experimental immersion challenge system for the study of abalone herpes virus infections in Australian abalone. *Virus Research* 165: 207– 213.

Corbeil S, Williams LM, Bergfield J, Crane MJ (2012b). Abalone herpes virus stability in sea water and susceptibility to chemical disinfectants. *Aquaculture* 326-329: 20-26.

Corbeil S, Williams LM, Warner S, Fegan M, Moody NJG, Mohammad I, Ellard K, Caraguel C, Deveney M, Cowley J, Crane MJ (2014). Aquatic Animal Health Subprogram: Characterisation of abalone herpes-like virus infections in abalone. FRDC Project No 2009/032. 57 pgs.

Corbeil S, Williams LM, McColl, K, Crane MJ (2016). Australian abalone (*Haliotis laevigata*, *H. rubra* and *H. conicopora*) are susceptible to infection by multiple abalone herpesvirus genotypes. *Diseases of Aquatic Organisms* 119: 101-106.

Corbeil S, McColl, K, Williams LM, Slater J, Crane MJ (2017). Innate resistance of New Zealand paua to abalone viral ganglioneuritis. *Journal of Invertebrate Pathology* 146: 31–35.

Coutts ADM, Dodgshun TJ (2007). The nature and extent of organisms in vessel sea-chests: A protected mechanism for marine bioinvasions. *Marine Pollution Bulletin* 54: 875–886.

Coventry R (2006). Hawkesbury River: Managing Salvinia on the Hawkesbury—a $1.8 million cooperative effort. In: Salvinia control manual. Management and control options for salvinia (*Salvinia molesta*) in Australia. Weeds of National Significance. NSW DPI publication, pgs 64-69. http://www.dpi.nsw.gov.au/__data/assets/pdf_file/0004/89554/salvinia-control-manual.pdf

Cowley JA, Corbeil S, Bulach D, Moody NJ and others (2012). Complete genome sequences of abalone herpes virus (AbHV) strains from Victoria and Tasmania provide insights into its origins and identity variations useful for epidemiology. In: Elliot N (ed) 8th International Abalone Symposium, 6–11 May 2012, Hobart, Tasmania. International Abalone Society, p 86 (Abstract).

Crane MJ, Fegan M, Corbeil S, Warner S (2009). Development of Molecular Diagnostic Procedures for the Detection and Identification of Herpes-like Virus of Abalone (*Haliotis* spp.). Final Report to the Fisheries Research and Development Corporation. Project Number 2007-006.

Crane MJ, Corbeil S, Williams LM, McColl KA, Gannon V (2013). Evaluation of Abalone Viral Ganglioneuritis resistance among wild abalone populations along the Victorian coast of Australia. *Journal of Shellfish Research* 32: 67–72.

Cranfield HJ, Dunn A, Doonan IJ, Michael KP (2005). *Bonamia exitiosa* epizootic in *Ostrea chilensis* from Foveaux Strait, southern New Zealand between 1986 and 1992. *ICES Journal of Marine Science* 62: 3-13.

Crawford C (2016). National review of *Ostrea angasi* aquaculture: historical culture, current methods and future priorities. Institute of Marine and Antarctic Studies Report. 43 pgs.

Creese RG, Davis AR, Glasby TM (2004). Eradicating and preventing the spread of the invasive alga *Caulerpa taxifolia* in NSW. Project No. 35593, June 2004. NSW Fisheries Final Report Series No. 64, 104 pgs.

Creighton C (2013). Revitalising Australia's Estuaries. Fisheries Research and Development Corporation, Canberra http://www.frdc.com.au/Archived-Reports/FRDC%20Projects/2012-036-DLD.pdf.

Cribb J (2010). Science brings salvation for oyster farmer. FRDC News *FISH* 18(1): 4-5.

Crockford M, Jones B (2012). Aquatic Animal Health Subprogram: Investigation of Chlamydiales-like organisms in pearl oysters, *Pinctada maxima*. Final Report FRDC Project No. 2008/031. Fisheries Research Report No. 232. Department of Fisheries, Western Australia. 36 pgs.

Cross ME, Bradley CR, Cross TF, Culloty S, Lynch S, McGinnity P, O'Riordan RM, Vartia S, Prodöhl PA (2016). Genetic evidence supports recolonisation by *Mya arenaria* of Western Europe from North America. *Marine Ecology Progress Series* 549: 99-112.

Crosson LM, Friedman CS (2018). Withering Syndrome susceptibility of northeastern Pacific abalones: A complex relationship with phylogeny and thermal experience. *Journal of Invertebrate Pathology* 151: 91-101.

Crosson LM, Wight N, VanBlaricom GR, Kiryu I, Moore JD, Friedman CS (2014). Abalone withering syndrome: distribution, impacts, current diagnostic methods and new findings. *Diseases of Aquatic Organisms* 108: 261-270.

Crosson LM, Lottsfeldt NS, Weavil-Abueg ME, Friedman CS (2020). Abalone withering syndrome disease dynamics: Infectious dose and temporal stability in seawater. *Journal of Aquatic Animal Health* doi: 10.1002/aah.10102.

Cruz-Flores R, Cruz-Flores R, Cáceres-Martínez J, Muñoz-Flores M, Vásquez-Yeomans R, Rodriguez MH, Del Río-Portilla MÁ, Rocha-Olivares A, Castro-Longoria E (2016). Hyper-parasitism by the bacteriophage (Caudovirales) infecting *Candidatus* Xenohaliotis californiensis (Rickettsiales-like prokaryote) parasite of wild abalone *Haliotis fulgens* and *Haliotis corrugata* from the Peninsula of Baja California, Mexico. *Journal of Invertebrate Pathology* 140: 58-67.

Cruz-Flores R, Cáceres-Martínez J, DelRío-Portilla MÁ, Licea-Navarro AF, Gonzales-Sánchez R, Guerrero A (2018). Complete genome sequence of a phage hyperparasite of *Candidatus* Xenohaliotis californiensis (Rickettsiales) - a pathogen of *Haliotis* spp (Gasteropoda). *Archives of Virology* 163: 1101-1104.

Culloty SC, Novoa B, Pernas M, Longshaw M, Mulcahy MF, Feist SW, Figueras A (1999). Susceptibility of a number of bivalve species to the protozoan parasite *Bonamia ostreae* and their ability to act as vectors for this parasite. *Diseases of Aquatic Organisms* 37: 73-80.

Culloty SC, Carnegie R, Diggles BK, Deveney M, Michael K, Farnsworth M (2019). *Bonamia* Response 2015: Report of the Technical Advisory Group 2019 on a return to flat oyster farming, 23–17 September 2019. Report date: 30 November 2019. Technical Advisory Report prepared for the Ministry for Primary Industries, New Zealand Government. 52 pgs.

Currie DR, Parry GD (1999). Changes to benthic communities over 20 years in Port Phillip Bay, Victoria, Australia. *Marine Pollution Bulletin* 38: 36-43.

DAFF (2003). Importation of pilchards (*Sardinops sagax*) for direct introduction into natural waters. Biosecurity policy review of viral haemorrhagic septicaemia virus (VHSV). Draft Report June 2003.

DAFF (2011). *Import Risk Analysis Handbook 2011*. Australian Government Department of Agriculture, Fisheries and Forestry, Canberra.

Dang C, Cribb TH, Cutmore SC, Chan J, Hénault O, Barnes AC (2013). Parasites of QX-resistant and wild-type Sydney rock oysters (*Saccostrea glomerata*) in Moreton Bay, SE Queensland, Australia: Diversity and host response. *Journal of Invertebrate Pathology* 112: 273-277.

Dang C, Dungan CF, Scott GP, Reece KS (2015). *Perkinsus* sp. infections and *in vitro* isolates from *Anadara trapezia* mud arks of Queensland. Australia. *Diseases of Aquatic Organisms* 113: 51–58.

Dang C, Miller T, Grounds G, Bannister J, Stephens F, Nolan D, Hillier P, Caraguel C, Go J, Bogema D, Jenkins C. (2017). Perkinsosis in molluscs from Western Australia. In: Fourth FRDC Australasian Scientific Conference on Aquatic Animal Health & Biosecurity, Pullman Reef Hotel Cairns 10-14 July 2017. p 29.

Dann AL, Ellard K, Grove SJ, Willan SC (2020). Genetic confirmation of *Mya japonica* Jay, 1857 (Bivalvia: Myidae) in Tasmania, Australia: first record of any species of *Mya* in the southern hemisphere. *BioInvasions Records* 9: 103–108.

Darbyson EA, Hanson JM, Locke A, Willison JH (2009). Survival of European green crab (*Carcinus maenus* L.) exposed to simulated overland and boating-vector transport conditions. *Journal of Shellfish Research* 28: 377–382.

D'Archino R, Nelson WA, Zuccarello GC (2007). Invasive marine red alga introduced to New Zealand waters: First record of *Grateloupia turuturu* (Halymeniaceae, Rhodophyta). *New Zealand Journal of Marine and Freshwater Research* 41: 35-42.

Darwin C (1854) A monograph on the sub-class Cirripedia. The Balanidae, or Sessile Cirripedes, the Verrucidae. Ray Society, London.

Dauer DM, Maybury CA, Ewing RM (1981). Feeding behavior and general ecology of several spionid polychaetes from the Chesapeake Bay. *Journal of Experimental Marine Biology and Ecology* 54: 21-38.

Daume S, Fromont J, Parker F, Davidson M, Murphy D, Hart A (2010). Quantifying sponge erosions in Western Australian pearl oyster shells. *Aquaculture Research* 41: e260-e267.

Davison AJ, Trus BL, Cheng N, Steven A, Watson MS, Cunningham C, Le Deuff RM, Renault T (2005). A novel class of herpesvirus with bivalve hosts. *Journal of General Virology* 86: 41-53.

Davison A, Eberle R, Ehlers B, Hayward G, McGeoch D, Minson A, Pellett P, Roizman B, Studdert M, Thiry E (2009). The order Herpesvirales. *Archives of Virology* 154: 171-177.

DAWR (2015a). Review of national marine pest biosecurity. Department of Agriculture and Water Resources, October 2015. 116 pgs. https://www.agriculture.gov.au/sites/default/files/sitecollectiondocuments/pests-diseases-weeds/marine-pests/review-national-marine-pest-biosecurity.pdf

DAWR (2015b). Rapid response manual for *Carcinus maenas*. Version 1.0, December 2015. https://www.marinepests.gov.au/sites/default/files/Documents/empplan-rapid-response-manual-carcinus-maenas.pdf

DAWR (2019). Australian priority marine pest list. Process and outcomes. Prepared by ABARES for the Marine Pest Sectoral Committee Secretariat of Department of Agriculture and Water Resources. 217 pgs. https://www.marinepests.gov.au/sites/default/files/Documents/apmpl-process-outcomes.pdf

Dégremont L (2011). Evidence of herpesvirus (OsHV-1) resistance in juvenile *Crassostrea gigas* selected for high resistance to the summer mortality phenomenon. *Aquaculture* 317: 94-98.

Dégremont L, Ernande B, Bédier E, Boudry P (2007). Summer mortality of hatchery-produced Pacific oyster spat (*Crassostrea gigas*). I. Estimation of genetic parametres for survival and growth. *Aquaculture* 262: 41–53.

Dégremont L, BédierE, Boudry P (2010a). Summer mortality of hatchery-produced Pacific oyster spat (*Crassostrea gigas*). II. Response to selection for survival and its influence on growth and yield. *Aquaculture* 299: 21–29.

Dégremont L, Soletchnik P, Boudry P (2010b). Summer mortality of selected juvenile Pacific oyster *Crassostrea gigas* under laboratory conditions and in comparison with field performance. *Journal of Shellfish Research* 29: 847-856.

de Kantzow M, Hick P, Becker JA, Whittington RJ (2016). Effect of water temperature on mortality of Pacific oysters *Crassostrea gigas* associated with microvariant ostreid herpesvirus 1 (OsHV-1 µVar). *Aquaculture Environment Interactions* 8: 419–428.

de Kantzow MC, Hick PM, Dhand NK, Whittington RJ (2017). Risk factors for mortality during the first occurrence of Pacific oyster mortality syndrome due to Ostreid herpesvirus-1 in Tasmania, 2016. *Aquaculture* 468: 328-336.

de Nys R, Ison O (2004). Evaluation of antifouling products developed for the Australian pearling industry. FRDC Project No. 2000/254, 86 pgs.

Denny CM (2008). Development of a method to reduce the spread of the ascidian *Didemnum vexillum* with aquaculture transfers. *ICES Journal of Marine Science* 65: 805–810.

Department of Agriculture (2014). Disease strategy: Abalone viral ganglioneuritis (Version 1.0). In: *Australian Aquatic Veterinary Emergency Plan (AQUAVETPLAN)*, Australian Government Department of Agriculture, Canberra, ACT. 44 pgs.

Desportes I, Perkins FO (1990). Phylum Paramyxea. In: Handbook of Protoctista, L. Margulis, J. O. Corliss, M. Melkonian and D.J. Chapman, (eds.). Jones and Bartlett Publ., Boston, pgs. 30-35.

Deveney MR, Roberts S, Moody N, Crane M, Ellard K (2017). Biofouling as a long distance vector for pathogens. In: Fourth FRDC Australasian Scientific Conference on Aquatic Animal Health & Biosecurity, Pullman Reef Hotel Cairns 10-14 July 2017. (abstract) p 35.

Deveney MR, Wiltshire KH, Buss JJ, Lieu YN, Harris JO, Speck P, Yeadon PJ, Ellard K, Mohr PG, Moody NJ, Bansemer MS, Roberts SD (2019). OsHV-1 microvariant in the Port River, South Australia: An unnatural history. In: Fifth FRDC Australasian Scientific Conference on Aquatic Animal Health & Biosecurity, Pullman Reef Hotel Cairns 8-12 July 2019. (abstract), p 22.

Diederich CM, Pechenik JA (2013). Thermal tolerance of *Crepidula fornicata* (Gastropoda) life history stages from intertidal and subtidal subpopulations. *Marine Ecology Progress Series* 486: 173-187.

Diederich CM, Bashevkin SM, Chaparro OR, Pechenik JA (2015). Desiccation tolerance and lifting behavior in *Crepidula fornicata* (Gastropoda). *Marine Ecology Progress Series* 528: 235-243.

Diederich S, Nehls G, van Beusekom JEE, Reise K (2005). Introduced Pacific oysters (*Crassostrea gigas*) in the northern Wadden Sea: invasion accelerated by warm summers? *Helgoland Marine Research* 59: 97–106.

Diggles BK (2003). Shellfish Disease Surveillance Programme – Final Report June 2003. NIWA Client Report AUS2002-018 for NIWA Project NAU03911 prepared for Primary Industries and Resources South Australia (PIRSA). 22 pgs.

Diggles BK (2011a). Risk Analysis. Aquatic animal diseases associated with domestic bait translocation. Final report prepared for the Australian Government Department of Agriculture, Fisheries and Forestry, Canberra, FRDC Project No. 2009/072. 296 pgs. http://frdc.com.au/research/Final_Reports/2009-072-DLD.pdf

Diggles BK (2011b). Abalone Farms Australia biosecurity assessment and disease risk analysis. DigsFish Services Client Report DF11-05, December 2011. 58 pgs.

Diggles BK (2013). Historical epidemiology indicates water quality decline drives loss of oyster (*Saccostrea glomerata*) reefs in Moreton Bay, Australia. *New Zealand Journal of Marine and Freshwater Research* 47: 561-581.

Diggles BK (2017). Northern Australia Biosecurity Initiative – Marine Pest and Diseases Risk Assessment. DigsFish Services Report No. DF17-02, 30 May 2017, prepared for The Northern Australia Biosecurity Initiative, Department of Agriculture and Water Resources. 194 pgs.

Diggles BK (2018). Making modern middens at Ningi Ningi (place of many oysters). Australian Shellfish Reef Restoration Network & 19th International Conference on Shellfish Restoration, Adelaide 19-21 February 2018, University of Adelaide, South Australia. pge 17. http://www.oyster-restoration.org/wp-content/uploads/2018/06/Shellfish-Restoration-Conference-Program-2018.pdf

Diggles BK (2020a). CRC NA – Pathogen risk analysis for tropical rock oysters. DigsFish Services Client Report DF20-02, 30 April 2020. 150 pgs.

Diggles BK (2020b). White spot disease in Australian crustaceans. *Animal Health Surveillance Quarterly January to March 2020*, 25(1): 17-21.

Diggles BK, Hine PM (2002). *Bonamia exitiosus* epidemiology in Foveaux Strait oysters. Final research report, OYS1999/01, Ministry of Fisheries, New Zealand. 51 pgs. http://www.digsfish.com/DigglesandHine2002OYS1999-01A.pdf .

Diggles BK, Arthur JR (2010). Pathogen risk analysis for aquatic animals: experiences from nine case studies , pp. 271-290. *In* Bondad-Reantaso, M.G., Jones, J.B., Corsin, F. and Aoki, T. (eds.). Diseases in Asian Aquaculture VII. Fish Health Section, Asian Fisheries Society, Selangor, Malaysia. 385 pgs.

Diggles BK, Nichol J, Hine PM, Wakefield S, Cochennec-Laureau N, Roberts RD, Friedman CS (2002). Pathology of cultured paua (*Haliotis iris* Martyn, 1784) infected by a novel haplosporidian parasite, with some observations on the course of disease. *Diseases of Aquatic Organisms* 50: 219-231.

Diggles BK, Cochennec-Laureau N, Hine M (2003). Comparison of diagnostic techniques for *Bonamia exitiosus* from flat oysters *Ostrea chilensis* in New Zealand. *Aquaculture* 220: 145-156.

Diggles BK, Hine PM, Carson J (2007). The Cook Islands Experience: pearl oyster health investigations. pp. 71-85. In: Bondad-Reantaso MG et al. (eds), Pearl oyster health management: a manual. FAO Fisheries Technical Paper No. 503, Rome FAO, 120 pgs.

Di Giorgio G, Gilardoni C, Ituarte C (2014). Pathology of *Haplosporidium patagon* affecting siphonariid gastropods in Patagonia. *Diseases of Aquatic Organisms* 112: 59-67.

Dinamani P (1971). Occurrence of the Japanese oyster *Crassostrea gigas* (Thunberg) in Northland, New Zealand. *New Zealand Journal of Marine and Freshwater Research* 5: 352-357.

Dinamani P, Hine PM, Jones JB (1987). Occurrence and characteristics of the haemocyte parasite *Bonamia* sp. in the New Zealand dredge oyster *Tiostrea lutaria*. *Diseases of Aquatic Organisms* 3: 37-44.

Ding Z, Yao Y, Zhang F, Wan J, Sun M, Liu H, Zhou G, Tang J, Pan J, Xue H, Zhao Z (2015). The first detection of white spot syndrome virus in naturally infected cultured Chinese mitten crabs, *Eriocheir sinensis* in China. *Journal of Virological Methods* 220: 49-54.

Dommisse M, Hough D (2004). Controlling the Northern Pacific seastar (*Asterias amurensis*) in Australia. Final report for the Australian Government Department of the Environment and Heritage, 52 pgs.

Doroudi MS (1996). Infestation of the pearl oysters by the boring and fouling organisms in the northern coast of Persian Gulf. *Iranian Scientific Fisheries Journal* 4: 15-30.

Dove MC, Nell JA, O'Connor WA (2013). 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*). *Aquaculture Research* 44: 1791–1800.

Dundon WG, Arzul I, Omnes E, Robert M, Magnabosco C, Zambon M, Gennari L, Toffan A, Terregino C, Capua I, Arcangeli G (2011). Detection of Type 1 Ostreid Herpes variant (OsHV-1 µvar) with no associated mortality in French-origin Pacific cupped oyster *Crassostrea gigas* farmed in Italy. *Aquaculture* 314: 49-52.

Dungan CF, Reece KS (2006). *In vitro* propagation of two *Perkinsus* spp. parasites from Japanese Manila clams *Venerupis philippinarum* and description of *Perkinsus honshuensis* n. sp. *Journal of Eukaryotic Microbiology* 53: 316–326.

Dunphy BJ, Wells RMG, Jeffs AG (2005). Polydorid infestation in the flat oyster, *Tiostrea chilensis*: hyposaline treatment for an aquaculture candidate. *Aquaculture International* 13: 351–358.

Dybdahl R, Pass DA (1985). An investigation of mortality of the pearl oyster, *Pinctada maxima*, in Western Australia. *Western Australian Fisheries Department Report* No. 71. 78 pp.

Edmondson DG, Hu B, Norris SJ (2018). Long-term *in vitro* culture of the syphilis spirochete *Treponema pallidum* subsp. *pallidum*. *MBio* 9: e01153-18.

Eide KE, Miller-Morgan T, Heidel JR, Kent ML, Bildfell RJ, LaPatra S, Watson G, Jin L (2011). Investigation of koi herpesvirus latency in koi. *Journal of Virology* 85: 4954-4962.

Ellard K (2011). Tasmanian Abalone Viral Ganglioneuritis 2010 emergency aquatic animal disease response: Summary of findings, March 2011. (Draft report, Department of Primary Industries, Parks, Water and Environment). 5 pgs

Ellard K, Andrewartha R, Baulch T (2011). "Putting the puzzle together". Findings of AVG disease investigations in Tasmania. Powerpoint presentation. Department of Primary Industries, Parks, Water and Environment. 15 pgs

Elston R (1979). Virus-like particles associated with lesions in larval Pacifc oysters (*Crassostrea gigas*). *Journal of Invertebrate Pathology* 33: 71-74.

Elston RA (1997). Special topic review: bivalves mollusc viruses. *World Journal of Microbiology and Biotechnology* 13: 393-403.

Elston RA, Wilkinson MT (1985). Pathology, management and diagnosis of oyster velar virus disease (OVVD). *Aquaculture* 48: 189-210.

Elston RA, Farley CA, Kent ML (1986). Occurrence and significance of bonamiasis in European flat oysters *Ostrea edulis* in North America. *Diseases of Aquatic Organisms* 2: 49–54.

Elston RA, Beattie JH, Friedman C, Hedrick RP, Kent ML (1987). Pathology and significance of fatal inflammatory bacteraemia in the Pacific oyster, *Crassostrea gigas*. *Journal of Fish Diseases* 10: 121-132.

Elston RA, Moore J, Abbott CL (2012). Denman Island disease (causative agent *Mikrocytos mackini*) in a new host, Kumamoto oysters *Crassostrea sikamea*. *Diseases of Aquatic Organisms* 102: 65-71.

Emilia Croce M, Parodi ER (2014). The Japanese alga *Polysiphonia morrowii* (Rhodomelaceae, Rhodophyta) on the South Atlantic Ocean: first report of an invasive macroalga inhabiting oyster reefs. *Helgoland Marine Research* 68: 241–252.

Engelsma MY, Roozenburg I, Joly JP (2008). First isolation of *Nocardia crassostreae* from Pacific oyster *Crassostrea gigas* in Europe. *Diseases of Aquatic Organisms* 80: 229-234.

Engelsma MY, Culloty SC, Lynch SA, Arzul I, Carnegie RB (2014). *Bonamia* parasites: a rapidly changing perspective on a genus of important mollusc pathogens. *Diseases of Aquatic Organisms* 110: 5-23.

Engell-Sorensen K, Andersen P, Holmstrup M (2009). Preservation of the invasive ctenophore *Mnemiopsis leidyi* using acidic Lugol's solution. *Journal of Plankton Research* 31: 917-920.

European Food Safety Authority (EFSA) (2010). Scientific opinion on the increased mortality events in Pacific oysters, *Crassostrea gigas*. *EFSA Journal* 8: 1894. 60 pgs.

Evans O, Hick P, Whittington RJ (2016). Distribution of Ostreid herpesvirus-1 (OsHV-1) microvariant in seawater in a recirculating aquaculture system. *Aquaculture* 458: 21–28.

Evans O, Paul-Pont I, Whittington RJ (2017a). Detection of ostreid herpesvirus 1 microvariant DNA in aquatic invertebrate species, sediment and other samples collected from the Georges River estuary, New South Wales, Australia. *Diseases of Aquatic Organisms* 122: 247-255.

Evans O, Hick P, Whittington RJ (2017b). Detection of Ostreid herpesvirus-1 microvariants in healthy *Crassostrea gigas* following disease events and their possible role as reservoirs of infection. *Journal of Invertebrate Pathology* 148: 20–33.

Ewart JW, Ford SE (1993). History and impact of MSX and dermo diseases on oyster stocks in the Northeast Region. *North East Regional Aquaculture Centre Fact Sheet* No. 200.

Ezgeta-Balic D, Santic D, Segvic-Bubic T, Bojanic N, Buzancic M, Vidjak O, Bojanic Varezic D, Staglicic N, Kundid P, Peharda M, Zuzul I, Grubisic L, Briski E (2020). Competitive feeding interactions between native *Ostrea edulis* and non-native *Crassostrea gigas* with implications of introducing *C. gigas* into commercial aquaculture in the eastern Adriatic Sea. *Marine Environmental Research* 160: 105051.

Farley CA, Banfield WG, Kasnic G, Foster WS (1972). Oyster Herpes-type virus. *Science* 178: 759-760.

Farley CA, Wolff PH, Elston RA (1988). A longterm study of 'microcell' disease in oysters with a description of a new genus, *Mikrocytos* (g.n.), and two new species, *Mikrocytos mackini* (sp.n.) and *Mikrocytos roughleyi* (sp.n.). *Fishery Bulletin* 86: 581−593.

Feist SW, Hine PM, Bateman KS, Stentiford GD, Longshaw M (2009). *Paramarteilia canceri* sp. n. (Cercozoa) in the European edible crab (*Cancer pagurus*) with a proposal for the revision of the order Paramyxida Chatton, 1911. *Folia Parasitologica* 56: 73–85.

Ferguson R (2000). The effectiveness of Australia's response to the black striped mussel incursion in Darwin, Australia. A report of the Marine Pest Incursion Management Workshop – 27-28 August 1999. Department of Environment and Heritage, Canberra. https://www.environment.gov.au/marine/publications/effectiveness-Australia's-response-black-striped-mussel-incursion-darwin-australia

Ferguson LF, Davidson JD, Landry T, Clements JC, Therriault TW (2017). *Didemnum vexillum*: invasion potential via harvesting and processing of the Pacific oyster (*Crassostrea gigas*) in British Columbia, Canada. *Management of Biological Invasions* 8: 553–558.

Fitridge I, Dempster T, Guenther J, de Nys R (2012). The impact and control of biofouling in marine aquaculture: a review. *Biofouling* 28: 649-669.

Flannery G, Lynch SA, Carlsson J, Cross TF, Culloty SC (2014). Assessment of the impact of a pathogen, *Bonamia ostreae*, on *Ostrea edulis* oyster stocks with different histories of exposure to the parasite in Ireland. *Aquaculture* 432: 243–251.

Fletcher LM, Forrest BM, Bell JJ (2013). Natural dispersal mechanisms and dispersal potential of the invasive ascidian *Didemnum vexillum*. *Biological Invasions* 15: 627–643.

Fofonoff PW, Ruiz GM, Steves B, Simkanin C, Carlton JT (2018). National Exotic Marine and Estuarine Species Information System. http://invasions.si.edu/nemesis/, accessed 31 July 2020.

Ford JR, Hamer P (2016). The forgotten shellfish reefs of coastal Victoria: documenting the loss of a marine ecosystem over 200 years since European settlement. *Proceedings of the Royal Society of Victoria* 128: 87-105.

Ford SE (1985). Effects of salinity on survival of the MSX parasite *Haplosporidium nelsoni* (Haskin, Stauber, and Mackin) in oysters. *Journal of Shellfish Research* 5: 85–90.

Ford SE, Haskin HH (1982). History and epizootiology of *Haplosporidium nelsoni* (MSX), an oyster pathogen in Delaware Bay, 1957-1980. *Journal of Invertebrate Pathology* 40: 118- 141.

Ford SE, Bushek D (2012). Development of resistance to an introduced marine pathogen by a native host. *Journal of Marine Research*, 70: 205–223.

Ford SE, Xu Z, Debrosse G (2001). Use of particle filtration and UV irradiation to prevent infection by *Haplosporidium nelsoni* (MSX) and *Perkinsus marinus* (Dermo) in hatchery reared larval and juvenile oysters. *Aquaculture* 194: 37-49.

Ford SE, Allam B, Xu Z (2009). Using bivalves as particle collectors with PCR detection to investigate the environmental distribution of *Haplosporidium nelsoni*. *Diseases of Aquatic Organisms* 83: 159-168.

Ford SE, Paterno J, Scarpa E, Stokes NA, Kim Y, Powell EN, Bushek D (2011). Widespread survey finds no evidence of *Haplosporidium nelsoni* (MSX) in Gulf of Mexico oysters. *Diseases of Aquatic Organisms* 93: 251-256.

Ford SE, Stokes NA, Alcox KA, Flores Kraus BS, Barber RD, Carnegie RB, Burreson EM (2018). Investigating the life cycle of *Haplosporidium nelsoni* (MSX): A review. *Journal of Shellfish Research* 37: 679-693.

Forrest B, Blakemore KA (2006). Evaluation of treatments to reduce the spread of a marine plant pest with aquaculture transfers. *Aquaculture* 257: 333-345.

Forrest BM, Hopkins GA (2013). Population control to mitigate the spread of marine pests: insights from management of the Asian kelp *Undaria pinnatifida* and colonial ascidian *Didemnum vexillum*. *Management of Biological Invasions* 4: 317–326.

Forrest B, Hopkins G, Dodgshun T, Gardner J (2007). Efficacy of acetic acid treatments in the management of marine biofouling. *Aquaculture* 262: 319-332.

Forsström T, Fowler AE, Manninen I, Vesakoski O (2015). An introduced species meets the local fauna: predatory behavior of the crab *Rhithropanopeus harrisii* in the Northern Baltic Sea. *Biological Invasions* 17: 2729–2741.

Foster BA (1971). Desiccation as a factor in the intertidal zonation of barnacles. *Marine Biology* 8: 12–29.

Foster BA, Willan RC (1979). Foreign barnacles transported to New Zealand on an oil platform. *New Zealand Journal of Marine and Freshwater Research* 13(1):143-149.

Fowler AE, McLay CL (2013). Early stages of a New Zealand invasion by *Charybdis japonica* (A. Milne-Edwards, 1861) (Brachyura: Portunidae) from Asia: Population demography. *Journal of Crustacean Biology* 33: 224–234.

Fowler AE, Forsström T, von Numers M, Vesakoski O (2013). The North American mud crab *Rhithropanopeus harrisii* (Gould, 1841) in newly colonized Northern Baltic Sea: distribution and ecology. *Aquatic Invasions* 8: 89–96.

Frances J, Kirkland P, Moloney B, Lyall I (2011). Pacific oyster mortality syndrome: The NSW experience. Proceedings of the First FRDC Australasian Aquatic Animal Health Scientific Conference, Pullman Reef Hotel, Wharf St, Cairns, QLD Australia 5-8 July 2011.

Friedman CS (1996). Haplosporidian infections of the Pacific oyster, *Crassostrea gigas* (Thunberg), in California and Japan. *Journal of Shellfish Research* 15: 597-600.

Friedman CS, Perkins FO (1994). Range extension of *Bonamia ostreae* to Maine, USA. *Journal of Invertebrate Pathology* 64: 179–181.

Friedman CS, Finley CA (2003). Anthropogenic introduction of the etiological agent of withering syndrome, into northern California abalone populations via conservation efforts. *Canadian Journal of Fisheries and Aquatic Science* 60: 1424–1431.

Friedman CS, Crosson LM (2012). Putative phage hyperparasite in the Rickettsial pathogen of abalone, "*Candidatus* Xenohaliotis californiensis". *Microbial Ecology* 64:1064-1072.

Friedman CS, McDowell T, Groff JM, Hollibaugh JT, Manzer D, Hedrick RP (1989). Presence of *Bonamia ostreae* among populations of the European flat oyster, *Ostrea edulis* Linne, in California, USA. *Journal of Shellfish Research* 8: 133-137.

Friedman CS, Beattie, JH, Elston RA, Hedrick RP (1991). Investigation of the relationship between the presence of a Gram positive bacterial infection and summer mortality of the Pacific oyster, *Crassostrea gigas* Thunberg. *Aquaculture* 94: 1–15.

Friedman CS, Thomson M, Chun C, Haaker PL, Hedrick RP (1997). Withering syndrome of the black abalone, *Haliotis cracherodii* (Leach): water temperature, food availability, and parasites as possible causes. *Journal of Shellfish Research* 16: 403−411.

Friedman CS, Beaman BL, Chun J, Goodfellow M, Hedrick RP (1998). *Nocardia crassostreae* sp. nov., the causal agent of nocardiosis in Pacific oysters. *International Journal of Systematic Bacteriology* 48: 237-246.

Friedman CS, Andree KB, Beauchamp KA, Moore JD, Robbins TT, Shields JD, Hedrick RP (2000). *'Candidatus Xenohaliotis californiensis'*, a newly described pathogen of abalone, *Haliotis* spp., along the west *International Journal of Systematic and Evolutionary Microbiology* 50: 847–855.

Friedman CS, Biggs W, Shields JD, Hedrick RP (2002). Transmission of withering syndrome in black abalone, *Haliotis cracherodii* Leach. *Journal of Shellfish Research* 21: 817-824.

Friedman CS, Trevelyan G, Robbins TT, Mulder EP, Fields R (2003). Devlopment of an oral administration of oxytetracycline to control losses due to withering syndrome in cultured red abalone *Haliotis rufescens*. *Aquaculture* 224: 1-23.

Friedman CS, Estes RM, Stokes NA, Burge CA, Hargove JS, Barber BJ, Elston RA, Burreson EM, Reece KS (2005). Herpes virus in juvenile Pacific oysters *Crassostrea gigas* from Tomales Bay, California, coincides with summer mortality episodes. *Diseases of Aquatic Organisms* 63: 33-41.

Friedman CS, Scott BB, Strenge RE, Vadopalas B, McCormick TB (2007). Oxytetracycline as a tool to manage and prevent losses of the endangered white abalone, *Haliotis sorenseni*, caused by withering syndrome. *Journal of Shellfish Research* 26: 877–885.

Friedman CS, Wight N, Crosson LM, VanBlaricom GR, Lafferty KD (2014). Reduced disease in black abalone following mass mortality: phage therapy and natural selection. *Frontiers of Microbiology* 5(78): 1-9.

Friedman CS, Wight N, Crosson LM, VanBlaricom GR, Lafferty KD (2014). Reduced disease in black Fromont J, Craig R, Rawlinson L, Alder J (2005). Excavating sponges that are destructive to farmed pearl oysters in Western and Northern Australia. *Aquaculture Research* 36: 150-162.

Fusianto C, Hick PM, Becker JA (2019). Stability of Infectious spleen and kidney necrosis virus and susceptibility to physical and chemical disinfectants. *Aquaculture* 506: 104-111.

Gagné N, Cochennec N, Stephenson M, Mc Gladdery S, Meyer GR, Bower S. (2008). First report of a *Mikrocytos*-like parasite in European oysters *Ostrea edulis* from Canada after transport and quarantine in France. *Diseases of Aquatic Organisms* 80: 27–35.

Galinou-Mitsoudi S, Sinis AI (1994). Reproductive cycle and fecundity of the date mussel, *Lithophaga lithophaga* (Bivalvia: Mytilidae). *Journal of Molluscan Studies* 60: 371–385.

Galinou-Mitsoudi S, Sinis AI (1995). Age and growth of *Lithophaga lithophaga* (Linnaeus, 1758) (Bivalvia: Mytilidae), based on annual growth lines in the shell. *Journal of Molluscan Studies* 61: 435–453.

Galinou-Mitsoudi S, Sinis AI (1997). Population dynamics of the date mussel, *Lithophaga lithophaga* (L., 1758) (Bivalvia : Mytilidae), in the Evoikos Gulf (Greece). *Helgolander Meeresuntersuchungen* 51: 137-154.

Gallucci F, Hutchings P, Gribben PE, Fonseca G (2012). Habitat alteration and community-level effects of an invasive ecosystem engineer: a case study along the coast of NSW, Australia. *Marine Ecology Progress Series* 449: 95-108.

Garcia C, Robert M, Arzul I, Chollet B, Joly JP, Miossec L, Comtet T, Berthe F (2006). Viral gametocytic hypertrophy of *Crassostrea gigas* in France: from occasional records to disease emergence? *Diseases of Aquatic Organisms* 70: 193-199.

Garcia C, Thébault A, Dégremont L, Arzul I, Miossec L, Robert M, Chollet B, François C, Joly J, Ferrand S, Kerdudou N, Renault T (2011). Ostreid herpesvirus 1 detection and relationship with *Crassostrea gigas* spat mortality in France between 1998 and 2006. *Veterinary Research* 42: 73.

Garcia C, Haond C, Chollet B, Nerac M, Omnes E, Joly JP, Dubreui C, Serpin D, Langlade A, Le Gal D, Terre-Terrillon A, Courtois O, Guichard B, Arzul I (2018). Descriptions of *Mikrocytos veneroïdes* n. sp. and *Mikrocytos donaxi* n. sp. (Ascetosporea: Mikrocytida: Mikrocytiidae), detected during important mortality events of the wedge clam *Donax trunculus* Linnaeus (Veneroida: Donacidae), in France between 2008 and 2011. *Parasites and Vectors* 11:119.

Gavine F, Ingram B, Doroudi M (2009). Development of management strategies for herpes-like virus infection in Abalone. FRDC Project No. 2006/243, May 2009. https://www.frdc.com.au/Archived-Reports/FRDC%20Projects/2006-243-DLD.PDF

Gaughan DJ (2002). Disease-translocation across geographic boundaries must be recognized as a risk even in the absence of disease identification : the case with Australian *Sardinops*. *Reviews in Fish Biology and Fisheries* 11: 113-123.

GESAMP (Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection) (1997). Opportunistic settlers and the problem of the ctenophore *Mnemiopsis leidyi* invasion in the Black Sea. *Reports and Studies GESAMP (58)*. 84 pgs . https://wedocs.unep.org/bitstream/handle/20.500.11822/9177/opportunistic_settlers.pdf?sequence=1&isAllowed=y

Gibbons MJ, Boero F, Brotz L (2016). We should not assume that fishing jellyfish will solve our jellyfish problem. *ICES Journal of Marine Science* 73: 1012-1018.

Gillies CL, Creighton C, McLeod IM (Eds.) (2015a). Shellfish Reef Habitats: a synopsis to underpin the repair and conservation of Australia's environmentally, socially and economically important bays and estuaries. Report to the National Environmental Science Programme. Marine Biodiversity Hub. Centre for Tropical Water and Aquatic Ecosystem Research (TropWATER) Publication, James Cook University, Townsville.

Gillies CL, Fitzsimons JA, Branigan S, Hale L, Hancock B, Creighton C, Alleway H, Bishop MJ, Brown S, Chamberlain D, Cleveland B, Crawford C, Crawford M, Diggles BK, Ford JR, Hamer P, Hart A, Johnston E,

McDonald T, Macleod I, Pinner B, Russell K, Winstanley R (2015b). Scaling-up marine restoration efforts in Australia. *Ecological Management and Restoration* 16: 84-85.

Gillies CL, McLeod IM, Alleway HK, Cook P, Crawford C, Creighton C, Diggles BK, Ford J, Hamer P, Heller-Wagner G, Lebrault E (2018). Australian shellfish ecosystems: past distribution, current status and future direction. *PloS One* 13 (2), e0190914.

Gillies CL, Castine SA, Alleway HK, Crawford C, Fitzsimons JA, Hancock B, Koch P, McAfee D, McLeod IM, zu Ermgassen PS (2020). Conservation status of the oyster reef ecosystem of southern and eastern Australia. *Global Ecology and Conservation* 22: e00988

Glasby TM (2013). *Caulerpa taxifolia* in seagrass meadows: killer or opportunistic weed? *Biological Invasions* 15: 1017-1035.

Glasby TM, Creese RG, Gibson PT (2005). Experimental use of salt to control the invasive marine alga *Caulerpa taxifolia* in New South Wales, Australia. *Biological Conservation* 122: 573–580.

Global Invasive Species Database (GISD) (2015). Species profile *Perna perna*. http://www.iucngisd.org/gisd/species.php?sc=742 (Accessed 31 July 2020).

Go J, Whittington RJ (2006). Experimental transmission and virulence of a megalocytivirus (Family *Iridoviridae*) of Dwarf gourami (*Colisa lalia*) from Asia in Murray cod (*Maccullochella peelii peelii*) in Australia. *Aquaculture* 258: 140–149.

Goggin CL (1998) Proceedings of a meeting on the biology and management of the introduced seastar *Asterias amurensis* in Australian waters. Centre for Research on Introduced Marine Pests. Technical Report no. 15. CSIRO Marine Research, Hobart, 67 pgs

Goggin CL, Lester RJG (1987). Occurrence of *Perkinsus* species (Protozoa, Apicomplexa) in bivalves from the Great Barrier Reef. *Diseases of Aquatic Organisms* 3: 113-117.

Goggin CL, Barker SC (1993). Phylogenetic position of the genus *Perkinsus* (Protista, Apicomplexa) based on small subunit ribosomal RNA. *Molecular and Biochemical Parasitology* 60: 65-70.

Goggin CL, Lester RJG (1995). *Perkinsus*, a protistan parasite of abalone in Australia: a review. *Marine and Freshwater Research* 46: 639-646.

Goggin CL, Sewell KB, Lester RJG (1989). Cross-infection experiments with Australian *Perkinsus* species. *Diseases of Aquatic Organisms* 7: 55-59.

Goggin CL, Sewell KB, Lester RJG (1990). Tolerances of *Perkinsus* spp. (Protozoa, Apicomplexa) to temperature, chlorine and salinity. *Journal of Shellfish Research* 9: 145–148.

Goncalves P, Raftos D, Jones D, Anderson K, Jones B, Snow M (2017). Identifying the cause of Oyster Oedema Disease (OOD) in pearl oysters (*Pinctada maxima*), and developing diagnostic tests for OOD. FRDC Project No. 2013/002. 81 pgs.

González R, Lohrmann KB, Pizarro J, Brokordt K (2014). Differential susceptibility to the Withering Syndrome agent and renal coccidia in juvenile *Haliotis rufescens, Haliotis discus hannai* and the interspecific hybrid. *Journal of Invertebrate Pathology* 116: 13-17.

Grabowski JH, Peterson CH (2007). Restoring oyster reefs to recover ecosystem services. In: Cuddington K, Byers J, Wilson W, Hastings A eds. Ecosystem engineers: plants to protists. Boston, MA, Academic Press. pp. 281-298.

Green B (2011). Living Marine Resources Management Act 1995. Fisheries (Biosecurity) Order (No. 1) 2011. Minister for Primary Industries and Water. 6 July 2011.

Green TJ, Barnes AC (2010). Reduced salinity, but not estuarine acidification, is a cause of immune-suppression in the Sydney rock oyster *Saccostrea glomerata*. *Marine Ecology Progress Series* 402: 161-170.

Green TJ, Raftos D, O'Connor W, Adlard RD, Barnes AC (2011). Disease prevention strategies for QX disease (*Marteilia sydneyi*) of Sydney rock oysters (*Saccostrea glomerata*). *Journal of Shellfish Research* 30: 47-53.

Greenhill JF (1965). New records of marine Mollusca from Tasmania. *Papers and Proceedings of the Royal Society of Tasmania* 99: 67-69.

Gregory LP, Campbell ML, Primo C, Hewitt CL (2012). Biotic and abiotic factors affecting the Tasmanian distribution and density of the introduced New Zealand porcelain crab *Petrolisthes elongatus*. *Aquatic Invasions* 7: 491-501.

Gribben PE, Wright JT, Byers JE, Glasby TM (2013). Positive versus negative effects of an invasive ecosystem engineer on different components of a marine ecosystem. *Oikos* 122: 816–824.

Grizel H (1985). Etude des recentes epizooties de l'huitre plate *Ostrea edulis* Linne et de leur impact sur l'osteiculture Bretonne. These, Academie de Montpellier, Universite des Sciences et Techniques du Languedoc.

Grizel H, Héral M (1991). Introduction in France of the Japanese oyster (*Crassostrea gigas*). *J. Cons. Int. Explor. Mer* 47: 399-403.

Grizel H, Comps M, Bonami JR, Cousserans F, Duthoit JL, LePennec MA (1974). Research on the agent of digestive gland disease of *Ostrea edulis* Linné. *Science et Pêche. Bulletin d'Information et de Documentation de l'Institut Scientifique et Technique des Pêches Maritimes* 240: 7-30.

Grizel H, Comps M, Raguenes D, Leborgne Y, Tige G, Martin A (1982). Bilan des essais d'acclimatation d'*Ostrea chilensis* sur les cotes de Bretagne. *Revue des Travaux de l'Institut des Pêches Maritimes* 46: 209-225.

Gröner F, Lenz M, Wahl M, Jenkins SR (2011). Stress resistance in two colonial ascidians from the Irish Sea: The recent invader *Didemnum vexillum* is more tolerant to low salinity than the cosmopolitan *Diplosoma listerianum*. *Journal of Experimental Marine Biology and Ecology* 409: 48–52.

Grove S, Willan R, Ellard K, Dann A (2018). Detection of the invasive exotic Japanese softshell clam *Mya japonica* Jay, 1857 (Bivalvia: Myidae) in Tasmania. *The Malacological Society of Australasia Newsletter* 167: 3-5.

Growcott A, Kluza D, Georgiades E (2016). Literature review: In-water systems to remove or treat biofouling in vessel sea chests and internal pipework. New Zealand MPI Technical Paper No: 2016-16. March 2016, 58 pgs.

Guenther J, Southgate PC, de Nys R (2006). The effect of age and shell size on accumulation of fouling organisms on the Akoya pearl oyster *Pinctada fucata* (Gould). *Aquaculture* 253: 366-373.

Haaker PL, Parker DO, Togstad H, Richards D, Davis GE, Friedman CS (1992). Mass mortality and withering foot syndrome in black abalone, *Haliotis cracherodii*, in California. In: Abalone of the World: Biology, Fisheries and Culture, Shepard SA, Tegner MJ, del Proo Guzman SA (eds), Fishing News Books, Cambridge, UK, vol. 214-224.

Hampson DJ (2017). The Spirochete *Brachyspira pilosicoli*, enteric pathogen of animals and humans. *Clinical Microbiology Reviews* 31: e00087-17.

Hand RE, Nell JA, Smith IR, Maguire GB (1998). Studies on triploid oysters in Australia. XI. Survival of diploid and triploid Sydney rock oysters, *Saccostrea commercialis* (Iredale and Roughley) through outbreaks of winter mortality caused by *Mikrocytos roughleyi* infestation. *Journal of Shellfish Research* 17: 1129-1135.

Handlinger J, Bastianello S, Carson J, Callinan R, Creeper J, Deveney M, Freeman K, Forsyth M, Hooper C, Jones B, Lancaster M, Landos M, Loh R, Oyay BS, Phillips P, Pyecroft S, Stephens F (2005). Abalone Aquaculture Subprogram: A national survey of diseases of commercially exploited abalone species to support trade and translocation issues and the development of health surveillance programs. FRDC Project No. 2002/201, Fisheries Research and Development Corporation, Canberra, ACT, Australia.

Handley SJ (2000). Larval development of *Boccardia knoxi*, a shell infesting spionid polychaete. *New Zealand Journal of Marine and Freshwater Research* 34: 681-686.

Hanfling B, Edwards F, Gherardi F (2011). Invasive alien Crustacea: dispersal, establishment, impact and control. *BioControl* 56: 573-595.

Harding JM (2003). Predation by blue crabs, *Callinectes sapidus*, on rapa whelks, *Rapana venosa*: possible natural controls for an invasive species. *Journal of Experimental Marine Biology and Ecology* 297: 161-177.

Harding JM, Mann R (2016). Habitat disturbance combined with life history traits facilitate establishment of *Rapana venosa* in the Chesapeake Bay. *Journal of Shellfish Research* 35: 885-910.

Harding JM, Unger MA, Jestel EA, Mann R (2017). Sex and site-specific trends in veined rapa whelk (*Rapana venosa*) tributyltin bioaccumulation: considerations for biomonitoring. *Journal of the Marine Biological Association of the United Kingdom* 97: 495-1504.

Hardy-Smith P (2006). Report on the events surrounding unusually high mortalities of farmed abalone in Victoria. 29 August 2006. Panaquatic Health Solutions.

Hartikainen H, Ashford OS, Berney C, Okamura B, Feist SW, Baker-Austin C, Stentiford GD, Bass D (2014a). Lineage-specific molecular probing reveals novel diversity and ecological partitioning of haplosporidians. *The International Society for Microbial Ecology Journal* 8: 177–186.

Hartikainen H, Stentiford GD, Bateman KS, Berney C, Feist SW, Longshaw M, Okamura B, Stone D, Ward G, Wood C, Bass D (2014b). Mikrocytids are a broadly distributed and divergent radiation of parasites in aquatic invertebrates. *Current Biology* 24: 807–812.

Haskin HH, Stauber LA, Mackin JA (1966). *Minchinia nelsoni* n. sp. (Haplosporida, Haplosporidiidae): Causative agent of the Delaware Bay oyster epizoötic. *Science* 153: 1414-1416.

Haskin HH, Ford SE (1982). *Haplosporidium nelsoni* (MSX) on Delaware Bay seed oyster beds: A host-parasite relationship along a salinity gradient. *Journal of Invertebrate Pathology* 40: 388-405.

Haskin HH, Andrews JD (1988). Uncertainties and speculations about the life cycle of the eastern oyster pathogen *Haplosporidium nelsoni* (MSX). In: Fisher WS (ed) Disease processes in marine bivalve molluscs. *American Fisheries Society Special Publication* 18: 5-22.

Haswell WA (1885). On a destructive parasite of the rock oyster. *Proceedings of the Linnean Society of New South Wales* 10: 272-275.

Hatanaka M, Kosaka M (1959). Biological studies on the population of the starfish, *Asterias amurensis*, in Sendai Bay. *Tohoku Journal of Agricultural Research* 9: 159-173.

Hayes KR, Sliwa C, Migus S, McEnnulty F, Dunstan P (2005). National priority pests: Part II. Ranking of Australian marine pests. CSIRO Marine Research, Hobart. February 2005. 94 pgs

Heasman M, Diggles BK, Hurwood D, Mather P, Pirozzi I, Dworjanyn S (2004). Paving the way for continued rapid development of the flat oyster (*Ostrea angasi*) farming industry in New South Wales. Final Report to the Department of Transport & Regional Services Project No. NT002/0195 June 2004 NSW

Fisheries Final Report Series No. 66. NSW Fisheries, Nelson Bay. https://www.dpi.nsw.gov.au/__data/assets/pdf_file/0003/134481/Output-448.pdf

Heersink D, Paini D, Caley P, Barry S (2014). Asian Green Mussel: estimation of approach rate and probability of invasion via biofouling. Plant Biosecurity CRC - Research Services Final Report 28 pgs. https://publications.csiro.au/rpr/pub?pid=csiro:EP172373

Heersink DK, Caley P, Paini D, Barry SC (2020). When exotic introductions fail: updating invasion beliefs. *Biological Invasions* 22: 1097-1107.

Hewitt CL, Campbell ML, Thresher RE, Martin RB (1999). Marine biological invasions of Port Phillip Bay, Victoria. Centre for Research on Introduced Marine Pests, Technical Report No. 20. CSIRO Marine Research, Hobart, 344 pgs.

Hewitt CL, Campbell ML, Thresher RE, Martin RB, Boyd S, Cohen BF, Currie DR, Gomon MF, Keough MJ, Lewis JA, Lockett MM, Mays N, McArthur MA, O'Hara TD, Poore GCB, Ross DJ, Storey MJ, Watson JE, Wilson RS (2004). Introduced and cryptogenic species in Port Phillip Bay, Victoria, Australia. *Marine Biology* 144: 183–202.

Herrbach B (1971). Sur une affection parasitaire de la glande digestive de l'huître plate, *Ostrea edulis* Linné. *Revue des Travaux de I'Institut des Pêches Maritimes* 35: 79-87.

Hervio D, Bachere E, Boulo V, Cochennec N, Vuillemin V, LeCoguic Y, Cailletaux G, Mazurie J, Mialhe E (1995). Establishment of an experimental infection protocol for the flat oyster, *Ostrea edulis*, with the intrahaemocytic protozoan parasite, *Bonamia ostreae*: application in the selection of parasite-resistant oysters. *Aquaculture* 132: 183-194.

Hervio D, Bower S, Meyer G (1996). Detection, isolation, and experimental transmission of *Mikrocytos mackini*, a microcell parasite of Pacific oysters *Crassostrea gigas* (Thunberg). *Journal of Invertebrate Pathology* 67: 72−79.

Hick P, Evans O, Looi R, English C, Whittington RJ (2016). Stability of Ostreid herpesvirus-1 (OsHV-1) and assessment of disinfection of seawater and oyster tissues using a bioassay. *Aquaculture* 450: 412–421.

Hick PM, Evans O, Rubio A, Dhand NK, Whittington RJ (2018). Both age and size influence susceptibility of Pacific oysters (*Crassostrea gigas*) to disease caused by Ostreid herpesvirus-1 (OsHV-1) in replicated field and laboratory experiments. *Aquaculture* 489: 110–120.

Hill KM, Carnegie RB, Aloui-Bejaoui N, Gharsalli RE, White DM, Stokes NA, Burreson EM (2010). Observation of a *Bonamia* sp. infecting the oyster *Ostrea stentina* in Tunisia, and a consideration of its phylogenetic affinities. *Journal of Invertebrate Pathology* 103: 179-185.

Hill KM, Stokes NA, Webb SC, Hine PM, Kroeck MA, Moore JD, Morley MS, Reece KS, Burreson EM, Carnegie RB (2014). Phylogenetics of *Bonamia* parasites based on small subunit and internal transcribed spacer region ribosomal DNA sequence data. *Diseases of Aquatic Organisms* 110: 33–54.

Hillock KA, Costello MJ (2013). Tolerance of the invasive tunicate *Styela clava* to air exposure. *The Journal of Bioadhesion and Biofilm Research,* DOI: 10.1080/08927014.2013.832221

Hill-Spanik KM, McDowell JR, Stokes NA, Reece KS, Burreson EM, Carnegie RB (2015). Phylogeographic perspective on the distribution and dispersal of a marine pathogen, the oyster parasite *Bonamia exitiosa*. *Marine Ecology Progress Series* 536: 65-75.

Hine PM (1991a). The annual pattern of infection by *Bonamia* sp. in New Zealand flat oysters, *Tiostrea chilensis*. *Aquaculture* 93: 241-251.

Hine PM (1991b). Ultrastructural observations on the annual infection pattern of *Bonamia* sp. in flat oysters *Tiostrea chilensis*. *Diseases of Aquatic Organisms* 11: 163-171.

Hine PM (1996a). The ecology of *Bonamia* and decline of bivalve molluscs. *New Zealand Journal of Ecology* 20: 109-116.

Hine PM (1996b). Southern hemisphere mollusc diseases and an overview of associated risk assessment problems. *Revue Scientifique Technique de Office International Epizooties* 15(2): 563-577.

Hine PM (1997). Health status of commercially important molluscs in New Zealand. *Surveillance* 24: 24-28.

Hine PM (2002). Severe apicomplexan infection in the oyster *Ostrea chilensis*: a possible predisposing factor in bonamiosis. *Diseases of Aquatic Organisms* 51: 49-60.

Hine PM (2020). Haplosporidian host-parasite interactions. *Fish and Shellfish Immunology* 103: 190-199.

Hine PM, Jones JB (1994). *Bonamia* and other aquatic parasites of importance to New Zealand. *New Zealand Journal of Zoology* 21: 49-56.

Hine PM, Thorne T (1997). Replication of herpes-like viruses in haemocytes of adult flat oysters *Ostrea angasi*: an ultrastructural study. *Diseases of Aquatic Organisms* 29: 189-196.

Hine PM, Wesney B (1997). Virus-like particles associated with cytopathology in the digestive gland epithelium of scallops *Pecten novaezelandiae* and toheroa *Paphies ventricosum*. *Diseases of Aquatic Organisms* 29: 197-204.

Hine PM, Thorne T (1998). *Haplosporidium* sp. (Haplosporidia) in hatchery-reared pearl oysters, *Pinctada maxima* (Jameson, 1901), in north Western Australia. *Journal of Invertebrate Pathology* 71: 48–52.

Hine PM, Thorne T (2000). A survey of some parasites and diseases of several species of bivalve mollusc in northern Western Australia. *Diseases of Aquatic Organisms* 40: 67-78.

Hine PM, Thorne T (2002). *Haplosporidium* sp. (Alveolata: Haplosporidia) associated with mortalities among rock oysters *Saccostrea cucullata* in north Western Australia. *Diseases of Aquatic Organisms* 51: 123–133.

Hine PM, Diggles BK (2002a). Prokaryote infections in the New Zealand scallops *Pecten novaezelandiae* and *Chlamys delicatula*. *Diseases of Aquatic Organisms* 50: 137-144.

Hine PM, Diggles BK (2002b). The distribution of *Perkinsus olseni* in New Zealand bivalve molluscs. *Surveillance* 29: 8-11.

Hine PM, Wesney B, Hay BE (1992). Herpesviruses associated with mortalities among hatchery reared larval Pacific oysters *Crassostrea gigas*. *Diseases of Aquatic Organisms* 12: 135-42.

Hine PM, Wesney B, Besant P (1998). Replication of a herpes-like virus in larvae of the flat oyster *Tiostrea chilensis* at ambient temperatures. *Diseases of Aquatic Organisms* 32: 161-171.

Hine PM, Cochennec-Laureau N, Berthe FCJ (2001a). *Bonamia exitiosus* n. sp. (Haplosporidia) infecting flat oysters *Ostrea chilensis* in New Zealand. *Diseases of Aquatic Organisms* 47: 63−72.

Hine PM, Bower SM, Meyer GR, Cochennec-Laureau N, Berthe FCJ (2001b). Ultrastructure of *Mikrocytos mackini*, the cause of Denman Island disease in oysters *Crassostrea* spp. and *Ostrea* spp. in British Columbia, Canada. *Diseases of Aquatic Organisms* 45: 215–227.

Hine PM, Wakefield S, Diggles BK, Webb VL, Maas EW (2002a). The ultrastructure of a haplosporidian containing Rickettsiae, associated with mortalities among cultured paua *Haliotis iris*. *Diseases of Aquatic Organisms* 49: 207-219.

Hine PM, Diggles BK, Parsons M, Pringle A, Bull B (2002b). The effects of stressors on the dynamics of *Bonamia exitiosus* Hine, Cochennec-Laureau & Berthe, infections in flat oysters *Ostrea chilensis* (Philippi). *Journal of Fish Diseases* 25: 545-554.

Hine PM, Carnegie RB, Burreson EM, Engelsma MY (2009). Inter-relationships of haplosporidians deduced from ultrastructural studies. *Diseases of Aquatic Organisms* 83: 247-256.

Hirano M, Kanai K, Yoshikoshi Y (2002). Contact infection trials failed to reproduce the disease condition of mass mortality in cultured pearl oyster *Pinctada fucata martensii*. *Fisheries Science* 68: 700–702.

Hirano M, Sugishita Y, Mobin SM, Kanai K, Yoshikoshi K (2005). An investigation of the pathology associated with mass mortality events in the cultured Japanese pearl oyster *Pinctada fucata martensii* at four farms in western Japan. *Journal of Aquatic Animal Health* 17: 323–337.

Hoese HD (1964). Studies on oyster scavengers and their relation to the fungus *Dermocystidium marinum*. *Proceedings of the National Shellfisheries Association* 53: 161-174.

Honkoop PJ, Bayne BL (2002). Stocking density and growth of the Pacific oyster (*Crassostrea gigas*) and the Sydney rock oyster (*Saccostrea glomerata*) in Port Stephens, Australia. *Aquaculture* 213: 171-186.

Hooper C, Hardy-Smith P, Handlinger J (2007). Ganglioneuritis causing high mortalities in farmed Australian abalone (*Haliotis laevigata* and *Haliotis rubra*). *Australian Veterinary Journal* 85: 188-193.

Hopkins GA, Prince M, Cahill PL, Fletcher LM, Atlah J (2016). Desiccation as a mitigation tool to manage biofouling risks: trials on temperate taxa to elucidate factors influencing mortality rates, *Biofouling* 32: 1-11.

Horn PL (1982). Adaptations of the chiton *Sypharochiton pelliserpentis* to rocky and estuarine habitats. New *Zealand Journal of Marine and Freshwater Research* 16 : 253-261.

Howard AE (1994). The possibility of long distance transmission of *Bonamia* by fouling on boat hulls. *Bulletin of the European Association of Fish Pathologists* 14: 211-212.

Hummel H, Fortuin AW, De Wolf L, Meijboom A (1988). Mortality of intertidal benthic animals after a period of prolonged emersion. *Journal of Experimental Marine Biology and Ecology* 121: 241-254.

Humphrey JD, Norton JH (2005). The pearl oyster (*Pinctada maxima* Jameson, 1901). An atlas of functional anatomy, pathology and histopathology. NT Department of Primary Industries, 110 pgs.

Humphrey JD, Barton MA (2009). An unsuccessful attempt to experimentally transmit "oyster oedema disease syndrome" to pearl oyster spat. In: NT Government Technote 125, May 2009. pp. 1-7.

Humphrey JD, Norton JH, Jones JB, Barton MA, Connell MT, Shelley CC, Creeper JH (1998). Pearl oyster (*Pinctada maxima*) aquaculture: Health survey of Northern Territory, Western Australia and Queensland pearl oyster beds and farms. Fisheries Research and Development Corporation (FRDC) Project No. 94/079, Final Report. 108 pp.

Husmann, G, Gerdts G, Wichels A (2010). Spirochetes in crystalline styles of marine bivalves: Group-specific PCR detection and 16S rRNA sequence analysis. *Journal of Shellfish Research* 29: 1069-1075.

ICES (2004). Alien Species Alert: *Rapana venosa* (veined whelk). Mann R, Occhipinti A, Harding JM (eds). ICES Cooperative Research Report No. 264. 14 pgs.

ICES (2010). MSX disease of oysters caused by *Haplosporidium nelsoni*. Original by Jay D Andrews, revised and updated by Susan E. Ford. ICES Identification Leaflets for Diseases and Parasites of Fish and Shellfish. Leaflet No. 38. 4 pp.

Imanaka S, Itoh N, Ogawa K, Wakabayashi H (2001). Seasonal fluctuations in the occurrence of abnormal enlargement of the ovary of Pacific oyster *Crassostrea gigas* at Gokasho Bay Mie, Japan. *Fish Pathology* 36: 83–91.

Inglis SD, Kristmundsson A, Freeman MA, Levesque M, Stokesbury K (2016). Gray meat in the Atlantic sea scallop, Placopecten magellanicus, and the identification of a known pathogenic scallop apicomplexan. *Journal of Invertebrate Pathology* 141: 66–75.

Itoh N, Oda T, Ogawa K, Wakabayashi H (2002). Identification and development of a paramyxean ovarian parasite in the Pacific oyster *Crassostrea gigas*. *Fish Pathology* 37: 23–28.

Itoh N, Komiyama H, Ueki N, Ogawa K (2004a). Early developmental stages of a protozoan parasite, *Marteilioides chungmuensis* (Paramyxea), the causative agent of the ovary enlargement disease in the Pacific oyster, *Crassostrea gigas*. *International Journal for Parasitology* 34: 1129–1135.

Itoh N, Tun TL, Komiyama H, Ueki N, Ogawa K (2004b). An ovarian infection in the Iwagaki oyster, *Crassostrea nippona*, with the protozoan parasite *Marteilioides chungmuensis*. *Journal of Fish Diseases* 27: 311-314.

Itoh N, Yamamoto T, Kang HS, Choi KS, Green TJ, Carrasco N, Awaji M, Chow S (2014). A novel paramyxean parasite, *Marteilia granula* sp. nov. (Cercozoa), from the digestive gland of Manila clam, *Ruditapes philippinarum*, in Japan. *Journal of Fish Pathology* 49: 181–193.

Itoh N, Komatsu Y, Maeda K, Hirase S, Yoshinaga T (2019). First discovery of *Perkinsus beihaiensis* in Mediterranean mussels (*Mytilus galloprovincialis*) in Tokyo Bay, Japan. *Journal of Invertebrate Pathology* 166: 107226.

Ituarte C, Bagnato E, Siddall ME, Cremonte F (2014). A new species of *Haplosporidium* Caullery & Mesnil, 1899 in the marine false limpet *Siphonaria lessonii* (Gastropoda: Siphonariidae) from Patagonia. *Systematic Parasitology* 88: 63−73.

Javidpour J, Molinero JC, Lehmann A, Hansen T, Sommer U (2009a). Annual assessment of the predation of *Mnemiopsis leidyi* in a new invaded environment, the Kiel Fjord (Western Baltic Sea): a matter of concern? *Journal of Plankton Research* 31: 729-738.

Javidpour J, Molinero JC, Peschutter J, Sommer U (2009b). Seasonal changes and population dynamics of the ctenophore *Mnemiopsis leidyi* after its first year of invasion in the Kiel Fjord, western Baltic Sea. *Biological Invasions* 11: 873-882.

Jeffs A, Hancock B, zu Ermgassen PS, Pogoda B (2019). Biosecurity and permitting in shellfish reef restoration. In: Restoration Guidelines for Shellfish Reefs (eds. Fitzsimons J, Branigan S, Brumbaugh RD, McDonald T, zu Ermgassen PS), pp. 30-35. The Nature Conservancy, Arlington VA, USA.

Jenkins C, Hick P, Gabor M, Spiers Z, Fell SA, Gu X, Read A, Go J, Dove M, O'Connor M, Kirkland PD, Frances J (2013). Identification and characterisation of an ostreid herpesvirus-1 microvariant (OsHV-1 µ-var) in *Crassostrea gigas* (Pacific oysters) in Australia. *Diseases of Aquatic Organisms* 105: 109-126.

Johnston C, Keeling S, Bingham P, Brosnahan C, Gais E, McDonald W, O'Keefe J (2011). Aspects of an ostreid herpesvirus outbreak in New Zealand. Proceedings of the First FRDC Australasian Aquatic Animal Health Scientific Conference, Pullman Reef Hotel, Wharf St, Cairns, QLD Australia 5-8 July 2011.

Jones JB (2007a). Review of pearl oyster mortalities and disease problems, pp. 61-70. In: Bondad-Reantaso MG et al. (eds), Pearl oyster health management: a manual. FAO Fisheries Technical Paper No. 503, Rome FAO, 120 pgs.

Jones JB (2007b). The Australian experience: pearl oyster mortalities and disease problems, pp. 87-93. In: Bondad-Reantaso MG et al. (eds), Pearl oyster health management: a manual. FAO Fisheries Technical Paper No. 503, Rome FAO, 120 pgs.

Jones JB, Creeper J (2006). Diseases of pearl oysters and other molluscs: A Western Australian perspective. *Journal of Shellfish Research* 25: 233-238.

Jones JB, Stephens F (2006). Aquatic Animal Health Subprogram: development of a national translocation policy using abalone and prawns as templates for other aquatic species. FRDC Project 2004/080. 86 pgs.

Jones JB, Scotti PD, Dearing SC, Wesney B (1996). Virus-like particles associated with marine mussel mortalities in New Zealand. *Diseases of Aquatic Organisms* 25: 143-149.

Jones JB, Crockford M, Creeper J, Stephens F (2010). Histopathology of oedema in pearl oysters *Pinctada maxima*. *Diseases of Aquatic Organisms* 91: 67-73.

Jones MB, Greenwood JG (1982). Water loss of a porcelain crab *Petrolisthes elongatus* (Milne Edwards, 1837) (Decapoda, Anomura) during atmospheric exposure. *Comparative Biochemistry and Physiology A* 72: 631-636.

Jute A, Dunphy BJ (2017). The potential efficacy and application of freshwater and hypersaline immersion to control the spread of a marine invasive species. *Biological Invasions* 19: 1137–1141.

Kamaishi T, Yoshinaga T (2002). Detection of *Haplosporidium nelsoni* in Pacific oyster *Crassostrea gigas* in Japan. *Fish Pathology* 37: 193-195.

Kang HS, Yang HS, Reece KS, Hong HK, Park KI, Choi KS (2016). First report of *Perkinsus honshuensis* in the variegated carpet shell clam *Ruditapes variegatus* in Korea. *Diseases of Aquatic Organisms* 122: 35-41.

Kang HS, Itoh N, Limpanont Y, Lee HM, Whang I, Choi KS (2019). A novel paramyxean parasite, *Marteilia tapetis* sp. nov. (Cercozoa) infecting the digestive gland of Manila clam *Ruditapes philippinarum* from the southeast coast of Korea. *Journal of Invertebrate Pathology* 163: 86-93.

Kasai H, Muto Y, Yoshimizu M (2005). Virucidal effects of ultraviolet, heat treatment and disinfectants against Koi Herpesvirus (KHV). *Fish Pathology* 40: 137-138.

Katayama K, Ikeda Z (1987). Tolerance of fresh water, hot water, and sun-drying by *Didemnum moseleyi*, fouling organisms attached to culture oyster. *Bulletin of the Fisheries Experiment Station, Okayama Prefecture* 2: 104–106.

Katkansky SC, Dahlstrom WA, Warner RW (1969). Observations on survival and growth of the European flat oyster, *Ostrea edulis*, in California. *California Fish and Game* 55: 69-74.

Katkansky SC, Warner RW (1974). Pacific oyster disease and mortality studies in California. Marine Resources Technical Report No. 25, California Dept. of Fish and Game, Long Beach. 51 pgs.

Kauano RV, Roper JJ, Rocha RM (2017). Small boats as vectors of marine invasion: experimental test of velocity and desiccation as limits. *Marine Biology* 164: 27.

Keeling SE, Brosnahan CL, Williams R, Gias E, Hannah M, Bueno R, McDonald WL, Johnston C (2014). New Zealand juvenile oyster mortality associated with ostreid herpesvirus 1- an opportunistic longitudinal study. *Diseases of Aquatic Organisms* 109: 231-239.

Kefi FJ, Boubaker S, El Menif NT (2014). Relative growth and reproductive cycle of the date mussel *Lithophaga lithophaga* (Linnaeus, 1758) sampled from the Bizerte Bay (Northern Tunisia). *Helgoland Marine Research* 68: 439–450.

Kerr R, Ward GM, Stentiford GD, Alfjorden A, Mortensen S, Bignell JP, Feist SW, Villalba A, Carballa MJ, Cao A, Arzul I, Ryder D, Bass D (2018). *Marteilia refringens* and *Marteilia pararefringens* sp. nov. are distinct parasites of bivalves and have different European distributions. *Parasitology* 145: 1483–1492.

Kershaw RC (1955). A systematic list of the Mollusca of Tasmania, Australia. *Papers and Proceedings of the Royal Society of Tasmania* 89: 289–355.

Kideys AE, Finenko GA, Anninsky BE, Shiganova TA, Roohi A, Tabari MR, Youseffan M, Rostamiya MT (2004). Chapter 9. Feeding, respiration and growth of ctenophore *Beroe* cf *ovata* in the low salinity conditions of the Caspian Sea. In:. Dumont DJ, Shiganova TA, Niermann U (eds), Aquatic Invasions in the Black, Caspian, and Mediterranean Seas. The Ctenophores *Mnemiopsis leidyi* and Beroe in the Ponto-Caspian and other Aquatic Invasions. Kulwer Academic Publishers. 313 pgs.

Kideys AE, Roohi A, Bagheri S, Finenko G, Kamburska L (2005). Impacts of invasive ctenophores on the fisheries of the Black Sea and Caspian Sea. *Oceanography* 18: 76-85.

Kim HJ, Jun JW, Giri SS, Yun S, Kim SG, Kim SW, Kang JW, Han SJ, Kwon J, Oh WT, Jeon HB, Chi C, Jeong D, Park SC (2019). Mass mortality in Korean bay scallop (*Argopecten irradians*) associated with Ostreid Herpesvirus-1 μVar. *Transboundary and Emerging Diseases* 66: 1442–1448.

King R (1997). Systematic relationships between Tasmanian and New Zealand populations of *Petrolisthes elongatus* (Crustacea: Anomura: Porcellanidae). In: Zoology Department. The University of Melbourne, Melbourne, 37 pp.

King SL (2017). Managing the unwanted organism *Sabella spallanzanii* (Gmelin, 1791): investigating how desiccation, fragmentation and acetic acid can be used to manage this pest on aquaculture facilities. Masters Thesis, The University of Waikato. 64 pgs

Kirby M (2004). Fishing down the coast: historical expansion and collapse of oyster fisheries along continental margins. *Proceedings of the National Acadamy of Science USA* 101: 13096-13099.

Kiryu I, Kurita J, Yuasa K, Nishioka T, Shimahara Y, Kamaishi T, Ototake M, Oseko N, Tange N, Inoue M, Yatabe T, Friedman CS (2013). First detection of *Candidatus* Xenohaliotis californiensis, the causative agent of withering syndrome, in Japanese black abalone *Haliotis discus discus* in Japan. *Fish Pathology* 48: 35−41.

Kleeman SN, Adlard RD, Lester RJG (2002a). Detection of the initial infective stages of the protozoan parasite *Marteilia sydneyi* in *Saccostrea glomerata* and their development through to sporogenesis. *International Journal for Parasitology* 32: 767-784.

Kleeman SN, Le Roux F, Berthe F, Adlard RD (2002b). Specificity of PCR and *in situ* hybridization assays designed for detection of *Marteilia sydneyi* and *M. refringens*. *Parasitology* 125: 131-141.

Kleeman SN, Adlard RD, Zhuc X, Gasser RB (2004). Mutation scanning analysis of *Marteilia sydneyi* populations from different geographical locations in eastern Australia. *Molecular and Cellular Probes* 1: 133–138.

Kleemann KH (1980). Boring bivalves and their host corals from the Great Barrier Reef. *Journal of Molluscan Studies* 46: 13-54.

Kleemann KH (1984). *Lithophaga* (Bivalvia) from dead coral from the Great Barrier Reef, Australia. *Journal of Molluscan Studies* 50: 192-230.

Kleeman KH (1995). Associations of coral and boring bivalves: Lizard Island (Great Barrier Reef, Australia) versus Safaga (N Red Sea). *Beitrage zur Paläontologie* 20: 31-39.

Kobayashi T, Nozawa N, Miyazaki T (1999). Studies on akoya-virus disease associated with mass mortalities in Japanese pearl oyster *Pinctada fucata martensii*. Fourth Symposium on Diseases in Asian Aquaculture, Cebu City, Philippines , November 22–26. Book of Abstracts.

Koganezawa A (1974). Present status of studies on the mass mortality of cultured oysters in Japan and its prevention. In: Proceedings of the Third U.S.–Japan Meeting on Aquaculture, Tokyo, Japan.

Kott P (1990). The Australian Ascidiacea Pt 2, Aplousobranchia (1). *Memoirs of the Queensland Museum* 29: 1–266.

Kotta J, Ojaveer H (2012). Rapid establishment of the alien crab *Rhithropanopeus harrisii* (Gould) in the Gulf of Riga. *Estonian Journal of Ecology* 61: 293-298.

Krassoi FR, Brown KR, Bishop MJ, Kelaher BP, Summerhayes S (2008). Condition-specific competition allows coexistence of competitively superior exotic oysters with native oysters. *Journal of Animal Ecology* 77: 5–15.

Kristmundsson A, Freeman MA (2018). Harmless sea snail parasite causes mass mortalities in numerous commercial scallop populations in the northern hemisphere. *Scientific Reports* 8: 7865.

Kristmundsson, A, Helgason S, Bambir SH, Eydal M, Freeman MA (2011a). *Margolisiella islandica* sp. nov. (Apicomplexa:Eimeridae) infecting Iceland scallop *Chlamys islandica* (Müller, 1776) in Icelandic waters *Journal of Invertebrate Pathology* 108: 139–146.

Kristmundsson, A, Helgason S, Bambir SH, Eydal M, Freeman MA (2011b). Previously unknown apicomplexan species infecting the Iceland scallop *Chlamys islandica* (Muller, 1776), a queen scallop, *Aequipecten opercularis*, L and king scallop, *Pecten maximus*. L. *Journal of Invertebrate Pathology* 108: 147–155.

Kristmundsson A, Erlingsdóttir A, Freeman MA (2015). Is an apicomplexan parasite responsible for the collapse of the Iceland scallop (*Chlamys islandica*) stock? *PLoS ONE* 10: e0144685.

Kuchel RP, O'Connor WA, Raftos DA (2011). Environmental stress and disease in pearl oysters, focusing on the Akoya pearl oyster (*Pinctada fucata* Gould 1850). *Reviews in Aquaculture* 3: 138–154.

Kurokawa T, Suzuki T, Okauchi M, Miwa S, Nagai K, Nakamura K, Honjo T, Nakajima K, Ashida K, Funakoshi S (1999). Experimental infection of a disease causing mass mortalities of Japanese pearl oyster *Pinctada fucata martensii* by tissue transplantation and cohabitation. *Bulletin of the Japanese Society of Scientific Fisheries* 65: 241–251.

Lafferty KD, Kuris AM (1993). Mass mortality of abalone *Haliotis cracherodii* on the California Channel Islands: tests of epidemiological hypotheses. *Marine Ecology Progress Series* 96: 239-248.

Lafferty KD, Ben-Horin T (2013). Abalone farm discharges the withering syndrome pathogen into the wild. *Frontiers in Microbiology* 4(373): 1-9.

Laing I, Dunn P, Peeler EJ, Feist SW, Longshaw M (2014). Epidemiology of *Bonamia* in the UK, 1982 to 2012. *Diseases of Aquatic Organisms* 110: 101-111.

Lambert G (2009). Adventures of a sea squirt sleuth: unraveling the identity of *Didemnum vexillum*, a global ascidian invader. *Aquatic Invasions* 4: 5-28.

Lane HS, Webb SC, Duncan J (2016). *Bonamia ostreae* in the New Zealand oyster *Ostrea chilensis*: a new host and geographic record for this haplosporidian parasite. *Diseases of Aquatic Organisms* 118: 55-63.

Langdon JS (1989). Experimental transmission and pathogenicity of epizootic haematopoietic necrosis virus (EHNV) in redfin perch, *Perca fluviatilis* L., and 11 other teleosts. *Journal of Fish Diseases* 12: 295–310.

Laramore SE, Krebs W, Lave AL, Gallagher K (2017). Survey of bivalve molluscs for *Bonamia* spp. and other parasitic pathogens in Florida east coast lagoons. *Journal of Shellfish Research* 36: 379-390.

Larsson JIR, Koie M (2005). Ultrastructual study and description of *Paramyxoides nephtys* gen. n., sp. n. a parasite of *Nephtys caeca* (Fabricius, 1780) (Polychaeta: Nephtyidae). *Acta Protozoology* 44: 175- 187.

Leach A (2011). *Testing the efficacy of heated seawater for managing biofouling in ships' sea chests*. Honours Thesis, University of Wollongong, Australia. 70 pgs. . https://ro.uow.edu.au/cgi/viewcontent.cgi?article=1021&context=thsci

Le Deuff RM, Renault T, Gérard A (1996). Effects of temperature on herpes-like virus detection among hatchery-reared larval Pacific oyster *Crassostrea gigas*. *Diseases of Aquatic Organisms* 24: 149-157.

Lester RJG (1986). Abalone dieback caused by protozoan infection? *Australian Fisheries* 45: 2627.

Lester RJG (1989). Diseases of cultured molluscs in Australia. In: Advances in Tropical Aquaculture, Tahiti Feb 20 – Mar 4 1989. AQUACOP IFREMER Actes de Colloque 9: 207-216.

Lester RJG, Davis GHG (1981). A new *Perkinsus* species (Apicomplexa, Perkinsea) from the abalone *Haliotis ruber*. *Journal of Invertebrate Pathology* 37: 181187.

Lester RJG, Hayward CJ (2005). Control of *Perkinsus* disease in abalone. Final Report for FRDC Project no. 2000/151. 41 pgs.

Lester RJG, Hine PM (2017). Paramyxida. In: JM Archibald et al. (eds.), Handbook of the Protists, Springer International Publishing, pgs. 1-18.

Levine ND (1978). *Perkinsus* gen. n. and other new taxa in the protozoan phylum Apicomplexa. *Journal of Parasitology* 64: 549.

Lewis JA (1999). A review of the occurrence of exotic macroalgae in southern Australia, with an emphasis on Port Phillip Bay, Victoria. Pgs 61-87, in: Hewitt CL; Campbell ML, Thresher RE, Martin RB (1999). Marine biological invasions of Port Phillip Bay, Victoria. Centre for Research on Introduced Marine Pests, Technical Report No. 20. CSIRO Marine Research, Hobart, 344 pgs.

Liggins GW, Upston J (2010). Investigating and managing the *Perkinsus*-related mortality of blacklip abalone in NSW. Department of Industry and Investment (Industry & Investment NSW) and the Fisheries Research & Development Corporation, FRDC Project Report No. 2004/084, June 2010. 182 pgs.

Link H, Nishi E, Tanaka K, Bastida-Zavala R, Kupriyanova EK, Yamakita T (2009). *Hydroides dianthus* (Polychaeta: Serpulidae), an alien species introduced into Tokyo Bay, Japan. *JMBA2 Biodiversity Records* 6430: 1-6.

Liu YJ, Wu XZ, Zhu MZ, Wang C, Zhang QZ, Pan JP (2002). Ultrastructural observation and cytopathology of spherical virus in *Chlamys farreri* (Jones and Preston). *Journal of Tropical Oceanography* 21: 76–79.

Liu O, Paul-Pont I, Rubio A, Dhand N, Whittington RJ (2020). Detection of ostreid herpesvirus-1 in plankton and seawater samples at an estuary scale. *Diseases of Aquatic Organisms* 138: 1-15.

Liversage K, Kotta J (2019). A rare example of non-native chitons: broad intertidal habitat range and large densities of *Sypharochiton pelliserpentis* show no evidence of habitat engineering effect in South Australia. *Aquatic Invasions* 14: 267–279.

Lleonart M (2002). Management of spionid mud worm infestations of Tasmanian cultured abalone. PhD thesis, School of Aquaculture, University of Tasmania. 240 pgs.

Lleonart M, Handlinger J, Powell M (2003a). Treatment of spionid mud worm (*Boccardia knoxi* Rainer) infestation of cultured abalone. *Aquaculture* 217: 1-10.

Lleonart M, Handlinger J, Powell M (2003b). Spionid mudworm infestation of farmed abalone (*Haliotis* spp.). *Aquaculture* 221: 85-96.

Locke A (2009). A screening procedure for potential tunicate invaders of Atlantic Canada. *Aquatic Invasions* 4: 71-79.

Lohrmann KB, Hine PM, Campalans M (2009). Ultrastructure of *Bonamia* sp. in *Ostrea chilensis* in Chile. *Diseases of Aquatic Organisms* 85: 199-208.

Lom J, Dykova I (1992). Protozoan Parasites of Fishes. Developments in Aquaculture and Fisheries Science 26. Elsevier. 315 pgs.

Longshaw M, Feist SW, Matthews RA, Figueras A (2001). Ultrastructural characterisation of *Marteilia* species (Paramyxea) from *Ostrea edulis*, *Mytilus edulis* and *Mytilus galloprovincialis* in Europe. *Diseases of Aquatic Organisms* 44: 137-142.

Lopez-Flores I, Garrido-Ramos MA, de la Herran R, Ruiz-Rejon C, Ruiz-Rejo´M, Navas JI (2008a). Identification of *Marteilia refringens* infecting the razor clam *Solen marginatus* by PCR and *insitu* hybridization. *Molecular and Cellular Probes* 22: 151–155.

López-Flores I, Robles F, Valencia JM, Grau A, Villalba A, de la Herrán R, Garrido-Ramos MA, Ruiz-Rejón C, Ruiz-Rejón M, Navas JI (2008b). Detection of *Marteilia refringens* using nested PCR and in situ hybridisation in *Chamelea gallina* from the Balearic Islands (Spain). *Diseases of Aquatic Organisms* 82: 79-87.

Lynch SA, Armitage DV, Coughlan J, Mulcahy MF, Culloty SC (2007). Investigating the possible role of benthic macroinvertebrates and zooplankton in the life cycle of the haplosporidian *Bonamia ostreae*. *Experimental Parasitology* 115: 359–368.

Lynch SA, Abollo E, Ramilo A, Cao A, Culloty SC, Villalba A (2010). Observations raise the question if the Pacific oyster, *Crassostrea gigas*, can act as either a carrier or a reservoir for *Bonamia ostreae* or *Bonamia exitiosa*. *Parasitology* 137: 1515−1526.

Lynch SA, Flannery G, Hugh-Jones T, Hugh-Jones D, Sulloty SC (2014). Thirty-year history of Irish (Rossmore) *Ostrea edulis* selectively bred for disease resistance to *Bonamia ostreae*. *Diseases of Aquatic Organisms* 110: 113–121.

Lyons DA, Scheibling RE (2009). Range expansion by invasive marine algae: rates and patterns of spread at a regional scale. *Diversity and Distributions* 15: 762–775.

McColl K, Corbeil S, Williams L, Lancaster M, Crane M (2007). Abalone ganglioneuritis—a transmission trial, and time-course study. Proceedings of the third national FRDC Aquatic Animal Health Scientific Conference, 25–27 July 2007, Cairns.

McCulloch M, Fallon S, Wyndham T, Hendy E, Lough J, Barnes D (2003). Coral record of increased sediment flux to the inner Great Barrier Reef since European settlement. *Nature* 421: 727–730.

McDonald J (2004). The invasive pest species *Ciona intestinalis* (Linnaeus, 1767) reported in a harbour in southern Western Australia. *Marine Pollution Bulletin* 49: 868-870.

McDonald JI, Bridgwood S, Hourston M (2015a). Likelihood of marine pest introduction to the Indian Ocean territories. Fisheries Research Report No. 264. Department of Fisheries, Western Australia. 40 pgs.

McDonald JI, Huisman JM, Hart FN, Dixon RR, Lewis JA (2015b). The first detection of the invasive macroalga *Codium fragile* subsp. *fragile* (Suringar) Hariot in Western Australia. *BioInvasions Records* 4: 75–80.

McDougall C, Nenadic N, Healy J (2020). Guide to Queensland's intertidal oysters. Version 1, September 2020. Griffith University, Brisbane, QLD, Australia. 9 pgs.

McKindsey CW, Landry T, O'Beirn FX, Davies IM (2007). Bivalve aquaculture and exotic species: A review of ecological considerations and management issues. *Journal of Shellfish Research* 26: 281-294.

McLeod IM, Bostrom-Einarsson L, Creighton C, D'Anastasi B, Diggles BK, Dwyer PG, Firby L, Le Port A, Luongo A, Martinez-Baena F, McOrrie S, Heller-Wagner G, Gillies CL (2019). Habitat value of Sydney rock oyster (*Saccostrea glomerata*) reefs on soft sediments. *Marine and Freshwater Research* https://doi.org/10.1071/MF18197

Madin, B. (2007). An investigation into a pearl oyster (*Pinctada maxima*) mortality event. Report prepared for the Department of Fisheries, Western Australia by AusVet Animal Health Services Pty Ltd. 29 pgs.

Mackin JG and Ray SM (1966). The taxonomic relationships of *Dermocystidium marinum* Mackin, Owen, and Collier. *Journal of Invertebrate Pathology* 8: 544-545.

Mackin JG, Owen HM, Collier A (1950). Preliminary note on the occurrence of a new protistan parasite, *Dermocystidium marinum* n. sp. in *Crassostrea virginica* (Gmelin). *Science*. 111: 328-329.

Mann R, Harding JM (2000). Invasion of the North American Atlantic Coast by a large predatory Asian mollusc. *Biological Invasions* 2: 7-22.

Mann R, Harding JM (2003). Salinity tolerance of larval *Rapana venosa*: Implications for dispersal and establishment of an invading predatory gastropod on the North American Atlantic coast. *Biological Bulletin* 204: 96-103.

Mariani S, Uriz MJ, Turon X (2000). Larval bloom of the oviparous sponge *Cliona viridis*: coupling of larval abundance and adult distribution. *Marine Biology* 137: 783–790.

Marine Pest Sectoral Committee (2015). Rapid response manual for '*Asterias amurensis'*, Version 1.0, December 2015. Department of Agriculture and Water Resources, Canberra, December. 53 pgs. https://www.marinepests.gov.au/sites/default/files/Documents/empplan-rapid-response-manual-asterias-amurensis.pdf

Marquis ND, Bishop TJ, Record NR, Countway PD, Fernández Robledo JA (2020). A qPCR-based survey of *Haplosporidium nelsoni* and *Perkinsus* spp. in the eastern oyster, *Crassostrea virginica* in Maine, USA. *Pathogens* 9: 256, doi:10.3390/pathogens9040256

Martenot C, Oden E, Travaillé E, Malas JP, Houssin M (2011). Detection of different variants of ostreid herpesvirus 1 in the Pacific oyster, *Crassostrea gigas* between 2008 and 2010. *Virus Research* 160: 25–31.

Martenot C, Lethuillier O, Fourour S, Oden E, Trancart S, Travaillé E, Houssin M (2015). Detection of undescribed ostreid herpesvirus 1 (OsHV-1) specimens from Pacific oyster, *Crassostrea gigas*. *Journal of Invertebrate Pathology* 132: 182–189.

Matsusato T, Masumura K (1981). Abnormal enlargement of the ovary of oyster, *Crassostrea gigas* (Thunberg) by an unidentified parasite. *Fish Pathology* 15: 207-212.

Matsusato T, Hoshina T, Arakawa Y, Matsumura K (1977). Study of the so-called abnormal egg-mass of Japanese oyster *Crassostrea gigas* (Thunberg)-1. *Bulletin Hiroshima Fisheries Exp. St*. 8: 9-25.

Matsuyama T, Yasuike M, Fujiwara A, Nakamura Y, Takano T, Takeuchi T, Satoh N, Adachi Y, Tsuchihashi Y, Aoki H, Odawara K, Iwanaga S, Kurita J, Kamaishi T, Nakayasu C (2017). A Spirochaete is suggested as the causative agent of Akoya oyster disease by metagenomic analysis. *PLoS ONE* 12(8): e0182280.

Matsuyama T, Matsuura Y, Inada M, Takano T, Nakayasu C, Sakai T, Terashima S, Yasuike M, Fujiwara A, Nakamura Y (2018). An epidemiological study of akoya oyster disease using polymerase chain reaction targeting Spirochaetes genes. *Fish Pathology* 53: 63–70.

Matsuyama T, Takano T, Nakayasu C, Odawara K, Tsuchihashi Y, Tanaka S, Yasuike M, Fujiwara A, Nakamura Y, Masaoka T (2019). Spatiotemporal dynamics of Spirochaeta, the putative etiologic agent of Akoya oyster disease in pearl oysters, as determined by quantitative PCR. *Aquaculture* 513: 734433.

Maximov A, Bonsdorff E, Eremina T, Kauppi L (2015). Context-dependent consequences of *Marenzelleria* spp. (Spionidae: Polychaeta) invasion for nutrient cycling in the Northern Baltic Sea. *Oceanologia* 57: 342-348.

Mayfield S, McGarvey R, Gorfine HK, Peeters H, Burch P, Sharma S (2011). Survey estimates of fishable biomass following a mass mortality in an Australian molluscan fishery. *Journal of Fish Diseases* 34: 287-302.

Menge BA, Daley BA, Sanford E, Dahlhoff EP, Lubchenco J (2007). Mussel zonation in New Zealand: an integrative eco-physiological approach. *Marine Ecology Progress Series* 345: 129-140.

Messerman NA, Bowden TJ (2016). Survey of potential reservoir species for the oyster parasite Multinucleate Sphere X (*Haplosporidium nelsoni*) in and around oyster farms in the Damariscotta River estuary, Maine. *Journal of Shellfish Research* 35: 851–856.

Meyers TR (1981). Endemic diseases of cultured shellfish of Long Island, New York: adult and juvenile American oysters (*Crassostrea virginica*) and hard clams (*Mercenaria mercenaria*). *Aquaculture* 22: 305-330.

Meyers TR, Burton T (2009). *Diseases of wild and cultured shellfish in Alaska*. Alaska Department of Fish and Game, February 2009, 130 pgs.

Meyers TR, Burton T, Evans W and Starkey N (2009). Detection of viruses and virus-like particles in four species of wild and farmed bivalve molluscs in Alaska, USA, from 1987 to 2009. *Diseases of Aquatic Organisms* 88: 1-12.

Miller AN, Strychar KB, Shirley TC, Rützler K (2010). Effects of heat and salinity stress on the sponge *Cliona celata*. *International Journal of Biology* 2: 3-16.

Mineur F, Belsher T, Johnson MP, Maggs CA, Verlaque M (2007). Experimental assessment of oyster transfers as a vector for macroalgal introductions. *Biological Conservation* 137: 237-247.

Mineur F, Johnson MP, Maggs CA (2008). Macroalgal introductions by hull fouling on recreational vessels: Seaweeds and sailors. *Environmental Management* 42: 667–676.

Mineur F, Provan J, Arnott G (2015). Phylogeographical analyses of shellfish viruses: inferring a geographical origin for ostreid herpesviruses OsHV-1 (Malacoherpesviridae). *Marine Biology* 162: 181–192.

Miossec L, Le Deuff RM, Goulletquer P (2009). Alien species alert: *Crassostrea gigas* (Pacific oyster). ICES Cooperative Research Report No. 299. 42 pgs.

Miyazaki T, Goto K, Kobayashi T, Kageyama T, Miyata M (1999). Mass mortalities associated with a virus disease in Japanese pearl oysters *Pinctada fucata martensii*. *Diseases of Aquatic Organisms* 37: 1-12.

Molloy DP, Giambérini L, Stokes NA, Burreson EM, Ovcharenko MA (2012). *Haplosporidium raabei* n. sp. (Haplosporidia): a parasite of zebra mussels, *Dreissena polymorpha* (Pallas, 1771). *Parasitology* 139: 463−477.

Moore JD, Robbins TT, Friedman CS (2000) Withering syndrome in farmed red abalone, *Haliotis rufescens*: thermal induction and association with a gastrointestinal Rickettsiales- like procaryote. *Journal of Aquatic Animal Health* 12: 26−34.

Moore JD, Robbins TT, Hedrick RP, Friedman CS (2001). Transmission of the Rickettsiales-like prokaryote "Candidatus *Xenohaliotis californiensis*" and its role in withering syndrome of California abalone, *Haliotis* spp. *Journal of Shellfish Research* 20: 867-874.

Moore JD, Juhasz CI, Robbins TT, Vilchis LI (2009). Green abalone, *Haliotis fulgens* infected with the agent of withering syndrome do not express disease signs under a temperature regime permissive for red abalone, *Haliotis rufescens*. *Marine Biology* 156: 2325−2330.

Moore JD, Marshman BC, Chun CC (2011). Health and survival of red abalone *Haliotis rufescens* from San Miguel Island, California, USA, in a laboratory simulation of La Niña and El Niño conditions. *Journal of Aquatic Animal Health* 23: 78–84.

Moore JD, Byron SN, Marshman BC, Snider JP (2019). An oxytetracycline bath protocol to eliminate the agent of withering syndrome, *Candidatus* Xenohaliotis californiensis, in captive abalone populations. *Aquaculture* 503: 267–274.

Morga B, Renault T, Faury N, Lerond S, Garcia C, Chollet B, Joly J-P, Lapègue S, Harrang E, Arzul I (2017). Contribution of *in-vivo* experimental challenges to understanding flat oyster *Ostrea edulis* resistance to *Bonamia ostreae*. *Frontiers in Cellular and Infection Microbiology* 7: 433.

Morizane T, Yamashita H, Fujita Y, Kawakami H, Ochi O, Maeno Y, Kamaishi T, Ito T, Kurita J, Nakajima K (2002). Experimental reproduction of a disease causing mass mortality of Japanese pearl oyster *Pinctada fucata martensii* by injection of diseased oyster haemolymph. *Fish Pathology*. 37: 149–151.

Morton B, Lam K, Slack-Smith S (2003). First report of the European flat oyster *Ostrea edulis*, identified genetically, from Oyster Harbour, Albany, south-western Western Australia. *Molluscan Research* 23: 199-208.

Moss JA, Burreson EM, Cordes JF, Dungan CF, Brown GD, Wang A, Wu X, Reece KS (2007). Pathogens in *Crassostrea ariakensis* and other Asian oyster species: implications for non-native oyster introduction to Chesapeake Bay. *Diseases of Aquatic Organisms* 77: 207-223.

Moss JA, Xiao J, Dungan CF, Reece KS (2008). Description of *Perkinsus beihaiensis* n. sp., a new *Perkinsus* sp. parasite in oysters of southern China. *Journal of Eukaryotic Microbiology* 55: 117–130.

Moulin FY, Guizien K, Thouzeau G, Chapalain G, Mülleners K, Bourg C (2007). Impact of an invasive species, *Crepidula fornicata*, on the hydrodynamics and transport properties of the benthic boundary layer. *Aquatic Living Resources* 20: 15-31.

Mountfort D, Hay CH, Taylor M, Buchanan S, Gibbs W (1999). Heat treatment of ships' ballast water: development and application of a model based on laboratory studies. *Journal of Marine Environmental Engineering* 5: 193–206.

Moutardier G, Gereva S, Mills SC, Adjeroud M, Beldade R, Ham J, Kakau, R, Dumas P (2015). Lime juice and vinegar injections as a cheap and natural alternative to control COTS outbreaks. PLoS ONE 10: e0137605.

Muellner P, Webb S, Janke A, King N, Kaspar H (2011). OsHV-1 in New Zealand – an epidemiological perspective. Proceedings of the First FRDC Australasian Aquatic Animal Health Scientific Conference, Pullman Reef Hotel, Wharf St, Cairns, QLD Australia 5-8 July 2011.

Munari C, Mistri M (2011). Short-term hypoxia modulates *Rapana venosa* (Muricidae) prey preference in Adriatic lagoons. *Journal of Experimental Marine Biology and Ecology* 407: 166-170.

Munoz J, McDonald J (2014). Potential eradication and control methods for the management of the ascidian Didemnum perlucidum in Western Australia. Fisheries Research Report No. 252. Department of Fisheries, Western Australia. 40 pgs.

Murphy B, Paini D (2010). CCIMPE Review: Update 2007-2009. CSIRO CCIMPE/NIMPIS Report. 326 pgs.

Murrell A., Kleeman SN, Barker SC, Lester RJG (2002). Synonymy of *Perkinsus olseni* Lester & Davis, 1981 and *Perkinsus atlanticus* Azevedo, 1989 and an update on the phylogenetic position of the genus *Perkinsus*. *Bulletin of the European Association of Fish Pathologists* 22: 258–265.

Mutlu, E. (2009). Recent distribution and size structure of gelatinous organisms in the southern Black Sea and their interactions with fish catches. *Marine Biology (Berlin)* 156(5): 935-957.

Nakajima K (1999). Mass mortality outbreaks associated with an infectious pathogen among pearl oyster *Pinctada fucata martensii* in Japan. *Fish Pathology* 34: 227.

Nakayasu C, Aoki H, Nakanishi M, Yamashita H, Okauchi M, Oseko N, Kumagai A (2004). Tissue distribution of the agent of akoya oyster disease in Japanese pearl oyster *Pinctada fucata martensii*. *Fish Pathology* 39: 203-208.

Naser MD, Rainbow PS, Clark PF, Yasser AG, Jones DS (2015). The barnacle *Amphibalanus improvisus* (Darwin, 1954), and the mitten crab *Eriocheir*: one invasive species getting off on another. *BioInvasions Records* 4: 205–209.

Neil KM, Hilliard R, Clark P, Russell BC (2005). A Situation and Gaps Analysis of IMS, Vectors, Nodes and Management Arrangements for the Northern Planning Area. An independent report undertaken for the National Ocean's Office Branch of the Department of Environment and Heritage by CRC Reef, URS and the MAGNT for National Oceans Office Branch of the Department of Environment and Heritage. 177 pp.

Nel R, Coetzee PS, Van Niekerk G (1996). The evaluation of two treatments to reduce mud worm (*Polydora hoplura* Claparède) infestations in commercially reared oysters (*Crassostrea gigas* Thunberg). *Aquaculture* 141: 31-39.

Nell JA (2001). The history of oyster farming in Australia. *Marine Fisheries Review* 63: 14-25.

Nell JA (2007). Controlling mudworm in oysters. Fishnote, NSW Fisheries, Port Stephens Fisheries Centre, Nelson Bay, NSW, Australia. 4 pp. .
http://www.dpi.nsw.gov.au/__data/assets/pdf_file/0005/164246/Controlling-mudworm-in-oysters.pdf

Nell JA, Hand RE (2003). Evaluation of the progeny of second-generation Sydney rock oysters *Saccostrea glomerata* (Gould, 1850) breeding lines for resistance to QX disease *Marteilia sydneyi*. *Aquaculture* 228: 27-35.

Nerlovic V, Peric L, Sliskovic M, Jelic Mrcelic GJ (2018). The invasive *Anadara transversa* (Say, 1822) (Mollusca: Bivalvia) in the biofouling community of northern Adriatic mariculture areas. *Management of Biological Invasions* 9: 239–251.

Newell RIE (1985). Physiological effects of the MSX parasite *Haplosporidium nelsoni* (Haskin, Stauber & Mackin) on the American oyster *Crassostrea virginica* (Gmelin). *Journal of Shellfish Research* 5: 91-95.

Newell RIE (2004). Ecosystem influences of natural and cultivated populations of suspension feeding bivalve molluscs: a review. *Journal of Shellfish Research* 23: 51-61.

Ngo TT, Berthe FCJ, Choi KS (2003). Prevalence and infection intensity of the ovarian parasite *Marteilioides chungmuensis* during an annual reproductive cycle of the oyster *Crassostrea gigas*. *Diseases of Aquatic Organisms* 56: 259–267.

Niermann U (2004). Chapter 1. *Mnemiopsis leidyi*: distribution and effect on the Black Sea ecosystem during the first years of invasion in comparison with other gelatinous blooms. In:. Dumont DJ, Shiganova TA, Niermann U (eds), Aquatic Invasions in the Black, Caspian, and Mediterranean Seas. The Ctenophores *Mnemiopsis leidyi* and Beroe in the Ponto-Caspian and other Aquatic Invasions. Kulwer Academic Publishers. 313 pgs.

Nicolas JL, Comps M, Cochennec N (1992). Herpes-like virus infecting Pacific oyster larvae, *Crassostrea gigas*. *Bulletin of the European Association of Fish Pathologists* 12: 11-13.

NOAA (2013). Decontamination of shells used for habitat restoration. 3 pgs. http://www.oyster-restoration.org/wp-content/uploads/2012/06/decontamination_shells-NOAA.pdf

Norton JH, Perkins FP, Ledua E (1993a). *Marteilia*-like infection in a giant clam, *Tridacna maxima*, in Fiji. *Journal of Invertebrate Pathology* 61: 328-330.

Norton JH, Shepherd MA, Perkins FP, Prior HC (1993b). *Perkinsus*-like infection in farmed golden lipped pearl oyster *Pinctada maxima* from the Torres Strait, Australia. *Journal of Invertebrate Pathology* 62: 105-106.

O'Brien AL, Ross DJ, Keough MJ (2006). Effects of *Sabella spallanzanii* physical structure on soft sediment macrofaunal assemblages. *Marine and Freshwater Research* 57: 363-371.

O'Donoghue PJ, Phillips PH, Shepherd SA (1991). *Perkinsus* (Protozoa: Apicomplexa) infections in abalone from South Australian waters. *Transactions of the Royal Society of South Australia* 115: 77-82.

Ogburn DM, White I, McPhee DP (2007). The disappearance of oyster reefs from eastern Australian estuaries impact of colonial settlement or mudworm invasion? *Coastal Management* 35: 271-287.

O'Hara TD (1999). Echinodermata of Port Phillip Bay: introduced species. Pgs 171-177, in: Hewitt CL; Campbell ML, Thresher RE, Martin RB (1999). Marine biological invasions of Port Phillip Bay, Victoria. Centre for Research on Introduced Marine Pests, Technical Report No. 20. CSIRO Marine Research, Hobart, 344 pgs.

OIE (2009). Chapter 2.2.10. Infection with *Haplosporidium nelsoni*. Manual of Diagnostic Tests for Aquatic Animals 2009.

OIE (2010a). Ostreid herpesvirus-1, New Zealand. Immediate notification. 7/12/2010.

OIE (2010b). Ostreid herpesvirus-1, New Zealand. Follow-up report No. 1. 14/12/2010.

OIE (2010c). Ostreid herpesvirus-1, New Zealand. Follow-up report No. 2. 21/12/2010.

OIE (2011a). Ostreid herpesvirus-1, New Zealand. Follow-up report No. 3. 20/01/2011.

OIE (2011b). Ostreid herpesvirus-1, New Zealand. Follow-up report No. 4. 25/08/2011.

OIE (2011c). Ostreid herpesvirus-1, Australia. Immediate notification. 07/01/2011.

OIE (2011d). Ostreid herpesvirus-1, Australia. Follow-up report No. 1. 16/02/2011.

OIE (2019). *Aquatic Animal Health Code*. Chapter 2.1 Import Risk Analysis. OIE Paris, France. http://www.oie.int/en/international-standard-setting/aquatic-code/access-online/

OIE (2020a). *Manual of Diagnostic Tests for Aquatic Animals* 2019. Chapter 2.4.5. Infection with Ostreid herpesvirus 1 microvariants. . https://www.oie.int/index.php?id=2439&L=0&htmfile=chapitre_ostreid_herpesvirus_1.htm

OIE (2020b). Manual of Diagnostic Tests for Aquatic Animals 2019. Chapter 2.3.8. Red sea bream iridoviral disease. http://www.oie.int/index.php?id=2439&L=0&htmfile=chapitre_rsbid.htm

OIE (2020c). Manual of Diagnostic Tests for Aquatic Animals 2019. Chapter 2.4.1, Infection with Abalone Herpesvirus. http://www.oie.int/index.php?id=2439&L=0&htmfile=chapitre_abalone_herpesvirus.htm

OIE (2020d). Manual of Diagnostic Tests for Aquatic Animals 2019. Chapter 2.4.8, Infection with *Xenohaliotis californiensis*. . https://www.oie.int/fileadmin/Home/eng/Health_standards/aahm/current/chapitre_xenohaliotis_californiensis.pdf

Ojaveer H, Galil BS, Carlton JT, Alleway H, Goulletquer P, Lehtiniemi M, Marchini A, Miller W, Occhipinti-Ambrogi A, Peharda M, Ruiz GM, Williams SL, Zaiko A (2018). Historical baselines in marine bioinvasions: Implications for policy and management. *PLOS One* 13(8): e0202383.

O'Loughlin E, McCloud C, Sierp M, Westphalen G (2006). Temperature and salinity tolerances of priority marine pests. Prepared for PIRSA Biosecurity. South Australian Research and Development Institute (AquaticSciences). SARDI Publication Number RD06/0751, Adelaide, 45 pgs. https://pir.sa.gov.au/__data/assets/pdf_file/0009/231597/TempandSalTolerances_Final_Report.pdf

Pagenkopp Lohan KM, Hill-Spanik KM, Torchin ME, Fleischer RC, Carnegie RB, Reece KS, Ruiz GM (2018). Phylogeography and connectivity of molluscan parasites: *Perkinsus* spp. in Panama and beyond. *International Journal for Parasitology* 48: 135-144.

Park MS, Kang CK, Choi DL, Jee BY (2003). Appearance and pathogenicity of ovarian parasite *Marteilioides chungmuensis* in the farmed Pacific oysters, *Crasssostrea gigas*, in Korea. *Journal of Shellfish Research* 22: 475–479.

Park KI, Park JK, Lee J, Choi KS (2005). Use of molecular markers for species identification of Korean *Perkinsus* sp. isolated from Manila clams *Ruditapes philippinarum*. *Diseases of Aquatic Organisms* 66: 255-263.

Parry GD, Cohen BF (2001). The distribution, abundance and population dynamics of the exotic seastar *Asterias amurensis* during the first three years of its invasion of Port Phillip Bay (incorporating a report on the Bay Pest Day, 2 April 2000). Marine and Freshwater Resources Institute Report No. 33. 64 pgs.

Pascual M, Martin AG, Zampatti, Coatanea D, Defossez J, Robert R. (1991). Testing of the Argentina oyster, *Ostrea puelchana*, in several French oyster farming sites. *Cons. Inter. Explor. Mer* CM 1991/K30.

Pass DA, Dybdahl R, Mannion MM (1987). Investigations into the causes of mortality of the pearl oyster, *Pinctada maxima* (Jamson), in Western Australia. *Aquaculture* 65: 149-169.

Pass DA, Perkins FO, Dybdahl R (1988). Virus-like particles in the digestive gland of the pearl oyster *Pinctada maxima*. *Journal of Invertebrate Pathology* 51: 166-167.

Paterson KJ, Schreider MJ, Zimmerman KD (2003). Anthropogenic effects on seston quality and quantity and the growth and survival of Sydney rock oyster (*Saccostrea glomerata*) in two estuaries in NSW, Australia. *Aquaculture* 221: 407–426.

Patton T, Newman C, Sager C, Mauck M (2010). Occurrence of Harris mud crab *Rhithropanopeus harrisii* in Oklahoma (Lake Texoma), the furthest inland report to date. *Proceedings of the Oklahoma Acadamy of Sciences* 90: 75-82.

Paul-Pont I, Evans O, Dhand NK, Rubio A, Coad P, Whittington RJ (2014). Descriptive epidemiology of mass mortality due to Ostreid herpesvirus-1 (OsHV-1) in commercially farmed Pacific oysters (*Crassostrea gigas*) in the Hawkesbury River estuary, Australia. *Aquaculture* 422–423: 146–159.

Pepin JF, Riou A, Renault T (2008). Rapid and sensitive detection of ostreid herpesvirus 1 in oyster samples by real-time PCR. *Journal of Virological Methods* 149: 269-276.

Perkins FO (1968). Fine structure of the oyster pathogen *Minchinia nelsoni* (Haplosporida, Haplosporidiidae). *Journal of Invertebrate Pathology* 10: 287-307.

Perkins FO (1969). Ultrastructure of vegetative stages in *Labyrinthomyxa marina* (=*Dermocystidium marinum*), a commercially significant oyster pathogen. *Journal of Invertebrate Pathology* 13: 199-222.

Perkins FO (1976a). Ultrastructure of sporulation in the European flat oyster pathogen, *Marteilia refringens* - Taxonomic implications. *Journal of Protozoology* 23: 64-74.

Perkins FO (1976b). Zoospores of the oyster pathogen, *Dermocystidium marinum*. I. Fine structure of the conoid and other sporozoan–like organelles. *Journal of Parasitology* 62: 959–974.

Perkins FO (1996). Shell disease in the gold lip pearl oyster, *Pinctada maxima* and the eastern oyster, *Crassostrea virginica*. *Aquatic Living Resources* 9: 159-168.

Perkins FO, Wolf PH (1976). Fine structure of *Marteilia sydneyi* sp. n. haplosporidan pathogen of Australian oysters. *The Journal of Parasitology* 62: 528538.

Peters R, Raftos DA (2003). The role of phenoloxidase suppression in QX disease outbreaks among Sydney rock oysters (*Saccostrea glomerata*). *Aquaculture* 223: 29-39.

Petersen KS, Rasmussen KL, Heinemeler J, Rud N (1992). Clams before Columbus? *Nature* 359: 679.

Petton B, Pernet F, Robert R, Boudry P (2013). Temperature influence on pathogen transmission and subsequent mortalities in juvenile pacific oysters *Crassostrea gigas*. *Aquaculture Environment Interactions* 3: 257-273.

Pickup J, Ironside JE (2018). Multiple origins of parasitic feminization: thelygeny and intersexuality in beach-hoppers are caused by paramyxid parasites, not microsporidia. *Parasitology* 145: 408-415.

Piola R, Hopkins G (2012). Thermal treatment as a method to control transfers of invasive biofouling species via vessel sea chests. *Marine Pollution Bulletin* 64: 1620-1630.

Pomponi SA (1980). Cytological mechanisms of calcium carbonate excavation by boring sponges. *International Reviews in Cytology* 65: 301–319.

Powell EN, Klinck JM, Ford SE, Hofmann EE, Jordan SJ (1999). Modeling the MSX parasite in eastern oyster (*Crassostrea virginica*) populations. III. Regional application and the problem of transmission. *Journal of Shellfish Research* 18: 517–537.

Prince J (2007). A Review of the Outbreak of a Herpes-like Virus in the Abalone Stocks of Western Zone Victoria and the Lessons to be Learnt. Biospherics P/L PO Box 168 South Fremantle WA 6162. 34 pgs.

Probst TA, Crawford CM (2008). Population characteristics and planktonic larval stage of the New Zealand screwshell *Maoricolpus roseus. Journal of Molluscan Studies* 74: 191–197.

Provan J, Booth D, Todd NP, Beatty GE, Maggs CA (2008). Tracking biological invasions in space and time: elucidating the invasive history of the green alga *Codium fragile* using old DNA. *Diversity and Distributions* 14: 343–354.

Purcell JE, Shiganova TA, Decker MB, Houde ED (2001). The ctenophore *Mnemiopsis* in native and exotic habitats: U.S. estuaries versus the Black Sea basin. *Hydrobiologia* 451: 145-176.

Purcell JE, Uye S, Lo WT (2007). Anthropogenic causes of jellyfish blooms and their direct consequences for humans: a review. *Marine Ecology Progress Series* 350: 153-174.

Quayle DB (1961). Denman Island oyster disease and mortality, 1960. Ottawa: Fisheries Research Board of Canada; Ms Report Series Number 713, 10 pgs.

Quayle DB (1988). Pacific oyster culture in British Columbia. *Canadian Bulletin of Fisheries and Aquatic Sciences* 218: 1-241.

Rainer S, Hutchings P (1977). Nephtyidae (Polychaeta: Errantia) from Australia. *Records of the Australian Museum* 31: 307-347.

Rajagopal S, Nair KVK, Azariah A (1995a). Response of brown mussel, *Perna indica*, to elevated temperatures in relation to power plant biofouling control. *Journal of Thermal Biology* 20: 461-467.

Rajagopal S, Venugopalan VP, Azariah J, Nair KVK (1995b). Response of the green mussel *Perna viridis* (L.) to heat treatment in relation to power plant biofouling control. *Biofouling* 8: 313-330.

Rajagopal S, Van der Velde G, Jansen J, Van der Gaag M, Atsma G, Janssen-Mommen JP, Jenner HA (2005). Thermal tolerance of the invasive oyster *Crassostrea gigas*: feasibility of heat treatment as an antifouling option. *Water Research* 39: 4335-4342.

Rainer, S.F. (1981), Soft-bottom benthic communities in Otago Harbour and Blueskin Bay, New Zealand. New *Zealand Oceanographic Institute Memoir* 80: 1-18.

Ramilo A, Iglesias D, Abollo E, González M, Darriba S, Villalba A (2014). Infection of Manila clams *Ruditapes philippinarum* from Galicia (NW Spain) with a *Mikrocytos*-like parasite. *Diseases of Aquatic Organisms* 110: 71-79.

Ramilo A, Abollo E, Villalba A, Carballal MJ (2018). A *Minchinia mercenariae*-like parasite infects cockles *Cerastoderma edule* in Galicia (NW Spain). *Journal of Fish Diseases* 41: 41-48.

Ramsay A (2015a). Freshwater immersion to control the clubbed tunicate, *Styela clava*. http://www.gov.pe.ca/photos/original/FARD_AIN272015.pdf

Ramsay A (2015b). Freshwater immersion to control the vase tunicate, *Ciona intestinalis*. https://www.princeedwardisland.ca/sites/default/files/publications/af_ain262015.pdf

Ravara A, Wiklund H, Cunha MR, Pleijet F (2010). Phylogenetic relationships within the Nephtyidae (Polychaeta: Annelida). *Zoologica Scripta* 39: 394-405.

Ray SM (1952). A culture technique for the diagnosis of infection with *Dermocystidium marinum* Mackin, Owen, and Collier in oysters. *Science* 116: 360-361.

Ray SM (1954). Biological studies of *Dermocystidium marinum*. Rice Institute Pamphlet 41 (Special Issue): 1-114.

Ray SM (1966). A review of the culture method for detecting *Dermocystidium marinum*, with suggested modifications and precautions. *Proceedings of the National Shellfisheries Association* 54: 55-69.

Ray SM (1996). Historical perspective on *Perkinsus marinus* disease of oysters in the Gulf of Mexico. *Journal of Shellfish Research* 15: 9-11.

Ray SM, Chandler AC (1955). *Dermocystidium marinum*, a parasite of oysters. *Experimental Parasitology* 4: 172-200.

Rayyan A, Chintiroglou CC (2003). *Steinhausia mytilovum* in cultured mussels *Mytilus galloprovincialis* in the Thermaikos Gulf (northern Aegean Sea, Greece). *Diseases of Aquatic Organisms* 57: 271-273.

Read GB (2010). Comparison and history of *Polydora websteri* and *P. haswelli* (Polychaeta: Spionidae) as mud-blister worms in New Zealand shellfish. *New Zealand Journal of Marine and Freshwater Research* 44: 83-100.

Read GB, Inglis G, Stratford P, Ahyong ST (2011). Arrival of the alien fanworm *Sabella spallanzanii* (Gemlin, 1791) (Polychaeta: Sabellidae) in two New Zealand harbours. *Aquatic Invasions* 6: 273–279.

Reece KS, Siddall ME, Burreson EM, Graves JE (1997). Phylogenetic analysis of *Perkinsus* based on actin gene sequences. *The Journal of Parasitology* 83: 417-423.

Reece KS, Siddall ME, Stokes NA, Burreson EM (2004). Molecular phylogeny of the Haplosporidia based on two independent gene sequences. *Journal of Parasitology* 90: 1111−1122.

Reece KS, Scott GP, Dang C, Dungan CF (2017). A novel monoclonal *Perkinsus chesapeaki* in vitro isolate from an Australian cockle, *Anadara trapezia*. *Journal of Invertebrate Pathology* 148: 86–93.

Reid AP, Johnson CR, Ross DJ (2018). Impacts of the New Zealand screwshell *Maoricolpus roseus* on growth and condition of juvenile commercial scallops *Pecten fumatus*. *Marine Ecology Progress Series* 604: 173-185.

Reid DJ, Bone EK (2020). The rise and fall of oyster cultivation in the highly urbanized Georges River estuary, Sydney, Australia: A review of lessons learned. *Regional Studies in Marine Science* 35: 101246.

Ren W, Chen H, Renault T, Cai Y, Bai C, Wang C, Huang J (2013). Complete genome sequence of acute viral necrosis virus associated with massive mortality outbreaks in the Chinese scallop, *Chlamys farreri*. *Virology Journal* 10: 110.

Renault T (2011). Chapter 6. Viruses infecting Marine Molluscs. In: Hurst CL. Studies in Viral Ecology : Animal Host systems. Vol 2 , 1st Edition. pgs 153-176.

Renault T (2016). Chapter 37. Malacoherpesviruses of molluscs. In: Kibenge FSB, Godoy MG (eds), Aquaculture Virology, Academic Press, pgs 513-524.

Renault T, Novoa B (2004). Viruses infecting bivalve molluscs. *Aquatic Living Resources* 17: 397-409.

Renault T, Le Deuff RM, Cochennec N, Maffart P (1994). Herpesviruses associated with mortalities among Pacific oyster, *Crassostrea gigas*, in France -comparative study. *Revue Méd Vét* 145: 735-742.

Renault T, Cochennec N, Chollet B (1995a). Marteiliosis in American oysters *Crassostrea virginica* reared in France. *Diseases of Aquatic Organisms* 23: 161-164.

Renault T, Cochennec N, Grizel H (1995b). *Bonamia ostreae* parasite of the European flat oyster, *Ostrea edulis*, does not experimentally infect the Japanese oyster, *Crassostrea gigas*. *Bulletin of the European Association of Fish Pathologists* 15: 78−80.

Renault T, Stokes NA, Chollet B, Cochennec N, Berthe F, Gerad A, Burreson EM (2000a). Haplosporidosis in the Pacific oyster *Crassostrea gigas* from the French Atlantic coast. *Diseases of Aquatic Organisms* 42: 207-214.

Renault T, Le Deuff R-M, Lipart C, Delsert C (2000b). Development of a PCR procedure for the detection of a herpes-like virus infecting oysters in France. *Journal of Virological Methods* 88: 41-50.

Renault T, Lipart C, Arzul I (2001). A herpes-like virus infecting *Crassostrea gigas* and *Ruditapes philippinarum* larvae in France. *Journal of Fish Diseases* 24: 369-376.

Renault T, Moreau P, Faury N, Pepin JF, Segarra A, Webb S (2012). Analysis of clinical Ostreid Herpesvirus 1 (Malacoherpesviridae) specimens by sequencing amplified fragments from three virus genome areas. *Journal of Virology* 86: 5942– 5947.

Renault T, Bouquet AL, Maurice JT, Lupo C, Blachier P (2014). Ostreid herpesvirus 1 infection among Pacific oyster (*Crassostrea gigas*) spat: relevance of water temperature to virus replication and circulation prior to the onset of mortality. *Applied Environmental Microbiology* 80: 5419−5426.

Richmond L, Darbyshire R, Summerson R (2010). Determining the potential range of invasive marine pests for cost sharing. Bureau of Rural Sciences, Canberra. 54 pgs.

Riisgard HU, Bottiger L, Madsen CV, Purcell JE (2007). Invasive ctenophore *Mnemiopsis leidyi* in Limfjorden (Denmark) in late summer 2007 - assessment of abundance and predation effects. *Aquatic Invasions* 2: 395-401.

Rimmer AE, Whittington RJ, Tweedie A, Becker JA (2017). Susceptibility of a number of Australian freshwater fishes to dwarf gourami iridovirus (Infectious spleen and kidney necrosis virus). *Journal of Fish Diseases* 40: 293-310.

Rindi F, Guiry MD, Cinelli F (1999). Morphology and reproduction of the adventive Mediterranean rhodophyte *Polysiphonia setacea*. *Hydrobiologia* 398/399: 91–100.

Roche DG, Torchin ME (2007). Established population of the North American Harris mud crab, *Rhithropanopeus harrisii* (Gould, 1841) (Crustacea: Brachyura: Xanthidae) in the Panama Canal. *Aquatic Invasions* 2: 155-161.

Rolheiser KC, Dunham A, Switzer SE, Pearce CM, Therriault TW (2012). Assessment of chemical treatments for controlling *Didemnum vexillum*, other biofouling, and predatory sea stars in Pacific oyster aquaculture. *Aquaculture* 364-365: 53–60.

Roohi A, Yasin Z, Kideys AE, Hwai ATS, Khanari AG, Eker-Develi E (2008). Impact of a new invasive ctenophore (*Mnemiopsis leidyi*) on the zooplankton community of the Southern Caspian Sea. *Marine Ecology-an Evolutionary Perspective* 29: 421-434.

Ross DJ, Johnson CR, Hewitt CL, Ruiz GM (2004). Interaction and impacts of two introduced species on a soft-sediment marine assemblage in SE Tasmania. *Marine Biology* 144: 747-756.

Ross DJ, Johnson CR, Hewitt CL (2006). Abundance of the introduced seastar, *Asterias amurensis*, and spatial variability in soft sediment assemblages in SE Tasmania: Clear correlations but complex interpretation. *Estuarine, Coastal and Shelf Science* 67: 695-707.

Roubal, FR, Masel J, Lester RGJ (1989). Studies on *Marteilia sydneyi*, agent of QX disease in the Sydney rock oyster, *Saccostrea commercialis*, with implications for its life cycle. *Australian Journal of Marine and Freshwater Research* 40: 155-167.

Roughley TC (1926). An investigation of the cause of an oyster mortality on the Georges River, NSW, 1924-25. *Proceedings of the Linnaean Society of New South Wales* 51: 446-491.

Roughley TC (1939). A review of the scientific investigation of the fisheries of New South Wales [Presidential address to Linnean Society]. *Proceedings of the Linnean Society of New South Wales* 64: vi_xxvii.

Rubio A, Frances J, Coad P, Stubbs J, Guise K (2013). The onset and termination of the QX disease window of infection in Sydney rock oyster (*Saccostrea glomerata*) cultivated in the Hawkesbury River, NSW, Australia. *Journal of Shellfish Research* 32: 483-496.

Ruesink JL, Lenihan HS, Trimble AC, Heiman KW, Micheli F, Byers JE, Kay MC (2005). Introduction of non-native oysters: ecosystem effects and restoration implications. *Annual Review of Ecology, Evolution, and Systematics* 36: 643–689.

Ruiz M, López C, Lee RS, Rodríguez R, Darrib S (2016). A novel paramyxean parasite, *Marteilia octospora* n. sp. (Cercozoa) infecting the Grooved Razor Shell clam *Solen marginatus* from Galicia (NW Spain). *Journal of Invertebrate Pathology* 135: 34–42.

Sant N, Delgado O, Rodriguez-Prieto C and Ballesteros E (1996). The spreading of the introduced seaweed *Caulerpa taxifolia* (Vahl) C. Agardh in the Mediterranean Sea: testing the boat transportation hypothesis. *Botanica Marina* 39: 427–430.

Sasikumar N, Nair KVK, Azariah J (1992). Response of barnacles to chlorine and heat treatment: an experimental study for power plant biofouling control. *Biofouling* 6: 69–79.

Sato-Okoshi W, Okoshi K, Shaw J (2008). Polydorid species (Polychaeta: Spionidae) in south-western Australian waters with special reference to *Polydora uncinata* and *Boccardia knoxi*. *Journal of the Marine Biological Association of the United Kingdom* 88: 491-501.

Sato-Okoshi W, Abe H, Nishitani G, Simon CA (2017). And then there was one: *Polydora uncinata* and *Polydora hoplura* (Annelida: Spionidae), the problematic polydorid pest species represent a single species. *Journal of the Marine Biological Association of the United Kingdom* 97: 1675-1684.

Saunders GW, Withall RD (2006). Collections of the invasive species *Grateloupia turuturu* (Halymeniales, Rhodophyta) from Tasmania, Australia. *Phycologia* 45: 711-714.

Saville-Kent W (1891). Oysters and oyster fisheries of Queensland. Presented to both Houses of Parliament by Command. Department of Fisheries, Brisbane. 17 pgs plus plates.

Savin K, Cocks B, Wong F, Sawbridge T, Cogan N, Savage D, Warner S. (2010). A neurotrophic herpesvirus infecting the gastropod, abalone, shares ancestry with oyster herpesvirus and a herpesvirus associated with amphioxus genome. *Virology Journal* 7: 308.

Savini D, Occhipinti-Ambrogi A (2006). Consumption rates and prey preference of the invasive gastropod *Rapana venosa* in the Northern Adriatic Sea. *Helgoland Journal of Marine Research* 60: 153-159.

Sauvage C, Pepin JF, Lapegue S, Boudry P, Renault T (2009). Ostreid herpes virus 1 infection in families of the Pacific oyster, *Crassostrea gigas*, during a summer mortality outbreak: differences in viral DNA detection and quantification using real-time PCR. *Virus Research* 142: 181-187.

Scanes E, Johnston EL, Cole VJ, O'Connor WA, Parker LM, Ross PM 2016. Quantifying abundance and distribution of native and invasive oysters in an urbanised estuary. *Aquatic Invasions* 11: 425-436.

Schaffelke B, Deane D (2005). Desiccation tolerance of the introduced marine green alga *Codium fragile* ssp. *tomentosoides* – clues for likely transport vectors? *Biological Invasions* 7: 557–565.

Schaffelke B, Hewitt CL (2007). Impacts of introduced seaweeds. *Botanica Marina* 50: 397–417.

Schikorski D, Faury N, Pepin JF, Saulnier D, Tourbiez D, Renault T (2011a). Experimental Ostreid herpesvirus 1 (OsHV-1) infection of the Pacific oyster *Crassostrea gigas*: Kinetics of virus DNA detection by q-PCR in seawater and in oyster samples. *Virus Research* 155: 28-34.

Schikorski D, Renault T, Saulnier D, Faury N, Moreau P, Pépin JF (2011b). Experimental infection of Pacific oyster *Crassostrea gigas* spat by ostreid herpesvirus 1: demonstration of oyster spat susceptibility. *Veterinary Research* 42: 27.

Schueller GH, Peters AF (1994). Arrival of *Fucus evanescens* (Phaeophyceae) in Kiel Bight (Western Baltic). *Botanica Marina* 37: 471-477.

Sebesvari Z, Esser F, Harder T (2006). Sediment-associated cues for larval settlement of the infaunal spionid polychaetes *Polydora cornuta* and *Streblospio benedicti*. *Journal of Experimental Marine Biology and Ecology* 337: 109-120.

Segarra A, Pépin JF, Arzul I, Morga B, Faury N, Renault T (2010). Detection and description of a particular ostreid herpesvirus 1 genotype associated with massive mortality outbreaks of Pacific oysters, *Crassostrea gigas*, in France in 2008. *Virus Research* 153: 92-99.

Seki H (1934). The abnormal development of the oyster gonads in Hiroshima Bay. *Bulletin of the Japanese Society for Scientific Fisheries* 9: 93–99.

Shatkin G, Shumway SE, Hawes R (1997). Considerations regarding the possible introduction of the Pacific oyster (*Crassostrea gigas*) to the Gulf of Maine: A review of global experience. *Journal of Shellfish Research* 16: 463-477.

Shiganova TA, Bulgakova YV, Volovik SP, Mirzoyan ZA, Dudkin SI (2001). The new invader *Beroe ovata* Mayer 1912 and its effect on the ecosystem in the northeastern Black Sea. *Hydrobiologica* 451: 187-197.

Shiganova TA, Mikaelyan AS, Moncheva S, Stefanova K, Chasovnikov VK, Mosharova SA, Mosharova IN, Slabakova N, Mavrodieva R, Stefanova E, Zasko DN, Dzhurova B (2019). Effect of invasive ctenophores *Mnemiopsis leidyi* and *Beroe ovata* on low trophic webs of the Black Sea ecosystem. *Marine Pollution Bulletin* 141: 434–447.

Shivani Y, Subhash Y, Tushar L, Sasikala Ch, Ramana Ch (2015). *Spirochaeta lutea* sp. nov., isolated from marine habitats and emended description of the genus *Spirochaeta*. *Systematic and Applied Microbiology* 38: 110-114.

Sievers M, Dempster T, Keough MJ, Fitridge I (2019). Methods to prevent and treat biofouling in shellfish aquaculture. *Aquaculture* 505: 263-270.

Sims N (1993). Chapter 12. Pearl oysters. In Wright A, Hill L (eds). Nearshore marine resources of the South Pacific, Suva: Institute of Pacific Studies, Honiara: Forum Fisheries Agency and Halifax: International Centre for Ocean Development, pgs 409-430.

Smith G (1985). The Queensland oyster fishery – an illustrated history. Department of Primary Industries, Queensland, 109 pgs.

Smith PJ, Ozaki H, Fujio Y (1986). No evidence for reduced genetic variation in the accidentally introduced oyster *Crassostrea gigas* in New Zealand. *New Zeealand Journal of Marine and Freshwater Research* 20: 569-574.

Smith IR, Nell JA, Adlard RD (2000). The effect of growing height and growing method on winter mortality in diploid and triploid Sydney rock oysters, *Saccostrea glomerata* (Gould, 1850). *Aquaculture* 185: 197−205.

Song WB, Wang CM, Wang XH, Li Y (2001). New research progress on massive mortality of cultured scallop *Chlamys farreri*. *Marine Science* 25: 23–27.

South PM, Floerl O, Forrest BM, Thomsen MS (2017). A review of three decades of research on the invasive kelp *Undaria pinnatifida* in Australasia: An assessment of its success, impacts and status as one of the world's worst invaders. *Marine Environmental Research* 131: 243-257.

Southgate PC, Lucas JS (2008). The Pearl Oyster. Elsevier, Netherlands, 565 pgs.

Spiers ZB, Gabor M, Fell SA, Carnegie RB, Dove M, O'Connor W, Frances J, Go J, Marsh IB, Jenkins C (2014). Longitudinal study of winter mortality disease in Sydney rock oysters *Saccostrea glomerata*. *Diseases of Aquatic Organisms* 110: 151−164.

Sprague V (1978). Comments on trends in research on parasitic diseases of shellfish and fish. *Marine Fisheries Review* 40: 26-30.

Stafford H, Willan RC, Neil KM (2007). The invasive Asian Green Mussel, *Perna viridis* (Linnaeus, 1758) (Mollusca: Mytilidae), breeds in Trinity Inlet, tropical northern Australia. *Molluscan Research* 27: 105–109.

Stentiford GD, Feist SW, Bateman KS, Hine PM (2004). A haemolymph parasite of the shore crab *Carcinus maenas*: Pathology, ultrastructure and observations on crustacean haplosporidians. *Diseases of Aquatic Organisms* 59: 57–68.

Stentiford GD, Bateman KS, Stokes NA, Carnegie RB (2013). *Haplosporidium littoralis* sp. nov.: a crustacean pathogen within the Haplosporida (Cercozoa, Ascetosporea). *Diseases of Aquatic Organisms* 105: 243−252.

Stentiford GD, Ramilo A, Abollo E, Kerr R, Bateman KS, Feist SW, Bass D, Villalba A (2017). *Hyperspora aquatica* n.gn., n.sp. (Microsporidia), hyperparasitic in *Marteilia cochillia* (Paramyxida), is closely related to crustacean-infecting microspordian taxa. *Parasitology* 144: 186–199.

Strasser M (1999). *Mya arenaria* – an ancient invader of the North Sea coast. *Helgolander Meeresunters* 52: 309-324.

Stubler AD, Robertson H, Styron HJ, Carroll JM, Finelli CM (2017). Reproductive and recruitment dynamics of clionaid sponges on oyster reefs in North Carolina. *Invertebrate Biology* 136: 365-378.

Sudthongkong C, Miyata M, Miyazaki T (2002). Iridovirus disease in two ornamental tropical fresh-water fishes: African lampeye and dwarf gourami. *Diseases of Aquatic Organisms 48:* 163–173.

Sugishita Y, Hirano M, Tsutsumi K, Mobin SM, Kanai K, Yoshikoshi K (2005). Effects of exogenous lipid peroxides on mortality and tissue alterations in Japanese pearl oysters *Pinctada fucata martensii*. *Journal of Aquatic Animal Health* 17: 233–243.

Summerson R, Darbyshire R, Lawrence E (2007). *Invasive Marine Species Range Mapping*, Bureau of Rural Sciences, Canberra. June 2007 , 77 pgs.

Sunila I, Karolus J, Lang EP, Mroczka ME, Volk J (2000). Transmission of the haplosporidian parasite *Haplosporidium nelsoni* to the eastern oyster *Crassostrea virginica* in an upweller system. *Diseases of Aquatic Organisms* 42: 153-155.

Suong NT, Banks JC, Fidler A, Jeffs A, Wakeman KC, Webb S (2019). PCR and histology identify new bivalve hosts of Apicomplexan-X (APX), a common parasite of the New Zealand flat oyster *Ostrea chilensis*. *Diseases of Aquatic Organisms* 132: 181-189.

Svoboda J, StrandDA, Vrålstad T, Grandjean F, Edsman L, Kozak P, Kouba A, Fristad RF, Koca SB, Petrusek A (2014). The crayfish plague pathogen can infect freshwater inhabiting crabs. *Freshwater Biology* 59: 918–929.

Swan K (2006). The effect of mitigation strategies for the clubbed tunicate *Styela clava* on mussel collectors. Masters Thesis, University of Prince Edward Island. 83 pgs. . https://www.islandscholar.ca/islandora/object/ir%3A21818/datastream/PDF/download

Talman SG, Keough MJ (2001). Impact of an exotic clam, *Corbula gibba*, on the commercial scallop *Pecten fumatus* in Port Phillip Bay, south-east Australia: evidence of resource restricted growth in a subtidal environment. *Marine Ecology Progress Series* 221: 135-143.

Tamburri MN, Luckenbach MW, Breitburg DL, Bonniwell SM (2008). Settlement of *Crassostrea ariakensis* larvae: effects of substrate, biofilms, sediment and adult chemical cues. *Journal of Shellfish Research* 27: 601-608.

Tan J, Lancaster M, Hyatt A, van Driel R, Wong F, Warner S (2008). Purification of a herpes-like virus from abalone (*Haliotis* spp.) with ganglioneuritis and detection by transmission electron microscopy. *Journal of Virological Methods* 149: 338–341.

Tan TL, Paul-Pont I, Evans OM, Watterson D, Young P, Whittington R, Fougerouse A, Bichet H, Barnes AC, Dang C (2015). Resistance of black-lip learl oyster, *Pinctada margaritifera*, to infection by ostreid herpes virus 1 µVar under experimental challenge may be mediated by humoral antiviral activity. *Fish and Shellfish Immunology* 44: 232 -240.

Tang B, Liu B, Wang X, Yue X, Xiang J (2010). Physiological and immune responses of zhikong scallop *Chlamys farreri* to the acute viral necrobiotic virus infection. *Fish and Shellfish Immunology* 29: 42-48.

Tasmania DPIW (2007). Risk Assessment of Abalone Fishing and Farming Activities Using Abalone Viral Ganglioneuritis as a Case Study. November 2007, 30 pgs.

Taylor JJ, Southgate PC, Rose RA (1997). Fouling animals and their effect on the growth of silver-lip pearl oysters, *Pinctada maxima* (Jameson) in suspended culture. *Aquaculture* 153: 3l-40.

Theil M, Westphalen G, Collings G, Cheshire A (2007). *Caulerpa taxifolia* responses to hyposalinity stress. *Aquatic Botany* 87: 221-228.

Thresher R, Proctor C, Ruiz G, Gurney R, MacKinnon C, Walton W, Rodriguez L, Bax N (2003). Invasion dynamics of the European shore crab, *Carcinus maenas*, in Australia. *Marine Biology* 142: 867–876.

Thurstan RH, Diggles BK, Gillies CL, Strong MK, Kerkhove R, Buckley SM, King RA, Smythe V, Heller-Wagner G, Weeks R, Palin F, McLeod I (2020). Charting two centuries of transformation in a coastal social-ecological system: A mixed methods approach. *Global Environmental Change* 61: 102058.

Tilmans M, Mrugała A, Svoboda J, Engelsma MY, Petie M, Soes DM, Nutbeam-Tuffs S, Oidtmann B, Roessink I, Petrusek A (2014). Survey of the crayfish plague pathogen presence in the Netherlands reveals a new *Aphanomyces astaci* carrier. *Journal of Invertebrate Pathology* 120: 74-79.

Tomaru Y, Kawabata Z, Nakano S (2001). Mass-mortality of the Japanese pearl oyster *Pinctada fucata martensii* in relation to water temperature, chlorophyll a and phytoplankton composition. *Diseases of Aquatic Organisms* 44: 61-68.

Trowbridge CD (1998). Ecology of the green macroalga *Codium fragile* (Suringar) Hariot 1889: invasive and non-invasive subspecies. *Oceanography and Marine Biology – An Annual Review* 36: 1–64.

Tun KL, Itoh N, Shimizu Y, Yamanoi H, Yoshinaga T, Ogawa K (2008a). Pathogenicity of the protozoan parasite *Marteilioides chungmuensis* in the Pacific oyster *Crassostrea gigas*. *International Journal for Parasitology* 38: 211–217.

Tun KL, Shimizu Y, Yamanoi H, Yoshinaga T, Ogawa K (2008b). Seasonality in the infection and invasion of *Marteilioides chungmuensis* in the Pacific oyster *Crassostrea gigas*. *Diseases of Aquatic Organisms* 80: 157-165.

Urban-Malinga B, Warzocha J, Zalewski M, Norkko A, Norkko J (2013). Effects of the invasive polychaete *Marenzelleria* spp. on benthic processes and meiobenthos of a species-poor brackish system. *Journal of Sea Research* 80: 25–34.

Urrutia A, Bass D, Ward G, Ross S, Bojko J, Marigomez I, Feist SW (2019). Ultrastructure, phylogeny and histopathology of two novel haplosporidians parasitising amphipods, and importance of crustaceans as hosts. *Diseases of Aquatic Organisms* 136: 87-103.

Utari HB, Senapin S, Jaengsanong C, Flegel TW, Kruatrachue M (2012). A haplosporidian parasite associated with high mortality and slow growth in *Penaeus* (*litopenaeus*) *vannamei* cultured in Indonesia. *Aquaculture* 366–367: 85–89.

Van Blaricom GR, Ruediger JL, Friedman CS, Woodard DD, Hedrick RP (1993). Discovery of withering syndrome among black abalone *Haliotis cracherodii* Leach, 1814, populations at San Nicolas Island, California. *Journal of Shellfish Research* 12: 185−188.

Vater A, Byrne BA, Marshman BC, Ashlock LW, Moore JD (2018). Differing responses of red abalone (*Haliotis rufescens*) and white abalone (*H. sorenseni*) to infection with phage associated *Candidatus* Xenohaliotis californiensis. *Peer J* 6:e5104; DOI 10.7717/peerj.5104

Victoria DPI (2006). Bulletin for Abalone Industry – 9 May 2006, Update on herpes like virus in farmed abalone.

Vigneron V, Solliec G, Montanié H, Renault T (2004). Detection of Ostreid Herpesvirus 1 (OsHV-1) DNA in seawater by PCR: influence of water parametres in bioassays. *Diseases of Aquatic Organisms* 62: 35-44.

Villalba A., Reece KS, Ordas MC, Casa SM, Figueras A (2004). Perkinsosis in molluscs: a review. *Aquatic Living Resources* 17: 411–432.

Villalba A, Iglesias D, Ramilo A, Darriba S, Parada JM, No E, Abollo E, Molares J, Carballal MJ (2014). Cockle *Cerastoderma edule* fishery collapse in the Ría de Arousa (Galicia, NW Spain) associated with the protistan parasite *Marteilia cochillia*. *Diseases of Aquatic Organisms* 109: 55–80.

Wada KT (1997). Causative factors and characteristics of mass mortalities that have occurred in cultured pearl oysters since 1994. *Shinju-Kenkyu* 6: 2-23 (In Japanese).

Wada KT (2007). The Japanese Experience: pearl oyster mortalities and constraints, pp. 95-101. In: Bondad-Reantaso MG et al. (eds), Pearl oyster health management: a manual. FAO Fisheries Technical Paper No. 503, Rome FAO, 120 pgs.

Waki T, Takahashi M, Eki T, Hiasa M, Umeda K, Karakawa N, Yoshinaga T (2018). Impact of *Perkinsus olseni* infection on a wild population of Manila clam *Ruditapes philippinarum* in Ariake Bay, Japan. *Journal of Invertebrate Pathology* 153: 134-144.

Walker LM (2009). *Polydora* and *Dipolydora* (Polychaeta: Spionidae) of estuaries and bays of subtropical eastern Australia: A review and morphometric investigation of their taxonomy and distribution. MSc thesis, Southern Cross University, Lismore, NSW. 180 pgs.

Walker LM (2011). A review of the current status of the *Polydora*-complex (Polychaeta: Spionidae) in Australia and a checklist of recorded species. *Zootaxa* 2751: 40-62.

Wallis RL (1977). Some aspects of the thermal tolerance of *Trichomya hirsuta* (Mollusca: Bivalvia) of Eastern Australia. *Marine Biology* 43: 217-223.

Wang H, Guo X, Zhang G, Zhang F (2004). Classification of jinjiang oysters *Crassostrea rivularis* (Gould, 1861) from China, based on morphology and phylogenetic analysis. *Aquaculture* 242: 137–155.

Wang Y, Yoshinaga T, Itoh N (2018). New insights into the entrance of *Perkinsus olseni* in the Manila clam, *Ruditapes philippinarum*. *Journal of Invertebrate Pathology* 153: 117-121.

Wang Z, Lu X, Liang Y, Wang C (2010a). *Haplosporidium nelsoni* and *H. costale* in the Pacific oyster *Crassostrea gigas* from Chile's coasts. *Diseases of Aquatic Organisms* 89: 223–228.

Wang Z, Liang Y, Lu X (2010b). Use of histopathology, PCR and in situ hybridization methods to detect the parasite *Mikrocytos* sp. in Pacific oyster *Crassostrea gigas* from the northern coast of the Yellow Sea, China. *Aquatic Living Resources* 23: 125−130.

Warburton FE (1958). The effects of boring sponges on oysters. *Fisheries Research Board of Canada Progress Reports of the Atlantic Coastal Stations*, No. 68: 3-8.

Warburton FE (1966). The behavior of sponge larvae. *Ecology* 47: 672-674.

Ward GM, Bennett M, Bateman K, Stentiford GD, Kerr R, Feist SW, Williams ST, Berney C, Bass D (2016). A new phylogeny and environmental DNA insight into paramyxids: an increasingly important but enigmatic clade of protistan parasites of marine invertebrates. *International Journal for Parasitology* 46: 605-619.

Ward GM, Feist SW, Noguera P, Marcos-López M, Ross S, Green M, Urrutia A, Bignell JP, Bass D (2019). Detection and characterisation of haplosporidian parasites of the blue mussel *Mytilus edulis*, including description of the novel parasite *Minchinia mytili* n. sp. . *Diseases of Aquatic Organisms* 133: 57–68.

Wargo RN, Ford SE (1993). The effect of shell infestation by *Polydora* sp. and infection by *Haplosporidium nelsoni* (MSX) on the tissue condition of oysters, *Crassostrea virginica*. *Estuaries* 16: 229−234.

Wasson K, Zabin CJ, Bedinger L, Diaz MC, Pearse JS (2001). Biological invasions of estuaries without international shipping: the importance of intraregional transport. *Biological Conservation* 102: 143-153.

Wells FE (2017). If the Asian green mussel, *Perna viridis* (Linnaeus, 1758), poses the greatest invasive marine species threat to Australia, why has it not invaded? *Molluscan Research* 37: 167-174.

Wells FE (2018). A low number of invasive marine species in the tropics: a case study from Pilbara (Western Australia). *Management of Biological Invasions* 9: 227–237.

Wesche SJ (1995). Outbreaks of *Marteilia sydneyi* in Sydney rock oysters and their relationship with environmental pH. *Bulletin of the European Association of Fish Pathologists* 15: 23-27.

Wesche SJ, Adlard RD, Hooper JNA (1997). The first incidence of clionid sponges (Porifera) from the Sydney rock oyster *Saccostrea commercialis* (Iredale and Roughley, 1933). *Aquaculture* 157: 173−180.

Wesche SJ, Adlard RD, Lester RJG (1999). Survival of spores of the oyster pathogen *Marteilia sydneyi* (Protozoa, Paramyxea) as assessed using fluorogenic dyes. *Diseases of Aquatic Organisms* 36: 221–226.

Wetchateng T, Friedman CS, Wight NA, Lee PY, Teng PH, Sriurairattana S, Wongprasert K, Withyachumnarnkul B (2010). Withering syndrome in the abalone *Haliotis diversicolor* supertexta. *Diseases of Aquatic Organisms* 90: 69-76.

White ME, Powell EN, Ray SM, Wilson EA (1987). Host to host transmission of *Perkinsus marinus* in oyster (*Crassostrea virginica*) populations by the ectoparasitic snail *Boonea impressa* (Pyramidellidae). *Journal of Shellfish Research* 6: 1-5.

Whitelegge T (1890). Report on the worm disease affecting the oysters on the coast of New South Wales. *Records of the Australian Museum* 1: 1 – 15.

Whittington RJ, Becker JA, Dennis MM (2010). Iridovirus infections in finfish – critical review with emphasis on ranaviruses. *Journal of Fish Diseases* 33: 95-122.

Whittington RJ, Hick PM, Evans O, Rubio A, Alford B, Dhand N, Paul-Pont I (2015a). Protection of Pacific oyster (*Crassostrea gigas*) spat from mortality due to ostreid herpesvirus 1 (OsHV-1 μVar) using simple treatments of incoming seawater in land-based upwellers. *Aquaculture* 437: 10–20.

Whittington RJ, Dhand NK, Evans O, Paul-Pont I (2015b). Further observations on the influence of husbandry practices on OsHV-1 μVar mortality in Pacific oysters *Crassostrea gigas*: Age, cultivation structures and growing height. *Aquaculture* 438: 82–97.

Whittington RJ, Hick P, Evans O, Rubio Zuazo A, Dhand N, Paul-Pont I (2016). Pacific oyster mortality syndrome: a marine herpesvirus active in Australia. *Microbiology Australia* 37: 126-128.

Whittington RJ, Paul-Pont I, Evans O, Hick P, Dhand NK (2018). Counting the dead to determine the source and transmission of the marine herpesvirus OsHV-1 in *Crassostrea gigas*. *Veterinary Research* 49: 34.

Whittington RJ, Liu O, Hick PM, Dhand NK, Rubio A (2019). Long-term temporal and spatial patterns of Ostreid herpesvirus 1 (OsHV-1) infection and mortality in sentinel Pacific oyster spat (*Crassostrea gigas*) inform farm management. *Aquaculture* 513: 734395.

Wikström SA, Kautsky L (2004) Invasion of a habitat-forming seaweed: effects on associated biota. *Biological Invasions* 6: 141- 150.

Wikström SA, von Wachenfeldt T, Kautsky L (2002). Establishment of the exotic species *Fucus evanescens* C.Ag. (Phaeophyceae) in Öresund, Southern Sweden. *Botanica Marina* 45: 510-517.

Wilkie EM, Bishop MJ, O'Connor WA, McPherson RG (2013). Status of the Sydney rock oyster in a disease-afflicted estuary: persistence of wild populations despite severe impacts on cultured counterparts. *Marine and Freshwater Research* 64: 267–276.

Willan RC (1987). The mussel *Musculista senhousia* in Australasia; another aggressive alien highlights the need for quarantine at ports. *Bulletin of Marine Science* 41: 475-489.

Willan RC, Russell BC, Murfet NB, Moore KL, McEnnulty FR, Horneer SK, Hewitt CL, Dally GM, Campbell ML, Bourke ST (2000). Outbreak of *Mytilopsis sallei* (Récluz, 1849) (Bivalvia: Dreissenidae) in Australia. *Molluscan Research* 20: 25–30.

Wilson BR (1979). A revision of Queensland Lithophagine mussels (Bivalvia, Mytilidae, Lithophaginae). *Records of the Australian Museum* 32: 435–489.

Wiltshire KH, Deveney MR (2017) The introduced alga *Caulerpa taxifolia* in South Australia: infestation boundary surveys and feasibility of control for populations outside the containment area. Report for PIRSA Biosecurity SA, SARDI Aquatic Sciences, Adelaide. SARDI Publication No. F2017/000204-1. SARDI Research Report Series No. 946. 16 pgs.

Winkler FM, García R, Valdivia MV, Lohrmann KB (2018). Assessment of oxytetracycline baths as therapeutic treatment for the control of the agent of withering syndrome (WS) in red abalone (*Haliotis rufescens*). *Journal of Invertebrate Pathology* 153: 109-116.

Wolf PH (1967). Winter mortality hits state's oyster output. *The Fisherman* 2: 20–22.

Wolf PH (1972). Occurrence of a haplosporidan in Sydney rock oysters *Crassostrea commercialis* from Moreton Bay, Queensland, Australia. *Journal of Invertebrate Pathology* 19: 416-417.

Wolf PH (1977). An unidentified protistan parasite in the ova of the blacklipped oyster, *Crassostrea echinata*, from northern Australia. *Journal of Invertebrate Pathology* 29: 244-246.

Wolf PH (1979). Life cycle and ecology of *Marteilia sydneyi* in the Australian oyster, *Crassostrea commercialis*. *Marine Fisheries Review* 41: 70-72.

Wolf PH, Sprague V (1978). An unidentified protistan parasite of the pearl oyster *Pinctada maxima*, in tropical Australia. *Journal of Invertebrate Pathology* 31: 262-263.

Wolff MH, Schneweis KE (1973). UV inactivation of Herpes simplex viruses, types 1 and 2. *Zentralbl Bakteriol Orig A* 1973, 223:470-477.

Wotton DM, O'Brien C, Stuart MD, Fergus DJ (2004). Eradication success down under: heat treatment of a sunken trawler to kill the invasive seaweed *Undaria pinnatifida*. *Marine Pollution Bulletin* 49: 844-849.

Xia J., Bai C, Wang C, Song X, Huang J (2015). Complete genome sequence of Ostreid herpesvirus-1 associated with mortalities of *Scapharca broughtonii* broodstocks. *Virology Journal* 12: 110, DOI: 10.1186/s12985-015-0334-0.

Xiao J, Ford SE, Yang H, Zhang G, Zhang F, Guob X (2005). Studies on mass summer mortality of cultured zhikong scallops (*Chlamys farreri* Jones et Preston) in China. *Aquaculture* 250: 602-615.

Xin L, Li C, Bai C, Wang C (2018). Ostreid Herpesvirus-1 infects specific haemocytes in ark clam, *Scapharca broughtonii*. *Viruses* 10: 529, DOI:10.3390/v10100529.

Xin L, Wei Z, Bai C, Chen H, Huang B, Wang C (2020). Influence of temperature on the pathogenicity of Ostreid herpesvirus-1 in ark clam, *Scapharca broughtonii*. *Journal of Invertebrate Pathology* 169: 107299.

Yarish C, Edwards P, Casey S (1979). Acclimation responses to salinity to three estuarine red algae from New Jersey. *Marine Biology* 51: 289-294.

York PH, Booth DJ, Glasby TM, Pease BC (2006). Fish assemblages in habitats dominated by *Caulerpa taxifolia* and native seagrasses in south-eastern Australia. *Marine Ecology Progress Series* 312: 223-234.

Yoshinaga T, Kokonoe H (2018). Impact of akoya oyster disease on the pearl culture industry in Japan in twenty years after its emergence. *Fish Pathology* 53: 136-138.

Young AM, Elliott JA (2020). Life history and population dynamics of green crabs (*Carcinus maenas*). *Fishes* 5: 4. doi:10.3390/fishes5010004

Zalota AK, Spiridonov VA, Kolyuchkina GA (2016). *In situ* observations and census of invasive mud crab *Rhithropanopeus harrisii* (Crustacea: Decapoda: Panopeidae) applied in the Black Sea and the Sea of Azov. *Arthropoda Selecta* 25: 39–62.

Zhang J, Li Y, Ren W, Cai Y (2010) Studies on phytoplanktons carrying and spreading AVNV. *Journal of Fisheries of China* 34:1254–1259

Zhang H, Campbell DA, Sturm NR, Dungan CF, Lin S (2011). Spliced leader RNAs, mitochondrial gene frameshifts and multi-protein phylogeny expand support for the genus *Perkinsus* as a unique group of Alveolates. PLoS ONE 6(5): e19933. doi:10.1371/journal.pone.0019933.

Zolotarev V (1996). Black Sea ecosystem changes related to the introduction of new mollusc species. *Marine Ecology* 17: 227-236.

zu Ermgassen P, Gamble C, Debney A, Colsoul B, Fabra M, Sanderson WG, Strand A, Preston J (eds) (2020). European guidelines on biosecurity in native oyster restoration. The Zoological Society of London, UK. 30 pgs.

Appendix 1. Mollusc shell recycling questionnaire

DigsFish Services Pty Ltd
32 Bowsprit Cres, Banksia Beach
Bribie Island, QLD 4507,
AUSTRALIA

Ph/fax +61 7 3408 8443
mob 0403 773 592
ben@digsfish.com
www.digsfish.com

Shellfish Reef Restoration: Oyster shell recycling questionnaire

As part of FRDC Project 2019-005 "*Risk analysis to identify and minimise biosecurity risks arising from recycling bivalve mollusc shell waste during shellfish reef restoration projects in Australia*", we are compiling information on the existing legislation that controls recycling of mollusc shells for shellfish restoration in each State of Australia, as well as overseas. The information is being obtained to assist the hazard identification process to identify the various significant pests and diseases that may be associated with recycled mollusc shells, and determine what risk mitigation measures are currently in place in various jurisdictions.

This questionnaire asks shellfish reef restoration practitioners to provide information on the shellfish (mollusc) species permitted for recycling in your jurisdiction; the diseases and pests requiring mitigation in your jurisdiction; and the regulations and sanitary requirements (if any) surrounding shell recycling that are currently being applied to recycled mollusc shells so that the biosecurity risks posed by recycled mollusc shells are minimised to a level considered acceptable in your jurisdiction.

Please provide this information by filling out the relevant sections of the table below.

Jurisdiction (e.g. State/ Territory, including country if not Australia)	**Mollusc species permitted for recycling**	**Diseases and pests requiring mitigation**	**Sanitary requirements applied to shell**	**Relevant legislation/Acts controlling recycling**	**Other comments**

Please return this questionnaire to the conference organisers or via email to ben@digsfish.com

Name of respondent: Ben Diggles Email: ben@digsfish.com

I wish to be kept informed of the outcomes of the risk analysis (tick box ✓) ☐

Thanks for your time spent filling out this questionnaire. Project No. 2019-005 "*Risk analysis to identify and minimise biosecurity risks arising from recycling bivalve mollusc shell waste during shellfish reef restoration projects in Australia*" is supported by funding from the FRDC on behalf of the Australian Government, as well as cash contributions by The Nature Conservancy.