

AUSTRALIAN AQUATIC VETERINARY EMERGENCY PLAN

AQUAVETPLAN

Disease Strategy

Infection with

ostreid herpesvirus-1 microvariant

Version 1, 2015



AQUAVETPLAN is a series of manuals that outline Australia's approach to national disease preparedness and proposes the technical response and control strategies to be activated in a national aquatic animal disease emergency.

National Biosecurity Committee

This disease strategy forms part of:

AQUAVETPLAN

This strategy will be reviewed regularly. Suggestions and recommendations for amendments should be forwarded to:

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Approved citation: Department of Agriculture (2015). AQUAVETPLAN Disease Strategy: Infection with ostreid herpesvirus-1 microvariant (Version [1]). In: *Australian Aquatic Veterinary Emergency Plan (AQUAVETPLAN)*, Australian Government Department of Agriculture, Canberra, ACT.

Publication record:

Version [1, January, 2015]

AQUAVETPLAN is available on the internet at: http://www.agriculture.gov.au/animal-plant-health/aquatic/aquavetplan

ISBN 978-1-76003-002-5

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Cataloguing data

Department of Agriculture 2015, AQUAVETPLAN Disease Strategy: Infection with ostreid herpes virus-1 microvariant, Department of Agriculture, Canberra.

Internet

AQUAVETPLAN Disease Strategy: Infection with ostreid herpesvirus-1 microvariant is available at www.agriculture.gov.au.

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Being a guide only, outbreaks or suspected outbreaks must be assessed on a case by case basis and expert advice should be obtained to determine the most appropriate management plan in response to the risk.

IMPORTANT NOTE: Important regulatory information for infection with ostreid herpesvirus-1 microvariant is contained in the World Organisation for Animal Health (OIE) *Aquatic Animal Health Code* (OIE 2014), which is updated annually and is available on the internet at the OIE website:

http://www.oie.int/international-standard-setting/aquatic-code/access-online/

Further details are given in Appendix 1 of this manual.

DISEASE WATCH HOTLINE

 $1\,800\,675\,888$

The Disease Watch Hotline is a toll-free telephone number that connects callers to the relevant state or territory officer to report concerns about any potential emergency animal disease situation. Anyone suspecting an emergency disease outbreak should use this number to get immediate advice and assistance

Preface

This disease strategy for the control and eradication of infection with ostreid herpesvirus-1 microvariant, also called Pacific oyster mortality syndrome (POMS), is an integral part of the Australian Aquatic Veterinary Emergency Plan, or AQUAVETPLAN.

AQUAVETPLAN disease strategy manuals are response manuals and do not include information about preventing the introduction of disease.

The Department of Agriculture provides quarantine inspection for international passengers, cargo, mail, animals, plants and animal or plant products arriving in Australia, and inspection and certification for a range of agricultural products exported from Australia. Quarantine controls at Australia's borders minimise the risk of entry of exotic pests and diseases, thereby protecting Australia's favourable human, animal and plant health status. Information on current import conditions can be found at the Department of Agriculture ICON website (http://www.agriculture.gov.au/biosecurity/import/icon-icd).

This strategy sets out the disease control principles for use in an aquatic veterinary emergency incident caused by the suspicion or confirmation of POMS in Australia. The strategy was scientifically reviewed by the Sub Committee for Aquatic Animal Health of the Animal Health Committee, before being endorsed by:

the Animal Health Committee of the National Biosecurity Committee in October 2014; and

The National Biosecurity Committee in December 2014.

Infection with OsHV-1 microvariant is listed on Australia's *National List of Reportable Diseases of Aquatic Animals* (http://www.agriculture.gov.au/animal-plant-health/aquatic/reporting).

Detailed instructions for the field implementation of AQUAVETPLAN are contained in the disease strategies, operational procedures manuals and management manuals. Industry-specific information is given in the enterprise manual. The full list of AQUAVETPLAN manuals that may need to be accessed in an emergency is shown in the table:

Table 1 AQUAVETPLAN manuals

Disease strategies Individual strategies for each disease

Operational procedures manuals Disposal Destruction Decontamination Enterprise manual Includes sections on: – open systems – semi-open systems – semi-closed systems – closed systems

Management manual Control centres management

Aquatic Animal Diseases Significant to Australia: Identification Field Guide (Department of Agriculture 2012) is a source of information about the aetiology, diagnosis and epidemiology of infection with OsHV-1 microvariant and should be read in conjunction with this strategy.

This first edition of this manual was prepared by Marty Deveney, Shane Roberts, Mark Crane and Tom Lewis in 2013. The authors were responsible for drafting the strategy, in consultation with a wide range of stakeholders from aquaculture and government sectors throughout Australia. However, the text was amended at various stages of the consultation and endorsement process, and the policies expressed in this version do not necessarily reflect the views of the authors. Contributions made by others not mentioned here are also gratefully acknowledged.

The format of this manual was adapted from similar manuals in AUSVETPLAN (the Australian Veterinary Emergency Plan for terrestrial animal diseases). The format and content have been kept as similar as possible to these documents, in order to enable animal health professionals trained in AUSVETPLAN procedures to work efficiently with this document in the event of an aquatic veterinary emergency. The work of the AUSVETPLAN writing teams and the permission to use the original AUSVETPLAN documents are gratefully acknowledged.

The revised manual has been reviewed and approved by the following representatives of government and industry:

Government

CSIRO Australian Animal Health Laboratory Department of Primary Industries, New South Wales Department of Primary Industry and Fisheries, Northern Territory Department of Agriculture, Fisheries and Forestry, Queensland Department of Primary Industries, Parks, Water and Environment, Tasmania Department of Fisheries, Western Australia Department of Environment and Primary Industries, Victoria Department of Primary Industries and Regions, South Australia Biosecurity Animal Division, Department of Agriculture, Australian Government Department of the Environment, Australian Government

Industry

National Aquatic Animal Health Industry Reference Group South Australian Oyster Growers Association

The complete series of AQUAVETPLAN documents is available on the internet (http://www.agriculture.gov.au/animal-plant-health/aquatic/aquavetplan).

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AQUAVETPLAN OsHV 1 microvariant Disease Strategy Manual

1 Nature of the disease

Infection with ostreid herpesvirus type 1 microvariant (OsHV-1 microvariant), a specific genotypic group of ostreid herpesvirus type 1 (OsHV-1), causes Pacific oyster mortality syndrome (POMS) and substantial acute mortality in juvenile and adult Pacific oysters (*Crassostrea gigas*). Pacific oysters (and the Portuguese cupped oyster, *C. angulata*) are the only species known to develop clinical disease due to infection with OsHV-1 microvariant (OIE 2014). OsHV-1 microvariant was first detected in Australia in 2010 (Jenkins et al. 2013). By June 2014, it was known to occur in Australia in three estuaries: the Georges River–Botany Bay, Port Jackson–Sydney Harbour and Hawkesbury River–Brisbane Water estuaries. OsHV-1 has not been detected outside these areas. POMS has a substantial impact on the viability of businesses and regional productivity where it occurs. Maintaining freedom from infection in South Australia and Tasmania is a priority for Australian aquatic animal health authorities.

This manual, consistent with the principles of AQUAVETPLAN, outlines the proposed approaches to national OsHV-1 microvariant preparedness and proposes the technical response and potential control strategies to be activated in an OsHV-1 microvariant-related disease emergency.

1.1 Aetiology

OsHV-1 is the only member of the genus *Ostreavirus* (family *Malacoherpesviridae*, order *Herpesvirales*) (Davison et al. 2009) and is a double-stranded DNA virus with a virion that has a dense core, protein capsid and a lipid envelope (Le Deuff & Renault 1999). OsHV-1 microvariant is defined by unique substitutions in the ORF 4 coding region, particularly a deletion of 12 consecutive nucleotides upstream from ORF 4 and other variants with similar deletions (OIE 2013a).

OsHV-1 microvariant causes POMS, a clinical disease characterised by sudden, acute mortality in all age groups of Pacific oysters, in Australia (NSW DPI 2013), and summer mortality in Europe (Martenot et al. 2010). In contrast, other strains of OsHV-1 (Arzul et al. 2001; Davison et al. 2005), are reported to cause mortality primarily in larvae of Pacific oysters (Nicolas et al. 1992) and other bivalve species (Arzul et al. 2001). The relationships between strains of OsHV-1 appear complex (Martenot et al. 2012) and data about them are emerging. This document focuses on OsHV-1 microvariant but many aspects of its management are shared with other herpesviruses of bivalves. Information about other variants of OsHV-1 is included where appropriate to better understand OsHV-1 microvariant and to differentiate POMS from larval mortality caused by other OsHV-1 variants. Although this manual relates primarily to the detection of OsHV-1 microvariant, any detection of OsHV-1 outside the current known infected areas is considered significant, and should trigger a response guided by this manual.

Throughout this manual, 'OsHV-1 microvariant' refers to that specific variant; where 'OsHV-1' is used it refers generically to all other OsHV-1 strains.

1.2 Susceptible species

Pacific oysters (and the Portuguese cupped oyster) are the only species known to develop clinical disease due to infection with OsHV-1 microvariant (OIE 2014). In Australia, Pacific oysters are an introduced species and are widely cultured and naturalised (English et al. 2000).

Other strains of OsHV-1 are recorded from the Pacific oyster (*C. gigas*), Portuguese oyster (*C. angulata*), suminoe oyster (*C. ariakensis*), European flat oyster (*Ostrea edulis*), Manila clam

(*Venerupis* [*Ruditapes*] *philippinarum*), carpet shell clam (*R. decussates*) and scallop (*Pecten maximus*) (Arzul et al. 2001a; 2001b; Renault et al. 2000; OIE 2012). Natural infections have been recorded in *C. gigas, O. edulis, V. philippinarum, R. decussates* and *P. maximus* (see OIE 2012). There is evidence of cross-species infection in hatcheries where multiple species are produced (Arzul et al. 2001b).

Recent (2014) challenge trials suggest that the OsHV-1 microvariant does not infect flat oysters (*O. angasi*) or Sydney rock oysters (*Saccostrea glomerata*).

There is no evidence that any OsHV-1 variant can infect humans. Infected oysters may be fit for human consumption provided that other normal food safety requirements for filter-feeding bivalves can be met.

1.3 World distribution

OsHV-1 microvariant and the associated clinical disease are recorded from Europe, New Zealand and Australia (OIE 2013).

Other strains of OsHV-1 are recorded from Europe, the People's Republic of China, Korea, Japan, Morocco, Mexico, New Zealand and the United States (Renault et al. 2010; OIE 2012; Shimahara et al. 2012). The close relationships between OsHV-1 variants make understanding the distributions of those variants difficult. For example, Shimahara et al. (2012) found OsHV-1 in Japan that displayed the unique substitutions in the ORF 4 coding region characteristic of the OsHV-1 microvariant but, due to nucleotide variations in other regions, concluded that the virus was not identical to the OsHV-1 microvariant. The clinical disease associated with the OsHV-1 microvariant has not been described from Japan.

In Australia, OsHV-1 microvariant is restricted to New South Wales including the Georges River, Botany Bay; Parramatta River, Port Jackson and the Hawkesbury River. A 2011 national survey provided evidence (95% confidence of detecting a 2% infection) that areas of South Australia and Tasmania where Pacific oysters are cultured are free of infection by all types of OsHV-1 (Herbert 2011). Oysters with the OsHV-1 microvariant are subject to control measures in New South Wales aimed at containing infection in the affected estuaries. South Australia and Tasmania have enacted controls to mitigate the risk of entry and establishment of the virus. In South Australia and Tasmania, Pacific oysters comprise more than 99% of aquaculture production of edible oysters.

1.4 Diagnosis of infection with OsHV-1

Cell cultures are not available for isolation of oyster viruses. Histological examination can identify indicative nuclear abnormalities such as pyknosis and chromatin margination, which may occur in association with OsHV-1 infections (Friedman et al. 2005; Vásquez-Yeomans et al. 2010), but numerous other factors can cause these features (Webb et al. 2007), and histological examination cannot differentiate between the diseases caused by different OsHV-1 variants. Cowdry type A bodies are not reported (Renault et al. 1994a; 1994b; Webb et al. 2007). Thus, definitive diagnosis of OsHV-1 infection relies on molecular methods.

The following case definition can be used to define suspect and confirmed cases of OsHV-1 microvariant infection for the purposes of this manual.

Suspect case

- Rapid onset of high mortality in Pacific oysters; or
- Positive result for OsHV-1 using a quantitative polymerase chain reaction (qPCR) test (Martenot et al. 2010) from one or more appropriate samples.

Confirmed case

- Positive result for OsHV-1 using the qPCR test; and
- Positive result with the C2/C6 conventional PCR test (Segarra et al. 2010); and
- Sequence analysis to confirm genotype as OsHV-1 microvariant (OsHV-1 microvariant exhibits a systematic deletion of 12–15 base pairs in ORF 4 of the genome (encompassed by the C2/C6 primers) in comparison with OsHV-1 (GenBank # AY509253).

1.4.1 Field methods: clinical signs and gross pathology

There are no pathognomonic clinical or gross pathological changes. Oysters affected by OsHV-1 microvariant die rapidly and are usually found as empty shells or open shells containing decomposing tissue. Early cases show gaping and weak closure (Richard Whittington, University of Sydney, 2013, pers. comm., August) but these are general characteristics of diseased Pacific oysters and are insufficient for diagnosis.

1.4.2 Laboratory methods

Laboratory methods for sampling and testing for OsHV-1 microvariants are outlined in the OIE Manual of Diagnostic Tests for Aquatic Animals. The latest versions of methods are available online (http://www.oie.int/international-standard-setting/aquatic-manual/access-online).

Sample submission

On discovery of any unusual mortality of Pacific oysters or other bivalves, samples should be submitted to the relevant state or territory laboratory and reported to the relevant state or territory department. The laboratory should be contacted directly to ensure that samples are collected using techniques that will satisfy its requirements. In the event that the laboratory cannot be contacted for advice (e.g. out of hours), live oysters can be submitted chilled on ice. If a delay in getting samples to the diagnostic laboratory is anticipated or oysters are dead, tissue preserved in 80%–95% analytical reagent-grade ethanol should be submitted. Mantle tissue is best for PCR testing.

Sampling protocols are detailed in the OIE Manual chapter on OsHV-1 microvariant (OIE 2013). Small spat may be pooled for sampling. For histology, a part of the animal including the digestive gland, gill and mantle is used. Samples of tissue for histopathology should be fixed in 10% seawater formalin solution or Davidson's fixative.

Sampling equipment may be available on-farm or may be obtained from state or territory fisheries or agricultural officers (see the AQUAVETPLAN Enterprise Manual [http://www.agriculture.gov.au/animal-plant-health/aquatic/aquavetplan/enterprise] for contact details). Advice on packaging of samples for shipment is also available from state and territory veterinary laboratories and the Fish Diseases Laboratory at the Australian Animal Health Laboratory, Geelong.

Microscopy

Histopathology

Histological examination of the animal is not sufficient to identify infection with herpesvirus but may be a valuable part of the diagnostic process. Histopathological findings indicative of viral infection, such as cellular and nuclear pathology, should be followed with molecular testing specific for the OsHV-1 microvariant.

Electron microscopy

OsHV-1 can be detected by visualisation of herpes-like viral particles by electron microscopy (Farley et al. 1972; Green et al. 2013). Viral particles are observed mainly in connective tissues of all organs in which fibroblastic-like cells exhibit enlarged nuclei with perinuclear chromatin (Arzul et al. 2002; Lipart & Renault 2002; Renault et al. 1995; Schikorsky et al. 2011a). Electron microscopy cannot differentiate between OsHV-1 variants. These findings should be followed with molecular testing specific for the OsHV-1 microvariant.

Culture methods

There are no culture or isolation methods available for OsHV-1.

Molecular techniques

Polymerase chain reaction testing

Conventional and quantitative PCR tests have been developed for highly sensitive and specific detection of OsHV-1 (Pepin et al. 2008; Martenot et al. 2010; OIE 2012a). PCR products can be sequenced to differentiate between variants of OsHV-1 (Webb et al. 2007; Dundon et al. 2011). PCR detects the presence of the agent's deoxyribonucleic acid (DNA) but cannot determine if infectious agent or active infection is present. The OIE Manual of Diagnostic Tests for Aquatic Animals details PCR tests for OsHV-1.

In situ hybridisation

An in-situ hybridisation (ISH) test has been developed for detecting OsHV-1 (Barbosa-Solomieu et al. 2004). ISH localises the agent's deoxyribonucleic acid (DNA) in infected tissues, furnishing complementary information about the site(s) of viral foci. Current ISH assays do not differentiate between variants of OsHV-1.

1.4.3 Confirmation of infection

Infection is confirmed by a positive PCR result and sequence analysis.

1.4.4 Differential diagnosis

OsHV-1 infection is difficult to differentiate from other diseases of Pacific oysters and has no pathognomonic signs. OsHV-1 infection should be suspected when there is an unexplained high mortality of Pacific oysters and other susceptible bivalves, and is confirmed by positive PCR tests showing the presence of viral DNA and further testing using PCR product sequencing to identify the viral strain.

A viral load > 10^4 copies/mg tissue is associated with clinical disease and low viral loads are associated with subclinical infection (Oden et al. 2011; Paul-Pont et al. 2013a). Oden et al. (2011) found that high OsHV-1 microvariant loads in spat and juvenile oyster tissues were associated with summer mortality and that a viral load threshold of 8.8×10^3 copies/mg tissue is a useful threshold for oyster farmers in evaluating mortality risk.

1.5 Resistance and immunity

The relationships between environment, oyster condition and immunity and the effects of OsHV-1 are poorly understood. Juvenile Pacific oysters appear more susceptible than adult oysters to OsHV-1 microvariant disease (OIE 2012; Paul-Pont et al. 2013a).

There are no vaccines available for OsHV-1. Green and Montagnani (2013) showed experimentally that the synthetic viral analogue poly(I:C) induced an antiviral state in Pacific

oysters and made 89% of experimental oysters refractory to OsHV-1 microvariant infection. Practical application of this process to prevent clinical disease in farmed oysters has not been shown.

Research into development of POMS-resistant *C. gigas* is ongoing in France, New Zealand and Australia. Selective breeding in France has produced stocks with 19% mortality, as opposed to 56% mortality of unselected stock, in two weeks in field conditions (Dégremont et al. 2013).

1.6 Epidemiology

The pattern and severity of disease is reported to depend on risk factors which include the culture method, proximity to sites showing clinical disease and environmental stressors. In France, higher water temperatures have been reported to be an important risk factor contributing to disease outbreaks within infected populations (Petton et al. 2013).

OsHV-1 (Arzul et al. 2001; Moss et al. 2007; Sauvage et al. 2009) and OsHV-1 microvariant (Dundon et al. 2011) have been detected in clinically normal oysters. Archival material suggests that OsHV-1 microvariant was present in New Zealand in 2005, several years before disease outbreaks in 2010 (Renault et al. 2012). In Australia, a low prevalence and intensity of infection with OsHV-1 microvariant was detected in archived samples from several months before the initial outbreak in the Hawkesbury River in 2012–2013 (Paul-Pont et al. 2014).

Animals die within a few days of showing signs of disease, but it is unclear if those animals are recently infected or have had the virus for an extended period. Stress in oysters caused by elevated water temperature (Petton et al. 2013) or water temperature change (Bingham et al. 2013) has been linked to the onset of clinical disease. A mild reduction in salinity was seen before outbreaks in experimental oysters in the Georges River in the 2011–2012 summer (Paul-Pont et al. 2013a), although salinity was not considered significant in the onset of mortalities associated with OsHV-1 microvariant in Australia in 2013 (Paul-Pont 2014).

In Europe, water temperature is an important influence on OsHV-1 microvariant clinical disease. Oysters held at 13°C for 40 days after experimental exposure to OsHV-1 microvariant showed no mortality, were OsHV-1 qPCR test-negative, and did not transmit the disease to healthy oysters (Petton et al. 2013).

In Australia, water temperatures of over 17°C have been associated with clinical disease (Jane Frances, Department of Primary Industries NSW 2013, pers. comm., April). Temperature alone may not, however, be the sole factor in the onset of clinical disease in Australia (Richard Whittington, University of Sydney, 2013, pers. comm., September). In the Hawkesbury River, subclinical infections were present at temperatures above 20°C for several months before the outbreak of clinical disease in 2013 (Paul-Pont et al. 2014).

In New Zealand, rapid temperature change rather than any specific temperature threshold was linked to occurrence of clinical disease (Bingham et al. 2013).

Oysters that experience longer periods of emersion show lower mortality (AusVet 2011; Paul-Pont et al. 2013a). Oysters held in cloches (semi-closed coastal lagoons) in France show lower mortality than oysters cultured in open marine systems (Angus Cameron, AusVet Animal Health Services, 2013, pers. comm., January). Stress is likely to play a substantial role in susceptibility to infection and/or development of clinical disease (Burge et al. 2007; AusVet 2011; Dégremont et al. 2013).

1.6.1 Incubation period

Substantial mortality of oysters may occur within a few days after exposure to OsHV-1 microvariant (AusVet 2011, Petton et al. 2013). The estimated incubation period for

expression of mass mortality in an Australian outbreak is four days (Paul-Pont et al. 2014). Mortalities of OsHV-1 microvariant-naïve oysters occurred three days after exposure to subclinically infected oysters in experimental conditions (Dégremont et al. 2013).

Burge & Friedman (2012) found that larvae die within six to 10 days of exposure to OsHV-1.

1.6.2 Persistence of the pathogen

Vigneron et al. (2004) showed that OsHV-1 DNA could be detected in seawater spiked with macerated oyster larvae for up to 22 days at 4°C and 12 days at 20°C. However, survival times of infectious virus may be shorter and the relationship between detection of DNA by PCR and infectivity of the virus is unknown. Survival of most aquatic viruses outside the host is greatest at lower temperatures (Corbeil et al. 2012). Clinically normal hosts are likely environmental reservoirs of OsHV-1.

Herpesviruses have a lipid envelope and are of intermediate to large size. The lipid envelope makes these viruses susceptible to inactivation by many lipophilic compounds such as soaps and detergents (Maillard 2001). OsHV-1 is likely to be susceptible to common decontamination agents, desiccation and irradiation. The presence of organic material and suspended solids will decrease the efficacy of a number of decontamination methods (see AQUAVETPLAN Operational Procedures Manual — Decontamination [http://www.agriculture.gov.au/animal-plant-health/aquatic/aquavetplan/decontamination]).

1.6.3 Modes of transmission

OsHV-1 microvariant infection can occur horizontally (via the water) and has been shown to occur experimentally by injection and cohabitation. Viral DNA load in water increases with infection duration and can be up to 1×10^6 copies/mL (Schikorski et al. 2011b). Vertical transmission has not been shown. Live hosts and host products are assumed to be the most effective carriers. Pacific oysters are found as fouling on ship's hulls (Minchin & Gollasch 2003), increasing the potential for long-distance transmission. Fomites may be important for transmission; contaminated equipment from infected areas in France was implicated in the index case of OsHV-1 microvariant in the United Kingdom (AusVet 2011).

Epidemiological evidence from the Georges River was consistent with particle or vectorborne transmission and there was no evidence that oyster movements or farm equipment introduced the disease to the Hawkesbury River (Paul-Pont et al. 2014). Furthermore, oysterto-oyster transmission was inefficient and did not trigger mass mortality in all bays in the Hawkesbury River subsequent to the index case in 2013 (Paul-Pont et al. 2014). Burge and Friedman (2012) showed that transmission of OsHV-1 to larval oysters can occur when low copy numbers of virus are present.

1.6.4 Factors influencing transmission and expression of disease

Transmission of OsHV-1 microvariant occurs optimally at 16.2–21.9°C in Europe (Petton et al. 2013) and stress appears to enhance transmission (AusVet 2011; Bingham et al. 2013). Temperature increases of 3°C have preceded OsHV-1 mortalities in France and the US, but did not precede clinical disease in Australian outbreaks (Paul-Pont et al. 2014).

The factors that lead to subclinical carriage are unknown. Oysters that survive exposure can become carriers which may die if stressed (such as during spawning), and can infect OsHV-1 microvariant-naïve oysters held in the same aquaria (Dégremont et al. 2013).

1.7 Impact

Pacific oyster aquaculture is an important industry in Australia. The value of production in 2011 was \$4.52 million in New South Wales, \$35 million in South Australia and \$21 million in Tasmania (ABARES 2012; NSW DPI 2012). OsHV-1 microvariant substantially affects production, employment and business viability where it occurs. Losses of stock on affected premises are up to 100% (AusVet 2011).

OsHV-1 microvariant caused the French oyster industry's value to drop by 38% from €630 million in 2008 to €391 million in 2011 (CESER 2012). In New South Wales, \$2.4 million of Pacific oyster production value was lost in the Hawkesbury River (NSW DPI 2013) and the value of New South Wales Pacific oyster production will halve (NSW DPI, unpublished data). In the Hawkesbury River, 15 businesses are threatened and at least five ceased trading in late 2013. In New Zealand, the value of the Pacific oyster industry was halved, with NZ\$15 million of production value and nearly half the production tonnage lost. Over 40 farms have been affected, with many having ceased trading and substantial job losses occurring (Colin Johnston, Aquaculture New Zealand 2013, pers. comm., August). There would be substantial mortalities and loss of production, with subsequent economic and social effects, if OsHV-1 microvariant became established in any of the major Pacific oyster growing regions in South Australia or Tasmania.

Pacific oysters are a valuable aquaculture species but also form wild populations in Australia. In South Australia, they are sparse and occur at low population densities as a result of a control program instigated by the South Australian Department of Primary Industries and Regions, and industry. In New South Wales, they are common, form dense populations, are regarded as a pest and are listed as an invasive species. They are listed under the NSW Fisheries Management Act, 1994, as a Class 2 noxious species in all New South Wales waters except Port Stephens. In Tasmania, naturalised Pacific oysters are so widespread and numerous that they are regarded as an established exotic species that cannot be managed. These naturalised oysters are potential hosts for OsHV-1 microvariant. The ecological effects of the loss of this substantial filter feeding biomass subsequent to mortality of naturalised oysters are unknown.

2 Principles of control and eradication

2.1 Introduction

OsHV-1 microvariant poses a severe threat to Pacific oyster aquaculture and any detection outside the current known range is a disease emergency in Australia.

A national survey in 2011 found no evidence of OsHV-1 microvariant or other forms of OsHV-1. Although non-OsHV-1 microvariant strains are not nationally reportable agents and are associated with less severe disease, there are taxonomic uncertainties associated with OsHV-1 and the role of different strains in disease epizootics. For these reasons, serious consideration should be given to determine the significance of all OsHV-1 detections.

This section provides background information to enable the choice of the most appropriate response option following detection of OsHV-1 microvariant in Australia outside its current geographic range.

There are three disease control strategies that could be adopted if OsHV-1 microvariant is detected in new areas in Australia:

- Eradication: in principle, the scale of eradication may be national (eradicate from Australia), local (eradicate from a local farm) or somewhere in between (eradicate from a region or state). National eradication of OSHV-1 microvariant is not considered feasible and the virus is well established in some estuaries in New South Wales. For new outbreaks, local or state eradication remains an option in specific circumstances (e.g. hatcheries) but is likely to be unfeasible in open waters.
- Containment, control and zoning: includes measures to exclude OsHV-1 microvariant from defined geographic areas and unaffected populations (e.g. by quarantine) and contain the virus to areas with enzootic infection.
- Control and mitigation of disease: measures are aimed at managing the frequency and severity of disease episodes in infected populations and keeping them within acceptable levels.

The basic principles of eradication and other response options are described in the AQUAVETPLAN Enterprise Manual and the AQUAVETPLAN Control Centres Management Manual. The AQUAVETPLAN Enterprise Manual, Appendix 1, lists the state and territory legislation relating to disease control and eradication.

The most likely scenario is an outbreak of OsHV-1 microvariant in a semi-open aquaculture system. Controlling such an outbreak will depend on the proximity of other Pacific oyster farms and wild populations of susceptible hosts, and the connectivity of the marine or estuarine system where the farm is located.

An outbreak could also occur in a semi-closed or closed hatchery system containing broodstock, juvenile and larval oysters. This was the usual manifestation of the virus before the emergence of OsHV-1 microvariant (Batista et al. 2007). The capacity to eradicate OsHV-1 microvariant following such an outbreak is greatly increased relative to outbreaks in more open systems because of increased control over stock, other hosts, water and fomites.

2.2 Methods to prevent spread and eliminate pathogens

2.2.1 Quarantine and movement controls

The following quarantine and movement restrictions should be implemented immediately the OsHV-1 microvariant is suspected.

Establishment of quarantine areas

Establishment of specified areas (see AQUAVETPLAN Enterprise Manual Section A for more details), including:

- Declared area: includes restricted area and control area
- Restricted area: area around infected premises or area
- **Control area**: a buffer between the restricted area and free areas
- **Free area**: non-infected area (this area is not considered a 'declared area' and may include large areas of Australia in which the presence or absence of OsHV-1 microvariant remains unassessed).

Figure 1 Establishment of specified areas to control OsHV 1 microvariant.

In the declaration of quarantine areas, the following factors need to be taken into account:

- proximity of other Pacific oyster farms to the index farm
- proximity of other farms growing other filter-feeding bivalves
- proximity of wild susceptible host populations
- hydrology and oceanography of the receiving marine or estuarine system
- vectors such as vessel traffic and other commercial and recreational activities.

The following practices must be considered when implementing response options:

- movement of stock as part of normal management practices
- local sales and disposal of Pacific oyster products
- use of Pacific oyster products as recreational fishing bait
- other commercial aquaculture of potential hosts or species that could mechanically carry the virus
- processing of Pacific oysters and other potential hosts
- movements of fomites including farming infrastructure
- discharge of untreated processing waste
- disposal of shell and other normal byproducts of farming, processing and endconsumers
- other commercial, recreational or traditional fishing and aquaculture activities, particularly those that move infrastructure such as traps or pots

- commercial and recreational shipping biofouling and carriage of ballast water
- activities of scavengers
- movements of other potential fomites
- discharge water from hatcheries or land-based depuration systems.

Considerations for disease management in aquatic environments

The establishment of boundaries for quarantine areas or other movement control areas, also referred to as disease management areas (DMAs) during an aquatic emergency animal disease (EAD) event requires detailed consideration of factors that are different to those necessary for terrestrial animal disease control. Water movement through and around farms in aquatic environments represents a substantial risk for the spread of disease through transfer of infectious pathogens in the water column, movement of infected material (particularly suspended organic and inorganic matter), and any infected wild organisms. For example, although an infected area may be established around an individual land-based hatchery or farm, water bodies adjacent to the infected area as well as in the same catchment should be considered for monitoring and control measures.

Although oysters are sedentary, in a POMS EAD event, the establishment of the relevant DMA boundaries must take into account dispersal of water discharged from any infected semiclosed aquaculture systems (e.g. hatcheries) and how this influences adjacent oyster populations. Similarly, outbreaks in semi-open systems (marine farms) require consideration of all oceanographically connected areas and distribution of wild susceptible host populations. Spread of infected oyster material through scavenging by other species also needs to be considered. Thus, rather than property boundaries, the geography, water flow, distance between farming areas and the range of susceptible species will define where DMA boundaries are placed.

Establishment of DMA boundaries and their classification must also take into account potential mechanisms by which disease may move beyond these boundaries. In most circumstances it is advisable to overestimate the size of DMAs and change their area as the response takes effect or more knowledge about the disease becomes available.

Movement controls

Movement controls include:

- restrictions or bans on the movement of live or uncooked Pacific oysters and other bivalves from infected areas
- restrictions or bans on releasing bivalves and water from infected areas into unaffected river systems or other aquatic environments
- restrictions or bans on the movement of bivalves between different estuary or marine systems, other aquatic environments or farms
- restrictions or bans on the use and movement of vessels and equipment from infected estuaries to unaffected estuaries or marine systems and between farms.

Implementation of bans and restrictions will be a dynamic process, determined by the location and extent of the disease outbreak and whether the aim is to eradicate the disease agent or to control its spread. Some restrictions may be impractical or unnecessary but others will be of critical importance to eradication or control.

The feasibility of restrictions and bans and extent to which these are able to be enforced will depend on the location of infection, the location and type of enterprises affected and the control response option chosen.

Zoning

If OsHV-1 microvariant were to become endemic in specific regions of Australia, a national zoning policy specific for OsHV-1 microvariant may be necessary to protect non-infected areas and to prevent further spread of infection. Zones would be based on the distribution of OsHV-1 microvariant-susceptible species and of any vector species present (if appropriate), the geographical and hydrological characteristics of water bodies and landforms, and predictions of the most likely method of spread of infection. Zoning must rely on the identification of biogeographic barriers, the connectedness of estuaries and the normal commercial movements of shell and equipment. A corresponding surveillance and monitoring program for OsHV-1 microvariant would be required to support the zoning policy. Principles of zoning for infected and non-infected zones in Australia are outlined in the AOUAPLAN Zoning Policy Guidelines

(http://www.agriculture.gov.au/aquaticanimalhealth) and in the OIE Aquatic Animal Health Code (OIE 2013).

Such controls are in place to contain and manage the outbreaks of OsHV-1 microvariant in New South Wales.

2.2.2 Tracing

Tracing a disease outbreak is the process of retrospectively determining the method and pattern of disease spread. Tracing investigations are crucial in determining all confirmed and potential locations of the disease, as well as defining restricted and control areas. The information gathered from tracing will assist in determining the most appropriate response action. The immediate steps required are to trace back all contacts with infected Pacific oysters, premises and sites (to establish the origin of the outbreak) and to trace forward all contacts with infected Pacific oysters, premises and sites (to establish the origin of the outbreak) and to trace forward all contacts with infected Pacific oysters, premises and sites (to establish the current location and potential spread of infection).

The following items should be considered when developing tracing activities:

- live Pacific oysters: broodstock, spat and stock sold to processors, restaurants or individuals
- live bivalves of other species
- dead bivalves: uncooked Pacific oysters and other bivalves intended for consumption or for use as bait (if cooked, tracing is not required)
- effluent and waste products from processing and/or cooking
- water: intake and outlet
- vehicles: oyster vessels, transport vehicles and operators' and visitors' cars
- materials: infrastructure, cultivation materials, baskets, trays or other culture units, tools, instruments and other fomites
- personnel: farm workers, sales and other professional representatives, trades people, veterinarians, scientists, technicians and visitors
- vessels and shipping.

Neighbouring Pacific oyster and other bivalve farms and processing plants may be, or may become, infected. Maps showing the location of neighbouring farms, processing plants and waterways, and hydrographic data are necessary to monitor the potential spread of the pathogen. The location of susceptible Pacific oysters and vectors including other bivalves should also be noted both upstream and downstream of the infected site. Further sources of infection may be identified if several facilities share common water.

2.2.3 Surveillance

Surveillance is necessary to:

- define the extent of the infection
- detect new outbreaks
- establish restricted and control areas to which quarantine and movement restrictions are applied
- establish infected and non-infected areas and zones for an OsHV-1 microvariant zoning program
- monitor the progress and success of a control or eradication strategy.

Sudden high mortality is a defining characteristic of clinical disease caused by OsHV-1 microvariant (OIE 2013). The detection of new outbreaks may rely primarily on investigation of morbidity or mortality reported by farmers, or on visual inspections by emergency response personnel. Areas where oysters show signs of disease should be investigated immediately. In outbreak conditions, high infection intensity and prevalence (often > 60%) is expected.

When testing for OsHV-1 in populations that do not show signs of disease, standard survey design principles should be applied to define the extent of infection or show freedom from the pathogen. Principles for the design and conduct of surveys for infectious aquatic diseases are outlined in Cameron (2002). Detailed information on general requirements for surveillance for recognition of freedom from infection is provided in the OIE Aquatic Animal Health Code chapter on surveillance (Chapter 1.4) and the OIE Manual of Diagnostic Tests for Aquatic Animals chapter on Infection with Ostreid Herpesvirus 1 microvariants (Chapter 2.4.9) (http://www.oie.int/en/international-standard-setting/aquatic-manual/access-online). The manual also provides information on surveillance for OsHV-1 microvariant.

Similarly, veterinary epidemiological principles for EAD events were outlined by Paskin (2009).

Subclinical infection of OsHV-1 microvariant may occur at low prevalence and intensity in Pacific oyster populations, warranting careful design considerations to determine freedom from infection. Australia conducted a national survey for OsHV-1 in 2011. In accordance with OIE guidelines to demonstrate freedom from disease, the survey was designed to detect 2% prevalence with 95% confidence. The prevalence of OsHV-1 in healthy Pacific oysters in the Hawkesbury River before the 2012 outbreak was 2.7–15% up to three months before mass oyster mortalities (Paul-Pont et al. 2014). These parameters could be used as guidelines for testing to detect OsHV-1 in a disease situation, and to determine the extent of distribution in areas not showing clinical disease. Surveillance for subclinical OsHV-1 is likely to be difficult, however, because early infections may have lower prevalences and titres, be difficult to sample and show false negatives when tested. Active infections have high viral loads and are easy to detect.

If positive results are obtained from known disease-free areas, retesting the suspect tissue samples is recommended to confirm results. If further confirmation is required, re-sampling the suspect population is necessary. Detection of OsHV-1 in oysters is by conventional PCR followed by sequencing of PCR product to differentiate between variants of OsHV-1.

The 2012 national survey defined a suspect and confirmed case of OsHV-1 using the case definition in section 1.4. This case definition ensured that all variants of OsHV-1 could be detected by the national survey. Test specificity (based on using two tests in series) of close to 100%, and a sensitivity of 95%, was assumed for surveillance in clinically normal oyster populations. It must be emphasised that the tests have not been fully validated for this purpose and the values quoted are assumptions based on available evidence.

Pooled samples may be used when sampling in areas with disease outbreaks where virus titres are expected to be high. Pool sizes of three to six animals have been used in Australian studies (Paul-Pont et al. 2014). Pooling is not recommended for use in surveillance of

populations with subclinical infections because target viral material may be diluted and affect diagnostic sensitivity.

2.2.4 Treatment of infected host species

There are no effective commercially available prophylactic or curative treatments for OsHV-1 infections.

2.2.5 Treatment of host products and by-products

How long OsHV-1 microvariant survives in dead oysters is not documented, and any uncooked oysters, oyster products and shell liquor should be regarded as potentially infectious.

In some instances, it may be appropriate to harvest surviving marketable oysters, although appropriate risk mitigation strategies are required to ensure that such oysters marketed for human consumption do not provide an additional pathway for release of OsHV-1 microvariant to an unaffected estuary or waterway.

Trade regulations, market requirements, food safety standards and potential spread of the pathogen must be considered when determining the treatment, processing and destination of Pacific oyster products and byproducts.

2.2.6 Destruction of hosts

Molluscs may be destroyed either by exposing them to sufficient heat to cause the two shells to open (in the case of bivalve molluscs) or by leaving them out of water for a sufficient period to cause death. The exact period will depend on the ambient air temperature; the warmer the temperature, the sooner the mollusc will die. Exudates and leakage from molluscs in these conditions should be contained. Careful inspection is required due to the clumping nature of oysters and the high likelihood of survival of sheltered oysters for extended periods in favourable conditions. Molluscs in systems that can be contained can be killed using copper sulphate or other chemical agents. The AQUAVETPLAN Operational Procedures Manual—Destruction (http://www.agriculture.gov.au/animal-plant-health/aquatic/aquavetplan/destruction) should be consulted for further information on destruction.

Any chemicals used for destruction must be approved for that use by the Australian Pesticides and Veterinary Medicines Authority (see Appendix 2).

In addition, any chemical that is used directly or indirectly for the control of an animal disease is governed in its use by relevant 'control of use' legislation in each state and territory. The relevant state or territory authority should also be consulted for further information before the use of the chemical.

Discharge of chemicals into waters or their use in natural waters is also regulated in all states and territories by environmental regulations.

2.2.7 Disposal of hosts

Molluscs are most effectively disposed of by composting or burial. The thick shell decreases the efficacy of many other disposal methods and the volume of product that remains to be disposed of following, for example, incineration, remains substantial. The AQUAVETPLAN Operational Procedures Manual—Disposal should be consulted for further information on disposal.

If oysters and other infected waste are buried, the sites used need to be chosen carefully to avoid any waste entering waterways or groundwater and avoid carriage by vectors.

2.2.8 Decontamination

The lipid envelope of herpesviruses means that soaps and detergents are the preferred decontaminating agents.

Due to differences in farming enterprises, disinfection protocols may need to be determined on an individual basis involving the farm manager, the state or territory chief veterinary officer and/or the director of the fisheries department. The protocol should take into consideration the factors outlined in Section 1.6, in particular:

- source and location of infection
- type of enterprise (e.g. farm, processing plant, hatchery, grow-out ponds and water source)
- construction materials of the buildings and structures on the site
- design of the site and its proximity to other waterways or buildings
- current disinfection protocols
- workplace safety concerns
- environmental impact of the disinfectant protocol
- legislative requirements (occupational health and safety, environmental protection and chemical use)
- availability of approved, appropriate and effective disinfectants.

No definitive data are available on the persistence of OsHV-1 microvariant in water, sediments or other substrates likely to require decontamination. Thorough drying and cleaning of equipment using soap or detergents is needed before re-use. See the AQUAVETPLAN Operational Procedures Manual — Decontamination for details of decontamination methods and their indicators.

2.2.9 Vaccination

There are no effective vaccines available for OsHV-1 microvariant.

2.2.10 Vector control

Although controlling the spread of OsHV-1 microvariant between systems is likely to be challenging due to the variety of possible vectors, it is potentially feasible. The virus is spread by carrier Pacific oysters, by water, or by translocation on equipment, vessels or fomites. Removing infected Pacific oysters as quickly as possible will limit access by vectors to diseased stock.

Limiting access by scavengers to known infected sites is important for controlling spread. Seabirds and wading birds occur commonly around oyster farms and may be attracted to dead or moribund oysters. In the event of an outbreak of OsHV-1 microvariant, therefore, access of birds to diseased oysters in affected farms may need to be controlled. Netting of sites is by far the most effective deterrent, but usually is not feasible on oyster leases.

Noisemakers may be effective in deterring birds in the short term but they become habituated to them. Firearms can be used as an alternative to noisemakers and, if approved, killing of a limited number of birds can reinforce fear instincts within flocks (Littauer 1990). In most regions of Australia, however, the use of firearms would be a last resort measure, requires licensed shooters and further permits from state police departments and environment protection and/or national parks agencies (see AQUAVETPLAN Disposal Operational Procedures Manual). Appropriate safety procedures and communications must be provided before use of live ammunition.

2.3 Environmental considerations

Environmental considerations in the control of OsHV-1 microvariant include the following.

- The release of disinfectants could adversely affect aquatic fauna and flora, especially when used in quantities or concentrations higher than normal, as might be necessitated in a disease emergency situation. In such situations, state and territory environmental protection agencies should be consulted (see the AQUAVETPLAN Enterprise Manual).
- Any environmental impacts associated with the destruction and disposal of infected carcasses and material should be minimised while ensuring measures are met to avoid infection being disseminated.
- Discharge of infectious or potentially infectious effluent from a semi-closed or closed system into natural waterways will pose a serious risk of spreading infection more widely and could lead to susceptible host populations becoming reservoirs of infection.
- Activities should be coordinated to comply with relevant state and territory environmental regulations.

See the AQUAVETPLAN Operational Procedures Manual — Decontamination for details of decontamination methods.

2.4 Sentinel animals and restocking measures

Pacific oysters might be obtained from virus-free locations and used as sentinel animals to assess the effectiveness of site decontamination measures, and preceding any large-scale restocking of individual oyster farms or oyster farming regions affected by OsHV-1 microvariant. It is important that, before sampling, such sentinel oysters are adequately exposed to environmental conditions, particularly temperature, conducive to infection and formation of clinical disease. Juvenile oysters or spat should be used as sentinels as they are believed to be more susceptible to infection. Due to the likelihood of subclinical infection, qPCR would be necessary to determine if infection of sentinels has occurred, rather than judging by appearance of clinical disease. The numbers of sentinels required would be dependent on the age of sentinels used and the layout of the system being tested. Pooling of samples will show whether virus is present, and is often used for spat.

Site-fallowing durations before restocking will need to be assessed on a species and case-bycase basis to minimise the risks of reoccurrence of OsHV-1 microvariant. The duration will depend on the season, the extent of the outbreak, the numbers of sites with confirmed diagnoses and the features of these sites. The duration required for fallowing to adequately allow virus clearance is unknown, and is likely to depend on the proximity and density of wild susceptible species populations.

For any attempts to eradicate OsHV-1 microvariant, it is important that restocked oysters are free of infection. For areas declared free of OsHV-1 microvariant, this status can also only be retained if introduced oysters are similarly free of infection. The use of spat derived from OsHV-1 microvariant-free broodstock reared under strict biosecurity measures would also be valuable in avoiding the reintroduction of infections at individual farms, farm clusters or broader regions.

2.5 Public awareness

Public awareness campaigns should emphasise education, surveillance and cooperation at both industry and community levels so that information is broadly disseminated to avoid practices that might exacerbate the likelihood of OsHV-1 microvariant infections being

spread inadvertently. This includes the potential for translocation of OsHV-1 by travellers or holidaymakers who may carry live oysters and 'swim' them in coastal areas to rejuvenate them or prolong their shelf life during their travels.

The importance of not using oysters as bait or aquaculture feed and using appropriate waste streams for discarded shells during an outbreak, due to the substantial risks of spreading OsHV-1 microvariant infection, should be emphasised. Public awareness documents need to emphasise that OsHV-1 is harmless to humans.

While OSHV-1 poses no risk to humans, public perceptions of the term 'herpesvirus' mean that this term should not be used in public awareness material. 'POMS' is the preferred terminology for this disease for public information purposes.

2.6 Feasibility of control or eradication of OsHV-1 microvariant in Australia

The feasibility of controlling an outbreak of OsHV-1 microvariant depends on the nature and location of the outbreak and the management strategy adopted. Essentially, as outlined in Section 2.1, there are three response options:

- *eradication:* eradication of OsHV-1 microvariant at some scale within Australia
- *containment, control and zoning:* containment of the virus to areas where infection is endemic to prevent further spread to uninfected areas
- *control and mitigation of disease:* implementation of management practices that minimise the incidence and severity of clinical disease outbreaks.

The outbreaks in New South Wales were regarded as ineradicable. The likely persistence of virus in naturalised Pacific oysters or wild susceptible species and difficulties in controlling those populations make eradication unlikely to succeed except in areas where they do not exist. Eradication is likely to be feasible in semi-closed and closed systems.

A major objective of a disease response strategy may be to minimise the risk of disease spread, as a result of oyster farming activities (e.g. stock and infrastructure movements), to areas that are free from OsHV-1 microvariant infection.

2.6.1 Response Option 1: eradication

Despite there being no records of successful eradication of OsHV-1 microvariant infection, attempting to eradicate OsHV-1 microvariant could be justified if:

- the outbreak occurs in a closed or semi-closed system, e.g. in intensive hatchery conditions
- the outbreak occurs in a semi-open system where no viable populations of susceptible hosts occur, or where naturalised Pacific oysters can also be controlled
- in Australia, farms in eradication zones seeking to re-establish operations could be stocked with native, non-susceptible oysters (e.g. *S. glomerata* or *O. angasi*).

Any attempt to eradicate OsHV-1 microvariant infection from a farm in an infected zone will require consideration of the following measures:

- agreement, among oyster growers and other affected parties in an area, that measures to achieve eradication are justified
- destruction and disposal of all farmed and naturalised Pacific oysters in the eradication zone
- resources are available for surveying and destocking naturalised oysters and wild susceptible species in the immediate area
- extensive decontamination of equipment, fomites and infrastructure
- sources of OsHV-1 microvariant-free or resistant stock are available if business continuity is an important consideration for eradication.

Eradication is unlikely to be successful or feasible if epidemiological investigations determine that infection is widespread, has no point source, or is unable to be contained due to:

- lack of ability to understand subclinical infection, and particularly establishment of infection at levels that are difficult to detect
- lack of ability to control susceptible species populations
- unavailability of OsHV-1 microvariant-free or resistant stock because hatchery stock prove to be infected or due to movement restrictions.

For situations in which eradication was the aim, affected closed or semi-closed systems with strict biosecurity controls could resume production following decontamination if:

- water is filtered (to 5 μm) and decontaminated (using UV light)
- farms in such zones could source OsHV-1 microvariant-free stock.

Clinically normal oysters

Animals in an infected zone should be regarded as potentially infected. Options available for such oysters are:

- destruction and disposal as undertaken with diseased oysters
- prompt harvesting and sale though normal systems, provided that risk mitigation measures are in place to ensure that marketing of such potentially infected oysters does not present a biosecurity risk, and lead to additional pathways for release of OsHV-1 microvariant.

The end result of both options is the prompt removal of 'potentially' infected oysters, removal of potential hosts and decreased infectious loads at affected sites, with reduced risks of infection spread. The systems used in harvesting the oysters must limit any possibility of further spread of infection, and thus could include:

- disinfection of all equipment and personnel involved in harvesting and processing of oysters
- quarantine measures including procedures for personnel, equipment and vehicles when moving from the infected estuaries to unaffected areas
- on-site processing systems adequate to either kill the virus or keep it in the human food chain with adequate controls to prevent use as bait (e.g. shucking) or the product being returned to a farm or the natural environment
- holding, disinfection and safe disposal of all processed waste including oyster shells, shell liquor and processing water.

Clinically diseased oysters

Immediate collection, destruction and safe disposal of all diseased and dead Pacific oysters will be essential to the success of any eradication strategy. These oysters, along with potentially infectious waste (including cultivation water exposed to OsHV-1 microvariant) will be the main means by which OsHV-1 microvariant infection could spread. Dead oysters will also decrease water quality and potentially support secondary pathogen propagation.

Other filter-feeding bivalves

Pacific oysters and the Portuguese cupped oyster are the only species known to develop clinical disease due to infection with OsHV-1 microvariant (OIE 2014). Although there is evidence for cross-species transmission of OsHV-1 between several bivalve mollusc species (Arzul et al. 2001b), other filter-feeding bivalves may be infected without clinical signs or be able to carry the virus in gut contents, adhering to external surfaces and in shell liquor. Adequate controls need to be enacted to prevent exposed individuals of other species of

bivalves grown in affected areas from being translocated outside the affected zone and, in particular, to prevent them being translocated to other Pacific oyster growing regions. Community awareness needs to focus on preventing use of all filter-feeding species from infected zones as bait, berley or in other ways that pose a transmission risk.

2.6.2 Response Option 2: containment, control and zoning

There are no effective, commercially available means of curing Pacific oysters that have become infected by OsHV-1 microvariant. If virus eradication is deemed to be unfeasible after an outbreak of OsHV-1 microvariant, zoning and associated disease control measures should be implemented to mitigate virus spread to uninfected zones.

The restricted movement of infected or 'potentially' infected oysters will be paramount to the success of such measures. The feasibility of zoning will also depend on the ability of farms, and the industry as a whole, to adjust management practices; the extent to which infection has spread by the time quarantine measures are enforced; and the location and distribution of susceptible species, including wild, naturalised oysters, in the region.

The feasibility of containment, control and zoning in the event of an incursion of OsHV-1 microvariant will need to be assessed at that time. The implications of restrictions on movements of oysters, people, boats and equipment, as well as on market access for Pacific oyster, and other filter-feeding bivalve products and by-products will need to be considered.

In a declared infected area, controlled grow-out and harvesting of oysters might be feasible without risking further spread of infection, provided that production systems can be modified to minimise infection, and appropriate processing and waste disinfection systems are used.

Justification for attempting to contain and control OsHV-1 microvariant infection within a zone is based on knowledge that:

- tissue from moribund and dead oysters and water containing OsHV-1 microvariant discharged during outbreaks will be a source of infection to other farms and wild susceptible species
- farms in such zones could source OsHV-1 microvariant-free stock to aid in decreasing the viral load.

There are several containment, control and zoning options available, but these options will centre on measures to manage disease outbreaks when they occur within an infected zone. The aim is to decrease the viral load locally to limit further infection within the zone and to decrease the likelihood of further spread, with the long-term aim of containment and limiting losses. The option chosen should prevent further exposure of local wild susceptible species populations as well as infection spreading beyond the zone.

Exposed (or potentially exposed) clinically normal oysters

If containment, control and zoning strategies are implemented, oysters could be farmed within infected zones using management strategies to limit infection and under heightened hygiene and biosecurity systems designed to limit the risk of exposure to OsHV-1 microvariant from all potential sources. From a biosecurity perspective, any oysters being reared in a declared infected zone must be considered potentially infected, and restrictions thus imposed on movements of oysters, people, vessels and equipment to prevent any potential for virus spread to uninfected zones.

Communications to heighten awareness about the potential for dead, uncooked oysters to act as vectors for OsHV-1 microvariant are vital to prevent further spread.

2.6.3 Response Option 3: mitigation of disease

Justification for attempting to mitigate OsHV-1 microvariant infection within a zone is based on knowledge that:

- tissue from moribund and dead oysters and water containing OsHV-1 microvariant discharged during outbreaks will be a source of infection to other farms and susceptible species
- farms in such zones could source OsHV-1 microvariant-free stock
- farms could use OsHV-1 microvariant-free stock grown in virus-free waters and then translocated as adults for on-growing (as in New Zealand)
- altered management strategies may exist that decrease losses and allow farms to operate, albeit at reduced profitability
- outbreaks may be geographically self-limiting because of discontinuities in Pacific oyster growing regions and areas where Pacific oysters are naturalised.

All the principles outlined for a containment, control and zoning strategy apply to the strategy of infection mitigation, except:

- the establishment of formal free and infected zones
- not taking an aggressive approach to management of clinical disease where it occurs.

2.6.4 Trade and industry considerations

OsHV-1 microvariant was listed as an emerging disease by the OIE (OIE 2013) but was delisted at the 2014 general session of the OIE. Although some Australian Pacific oysters are exported to Asia and Europe, to date, few trade implications have emerged from the outbreaks in New South Wales. The main industry consideration for outbreaks of the disease is the substantial loss of productivity in infected areas.

Trade regulations, market requirements and food safety standards must be considered as part of a response strategy. Permits may be required from the relevant authorities to allow products from declared areas to be released and sold for human consumption.

Domestic markets

An adequately controlled approach is required for the harvest of exposed or potentially exposed product for the domestic market. Pacific oysters are farmed in geographically separate areas that are could be managed as OsHV-1 microvariant-free zones. Waste released from uncooked oysters and non-host filter-feeding bivalves could present a risk if it were discarded in waterways containing susceptible hosts. Decisions regarding the release of exposed oysters or oyster products to the domestic market will depend on the response strategy implemented.

National and international trade regulations, market requirements and food safety standards must be considered as part of a control strategy. For example, permits might be required from the relevant authorities to allow mollusc products derived from disease zones to be released and sold for human consumption.

Trade in live stock (particularly spat) also needs to be considered. Permits for interstate trade or trade from infected areas to uninfected areas should consider health status of source stock.

The end point of marketed stock needs to be considered. Where whole live oysters are sold, conditions should be in place to avoid them being transported out of the infected area. Translocation of OsHV-1 by travellers or holidaymakers is recognised as a potential issue where live oysters may be 'swum' to rejuvenate them or prolong their shelf life.

Export markets

OsHV-1 microvariant was listed by the OIE as an emerging disease (OIE 2013), but listing of emerging diseases ceased in 2015. The occurrence of significant trade implications of this listing is unlikely. The taxonomic complexities of OsHV-1 variants makes defining the range of OsHV-1 microvariant difficult, but it is endemic in much of Europe, northern New Zealand and part of Australia. Export of Australian Pacific oysters is limited, and market access restrictions seem unlikely and, if experienced, to be of low impact.

The Department of Agriculture is responsible for the health certification of all exports and should be consulted for detailed information about current export market requirements (contact export@agriculture.gov.au).

3 Preferred Australian response options

3.1 Overall policy for OsHV-1 microvariant

Pacific oyster mortality syndrome (POMS) is caused by ostreid herpesvirus (OsHV)-1 microvariant. It occurs in Australia but is restricted to three estuaries in New South Wales, as of June 2014. OsHV-1 microvariant was considered ineradicable from the affected estuaries. Measures were put in place to minimise the risk of spread to virus-free areas.

POMS would likely cause severe losses in Pacific oyster aquaculture in the main growing regions in South Australia and Tasmania should it spread to those areas. Control of any outbreaks in these regions would have substantial human and financial costs for industry and governments.

In the event of OsHV-1 microvariant occurring outside the current range, and following initial epidemiological investigations (see Section 3.3.3), the appropriate response option will be decided by the Director of Fisheries and/or the Chief Veterinary Officer (CVO) of the state or territory in which the outbreak or detection has occurred, in consultation with the Aquatic Consultative Committee on Emergency Animal Diseases (AqCCEAD).

The three possible response options for OsHV-1 microvariant control in new outbreaks in Australia are:

Option 1: *eradication* with the aim of returning a newly infected premises or area to freedom from OsHV-1 microvariant

Option 2: *containment, control and zoning* with the aim of placing restrictions in areas in which OsHV-1 microvariant infection is endemic to prevent its further spread to uninfected areas

Option 3: *control and mitigation* with the aim of mitigating the impacts of OsHV-1 microvariant if it is accepted that the virus will remain endemic in new outbreak areas

Each of these response options will involve the use of a combination of strategies, which might include:

quarantine and movement controls on molluscs and culture equipment within declared areas to prevent infection spreading

destruction of diseased molluscs in a quarantined estuary promptly to prevent further shedding of virus

decontamination of facilities, equipment and vehicles or vessels to eliminate and prevent virus spreading

surveillance to determine the source and distribution of infection and freedom of infection

zoning to define and assist in maintaining virus-free zones

hygiene and biosecurity measures to mitigate on-site impacts of OsHV-1 microvariant.

The nature of the response will be determined mainly by whether the outbreak is multifocal or localised, and the likelihood that eradication or containment can be achieved and is cost-effective. The most appropriate strategy must be chosen after epidemiological investigations have been conducted. The decision must be based on scientific effectiveness and financial feasibility.

For a description of the notification arrangements, order of procedures, management structures and roles of personnel following suspicion of the presence of OsHV-1 microvariant in Australia, see the AQUAVETPLAN Control Centres Management Manual.

The Director of Fisheries and/or the CVO in the state or territory in which the outbreak occurs will be responsible for developing an emergency animal response plan (EAD Response Plan). This plan will be submitted to the AqCCEAD, which will provide advice on the technical soundness of the plan and its consistency with AQUAVETPLAN.

Directors of Fisheries and/or CVOs will implement the disease control measures as agreed in the EAD Response Plan and in accordance with relevant legislation. They will make ongoing decisions on follow-up disease response measures in consultation with the AqCCEAD. The detailed response measures adopted will be determined using the principles of control and eradication (see Section 2), epidemiological information about the outbreak and the financial feasibility of the option.

For information on the responsibilities of the other state or territory disease control headquarters and local disease control centres, see the AQUAVETPLAN Control Centres Management Manual.

3.2 Response options

The circumstances surrounding an outbreak of OsHV-1 microvariant will greatly influence selection of the most suitable response option. Figure 2 details the actions that should occur on initial suspicion of OsHV-1 microvariant in a new area.

a - as appropriate in the affected jurisdiction

Figure 3 Determination of most appropriate response option.

The algorithm shown in Figure 3 has been developed to help identify the most appropriate response option. These decision trees are flexible, depending on the specific situations experienced. However, in Figure 3, an instance when a response is 'unknown' should be treated in a cautionary manner and the 'no' option should be followed.

3.2.1 Option 1–Eradication

Eradication has the highest short-term economic costs; however, if OsHV-1 microvariant were successfully eradicated, long-term economic benefits could outweigh the short-term costs in states with significant *C. gigas* resources or industries.

If epidemiological investigations determine an obvious point source of infection that can potentially be contained with minimal or no spread of the virus, an eradication strategy might be successful and should be attempted. Eradication could also be attempted if the outbreak is in a closed or semi-closed system, or in an area where there are no populations of susceptible species or they can be controlled.

Eradication is unlikely to be feasible or successful if infection is widespread, has no identifiable source, cannot be contained or is potentially widespread in wild susceptible species or other as yet unknown reservoirs.

Eradication measures include:

- establishment of specified zones (restricted, control and free)
- quarantine and movement controls and restrictions on Pacific oysters and other bivalves, water and any other potential vectors (including vessels, materials and equipment) in or from zones declared restricted or control to prevent the spread of infection
- destruction and disposal of all clinically diseased Pacific oysters
- processing of exposed or potentially exposed, but clinically normal Pacific oysters within the infected zone, or under strict biosecurity conditions, to prevent the spread of infection
- disinfection and safe disposal of processing effluent and waste (oyster shells, shell liquor and processing water)

- disinfection, decontamination and safe disposal where necessary of facilities, products, equipment, vessels and vehicles etc., to eliminate the virus from infected premises and to prevent spread
- control of scavenger access, particularly birds, to live and dead oysters
- tracing and surveillance to determine the source and extent of infection and to provide proof of freedom from the disease
- a public awareness campaign to encourage cooperation from industry and the community in application of other measures as applicable in the situation
- disinfection of contaminated water from hatcheries and depuration facilities.

3.2.2 Option 2 – Containment, control and zoning

If infection occurs in wild susceptible species, or at numerous disparate farms, or across a very broad geographic range, eradication is unlikely to be practical. In this situation, containment and prevention of further spread and the protection of uninfected areas is the preferred response. Containment, control and zoning will also apply outside of affected farms or localities when eradication is pursued.

A zoning program will help the Australian Pacific oyster industry to maintain productivity. Restrictions on the movement of Pacific oysters and products and a surveillance program will be necessary to support zoning.

Farms in infected zones will need to implement management practices to reduce the severity and impact of OsHV-1 microvariant outbreaks.

Measures for containment, control and zoning are similar to those for eradication, but will emphasise management of the disease in individual facilities. Procedures might include:

- zoning or compartments to define infected and disease-free areas
- quarantine and movement controls or restrictions on Pacific oysters and other bivalves, water and any other potential vectors (including materials and equipment) within the infected zone and to free zones
- surveillance, with destruction and safe disposal of any oysters shown to be PCR positive in the infected zone, followed by clean-up and disinfection
- testing of broodstock and spat for OsHV-1 microvariant
- compartmentalisation of selected facilities (such as hatcheries for production of OsHV-1 microvariant-free stock) may be a part of a control and mitigation strategy
- emphasis on high standards of hygiene (including decontamination and use of sentinel animals before restocking) and biosecurity (screening of incoming spat for OsHV-1 microvariant)
- tracing and surveillance to determine the source and extent of infection
- a public awareness campaign to encourage cooperation from industry and the community.

3.2.3 Option 3–Mitigation of disease

If infection is extensive, wild susceptible species are infected and widespread, or if OsHV-1 microvariant-free stock are not available, it might not be appropriate to institute the controls described in sections 3.2.1 and 3.2.2, and an industry-based program to mitigate the effects of the disease might be appropriate. Zoning would not be used under this level of control, which would be similar to control measures in countries where OsHV-1 microvariant is endemic.

In a mitigation strategy, it would be the responsibility mainly of individual producers to manage the disease in their facilities using recommended measures to reduce the likelihood and severity of outbreaks. Producers would be encouraged to adopt current best practice

through provision of enterprise-level standard operating procedures and quality assurance programs, possibly leading to the development of an accreditation scheme.

Mitigation can occur in two forms:

- 1. Employed in affected estuaries in combination with containment.
- 2. As the only management response, without any controls to prevent further spread.

The second option may be considered if natural spread is considered to be inevitable or if further mitigation measures to restrict spread are impractical.

Measures for mitigation include:

- best-practice management to minimise the effects of disease
- farm surveillance, with destruction and safe disposal of all clinically diseased Pacific oysters
- use of OsHV-1 microvariant-free spat where possible
- emphasis on high standards of hygiene (including decontamination and use of sentinels before restocking) and biosecurity (screening of incoming spat for OsHV-1 microvariant)
- best-practice management methods to minimise stress and hence the risk of an outbreak during grow-out of stock with subclinical infections
- compartmentalisation of selected facilities such as hatcheries.

Table 2 Summary of strategies for each response option for OsHV 1 microvariant. Each strategy adopted may or may not be applicable depending on the control method (eradication, containment, mitigation) adopted.

	Control method		
Strategy	Eradication	Containment	Mitigation
Quarantine and movement controls	Yes	Yes	No
Declared restricted/control areas	Yes	Yes	No
Zoning	N/A	Yes	No
Movement controls within declared area or infected zone	Yes	Optional	N/A
Movement controls out of declared area or infected zone	Yes	Yes	Yes
Destruction of clinically diseased oysters	Yes	Yes	Yes
Destruction of unexposed oysters	Optional	No	No
Destruction of wild oysters	Yes	No	No
Harvest with processing of exposed or potentially exposed but clinically normal market-size ovsters	Yes	Yes	Yes
Within-zone processing	Yes	Yes	N/A
Disposal of infected oysters and wastes	Yes	Yes	Yes
Decontamination	Required	Optional	Optional
Surveillance	Yes	Yes	Yes
Tracing	Yes	Optional	No
Screening of broodstock and spat for OsHV-1 microvariant	Yes	Yes	Optional
Optimised management on farms	N/A	Yes	Yes
Specific farm-level hygiene measures	Yes	Yes	Yes
Specific farm-level biosecurity measures	Yes	Yes	Yes
Management of environmental issues	Yes	Yes	Yes
Management of commercial issues	Yes	Yes	Yes

3.3 Criteria for proof of freedom

Proof of freedom from OsHV-1 microvariant can be shown at the aquaculture establishment, zone and country level. Criteria for proof of freedom at each level are given in the OIE Aquatic Animal Health Code (OIE 2012).

Criteria for establishing local freedom further to those found in the OIE Aquatic Animal Health Code (OIE 2012) require a better understanding of diagnostic test results and the status of clinically normal, infected animals.

3.4 Funding and compensation

There are currently (2015) no national cost-sharing agreements in place for emergency responses to outbreaks of OsHV-1 microvariant. It is the responsibility of the users of this publication to seek advice in relation to any relevant funding or compensation arrangements within the relevant jurisdiction.

However, during a national disease response exercise based on POMS (Roberts et al. 2013), it was agreed that government would be heavily reliant on industry resources (e.g. personnel such as divers, infrastructure, vessels and equipment). The in-kind contribution that industry would need to make during a response, particularly for open and semi-open systems, would be substantial to ensure an effective and efficient response. This co-resourced model is the most feasible model for POMS (Roberts et al. 2013).

Appendix 1 OIE Aquatic Animal Health Code and Manual of Diagnostic Tests for Aquatic Animals

OIE Aquatic Animal Health Code

The objective of the OIE Aquatic Animal Health Code (OIE 2014) is to prevent the spread of aquatic animal diseases, while facilitating international trade in aquatic animals and aquatic animal products. This annually updated volume is a reference document for use by veterinary departments, import and export services, epidemiologists and all those involved in international trade of aquatic animals and their products.

The current edition of the OIE Aquatic Code is available on the OIE website at:

http://www.oie.int/international-standard-setting/aquatic-code/access-online

OIE Manual of Diagnostic Tests for Aquatic Animals

The purpose of the OIE Manual of Diagnostic Tests for Aquatic Animals (OIE 2014) is to contribute to the international harmonisation of methods for the surveillance and control of the most important aquatic animal diseases. Standards are described for laboratory diagnostic tests and the production and control of biological products (principally vaccines) for veterinary use across the globe.

The current edition of the OIE Aquatic Manual is available on the OIE website at:

http://www.oie.int/en/international-standard-setting/aquatic-manual/access-online

Chapter 2.4.9 Infection with ostreid herpesvirus 1 microvariant is relevant to this manual.

Further information

Further information about the OIE Aquatic Code and Aquatic Manual is available on the OIE website at:

http://www.oie.int/international-standard-setting

Appendix 2 Approval of chemicals for use in Australia

The Australian Pesticides and Veterinary Medicines Authority (APVMA) evaluates, registers and regulates agricultural and veterinary chemicals. Before an antibiotic or vaccine can enter the Australian market, it must go through the APVMA's rigorous assessment process to ensure that it meets high standards of safety and effectiveness. (In addition, an import permit is required from the Department of Agriculture if a product containing biological material is to be sourced from overseas.)

Detailed data about the product and its proposed use pattern must be submitted to the APVMA with the application for registration or permits. Since the assessment process is so detailed, the evaluation may take some time to complete.

Minor use permit system

The minor use permit (MUP) system is a temporary approval system for the use of drugs and chemicals. The system was devised by the APVMA for Australia, and allows the restricted use of a limited amount of a drug or chemical in a specified species when inadequate data are available to satisfy APVMA requirements for registration. Conditions are applied to the permit, which often include the collection of data related to the use of the product. The MUP system aims to enable restricted use of a drug or chemical until sufficient data are available to enable full registration.

For example, the APVMA may set a temporary withholding period with a wide margin of safety for a MUP. This withholding period may have been extrapolated from data relating to the use of the product in other species. In such cases, a condition of the MUP will be the collection of residue testing data. Results from the data are assessed by the APVMA (usually after 12 months, the duration of most permits) and used to more accurately set a withholding period for the product.

Emergency use permits

The APVMA has a permit system for the emergency use of a product that is either unregistered in Australia or registered for use in a different species or for a different use pattern. The APVMA will verify with the appropriate state and territory coordinators that the emergency is genuine.

For further details or permit application forms, see the APVMA website (http://www.apvma.gov.au).

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