Australian Government
Department of Agriculture

Australian aquatic veterinary emergency plan (AQUAVETPLAN) for withering syndrome

Version 2.0, 2019

Cataloguing data

This publication (and any material sourced from it) should be attributed as: Department of Agriculture 2019, Australian aquatic veterinary emergency plan (AQUAVETPLAN) for withering syndrome (version 2.0), Canberra. CC BY 4.0.

ISBN 9781760032067

This publication is available at agriculture.gov.au/animal/aquatic/aquavetplan.

AQUAVETPLAN

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.

This strategy will be reviewed regularly. Forward suggestions and recommendations for amendments to:

AQUAVETPLAN Coordinator
Aquatic Pest and Health Policy, Animal Health Division
Department of Agriculture
GPO Box 858 Canberra ACT 2601
Telephone 1800 900 090
Web agriculture.gov.au

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

NOTE: Important regulatory information for withering syndrome is contained in the World Organisation for Animal Health Aquatic Animal Health Code, which is updated annually.

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 withering syndrome is an integral part of the Australian Aquatic Veterinary Emergency Plan (AQUAVETPLAN).

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

The Department of Agriculture provides biosecurity 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. Biosecurity controls at Australia's borders minimise the risk of entry of exotic pests and diseases, and protect Australia's favourable human, animal and plant health status. Information on current import conditions can be found at the Department of Agriculture's BICON website.

This strategy sets out the disease control principles for use in an aquatic veterinary emergency incident caused by the suspicion or confirmation of withering syndrome 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 April 2019.

Withering syndrome is listed by the OIE in the Aquatic Animal Health Code (OIE 2016a). Withering syndrome is listed on Australia's National List of Reportable Diseases of Aquatic Animals (Agriculture 2019).

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 are:

- disease strategies
  - individual strategies for each disease
- operational procedures manuals
  - disposal
  - destruction
  - decontamination
- enterprise manual, including sections on
  - open systems
  - semi-open systems
  - semi-closed systems
- management manuals
  - control centre manual.

The Aquatic Animal Diseases Significant to Australia: Identification Field Guide 5th edition (Department of Agriculture 2019) is a source of information about the aetiology, diagnosis and epidemiology of infection with infectious salmon anaemia and should be read in conjunction with this strategy.

The first edition of this manual was prepared by Karina Scott in 2006. This revision was prepared by Marty Deveney, Brian Jones and Matt Landos and completed in 2015. These authors were responsible for drafting the strategy, in consultation with a wide range of stakeholders from aquaculture, recreational fishing 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. The authors would like to thank staff at the South Australian Research and Development Institute Aquatic Sciences, Marine Innovation Southern Australia, Ministry of Primary Industries New Zealand and Future Fisheries Veterinary Services for assistance in preparing this revision. 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) and from the AQUAVETPLAN enterprise manual. The format and content have been kept as similar as possible to these documents, so animal health professionals trained in AUSVETPLAN procedures can 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 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 and Fisheries, Queensland
  - Department of Primary Industries, Parks, Water and Environment, Tasmania
  - Department of Fisheries, Western Australia
  - Department of Economic Development, Jobs, Transport and Resources, Victoria
  - Department of Primary Industries and Regions, South Australia
  - Biosecurity Animal Division, Department of Agriculture and Water Resources, Australian Government
  - Department of the Environment, Australian Government
- Industry
  - Australian Abalone Growers Association

The complete series of AQUAVETPLAN documents is available on the Department of Agriculture website.

Contents

Tables

Figures

1 Nature of the disease

Withering syndrome is a disease of abalone (*Haliotis* spp.) not known to occur in Australia. It is caused by infection with the rickettsia-like pathogen *Candidatus Xenohaliotis californiensis* that can cause substantial mortality of farmed and wild abalone. In the literature the taxonomic term *Candidatus* indicates that the causative agent cannot be cultured but that it has been given provisional status as a new species in conformity with the International Code of Bacterial Nomenclature. For the purposes of this AQUAVETPLAN manual, however, the prefix '*Candidatus*' will not be used when referring to *Xenohaliotis californiensis*. This ensures consistency with the terminology used by the OIE in the Aquatic Animal Health Code and the Manual of Diagnostic Tests for Aquatic Animals (OIE 2016a; OIE 2016b), and Australia's National List of Reportable Diseases of Aquatic Animals (Department of Agriculture 2019b).

Abalone infected with *X. californiensis* display a severely shrunken or 'withered' body in association with pathologic changes to digestive epithelia. The morphological changes in the gut result in physiological starvation, anorexia and absorption of pedal musculature (Moore et al. 2001; Crosson et al. 2014).

Xenohaliotis californiensis has not been reported in Australia, but if introduced it could cause high mortalities and production losses in abalone aquaculture and fisheries, assuming that the disease manifests in a similar manner in Australia to that observed overseas.

1.1 Aetiology

The aetiological agent of withering syndrome is the *Rickettsiales*-like obligate intracellular bacterium *Xenohaliotis californiensis*. *X. californiensis* is a new genus and new species of intracellular prokaryote in the family Rickettsiaceae (Berthe 2003; Bower 2004).

There is currently no information on whether there are different strains of this bacterium, but some *X. californiensis* are infected with a phage (Friedman & Crosson 2012; Crosson et al. 2014). The phage alters the host-pathogen relationship and appears to decrease disease and mortality (Friedman et al. 2014), but its relationship to virulence and pathogenicity remains poorly understood. *X. californiensis* has a dimorphic rod-to-spherical shape. The average size of the rod form is 332 nm by 1550 nm, and the spherical morphotype has a mean diameter of 1405 nm. Reproduction occurs in intracytoplasmic vacuoles 14 µm to 56 µm in diameter (Friedman et al. 2000a).

1.2 Susceptible species

Xenohaliotis californiensis infects abalone of the genus *Haliotis* (Vetigastropoda: Mollusca). Susceptibility varies between *Haliotis* species (Berthe 2002). Natural infection has been observed in black abalone (*H. cracherodii*), which display greatest susceptibility (Friedman et al. 2014), but also occurs in red abalone (*H. rufescens*), pinto abalone (*H. kamtschatkana*), pink abalone (*H. corrugata*), green abalone (*H. fulgens*), white abalone (*H. sorenseni*), Japanese abalone (*H. discus hannai* and *H. discus discus*), small abalone (*H. diversicolor supertexta*), European abalone (*H. tuberculata*) and flat abalone (*H. wallalensis*). Bower (2004) suggests that *H. midae* may also be susceptible to infection. *X. californiensis* can infect both wild and cultured abalone (Friedman et al. 2003).

Disease susceptibility profiles of other *Haliotis* species, including Australian species, to infection with *X. californiensis* have 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.

Xenohaliotis californiensis has not been reported as a human pathogen. Although infected abalone are suitable for human consumption, animals with advanced infection have an extremely atrophied foot muscle and may be rejected by processors for quality and aesthetic reasons (C. Friedman, School of Aquatic and Fishery Sciences, University of Washington, pers comm, September 2005).

1.3 World distribution

Xenohaliotis californiensis was first observed in 1985 in black abalone populations on the South shore of Santa Cruz Island, California. While the initial source of the pathogen is unknown (Ben-Horin et al. 2013), the disease agent is now considered endemic on the southwest coast of North America, from Baja California, Mexico, north to San Francisco County, California (Friedman et al. 2003). Because infected abalone have been transported to Chile, the People's Republic of China, Chinese Taipei, Iceland, Ireland, Israel, Japan, Spain and Thailand, the geographical range of the aetiological agent is suspected to be broad. Areas where California red abalone *H. rufescens* is cultured, or where native species have been exposed to red abalone, are also likely to be infected (Berthe 2003; OIE 2016b). Withering syndrome has been reported from Chile (Godoy & Muñoz 2003). The presence of *X. californiensis* (without presence of disease) was reported in Iceland in June 2004, on farms that imported broodstock from California in 1988 (OIE 2004).

Xenohaliotis californiensis has not been reported in Australia. There are tight restrictions on the import of live molluscs into Australia. Rickettsia-like organisms were found in Australian abalone during a national survey, however the organisms did not resemble *X. californiensis* and the histological changes observed in the digestive tubules were dissimilar from those described for withering syndrome (Handlinger et al. 2005).

1.4 Diagnosis of infection with *Xenohaliotis californiensis*

A number of methods are available to diagnose infection with *X. californiensis*. A positive result from any single method, however, is not considered confirmatory.

This manual refers to both suspect and confirmed cases of *X. californiensis* infection.

A case is defined as **suspect** when either:

- the observation of clinical signs consistent with withering syndrome (section 1.4.1)
- histology shows intracellular bacteria and cellular changes consistent with withering syndrome

  or
- one or more appropriate samples test positive for *X. californiensis* using a polymerase chain reaction (PCR) test (Andree et al. 2000) or *in situ* hybridisation (ISH) test (Antonio et al. 2000).

A case is defined as **confirmed** when both:

- visualisation of *X. californiensis* by histology shows intracellular bacteria and cellular changes consistent with withering syndrome, or positive ISH (Antonio et al. 2000)

  and

- samples show positive result for *X. californiensis* using a polymerase chain reaction (PCR) test (Andree et al. 2000) including sequencing of the amplified DNA product to confirm sequence identity with the target bacterium (GenBank Accession AF133090).

1.4.1 Field methods: clinical signs and gross pathology

One of the earliest clinical signs of adverse health linked to withering syndrome is decreased feeding rate (Moore et al. 2001). However, this is also a common response to other diseases. The primary clinical sign of withering syndrome is a decrease in body mass, as assessed by atrophy of the foot muscle and other soft tissues, and retraction of the mantle (Moore et al. 2000b). A mottled digestive gland in moribund abalone (dark brown with small foci of tan coloured tissue) provides further presumptive evidence of clinical infection with *X. californiensis* (Friedman et al. 2000a).

The clinical signs of withering syndrome may include:

- weakness (Moore et al. 2000b)
- lethargy (Moore et al. 2000b)
- mantle retraction, and atrophied (or greatly diminished) and flaccid foot muscle, so that it appears that the animal does not fit its shell (Friedman et al. 1997; Haaker et al. 1992; Vanblaricom et al. 1993)
- decreased response to tactile stimuli (Friedman et al. 1997; Haaker et al. 1992; Vanblaricom et al. 1993)
- easy detachment from the substrate by hand (Bower 2004)
- inability of individuals to right themselves when turned upside down (Bower 2004)
- in the wild, loose attachment of individuals to rocks; they may hang from the rocks and have greater vulnerability to dislodgement by waves (Haaker et al. 1992; Vanblaricom et al. 1993)
- poor, or lack of, gonadal development (Friedman et al. 1997; Moore et al. 2000b)
- death (Friedman et al. 2000a; Friedman et al. 1997; Moore et al. 2000b).

Collectively, these signs have been termed withering syndrome. Clinical signs alone are insufficient to definitively diagnose infection with *X. californiensis*, but foot withering is an important clinical sign of this disease.

1.4.2 Laboratory methods

Sample submission

Samples should firstly be submitted to the relevant state or territory laboratory. 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 example, out of hours),

formalin-fixed material should be submitted for histopathology and ISH. For analysis by polymerase chain reaction (PCR), frozen tissues (or, if this is not possible, tissue preserved in 80% ethanol) should be submitted (M Crane, Australian Animal Health Laboratory, pers. comm., August 2016).

Sampling equipment may be available on farm or may be obtained from state or territory fisheries or agricultural officers (see the AQUAVETPLAN Enterprise Manual, DA 2015, for contact details). Advice on packaging of samples for shipment is also available from state and territory laboratories and the Fish Diseases Laboratory at the Australian Animal Health Laboratory.

The best target tissue is the posterior oesophagus, although the digestive gland and intestine complex is also suitable to a lesser degree. Non-digestive tract tissues do not contain pathogen DNA and should be avoided (OIE 2016b).

Microscopy

Tissue imprints (from sections of the posterior oesophagus) may be used to detect moderate to high intensities of infection with *X. californiensis* but histology is more sensitive than tissue imprints.

Xenohaliotis californiensis infects the epithelial cells of the posterior oesophagus (post-oesophagus), digestive gland and, to a lesser extent, intestine (Berthe 2003). The main pathological lesions characterising the disease are the presence of intracellular bacteria in the digestive epithelia and morphological changes in these areas. The morphological changes in the digestive gland vary between species, but may include metaplasia and/or degeneration.

Degeneration is characterised by an increase in connective tissues, inflammation, and atrophy of the digestive tubules (Berthe 2003). Metaplasia refers to the substitution of one mature cell type with another. The metaplastic changes in abalone with withering syndrome involve the replacement of terminal secretory or absorptive acini with absorptive or transport ducts similar in appearance to those observed in the post oesophagus (Braid et al. 2005; Friedman et al. 2000a; Friedman et al. 2002; Gardner et al. 1995). Some hyperplasia of the absorptive or transport ducts may also be involved.

The foot of affected individuals contains fewer and less organised muscle bundles than in unaffected individuals, and abundant connective tissue. It may also contain more brown pigmented serous cells than in unaffected individuals (Vanblaricom et al. 1993).

Infection with *X. californiensis* may be detected through histological examination of sections of the post-oesophagus, digestive gland, and foot muscle. Presumptive diagnosis by histology must include the observation of morphological changes to the digestive gland and the presence of the bacterium.

Transmission electron microscopy can be used to confirm the presence of intracellular bacteria with rickettsia-like morphology. However, this method cannot differentiate *X. californiensis* from other nonpathogenic members of the group, which have also been detected in abalone (Diggles et al. 2002; Handlinger et al. 2005).

Detailed information on the conduct of these tests is available in the OIE Manual of Diagnostic Tests for Aquatic Animals (OIE 2016b).

Culture methods

Xenohaliotis californiensis has not been cultured (Crosson et al. 2014).

Molecular Techniques

A PCR test has been developed for detecting *X. californiensis* associated with withering syndrome (Andree et al. 2000). PCR detects the presence of the agent's deoxyribonucleic acid (DNA) but cannot determine whether viable agent or infection is present. A positive result provides a presumptive diagnosis but is not confirmatory.

In situ hybridisation techniques have been developed to detect *X. californiensis* associated with withering syndrome (Antonio et al. 2000). In situ hybridisation is a useful tool that allows a specific probe hybridised to the target organism to be visualised in infected tissues.

1.4.3 Differential diagnosis

The clinical signs of infection with *X. californiensis* are not specific to the disease. They are also seen in starvation (Moore et al. 2000b), poor food supply, poor environmental conditions and other diseases (Moore et al. 2001).

The presence of digestive gland metaplasia (where functional tissue, including secretory cells, is replaced with cells similar in appearance to those of transport ducts) appears to be typical of infection with *X. californiensis* and is pronounced in this condition (Gardner et al. 1995; Moore et al. 2001).

Rickettsia-like organisms have also been reported, without associated gut pathology, in perlemoen abalone (*Haliotis midae*) from aquaculture in South Africa (Mouton 2000), in paua (*H. iris*) from aquaculture in New Zealand (Diggles et al. 2002), and from abalone (*H. laevigata* and *H. rubra*) from southern Australia (Handlinger et al. 2005). The organisms from New Zealand have been shown to be negative for *X. californiensis* by ISH (C Friedman, School of Aquatic and Fishery Sciences; University of Washington; and B Diggles, Principal Consultant, DigsFish Services, pers. comm., August 2005), as have those from South Africa (J Handlinger, Senior Veterinary Pathologist (Aquatic Animals), DPIWE, Tasmania, pers. comm., August 2004). The histological appearance of the rickettsia-like organisms found in Australia was dissimilar to that of *X. californiensis*, and cellular changes characteristic of withering syndrome were absent (Handlinger et al. 2005).

1.5 Resistance and immunity

Red abalone (*H. rufescens*) appear less susceptible to withering syndrome than black abalone (*H. cracherodii*), although Moore et al. (2000a) recorded severe losses of red abalone with clinical signs of withering syndrome.

Stressors, including temperatures above physiological tolerance, reduce the immune function of abalone (Malham et al. 2003; Moore et al. 2000b) and increase susceptibility to *X. californiensis* (Braid et al. 2005).

Abalone haemocytes have a nonspecific immune function that involves chemotactic, phagocytic and neuroendocrine function (Ottaviani 2004). Friedman et al. (2000b) showed experimentally that this immune capacity is affected in abalone with withering syndrome: haemocytes displayed increased

chemotactic activity but also displayed a compromised ability to engulf and destroy foreign particles. This compromised ability may play a role in the mortality associated with withering syndrome. It has been suggested that the increase in chemotactic activity may be due to the degeneration of the digestive gland and the use of the foot muscle as an energy source (Friedman et al. 2000b).

Molluscs do not have B-type or T-type lymphocytes and do not produce antibodies (Berthe 2002). There is no evidence that molluscs can generate long-term acquired immunological memory comparable to that found in vertebrates (Benkendorff 2003).

1.6 Epidemiology

The first reports of withering syndrome came from commercial abalone fishers, who observed gross signs typical of the syndrome on the south side of Santa Cruz Island, California, around 1985 (Haaker et al. 1992; Lafferty & Kuris 1993). The aetiology of the syndrome remained undescribed until 2000 (Friedman et al. 2000a).

Withering syndrome was initially recorded in black abalone. It was reported in wild red abalone from San Miguel Island, California, in 1994, and was first observed in cultured red abalone during the 1997 to 1998 El Niño event (Friedman et al. 2003).

1.6.1 Incubation period

When infection results in disease, the incubation period is prolonged (245 days in black abalone, 130 days in red abalone) but comparable to incubation periods reported for other rickettsial diseases (Friedman et al. 2003). Death occurs within one month of development of clinical signs of withering syndrome at temperatures of 18°C to 20°C (Friedman 1996).

1.6.2 Persistence of the pathogen

Transmission experiments suggest that *X. californiensis* survives in sea water (Moore et al. 2001) for an undetermined time (Balseiro et al. 2006; Braid et al. 2005; Friedman et al. 2002; Friedman et al. 2007; Rosenblum et al. 2008). No data are available on the survival of *X. californiensis* in sediment or other environmental reservoirs.

Xenohaliotis californiensis belongs to a group of bacteria that are most easily inactivated, and it is therefore likely to be susceptible to most disinfecting agents (AQUAVETPLAN Operational Procedures Manual—Decontamination, DAFF 2008). *Xenohaliotis californiensis* is inactivated by immersion in less than 10% sodium hypochlorite (Friedman & Finley 2003; OIE 2016b). A marine laboratory with a flow-through system treated seawater containing the bacterium with greater than 10 mg/litre calcium hypochlorite, and disinfected equipment in 1% iodophore in freshwater for 1 hour, with no detection of the pathogen in adjacent abalone populations, indicating that the decontamination protocol was adequate to deactivate the pathogen (Friedman & Finley 2003; OIE 2016b). All disinfection protocols should be preceded by effective cleaning, as cleaning may remove more than 90% of the pathogen loading and greatly increase the efficacy of the subsequent disinfection phase (DAFF 2008).

Xenohaliotis californiensis has only been reported in *Haliotis* spp. PCR analysis suggests, however, that colonial ascidians may concentrate the bacterium via feeding, and it is possible that other filter feeders may mechanically concentrate the pathogen (OIE 2016b).

1.6.3 Modes of transmission

Horizontal spread

Transmission of *X. californiensis* is likely to occur by direct contact between infectious abalone or through exposure to contaminated seawater (Ben-Horin et al. 2013). Experimental transmission studies have also demonstrated that close or direct physical contact between infected and uninfected abalone is not required, confirming that contaminated seawater is a potential source of infection (Friedman et al. 2002; Moore et al. 2001). The movement of infected animals can introduce the causative agent to new areas. Friedman & Finley (2003) linked the release of hatchery-reared abalone for stock restoration in California with the distribution of *X. californiensis*, and Wetchateng et al. (2010) described the translocation of *X. californiensis* in infected red abalone seed from a Californian hatchery to Thailand, via China and Taiwan.

Lafferty & Ben-Horin (2013) demonstrated that *X. californiensis* DNA was detectable in the discharge effluent plume up to 20 km from an infected shore-based abalone farm, but also noted that a strong DNA signal was limited to less than one to a few kilometres from the discharge source.

Friedman et al. (2002) suggest that *X. californiensis* is transmissible via a waterborne faecal–oral route, and that the bacterium may be shed in the faeces of infected abalone. This is supported by occurrence of bacterial foci in the digestive epithelium and intact and lysed rickettsial foci in lumina of the digestive tract (Friedman et al. 2002). Moore et al. (2001) concluded that ingestion of food contaminated with *X. californiensis* is the most likely mode of transmission.

Vertical spread

No studies have examined the vertical transmission of *X. californiensis*. In the absence of any knowledge about potential vertical transmission, the movement of abalone eggs and larvae represent an unknown risk factor for spread of infection. Abalone larval field studies suggest highly variable dispersal potential in different abalone species (Morgan & Shepherd 2006), ranging from several kilometres in greenlip larvae (*H. laevigata*) to tens of metres in blacklip abalone (*H. rubra*) (Prince et al. 1988; Rodda et al. 1997). However, given that abalone larvae are lecithotrophic (that is, non-feeding), the risk of infection prior to settlement, metamorphosis and commencement of feeding is considered low (OIE 2016b). Abalone larvae would therefore only constitute a potential vector for disease spread if vertical transmission does occur.

1.6.4 Factors influencing transmission and expression of disease

Intensity of *Xenohaliotis californiensis* infection

The severity of withering syndrome (based on gross pathology and histopathological changes in affected tissues) is correlated with the intensity of *X. californiensis* infection (as measured by the number of bacterial foci observed at the 200× magnification field in targeted gastrointestinal tract tissues) in both cultured and wild Californian red abalone. In contrast, there was no correlation between intensity of *X. californiensis* infection and severity of withering syndrome in the more susceptible black abalone, other than a single positive correlate between infection intensity and degree of metaplasia in digestive gland architecture (Friedman et al. 2002).

Temperature

Increased mortality, expression of clinical signs and rickettsial burdens in cultured subclinical red abalone is associated with water temperatures above 18.5°C—an increase of at least 4.5°C from the average culture temperature of 14°C (Friedman et al. 2003; Moore et al. 2000b). In black abalone displaying clinical signs consistent with withering syndrome, animals held at 13°C experienced higher survival rates than animals held at 20°C over the 40-week study (Friedman et al. 1997). In a related experimental study, infection rates in black abalone were positively correlated with the magnitude of daily fluctuations in temperature. However, withering syndrome in infected black abalone only occurred at elevated water temperatures, suggesting that while large daily variations in water temperature increase susceptibility to infection, infected abalone remain subclinical until water temperatures reach a critical threshold for disease expression (Ben-Horin et al. 2013). Low temperature (less than 13° C) may limit spread of the disease agent (Crosson et al. 2014) by limiting transmission of the bacterium (Braid et al. 2005). Subclinical infections have been observed in the tropical abalone H. diversicolor supertexta raised at 27°C to 29°C (Vanblaricom et al. 2011) indicating that tropical *Haliotis* spp. are likely to be hosts of the pathogen, although they may not develop withering syndrome.

The relationship between temperature and clinical presentation of withering syndrome may also depend on host tolerance factors rather than the absolute temperature. Warm temperate/tropical small abalone (*H. diversicolor*) do not develop withering syndrome but are subclinical carriers of *X. californiensis* (Wetchateng et al. 2010). However, it is unclear if *H. diversicolor* is refractory to infection because it is within its normal thermal range and therefore not heat-stressed at 26°C to 28°C, or if these temperatures are outside the range at which the bacterium can reproduce. Green abalone (*H. fulgens*) exposed to elevated water temperatures typical of a southern California El Niño event (18.0°C) did not exhibit an increase in either infection intensity or clinical signs of disease compared to animals maintained at lower water temperatures typical of a La Niña event (14.4°C), which possibly reflected the fact that both experimental groups were held at temperatures well within the normal thermal range for this species (Moore et al. 2009).

Thermal stress and other physiological stressors that may increase susceptibility to infection with *X. californiensis* could differ in Australian abalone from those observed overseas. This will in part depend on normal local seawater temperatures and the natural thermotolerance range of the affected abalone species. Cool-water *Haliotis* spp. are prone to heat stress (Hooper et al. 2014) and heat stress is a predisposing factor for many diseases of abalone (Dang et al. 2012).

1.7 Impact

Since 1985, widespread mass mortalities associated with *X. californiensis* infection have caused significant reductions in the wild populations of black abalone on the mainland coast of central California. The largest decline was seen at the southernmost extent of abalone populations, with a 97% reduction in abalone populations between 1992 and 1995 (Altstatt et al. 1996).

Overfishing had depleted populations of pink (*H. corrugata*), green (*H. fulgens*), white (*H. sorenseni*) and red abalone (*H. rufescens*) in southern California, causing closure of fisheries for these species prior to the emergence of withering syndrome (Karpov et al. 2000). Although overfishing had already greatly reduced many black abalone populations in southern California, withering syndrome nearly

eliminated the remaining populations throughout the Channel Islands and off central California (Moore et al. 2002). During the 1997 to 1998 El Niño, red abalone farms from Mexico to central California experienced high mortality rates, and animals showed signs of withering syndrome (Moore et al. 2000b). Despite these serious impacts, there are no estimates of the economic loss caused by *X. californiensis* in California.

Abalone fisheries in Australia were valued at $138 million and abalone aquaculture was valued at $27 million in 2013–2014 (ABARES 2015). Mazur et al. (2010) predicted that by 2014–2015 approximately one-third of Australia's abalone production would be farmed. The most recent figures (2013–2014) confirm that farmed abalone has been increasing as a percentage of total production, from 12% in 2011–2012 to 18% in 2013–2014 (ABARES 2015).

Withering syndrome caused by *X. californiensis* can cause high mortality rates in both farmed and wild populations. The impact on Australian abalone industries if *X. californiensis* occurred here is difficult to predict because of knowledge gaps about local species' susceptibility, types of physiological or environmental stressors that might exacerbate development of clinical disease and the inherent difficulty in predicting where the disease might first be introduced or emerge.

It is likely, however, that if *X. californiensis* did occur in Australia that economic impacts may be significant at the regional level. Immediate impacts in a fishery would include loss of resource (abalone biomass) and reduced resource access (areas closed to fishing for disease control and subsequent quota reductions). Long-term impacts on abalone recruitment could occur as a result of reduced breeding populations. There is evidence that black abalone are recovering in California but signs of recovery have taken decades and populations have not yet recovered to levels that can support sustainable harvest (Crosson et al. 2014). Abalone are keystone grazers of turf and other algae (Scheibling 1994) but juveniles rely on crustose coralline algae for food and habitat (Shepherd & Turner 1985).

In areas where abalone populations have undergone mass mortality events, including those caused by disease, they may not recover (Mayfield et al. 2011). Loss of abalone can cause ecosystem shifts where crustose coralline algae are overgrown by turfs, preventing population recovery. In Western Australia, a marine heatwave is suspected to have killed almost all the Roe's abalone (*H. roei*) on approximately 200 km of reefs in the north of this species' range (Pearce & Feng 2013). Recovery has been slow, particularly in areas most severely affected by the heatwave (Fletcher & Santoro 2015). Prevention of entry and spread of disease to abalone populations remains an important mechanism to support sustainable fisheries and aquaculture (Mayfield et al. 2011).

The impact of *X. californiensis* on abalone farms would include loss of stock, direct costs of responding and production interruption. Long-term impacts would include decreased productivity and increased husbandry costs.

The flow-on effects of a serious disease in abalone on associated supply chains and service industries would be significant.

2 Principles of control and eradication

2.1 Introduction

The discovery of *Xenohaliotis californiensis* in Australia (with or without the clinical signs of withering syndrome) may present a serious threat to wild and farmed abalone and associated industries and ecosystems. Any confirmed detection of *X. californiensis* would constitute a disease emergency in Australia. Several control measures may be effective in minimising the impact of *X. californiensis* occurrence in Australia. This section provides background information to enable the choice of the most appropriate response option following detection of *X. californiensis* in Australia.

There are two main response options if *X. californiensis* is detected in Australia.

1) Eradication—the scale of eradication of *X. californiensis* may be national (eradicate from Australia), local (eradicate from a local farm) or somewhere in between (eradicate from a region or state) but is likely to be infeasible in open systems.
2) Control—measures to exclude *X. californiensis* from defined geographic areas and unaffected populations (for example, by quarantine) and contain the bacterium to areas with enzootic infection.

Measures to control and mitigate disease are aimed at managing the frequency and severity of disease episodes in infected populations and keeping them within acceptable levels. Control and mitigation is not favoured because it is likely to lead to spread and establishment of *X. californiensis* in farmed and wild abalone populations posing extreme risk to the economy and environment.

The basic principles of eradication and other control responses are described in the AQUAVETPLAN Enterprise Manual (DA 2015) and the AQUAVETPLAN Control Centres Management Manual (DAFF 2001). The AQUAVETPLAN Enterprise Manual lists the state and territory legislation relating to disease control and eradication (DA 2015). The principles of control and eradication for *X. californiensis* are also similar to those outlined in the AQUAVETPLAN response manual for abalone viral ganglioneuritis (AVG) (Department of Agriculture and Water Resources 2014).

The most likely scenario is difficult to predict. An outbreak could occur in any type of system. The capacity to eradicate *X. californiensis* following an outbreak or detection in a closed system is much greater than for outbreaks in more open systems because of increased control over water, stock, other hosts and fomites.

Within these overall options, the general principles for the control and eradication of *X. californiensis* include:

- rapid detection and identification of infection
- rapid definition of the nature and extent of the infection
- rapid definition and implementation of control measures
- prevention of spread of *X. californiensis* by controlling stock movement, within and between farms and water bodies

- maintenance of good husbandry practices and high standards of hygiene.

The most appropriate option will depend on:

- presence or absence, and location, of reservoirs of infection
- the probability of success of eradication
- level of risk accepted for future spread of infection
- short-term costs of control and disruption to production
- long-term costs of production with or without *X. californiensis*
- long-term costs of control if *X. californiensis* became established.

In Australia, abalone are fished from the wild or seeded onto structures on the sea floor (open systems), grown in floating barrels moored off ropes, or in plates in ring cages (semi-open systems), in flow-through systems on shore (semi-closed system) and in recirculation and other systems with controlled outlets (closed systems). The type of system in which the infection is found will be a major factor in determining the most appropriate response option.

2.1.1 Open systems

An outbreak of *X. californiensis* in an open system (such as wild fisheries) would be extremely difficult to eradicate. Manual removal of abalone from the affected area would be time consuming, and successful extraction of all exposed abalone could not be assured. Sustained heavy fishing pressure may reduce the abundance of abalone to a very low level, although an intensive fishing program on a reef in Victoria in 2008, targeting abalone of all sizes, was not successful in preventing the spread of abalone viral ganglioneuritis (AVG) (T Bradley, Department of Economic Development, Jobs, Transport and Resources, Victoria, pers. comm., January 2017).

The use of chemicals to aid removal of individuals may also be impractical, expensive and environmentally sensitive. Further, chemical control measures are subject to a complex regulatory framework in relation to both the approved use of the chemical agent and environmental impact assessment. For details, see Appendix B: Approval of chemicals for use in Australia, and the AQUAVETPLAN Operational Procedures Manual — Destruction (DAFF 2009a).

Dredging areas to remove hosts in an open system to control *Bonamia ostreae* in the European flat oyster *Ostrea edulis* in Holland had limited success (van Banning 1988). This technique was also trialled to minimise the spread of *Bonamia exitiosa* in *Ostrea chilensis* in New Zealand's Foveaux Strait oyster fishery in 1990. There was no evaluation of its efficacy (K Michael, National Institute of Water and Atmospheric Research, New Zealand, pers. comm., February 2005) and it was not included in subsequent fisheries management plans for the Foveaux Strait (Anonymous 1995). Due to their potential impact on benthic marine environments, dredging operations would likely need approval from the relevant environmental agency (Erftemeijer & Lewis 2006; Erftemeijer et al. 2012).

2.1.2 Semi-open systems

In semi-open systems (for example, sea-cages), the movement of abalone can be controlled, but there is little or no control over the flow of water. Treatment of abalone or equipment in semi-open systems may impact the surrounding environment and must be taken into consideration. Disease

spread beyond the index site is likely to occur rapidly and reduce the likelihood of success in an eradication response. However, consideration could be given for immediate removal and destruction of exposed abalone from affected sites in an effort to reduce the infectious load and limit further disease transmission.

2.1.3 Semi-closed systems

In semi-closed systems (for example, land-based hatcheries or grow-out systems) abalone movement can be controlled, and removal and destruction of exposed abalone can be considered as a response option. An additional measure would be to immediately stop the discharge of contaminated water from affected facilities. However, the capacity to contain incoming or outgoing water is typically low for abalone farms. Inability to manage water discharge from affected facilities will reduce the effectiveness of disease control and eradication efforts. Control of seawater temperature may reduce transmission of the bacterium and disease expression (Friedman et al. 2003), but semi-closed systems rarely have this capacity. The use of antimicrobials or disinfectants is more effective and practical in a semi-closed system than in more open systems. Disinfection of water exiting the facility may be feasible, but is not possible for farms with large discharge volumes and/or no capacity to store discharge water. Using pathogen-free seawater from a bore or other uncontaminated source to establish disease-free farms could also be investigated.

In both semi-open systems and semi-closed systems, the critical step in deciding how to respond to an outbreak of *X. californiensis* will likely depend on initial disease surveillance outside the affected aquaculture establishment and the extent to which the disease has spread.

2.1.4 Closed systems

In closed systems, the movement of abalone and water can be controlled. Control options can be more easily applied and eradication is more likely to be successful.

2.2 Methods to prevent spread and eliminate pathogens

2.2.1 Quarantine and movement controls

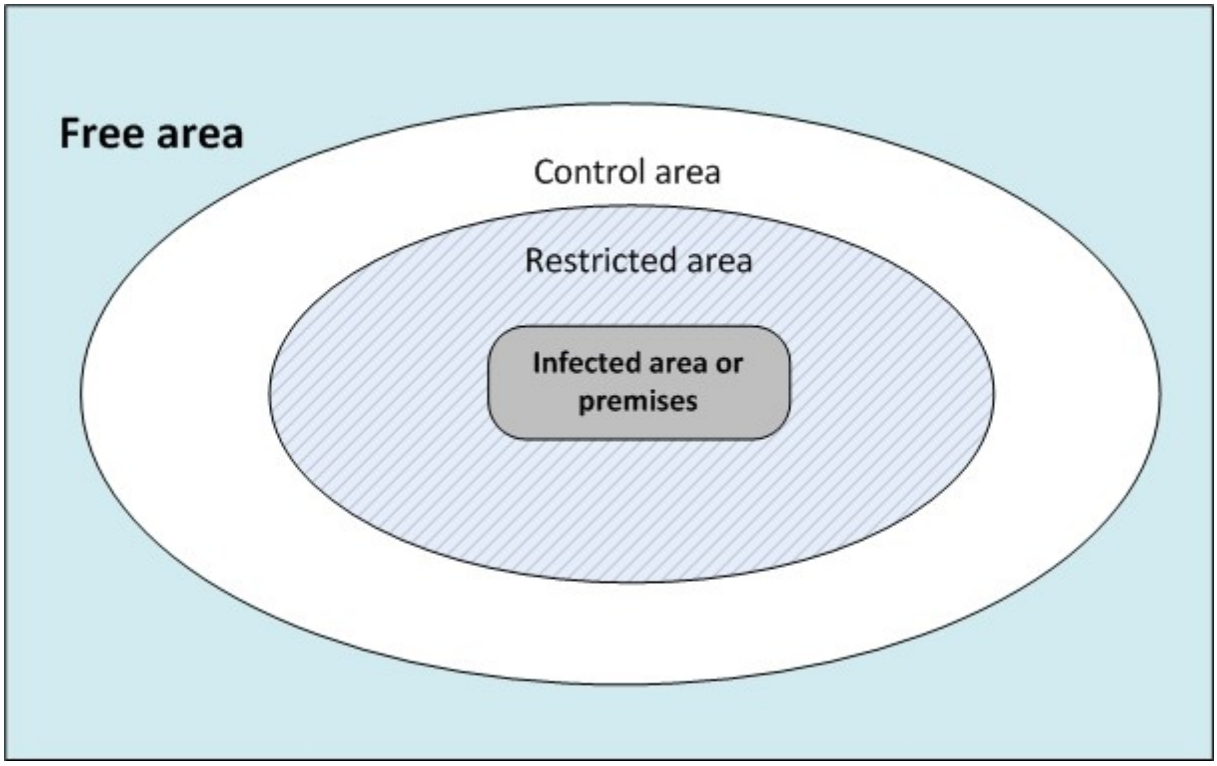
Quarantine and movement restrictions should be implemented immediately upon suspicion of *X. californiensis*.

Establishment of quarantine areas

Establishment of specified areas including:

- declared area—infected, restricted and control areas
  - infected area or premises—the premises (for example, farm) or area where the infection is present and the immediate vicinity
  - 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 *X. californiensis* remains unassessed).

Figure 1 Establishment of specified areas to control *X. californiensis*



In the declaration of quarantine areas, a range of factors need to be taken into account, including:

- movement of susceptible farmed stock as part of normal husbandry practices
- disposal of dead farm stock, shell and other normal by-products of farming
- movements of fomites including farming infrastructure
- discharge of water from farms or processors/holding facilities
- legal and illegal abalone harvesting and transport to processors
- abalone processing and discharge of processing waste
- recreational abalone fishing, discard of recreationally caught abalone waste and products and use of abalone products as fishing bait
- recreational marine activities
- proximity to the index site of filter-feeders and other mechanical accumulators that could carry the bacterium
- other 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.

Movement controls

Movement controls include:

- bans on the movement of live abalone and abalone products into, within, or out of the declared area
- bans on the movement of live abalone into uninfected areas
- restrictions on discharge of contaminated water from affected facilities in declared areas
- restrictions or bans on movement of people, vehicles or equipment within and between farms or water bodies containing abalone within the declared area
- suspension of recreational abalone fishing within the declared area.

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

See the AQUAVETPLAN Enterprise Manual (DA 2015) for more details on establishing and managing quarantine areas.

2.2.2 Zoning and compartmentalisation

Zoning

Zoning relies on the identification of biogeographic barriers or boundaries to describe an area to establish and maintain a subpopulation with a different health status within national boundaries.

A corresponding surveillance and monitoring program for *X. californiensis* would be required to support a zoning policy. Principles of zoning for infected and non-infected zones in Australia are outlined in the AQUAPLAN Zoning Policy Guidelines (DAFF 2000) and in the OIE Aquatic Animal Health Code (OIE 2016a).

Compartmentalisation

A compartment is defined as one or more aquaculture establishments under a common biosecurity management system. The compartment contains an aquatic animal population with a distinct health status with respect to a specific disease or diseases for which required surveillance and control measures are applied, and basic biosecurity conditions are met, for the purpose of international trade. Such compartments must be clearly documented by the competent authority.

A compartment does not have to be contiguous facilities—it can apply to a series of farms over a large area, including over several jurisdictions. A compartment must have in place a biosecurity management system that meets guidelines provided in chapters 4.1 and 4.2 of the OIE Aquatic Animal Health Code (OIE 2016a), and has been documented and approved by the competent authority (such as the veterinary authority of the jurisdiction). To be effective, the biosecurity

management systems would need to be recognised by all potentially affected jurisdictions, and by the Government of Australia if international recognition is necessary.

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 and farms adjacent to the infected area (as well as in the same catchment), should also be considered for monitoring and control measures.

Although adult abalone are relatively sedentary (Morgan & Shepherd 2006), in a *X. californiensis* EAD event the establishment of the relevant DMA boundaries must take into account dispersal of water discharged from any infected aquaculture systems and how this connects to adjacent abalone populations, including farmed animals. Similarly, outbreaks in semi-open systems require consideration of all oceanographically connected areas and distribution of wild susceptible host populations. Spread of infected abalone 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.

2.2.3 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. Tracing may be helped when zoning and compartments have been defined prior to the incursion.

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 abalone, premises and sites (to establish the origin of the outbreak), and to trace-forward all contacts with infected abalone, premises and sites (to establish the current location and potential spread of infection).

Items that must be traced are:

- live abalone—wild and farmed broodstock, spat and stock, and transport containers sold to processors, restaurants or individuals
- dead abalone—uncooked abalone and abalone products intended for consumption or for use as bait (if cooked, tracing is not required)
- effluent and waste products from processing

- water—intake and outlet
- vehicles—abalone fishing vessels, tenders for open and semi-open aquaculture, transport vehicles and operators' and visitors' cars
- materials—floating and submerged infrastructure, cultivation materials, raceways, trays or other culture units, tools, instruments and other fomites
- personnel—abalone fishers and recently fished sites, farm workers, sales and other professional representatives, trades people, veterinarians, scientists, technicians and visitors
- vessels and shipping.

Neighbouring abalone farms and processing plants may become, or may already be, infected. Maps showing the location of neighbouring abalone farms, processing plants and waterways, and hydrographic data, are necessary to monitor the potential spread of *X. californiensis*. The location of susceptible abalone species and potential animal reservoirs in the vicinity of the infected site should also be noted. Other sites of infection may be identified if a number of facilities share common water. The location of susceptible abalone and vectors should also be noted both upstream and downstream of the infected site.

Tracing an outbreak in an open system may be very challenging. Accurate oceanographic information, including prevailing currents and water temperature profiles for the affected area, will be particularly useful. Where they exist, hydrodynamic models (for example, CSIRO's Connie tool) may also be useful for simulating the likely directions of current flow and the possible rate and extent of spread of infectious particles from the known incursion area. Distribution maps of wild abalone species, commercial and recreational fishing zones, marine parks, and marine benthic habitat maps will further aid tracing and surveillance activities.

2.2.4 Surveillance

Surveillance is necessary to:

- define the extent of the infection
- support tracing activities
- detect new outbreaks
- establish restricted and control areas to which quarantine and movement restrictions are applied
- establish infected and non-infected areas for a *X. californiensis* emergency disease response
- monitor the progress and success of the selected emergency disease response
- provide evidence to support a self-declaration of disease freedom for trade purposes.

If possible, surveillance activities should target animals displaying clinical signs of disease, or populations recently exposed to a period of warm (greater than 18°C) water. Surveillance needs to be designed to meet the criteria for confirmation of infection (section 1.4). However, given the long incubation period in abalone infected with *X. californiensis*, and strong evidence that some abalone will remain subclinical carriers without developing clinical signs of disease (Ben-Horin et al. 2013;

OIE 2016b), the detection of new outbreaks will primarily rely on non-targeted surveillance. Detection of disease in surveyed populations is likely to indicate that the pathogen has been established for months or years.

When testing for *X. californiensis* 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 1.4—Aquatic Animal Health Surveillance (OIE 2016a) and the OIE Manual of Diagnostic Tests for Aquatic Animals Chapter 2.4.8—Infection with *Xenohaliotis californiensis* (OIE 2016b). The manual also provides specific information on surveillance for *X. californiensis*.

Veterinary epidemiological principles for EAD events were outlined by Paskin (2009).

Subclinical infection of *X. californiensis* may occur at low prevalence in abalone populations (OIE 2016b), warranting careful design considerations to determine freedom from infection. It is important to note that the positive predictive value of a diagnostic test is a function of disease prevalence, and even highly specific tests will return a high proportion of false positive results if disease prevalence is low. If positive results are obtained from known disease-free areas, retesting the suspect tissue samples should be the first step in confirming positive results. If the detection is based on a PCR test, sequencing of the amplification product should be undertaken, and additional confirmatory diagnostic testing using ISH should also be considered (section 1.4). If further confirmation is required, re-sampling the suspect population using an appropriate sample size for the estimated disease prevalence is necessary to ensure that presumed false positives are indeed false (OIE 2016a). It is strongly recommended that sampling regimes facilitate retesting and the provision of reference material. Samples can be stored in ethanol, embedded in wax or frozen (preferably at –80°C).

Individual samples may be pooled (n=5) for molecular diagnostic testing purposes if they are collected from disease outbreak areas where bacterial loads are expected to be high. Individual samples should be retained to enable their re-testing if there are any concerns about loss of test sensitivity as a result of pooling, or to allow retrospective testing of the constitutive individual samples from positive pools. Pooled samples are not recommended for surveillance of subclinical populations where bacterial loads are likely to be low, as dilution of target organisms may result in lower diagnostic test sensitivity. PCRs should be run in triplicate to account for possible pooling-related dilution of target DNA (OIE 2016b).

2.2.5 Treatment of infected host species

Reducing water temperatures may reduce bacterial (or pathogen) transmission and disease expression, but farms do not typically have the ability to regulate water temperatures (Friedman et al. 2003).

The decision to treat abalone with antibiotics depends on the control measures implemented in an EAD response and the availability of effective antibiotics. Considerations for the use of antibiotics against withering syndrome include:

- availability of the treatment—no antimicrobials are currently permitted or registered in Australia for use in farmed molluscs (Appendix B: Approval of chemicals for use in Australia). Note that some jurisdictions may allow for the 'off-label' use of veterinary pharmaceuticals in farmed aquatic animal species.
- cost of treatment, including labour, administration and equipment
- practicality of observing withholding periods.

Xenohaliotis californiensis is susceptible to tetracyclines. Twelve intramuscular doses of 21 mg kg^{-1} oxytetracycline putatively cleared infections in both red and black abalone as assessed by routine histology of target tissues, but note that bacterial shedding was not investigated in this study (Friedman et al. 2003). Oral administration of oxytetracycline-medicated feed (for 14 consecutive days) to infected but subclinical red abalone also controlled *X. californiensis* infection. The treatment caused significant and persistent long-term reductions in both the degree of morphological changes in the digestive gland and the intensity of bacterial infection (Friedman et al. 2003).

The potential use of oxytetracycline as a preventative treatment was also demonstrated in white abalone (Friedman et al. 2007). This study is significant for being the first to assess the relative diagnostic sensitivity of PCR versus histology in determining the infection status of abalone. Thirty percent of putatively non-infected abalone, as determined by standard histological examination of target tissues, were PCR-positive for *X. californiensis*. It is important to consider this when evaluating the purported efficacy of oxytetracycline in "curing" *X. californiensis* infection in earlier studies that were entirely reliant on histology to determine infection status; a reasonable proportion of non-infected abalone were likely false negative results due to lower test sensitivity. While the detection of pathogen DNA is not in itself evidence of a patent infection, the possibility that animals testing negative using histological methods are both infected and potentially infectious should not be discounted. Given the high degree of uncertainty about the efficacy of oxytetracycline treatment regimes in definitively clearing infection in abalone infected with *X. californiensis*, a cautious approach is advocated when considering the use of this antibiotic during an emergency animal disease response.

An additional qualifier for consideration of oxytetracycline use in infected abalone is the extent of disease progression prior to treatment administration. Once clinical signs of withering syndrome are observed, antimicrobials are ineffectual treatments; the administration of oxytetracycline to black abalone that had moderate to severe clinical signs of infection with *X. californiensis* did not improve survival (Friedman et al. 2003). Due to the chronic nature of withering syndrome and the influence of temperature on the development of clinical signs, treatment should also commence before the onset of warm water events. Treatment by injection would not be practical for large numbers of animals but farm populations can be treated with medicated feed (Friedman et al. 2003).

Before the administration of any treatment as part of response measures, appropriate diagnostic samples for the confirmation of *X. californiensis* infection must be obtained, and sent to the relevant

veterinary diagnostic laboratory. Administration of antimicrobial treatments to exposed animals can then commence immediately based on the presumptive diagnosis of infection with *X. californiensis*.

2.2.6 Treatment of host products and by-products

How long *X. californiensis* survives in dead abalone is not documented, and any uncooked abalone, abalone products and processing waste should be regarded as infectious.

In some instances, it may be appropriate to harvest surviving marketable abalone, although appropriate risk mitigation strategies are required to ensure that such abalone marketed for human consumption do not provide an additional pathway for release of *X. californiensis* to an unaffected zone.

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

2.2.7 Destruction of hosts

Harvest and destruction must be hygienic and there must be no spillage of the waste produced by these activities.

The most appropriate method of destruction (AQUAVETPLAN Operational Procedures Manual—Destruction, DAFF 2009a) will depend on the following factors:

- size and number of abalone
- deadline for harvest or destruction, which depends on the pressure of infection and the risk of further spread
- slaughter facilities—site, equipment and methods available
- experience and availability of personnel.

If necessary, an anaesthetic or relaxant may be administered to aid in harvesting abalone. Chemical approval processes are outlined in Appendix B: Approval of chemicals for use in Australia.

2.2.8 Disposal of hosts

The most effective means for disposal of molluscs is composting or burial. Other disposal methods are less effective due to the thickness of the shell, while the volume of product that needs to be handled following, for example, incineration, remains substantial.

Diseased and dead abalone are the main source of *X. californiensis* in the environment. They should be removed and disposed of, together with other waste, as soon as possible to prevent further spread of infection. If burial is used for disposal, selected sites need to be carefully chosen to avoid waste entering waterways or groundwater, and carriage by vectors, including scavengers.

The AQUAVETPLAN Operational Procedures Manual—Disposal (DAFF 2009b) should be consulted for further information on disposal.

2.2.9 Decontamination

Due to differences in farming enterprises, disinfection protocols may need to be determined on an individual basis involving the farm manager, the state/territory CVO and/or Director of Fisheries and potentially the responsible environmental protection agency.

The protocol should take into consideration the factors outlined in section 1.6, in particular the:

- source and location of infection
- type of enterprise (such as a farm, processing plant, hatchery, grow out ponds or water source)
- construction materials of the buildings/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, chemical use)
- availability of approved, appropriate and effective disinfectants.

See the AQUAVETPLAN Operational Procedures Manual—Decontamination (DAFF 2008) for details of decontamination methods and their indicators, and Appendix B: Approval of chemicals for use in Australia for an outline of chemical approval processes.

2.2.10 Vaccination

No vaccines or vaccine-like prophylactics are available for *X. californiensis*, or for use in abalone species in Australia.

2.2.11 Vector control

Although controlling the spread of *X. californiensis* between systems is likely to be challenging due to the variety of possible vectors, it may be feasible, particularly if the infection is confined to a farm. The bacterium is spread by carrier abalone, water and translocation on equipment, vessels or other fomites. Removing infected abalone 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. Birds, rodents and pets commonly occur around abalone farms and may be attracted to dead or moribund abalone. In the event of an outbreak of *X. californiensis* access of scavengers to diseased abalone in affected farms may need to be controlled. Control options for scavengers are outlined in the AQUAVETPLAN Operational Procedures Manual—Disposal (DAFF 2009b).

2.3 Environmental considerations

Environmental considerations in the control of withering syndrome include that the:

- discharge of infected, or potentially infected, effluent into catchment areas or natural waterways will probably lead to further spread of infection and the establishment of reservoirs of infection in wild abalone populations and waterways
- loss of wild populations of abalone could lead to irreversible environmental change
- use of disinfectants or antimicrobials may have impacts on the environment, especially if used in larger than normal quantities or concentrations, as is possible in a disease control situation. See Appendix B: Approval of chemicals for use in Australia and the AQUAVETPLAN Enterprise Manual (DA 2015)
- destruction of affected stock and disposal of infected material may have impacts on the environment, and this must be minimised while preventing the dissemination of infection.

See the AQUAVETPLAN Operational Procedures Manual—Decontamination (DAFF 2008) for details of decontamination methods.

2.4 Sentinel animals and restocking measures

Xenohaliotis californiensis-free abalone might be obtained from infection-free locations and used as sentinel animals to assess the efficacy of control measures preceding large-scale restocking of any abalone farms affected by *X. californiensis*. It is important to expose sentinel abalone to environmental conditions conducive to both infection and development of clinical disease. This could include sourcing potentially contaminated water from the local control area, and manipulating the temperature regime to increase likelihood of clinical expression of disease (for example, daily fluctuations at the thermotolerance limits for the affected species). The number of sentinels required would depend on the configuration of the system being assessed. Due to the long incubation period and possibility of subclinical infection, regular testing over a period of months, using a highly sensitive diagnostic test such as PCR, would be required to assess infection in sentinels. It may be difficult to interpret the absence of infection in sentinel abalone if exposure to the pathogen is limited by low population densities of infected abalone in the source water body.

Site-fallowing durations before restocking will need to be assessed on a species and case-by-case basis to minimise the risks of reoccurrence of *X. californiensis* infection. 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 clearance of the pathogen is unknown, and is likely to depend on the proximity and density of wild abalone populations.

For any attempts to eradicate *X. californiensis*, it is important that restocked abalone are free of infection. For areas declared free of *X. californiensis*, this status can also only be retained if introduced abalone are free of the pathogen. The use of spat derived from *X. californiensis*-free broodstock reared in strict biosecure conditions would also be valuable in avoiding the reintroduction of infections to farms or open systems.

2.5 Public awareness

Community engagement programs should be developed as part of a broader EAD response communication strategy. Community engagement programs should emphasise education, surveillance and cooperation at both industry and community levels, so that information is broadly disseminated to avoid practices that might increase the likelihood of *X. californiensis* being inadvertently spread during an outbreak. Particular emphasis should be placed on communicating the importance of not using abalone as bait or aquaculture feed, and using appropriate waste disposal for recreationally-caught discarded abalone gut and shells.

The public should be informed about specific zoonotic concerns, namely that:

- withering syndrome of abalone is not infective to humans

  and

- eating abalone that may have been exposed to *X. californiensis* is not considered a health risk.

2.6 Feasbility of control or eradication of *Xenohaliotis californiensis* in Australia

The feasibility of controlling an outbreak of *X. californiensis* in Australia depends upon both the nature of the outbreak (including whether it occurs in an open, semi-open or semi-closed system) and the control strategy adopted. As outlined in section 2.1, there are two broad control options for withering syndrome in Australia:

1) Eradication—eradication of *X. californiensis* from Australia
2) Containment and control via zoning and compartmentalisation—containment of *X. californiensis* to areas with endemic infection, prevention of further spread and protection of uninfected areas

Outbreaks in open systems may be ineradicable. The likely persistence of the pathogen in the environment and the long incubation period in abalone, combined with the difficulties in controlling wild populations, makes eradication unlikely to succeed, except in marine biogeographical areas completely isolated from susceptible wild abalone populations with respect to disease transmission. Eradication is more likely to be feasible in semi-closed and closed systems.

A major objective of any emergency disease response will be to minimise the risk of disease spread from infected to non-infected areas, particularly as a result of abalone farming or fishing activities (for example, stock and infrastructure movements).

2.6.1 Response option 1: eradication

Despite there being no records of successful eradication of *X. californiensis* infection, attempting to eradicate *X. californiensis* could be justified if the:

- outbreak occurs in a closed or semi-closed system, for example, in intensive hatchery conditions
- outbreak occurs in a semi-open system where no viable populations of susceptible abalone occur, or where wild abalone can also be controlled.

Any attempt to eradicate *X. californiensis* infection in an infected zone will require consideration of:

- agreement, among abalone industry participants and other affected parties in an area, that measures to achieve eradication are justified
- destruction and disposal (including emergency harvest) of all farmed and wild abalone in the eradication zone
- resource availability for surveying and destocking abalone in the infected zone
- extensive decontamination of equipment, fomites and infrastructure
- availability of *X. californiensis*-free stock if aquaculture 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. The feasibility of an eradication response may also be limited by:

- difficulties in detecting infection during the long incubation period, or inability to detect subclinical infection
- inability to control susceptible species populations
- lack of availability of *X. californiensis*-free stock because hatchery stock prove to be infected or are inaccessible due to movement restrictions.

Where eradication is the aim, affected closed or semi-closed systems with strict biosecurity controls may resume production following decontamination if:

- water is filtered (to 5 µm) and decontaminated
- farms in such zones could source *X. californiensis*-free stock.

Unexposed abalone

Pre-market size unexposed abalone in disease-free areas may be allowed to grow out, provided that future exposure to infection can be prevented. Market-size unexposed abalone may be harvested for human consumption.

A conservative destocking approach that involves the destruction of unexposed abalone populations located within declared control areas will potentially decrease the chance of infection spreading to even more isolated abalone populations in disease-free areas. However, given the high value of stocks, permitting grow out or harvesting of unexposed abalone must be weighed against the benefits to the eradication response associated with conservative destocking in control areas. Concurrent surveillance of abalone populations in control and disease-free areas must also be undertaken as part of the eradication response. Detection of *X. californiensis* infection in control or disease-free areas indicates that containment of the outbreak has failed. A decision will need to be made about whether disease eradication remains feasible, or whether the response needs to transition to a containment and control phase (section 2.6.2).

Exposed or potentially exposed abalone

All live abalone within an infected or restricted area are assumed to be exposed. Grow-out within the restricted area is therefore not an option as it would increase the likelihood of infection spreading to other farms or wild abalone stocks both within and beyond the restricted area.

All abalone must be removed from the water, destroyed and safely disposed of as soon as possible. Although such abalone are safe for human consumption, the emergency harvesting of both wild-caught and farmed abalone may jeopardise the success of an eradication strategy, unless it is carried out under strict control measures. Strict hygiene protocols for the farm, transportation and processing would be necessary to ensure there is no transfer of infection to unexposed abalone in disease-free areas.

On-farm processing may be preferable to prevent the spread of infection during transport to off-site processing plants. Abalone of particular value (broodstock or relict populations) may be individually treated with antimicrobials (Friedman et al. 2007), but given the degree of uncertainty about the efficacy of antimicrobial treatments to truly eliminate the pathogen all treated animals should be regarded as infected. For information about veterinary medicine use see Appendix B: Approval of chemicals for use in Australia.

Control measures necessary to prevent further spread of infection include:

- disinfection of all equipment and personnel involved in harvesting, destruction and processing
- application of quarantine restrictions and procedures to the infected site, including for personnel, equipment and vehicles
- on-site processing—possibly the only option if quarantine restrictions are in place
- strict movement and disinfection procedures for the transport of abalone to off-site processing plants, which will then become infected sites that are subject to quarantine procedures
- holding, treatment and safe disposal of harvest or processing effluent (including holding water and any waste material)
- ensuring that the final product will not result in the spread of infection.

Infected abalone

Clinically diseased and dead abalone (and the infectious waste they generate) are the main source of *X. californiensis* in the environment, and their immediate removal, destruction and disposal is essential to the success of an eradication response. Removal of these animals is the most effective means of decreasing the infectious load on a site and minimising further spread of infection. Antibiotic treatment for these abalone is not effective (Friedman et al. 2003) and should not be considered as part of an eradication response. Disposal methods and sites should be chosen carefully to prevent contact with waterways or vectors.

2.6.2 Response option 2: containment and control via zoning and compartmentalisation

Unexposed abalone

The implementation of a zoning policy, and associated control measures, to maintain uninfected zones/compartments would be necessary. Aquaculture and harvesting for human consumption can occur as normal in declared uninfected biogeographical areas or premises. Immediate destruction is also an option for unexposed abalone populations located within a de-stocking control zone/compartment, as it will decrease the chance of infection spreading to disease-free abalone stocks and therefore mitigate further propagation of the disease. There would be no restrictions on production in disease free zones/compartments.

Exposed or potentially exposed abalone

A successful containment and control response will rely on the implementation of movement restrictions for exposed or potentially exposed abalone to prevent spread of *X. californiensis* to uninfected zones/compartments. In particular, restrictions will need to be imposed on abalone products released for human consumption to prevent spread to uninfected zones/compartments of Australia.

Processing methods must ensure that abalone products are no longer infectious (for example, if destined for domestic human consumption in zones/compartments free of *X. californiensis*, the products must be cooked or otherwise processed to remove/inactivate viable bacteria). The feasibility of this response option will largely depend on fishing and aquaculture management practices, the extent of the outbreak, and the location of potential reservoirs of infection. This can only be assessed at the time of the outbreak, taking into account movement restrictions required on abalone, people, vehicles and boats, and market access for the abalone products and by products.

Exposed or potentially exposed abalone within an infected zone/compartment are assumed to be infected. Immediate destruction remains an option for ensuring disease containment and control. However, in a declared infected area, normal or controlled grow-out and slaughter is an option. Additional disease control strategies for consideration include reducing water temperatures, and the use of antimicrobial agents for both disease treatment and prevention, particularly in high-value broodstock not intended for human consumption (Friedman et al. 2007; Friedman et al. 2003). These measures are likely to be feasible only in aquaculture and in semi-closed or closed systems.

Infected abalone

Clinically diseased and dead abalone should be managed as for an eradication response (section 2.6.1). Within declared infected zones/compartments, it must be assumed that withering syndrome will become established in both wild and farmed abalone populations. The long-term commercial viability of existing abalone aquaculture or fishery operations within declared infected zones/compartments cannot be guaranteed. The viability of local native and/or endemic abalone populations would also be uncertain.

Given the potential role of abalone, abalone products, vehicles and boats as disease vectors, recreational harvest of abalone would also need to be tightly regulated within an infected zone/compartment. Aquaculture operations within an infected zone/compartment would likely need

to initiate strict biosecurity arrangements to minimise the risk of continual outbreaks in commercial stock, and undertake farm-level disease control programs when outbreaks do occur.

While the declaration of an infected zone/compartment is an acknowledgment that withering syndrome has become established in Australia, there would still be a requirement to aggressively manage disease outbreaks within infected zones/compartments as part of the broader regional strategy to ensure that uninfected zones/compartments remain disease-free.

2.6.3 Trade and industry considerations

The abalone industries are likely to be substantially impacted by an outbreak of *X. californiensis*. Following the initial outbreaks of abalone viral ganglioneuritis in Victoria in 2006, the total allowable catch in the Victorian Western Zone Abalone fishery was reduced by 95% with an associated $12 million decrease in landed value (Mayfield et al. 2011). Individual transferable quota licenses that were trading for US$5 to 6 million before the epizootic lost almost all of their value (Conrad & Rondeau 2014). Significant interruptions in production and loss of business continuity are also likely to occur in the abalone aquaculture sector.

Trade regulations, market requirements and food safety standards must be considered as part of any emergency disease response.

Export markets

Xenohaliotis californiensis is notifiable to the OIE and some countries may have import conditions in place related to the *X. californiensis*, such as requiring imports to be certified free of *X. californiensis*. The Department of Agriculture is responsible for the health certification of all exports and should be contacted for further information (export@agriculture.gov.au).

Domestic markets

A cautious approach is required for the salvage of exposed or potentially exposed but clinically normal abalone for the domestic market. Decisions regarding the release of abalone to the domestic market will depend on the selected emergency disease response option, but the primary aim must be to minimise the possible spread of disease from infected to uninfected areas. Abalone in the late stages of disease with severe clinical signs are not suitable for human consumption because the saleable foot muscle is withered by the infection; in affected abalone, meat quality and appearance are inferior.

Abalone without clinical signs that have undergone treatment or preventative antimicrobial therapy for *X. californiensis* infection may be harvested for human consumption, provided that appropriate drug withholding periods are observed. Provided that normal seafood safety practices are maintained, there are no public health risks associated with harvesting potentially infected abalone for human consumption. If healthy, potentially infected or infected abalone are destined for human consumption, the chief medical officer and health authority of the relevant state or territory should be informed that there are no human health concerns associated with *X. californiensis*, and that withering syndrome is not a zoonotic disease.

3 Preferred Australian response options

3.1 Overall policy for *Xenohaliotis californiensis*

Xenohaliotis californiensis could potentially cause severe, long-term production and economic losses in the abalone farming and fishery industries and associated production, sales and export industries. It will therefore be necessary to act immediately to control or eradicate the disease if it were to be detected in Australia.

The response to a detection of *X. californiensis* in Australia will depend on the nature of the outbreak. By drawing on the previous sections of this manual, Section 3 provides guidance to directors of fisheries and chief veterinary officers (CVOs) to choose and implement the most appropriate response option for the circumstances.

There are two preferred response options for *X. californiensis* in Australia:

1) Eradication of *X. californiensis* from Australia

2) Containment and control via zoning/compartmentalisation, with the aim of containing *X. californiensis* within known endemic areas, thus preventing further spread to uninfected areas.

Both these options involve a combination of strategies, which may include:

- quarantine and movement controls on abalone, abalone products and equipment in declared areas to prevent spread of infection
- destruction and disposal of presumed infected, clinically diseased and dead abalone to prevent release of *X. californiensis* into the environment
- decontamination of facilities
- surveillance to determine the extent of possible infection, and to provide proof of freedom from *X. californiensis*
- zoning/compartmentalisation to define non-infected areas
- restocking with sentinel abalone; and
- a public awareness campaign to encourage cooperation from the fishing and aquaculture industry and the community.

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 disease response plan (EAD Response Plan). This plan will be submitted to the Aquatic Consultative Committee on Emergency Animal Diseases (AqCCEAD), which will provide advice on its technical soundness and 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 AqCCEAD. The detailed response measures adopted will be determined using the principles of containment, control and eradication (section 2), epidemiological information about the outbreak, and the financial feasibility of the option.

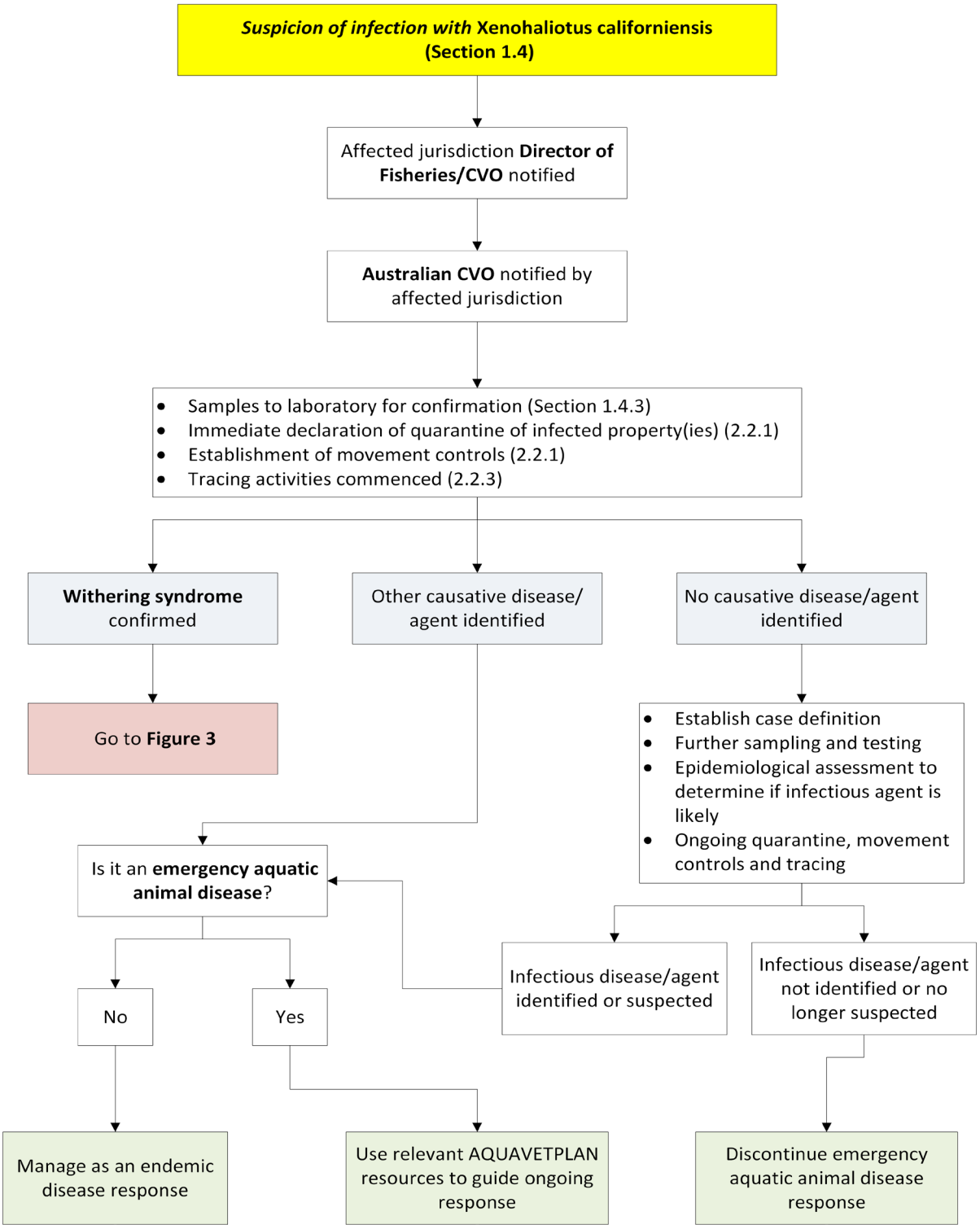
For information on the responsibilities of the state or territory disease control headquarters and local disease control centres, see the AQUAVETPLAN Control Centres management manual.

3.2 Response options

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 AqCCEAD. The detailed response measures adopted will be determined using the principles of disease eradication, containment, control and mitigation (section 2), depending on the epidemiological information about the outbreak and the financial and logistical feasibility of the selected option.

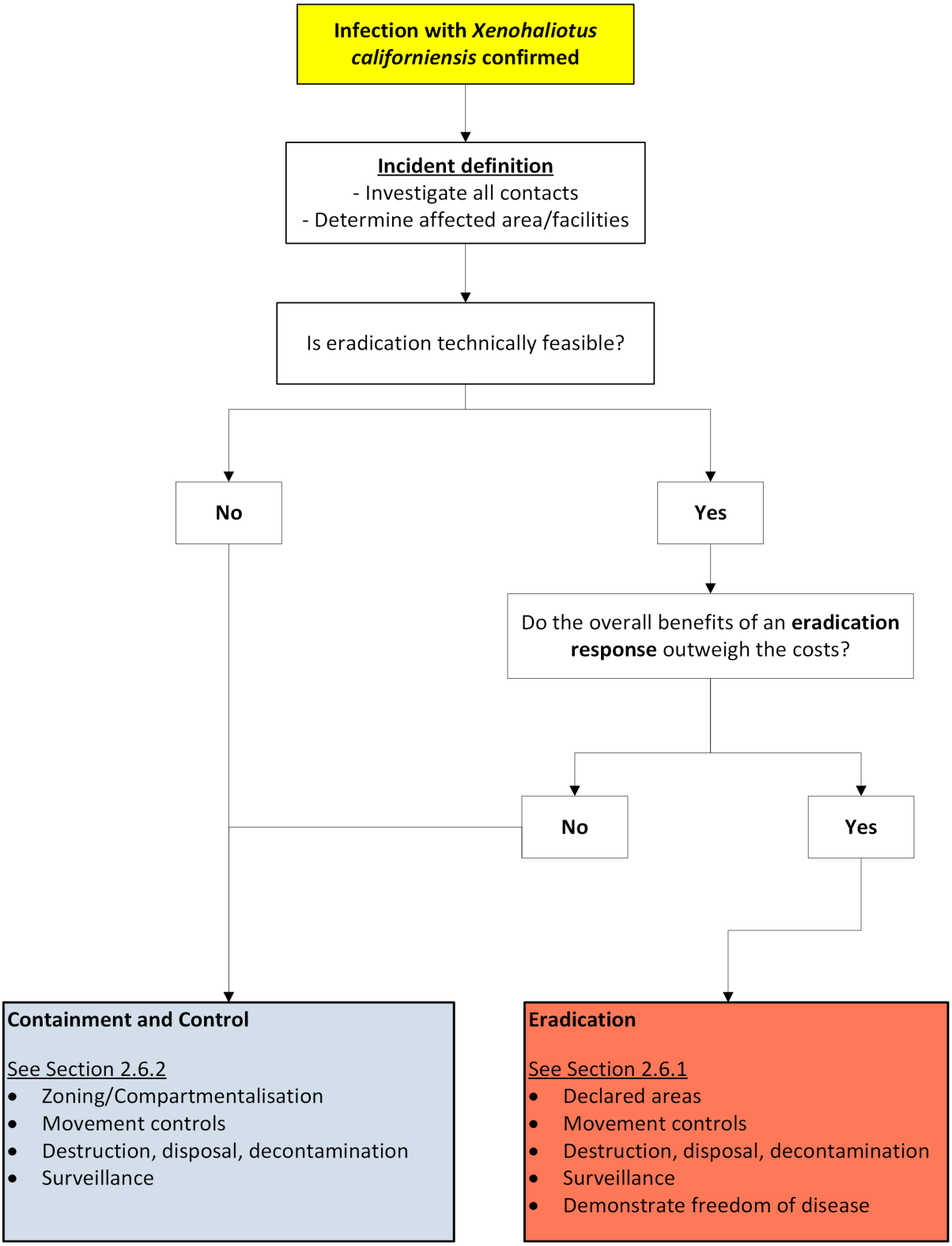
For information on the responsibilities of the other state or territory disease control headquarters and local disease control centres, see the AQUAVETPLAN Control Centres Management Manual (DAFF 2001).

Figure 2 Decision flow chart for suspected infection with *X. californiensis*



CVO Chief veterinary officer.

Figure 3 Determining response to outbreak or confirmed infectious with *X. californiensis*



3.2.1 Eradication

The long-term economic benefits of a successful eradication response for *X. californiensis* are likely to outweigh the short-term costs. If the outbreak is in a closed system or if epidemiological investigations determine an obvious point source of infection that could be contained with minimal or no spread of *X. californiensis*, an eradication response is likely to be successful and should be attempted. Eradication could also be attempted in a semi-closed, semi-open or open system if the outbreak occurred in an area where there were no adjacent populations of susceptible abalone species, or where exposed abalone populations could be effectively 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.

An eradication plan will include:

- establishment of specified areas (infected, restricted, control and free)
- quarantine and movement controls on abalone, water and any other potential vectors (including vessels, materials and equipment) from declared infected or restricted zones to control zones or disease-free areas to prevent the spread of infection
- destruction and disposal of all dead and clinically diseased abalone
- restriction on use of abalone products as bait and berley
- processing of exposed or potentially exposed, but clinically normal abalone within declared infected and restricted areas (or under strict biosecurity conditions in control or disease-free areas), to prevent the spread of infection
- disinfection and safe disposal of processing effluent and waste (abalone shells, shell liquor, all abalone tissue and processing water)
- disinfection, decontamination and safe disposal where necessary of facilities, products, equipment, vessels and vehicles etc., to eliminate *X. californiensis* from infected premises and to prevent spread
- control of scavenger access, particularly birds, to live and dead abalone
- 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 terms of their respective roles in preventing disease spread
- disinfection of contaminated water from hatcheries and depuration facilities

Restocking with sentinel abalone can occur only after the site has been thoroughly decontaminated.

3.2.2 Containment and control

If infection occurs in wild susceptible species, or at multiple disparate farms, or across a broad geographic range, eradication is unlikely to be feasible or successful. In this situation, containment and control aimed at preventing further spread of infection and the protection of uninfected areas is the preferred response. A zoning/compartmentalisation program will help the Australian abalone industry maintain long-term productivity, and potentially aid in ongoing access to key export markets. Restrictions on the movement of abalone and products and an ongoing surveillance program will be necessary to support zoning/compartmentalisation. Farms in infected zones/compartments will need to implement management practices to reduce the severity and impact of *X. californiensis* outbreaks.

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

- zoning/compartmentalisation to define infected and non-infected areas
- quarantine and movement controls or restrictions on abalone, water and any other potential vectors (including materials and equipment) both within infected areas, and from infected to uninfected areas
- ongoing surveillance activities in infected and uninfected zones/compartments
- testing of broodstock and spat for *X. californiensis*
- compartmentalisation of selected facilities (such as hatcheries for production of *X. californiensis* -free stock)
- emphasis on high standards of hygiene (including decontamination and use of sentinel animals before restocking) and biosecurity (screening of incoming spat for *X. californiensis*)
- a public awareness campaign to encourage cooperation from industry and the community.

Restocking with *X. californiensis*-free, marked, sentinel abalone is one method of ascertaining freedom from infection in open systems where isolated or dispersed populations of infected abalone could still remain. Large-scale restocking with susceptible species should occur only once the zone or compartment is considered to be, or likely to be, uninfected.

3.3 Criteria for proof of freedom

Proof of freedom from *X. californiensis*, which may be important for trade, can be demonstrated at the compartment, zone and country level. Criteria for proof of freedom at each level are given in the OIE Aquatic Animal Health Code (OIE 2016a).

3.4 Funding and compensation

There are no national cost-sharing agreements in place for emergency responses to *X. californiensis*, although government and industry are currently working together to develop industry-government emergency aquatic animal disease response arrangements (Activity 2.1 in AQUAPLAN 2014–2019, DA 2014). 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.

Recent disease response exercises (Roberts et al. 2013) identified that governments would be heavily reliant on industry resources in a response (for example, personnel such as divers, infrastructure, vessels and equipment). The 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 *X. californiensis*.

Table 1 Summary of strategies for each response option for *X. californiensis*

	Response option	
Strategy	**Eradication**	**Containment and control**
Quarantine and movement controls	Yes	Yes
Declared restricted/control areas	Yes	Yes
Zoning/compartmentalisation	N/A	Yes
Movement controls within declared area or infected zone/compartment	Yes	Optional
Movement controls out of declared area or infected zone/compartment	Yes	Yes
Destruction of clinically diseased abalone	Yes	Yes
Destruction of unexposed abalone	Optional	Optional
Destruction of wild abalone as necessary	Yes	Optional
Harvest with processing of exposed or potentially exposed but clinically normal abalone	Yes	Yes
Within-zone processing	Yes	Yes
Appropriate disposal of infected abalone and wastes	Yes	Yes
Decontamination	Yes	Optional
Surveillance	Yes	Yes
Tracing	Yes	Optional
Screening of broodstock and spat for *Ca X. californiensis*	Yes	Yes
Optimised farm husbandry	Yes	Yes
Specific farm-level hygiene measures	Yes	Yes
Specific farm-level biosecurity measures	Yes	Yes
Management of environmental issues	Yes	Yes
Management of commercial issues	Yes	Yes

N/A Not applicable. Note: Each strategy may or may not be applicable depending on the approach (eradication, containment and control) adopted.

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

OIE Aquatic Code

The objective of the OIE (World Organisation for Animal Health) Aquatic Animal Health Code (OIE 2016a) 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.

Chapter 11.7 Infection with Xenohaliotis californiensis of the 2016 OIE Aquatic Animal Health Code (19th edition) is specifically relevant to this manual.

OIE Aquatic Manual

The purpose of the OIE Manual of Diagnostic Tests for Aquatic Animals (OIE 2016c) 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. Chapter 2.3.5 is specifically relevant to this manual.

Appendix B: 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, Water and the Environment 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.

Registration

Registration is the default method for APVMA to allow the use of a veterinary chemical in Australia. Registration is time consuming and expensive and it may be necessary to apply for a minor or emergency use permit during an emergency.

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 an 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, visit the APVMA website.

References

ABARES, 2015, Australian fisheries and aquaculture statistics 2014, *Australian Bureau of Agricultural Resource Economics and Sciences*, Canberra, ACT, Australia.

Altstatt JM, Ambrose RF, Engle JM, Haaker PL, Lafferty KD, Raimondi PT, 1996, Recent declines of black abalone Haliotis cracherodii on the mainland coast of central California, *Marine Ecology Progress Series*, vol. 142, pp. 185–192, doi: 10.3354/meps142185.

Andree KB, 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*, vol. 19, pp. 213–218.

Anonymous, 1995, 'A draft plan for the Foveaux Strait oyster fishery', *Ministry of Fisheries Library*, Dunedin, New Zealand.

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*, vol. 75, pp. 180–182, doi: 10.1006/jipa.1999.4906.

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*, vol. 258, pp. 63–72, doi: 10.1016/j.aquaculture.2006.03.046.

Ben-Horin T, Lenihan HS, Lafferty KD, 2013, Variable intertidal temperature explains why disease endangers black abalone, *Ecology*, vol. 94, pp. 161–168, doi: 10.1890/11-2257.1.

Benkendorff K, 2003, 'Immune indicators for monitoring abalone health', in *Proceedings of the 10th Annual Abalone Aquaculture Workshop*, Fleming AE (eds), Fisheries Research and Development Corporation, Port Lincoln, Australia, pp. 131–138.

Berthe FCJ, 2002, Pacem in terries pathogenibus bonae voluntatis: molluscs–pathogen relationships prospects, *Bulletin of the European Association of Fish Pathologists*, vol. 22, pp. 52.

—— 2003, Disease card—Withering syndrome of Abalone, *IFREMER*, Paris, France.

Bower, SM 2004, Synopsis of Infectious Diseases and Parasites of Commercially Exploited Shellfish: Withering syndrome of Abalone, *Fisheries and Oceans Canada*, available online.

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*, vol. 89, pp. 219–231, doi: 10.1016/j.jip.2005.06.004.

Cameron AR, 2002, Survey tool for aquatic animal diseases: a practical manual and software package, *Australian Centre for International Agricultural Research*, Canberra, ACT, Australia.

Conrad JM, Rondeau D, 2014, Bioeconomics of a marine disease, Working Papers 162433, The University of Victoria, Resource and Economic Policy, Canada, doi: 10.22004/ag.econ.162433.

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*, vol. 108, pp. 261–270, doi: 10.3354/dao02713.

Department of Agriculture, 2019a, Aquatic Animal Diseases Significant to Australia: Identification Field Guide (5th Edition), Department of Agriculture, ACT, Canberra, Australia.

—— 2019b, Australia's national list of reportable diseases of aquatic animals, Department of Agriculture, Canberra, ACT, Australia.

—— 2014, AQUAPLAN Australia's National Strategic Plan for Aquatic Animal Health, Department of Agriculture, Canberra, ACT, Australia.

Department of Agriculture and Water Resources, 2014, AQUAVETPLAN—Disease Strategy Manual—Abalone Viral Ganglioneuritis, Department of Agriculture, Canberra, ACT, Australia.

DA, 2015, Enterprise manual (Version 2.0), Department of Agriculture, Canberra, ACT, Australia.

DAFF, 2000, Zoning Policy Guidelines (AQUAPLAN), Department of Agriculture, Fisheries and Forestry, Canberra, ACT, Australia.

—— 2001, Control centres management manual (Version 1.0), Department of Agriculture, Fisheries and Forestry, Canberra, ACT, Australia.

—— 2008, Operational procedures manual—Decontamination, Department of Agriculture Fisheries and Forestry, Canberra, ACT, Australia.

—— 2009a, Operational procedures manual—Destruction, Department of Agriculture, Fisheries and Forestry, Canberra, ACT, Australia.

—— 2009b, Operational procedures manual—Disposal, Department of Agriculture, Fisheries and Forestry, Canberra, ACT, Australia.

Dang VT, Speck P, Benkendorff K, 2012, Influence of elevated temperatures on the immune response of abalone, Haliotis rubra, *Fish & Shellfish Immunology*, vol. 32, pp. 732–740, doi: 10.1016/j.fsi.2012.01.022.

Diggles BK, Nichol J, Hine PM, Wakefield S, Cochennec-Laureau N, Roberts RD, Friedman CS, 2002, Pathology of cultured paua Haliotis iris infected with a novel haplosporidian parasite, with some observations on the course of disease, *Diseases of Aquatic Organisms*, vol. 50, pp. 219–231, doi: 10.3354/dao050219.

Erftemeijer PLA, Lewis RRR, 2006, Environmental impacts of dredging on seagrasses: A review, *Marine Pollution Bulletin*, vol. 52, pp. 1553–1572, doi: 10.1016/j.marpolbul.2006.09.006.

Erftemeijer PLA, Riegl B, Hoeksema BW, Todd PA, 2012, Environmental impacts of dredging and other sediment disturbances on corals: A review, *Marine Pollution Bulletin*, vol. 64, pp. 1737–1765, doi: 10.1016/j.marpolbul.2012.05.008.

Fletcher WJ, Santoro K, (Eds), 2015, Status Reports of the Fisheries and Aquatic Resources of Western Australia 2014/15: The State of the Fisheries, Western Australia, Department of Fisheries.

Friedman CS, 1996, 'Update on abalone withering syndrome', *Alolkoy*, vol. 9, no. Fall.

Friedman CS, Andree KB, Beauchamp KA, Moore JD, Robbins TT, Shields JD, Hedrick RP, 2000a, Candidatus Xenohaliotis californiensis', a newly described pathogen of abalone, Haliotis spp., along the west coast of North America, *International Journal of Systematic and Evolutionary Microbiology*, vol. 50, pp. 847–855, doi: 10.1099/00207713-50-2-847.

Friedman CS, Robbins TT, Jacobsen JL, Shields JD, 2000b, 'The cellular immune response of black abalone, *Haliotis cracherodii* Leach, with and without withering syndrome', *Journal of Shellfish Research*, vol. 19, pp. 514.

Friedman CS, Biggs W, Shields JD, Hedrick RP, 2002, Transmission of withering syndrome in black abalone, Haliotis cracherodii Leach, *Journal of Shellfish Research*, vol. 21, pp. 817–824.

Friedman CS, Crosson LM, 2012, Putative phage hyperparasite in the Rickettsial pathogen of abalone, Candidatus Xenohaliotis californiensis, *Microbial Ecology*, vol. 64, pp. 1064–1072, doi: 10.1007/s00248-012-0080-4.

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 Sciences*, vol. 60, pp. 1424–1431, doi: 10.1139/f03-121.

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*, vol. 26, pp. 877–885, doi: 10.2983/0730-8000(2007)26[877:OAATTM]2.0.CO;2.

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*, vol. 16, pp. 403–411.

Friedman CS, Trevelyan G, Robbins TT, Mulder EP, Fields R, 2003, Development of an oral administration of oxytetracycline to control losses due to withering syndrome in cultured red abalone Haliotis rufescens, *Aquaculture*, vol. 224, pp. 1–23, doi: 10.1016/S0044-8486(03)00165-0.

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 in Microbiology*, vol. 5, doi: 10.3389/fmicb.2014.00078.

Gardner GR, Harshbarger JC, Lake JL, Sawyer TK, Price KL, Stephenson MD, Haaker PL, Togstad HA, 1995, Association of prokaryotes with symptomatic appearance of withering syndrome in black abalone Haliotis cracherodii, *Journal of Invertebrate Pathology*, vol. 66, pp. 111–120, doi: 10.1006/jipa.1995.1072.

Godoy MG, Muñoz GP, 2003, 'Major diseases encountered in Japanese abalone (*Haliotis discus hannai*) and red abalone (*Haliotis rufescens*) reared in Chile', in 5th International Abalone Symposium, Qingdao, China, 12–17 October 2003.

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.

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.

Hooper, C, Day, R, Slocombe, R, Benkendorff, K, Handlinger, J & Goulias, J 2014, Effects of severe heat stress on immune function, biochemistry and histopathology in farmed Australian abalone (hybrid Haliotis laevigata × Haliotis rubra), *Aquaculture*, vol. 432, pp. 26–37, doi: 10.1016/j.aquaculture.2014.03.032.

Karpov KA, Haaker PL, Taniguchi IK, Rogers-Bennett L, 2000, 'Serial depletion and the collapse of the California abalone (*Haliotis* spp.) fishery', *Canadian Special Publications in Fisheries and Aquatic Science*, vol. 130, pp. 11–24.

Lafferty KD, Ben-Horin T, 2013, Abalone farm discharges the withering syndrome pathogen into the wild, *Frontiers in Microbiology*, vol. 4, pp. 1–5, doi: 10.3389/fmicb.2013.00373.

Lafferty KD, Kuris AM, 1993, Mass mortality of abalone Haliotis cracherodii on the California Channel Islands—tests of epidemiologic hypotheses, *Marine Ecology Progress Series*, vol. 96, pp. 239–248.

Malham SK, Lacoste A, Gelebart F, Cueff A, Poulet SA, 2003, Evidence for a direct link between stress and immunity in the mollusc Haliotis tuberculata, *Journal of Experimental Zoology Part A – Comparative Experimental Biology*, vol. 295A, pp. 136–144, doi: 10.1002/jez.a.10222.

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*, vol. 34, pp. 287–302, doi: 10.1111/j.1365-2761.2011.01241.x.

Mazur K, Curotti R, Perks C, Viera S, Pham T, George D, 2010, Australian fisheries—the global context, Australian Bureau of Agricultural Resource Economics and Sciences, Canberra, ACT, Australia.

Moore JD, Finley CA, Robbins TT, Friedman CS, 2002, Withering syndrome and restoration of Southern California abalone populations, CalCOFI Reports, vol. 43, pp. 112–117.

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*, vol. 156, pp. 2325–2330, doi: 10.1007/s00227-009-1260-8

Moore JD, Robbins TT, Friedman CS, 2000a, 'The role of a Rickettsia-like prokaryote in withering syndrome in California red abalone, *Haliotis rufescens'*, *Journal of Shellfish Research*, vol. 19, pp. 525–526.

Moore JD, Robbins TT, Friedman CS, 2000b, Withering syndrome in farmed red abalone Haliotis rufescens: Thermal induction and association with a gastrointestinal Rickettsiales-like prokaryote, *Journal of Aquatic Animal Health*, vol. 12, no. 1, pp. 26–34, doi: 0.1577/1548-8667(2000)012%3C0026:WSIFRA%3E2.0.CO;2.

Moore, JD, Robbins, TT & Hedrick, RP 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*, vol. 20, no. 2, pp. 867–874.

Morgan L, Shepherd SA, 2006, *Population and spatial structure of two common temperate reef herbivores: abalone and sea urchins*, in Marine metapopulations, Kritzer JP & Sale PF (eds), Elsevier, Amsterdam, pp. 205–246.

Mouton A, 2000, 'Health management and disease surveillance in abalone, *Haliotis midae*, in South Africa', *Journal of Shellfish Research*, vol. 19, pp. 526.

OIE 2004, 'Candidatus *Xenohaliotis californiensis* in Iceland', *Disease Information*, vol. 17, no. 25.

—— 2016a, Aquatic Animal Health Code, World Organisation for Animal Health (OIE), Paris, France.

—— 2016b, Manual of Diagnostic Tests for Aquatic Animals, World Organisation for Animal Health (OIE), Paris, France.

Ottaviani, E 2004, The mollusc as a suitable model for mammalian immune-neuroendocrine investigations, Invertebrate Survival Journal, vol. 1, pp. 2–4.

Paskin R, 2009, At the epicentre: essentials for epidemiologists in outbreaks. General principles of investigation and response, Victoria Department of Primary Industries, Melbourne, Victoria, Australia.

Pearce AF, Feng M, 2013, The rise and fall of the "marine heat wave" off Western Australia during the summer of 2010/2011, *Journal of Marine Systems*, vol. 111, pp. 139–156, doi: 10.1016/j.jmarsys.2012.10.009.

Prince JD, Sellers TL, Ford WB, Talbot SR, 1988, Confirmation of a relationship between the localized abundance of breeding stock and recruitment for Haliotis rubra leach (Mollusca, Gastropoda), *Journal of Experimental Marine Biology and Ecology*, vol. 122, pp. 91–104, doi 10.1016/0022-0981(88)90178-5.

Roberts S, Chalupa A, Bombardieri N, Kowalski D, Deveney M, Lauer P, Heaven C, Zippel B, Rahaley R, Doroudi M, 2013, Exercise Sea Fox: Testing aquatic animal disease emergency response capabilities within aquaculture, *Fisheries Research and Development Corporation*, Canberra, Australia.

Rodda KR, Keesing JK, Foureur BL, 1997, Variability in larval settlement of abalone on artificial collectors, *Molluscan Research*, vol. 18, pp. 253–264, doi: 10.1080/13235818.1997.10673699.

Rosenblum ES, Robbins TT, Scott BB, Nelson S, Juhasz C, Craigmill, A, Tjeerdema RS, Moore JD, Friedman CS, 2008, Efficacy, tissue distribution, and residue depletion of oxytetracycline in WS-RLP infected California red abalone Haliotis rufescens, *Aquaculture*, vol. 277, pp. 138–148, doi: 10.1016/j.aquaculture.2008.03.005.

Scheibling RE, 1994, Molluscan grazing and macroalgal zonation on a rocky intertidal platform at Perth, Western Australia, *Australian Journal of Ecology*, vol. 19, pp. 141–149, doi: 10.1111/j.1442-9993.1994.tb00477.x.

Shepherd SA, Turner JA, 1985, Studies on southern Australian abalone (genus Haliotis). 6. Habitat preference, abundance and predators of juveniles, *Journal of Experimental Marine Biology and Ecology*, vol. 93, pp. 285–298, doi: 10.1016/0022-0981(85)90245-X.

van Banning P, 1988, 'Management strategies to control diseases in the Dutch culture of edible oysters', *American Fisheries Society Special Publication*, vol. 18, pp. 43–245.

Vanblaricom GR, Friedman CS, Neuman M, 2011, 'Dynamics of endangered black abalone populations at San Nicolas Island, Naval Base Ventura County, California', *Natural Selection*, US Department of Defense, Legacy Natural Resource Management Program, vol. Spring.

Vanblaricom 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*, vol. 12, no. 2, pp. 185–188.

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*, vol. 90, pp. 69–76, doi: 10.3354/dao02221.