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Department of Agriculture, Water and the Environment

Australian aquatic veterinary emergency plan (AQUAVETPLAN) for crayfish plague

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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, Water and the Environment
GPO Box 858 Canberra ACT 2601
Telephone 1800 900 090
Web agriculture.gov.au

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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 crayfish plague 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 crayfish plague 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, Water and the Environment 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, Water and the Environment'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 crayfish plague 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 March 2020.

Crayfish plague is listed by the OIE in the Aquatic Animal Health Code. Crayfish plague 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, Water and the Environment 2019) is a source of information about the aetiology, diagnosis and epidemiology of infection with crayfish plague and should be read in conjunction with this strategy.

This first edition of this manual was prepared by Dr Frances Stephens in 2005, with the assistance of Ms Nicky Buller, Dr David Alderman (UK), and Drs Andrew Cameron, Mehdi Doroudi, Preston Suijdendorp, Marty Deveney and Rachel Bowater. This revision was completed in 2015 by Dr Ben Diggles. The author was 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 author. The author would like to thank Drs David Alderman, Trude Vrålstad and David Strand for allowing reproduction of their photographs, and illustrations of Aphanomyces astaci life history stages. 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 Resources, Northern Territory
  - Department of Agriculture, Water and the Environmentand Fisheries, Queensland
  - Department of Primary Industries, Parks, Water and Environment, Tasmania
  - Department of Primary Industries and Regional Development, 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, Water and the Environmentand Water Resources, Australian Government
  - Department of the Environment, Australian Government

The complete series of AQUAVETPLAN documents is available on the Department of Agriculture, Water and the Environment website.

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1 Nature of the disease

Crayfish plague is a fungal disease of freshwater crayfish that has the potential to cause large-scale mortality of freshwater crayfish in Australia. At present, the disease does not occur in Australia. However, it is important that State and Territory governments and the redclaw, yabby and marron aquaculture industries are aware of the dangers posed by this disease and are adequately prepared to manage a disease outbreak, because an incursion of the disease could devastate the freshwater crayfish aquaculture industry and wild populations of freshwater crayfish in Australia.

1.1 Aetiology

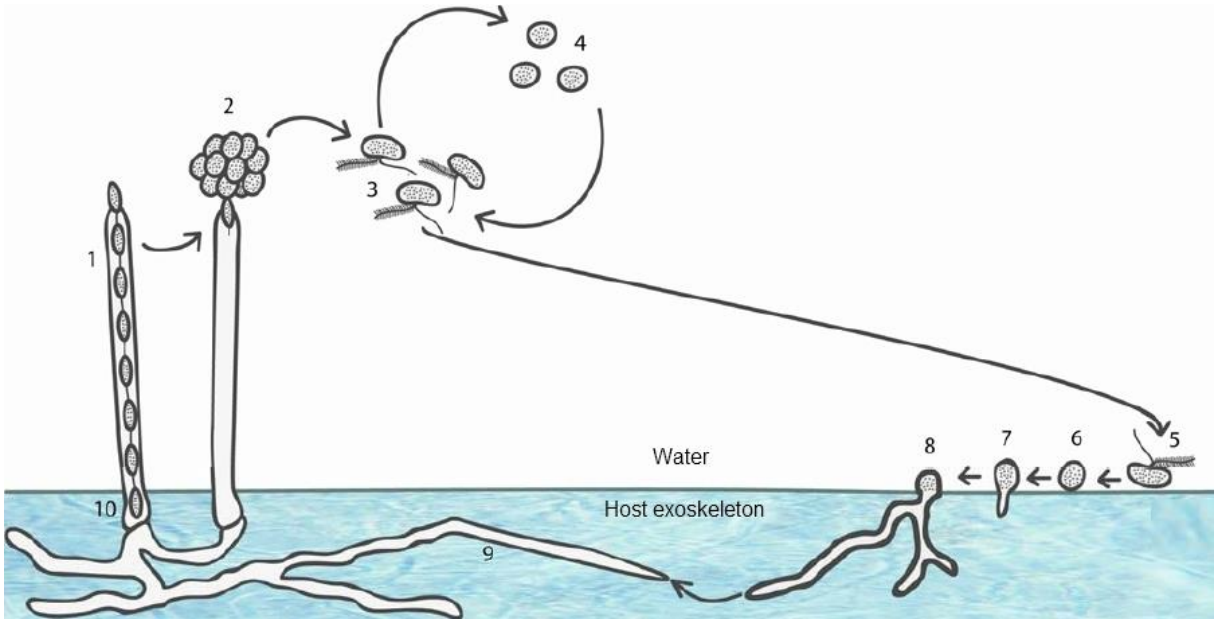
The aetiological agent of crayfish plague is an oomycete *Aphanomyces astaci* Schikora, also known as the krebspest (Schikora 1906). Oomycetes (commonly called water moulds) are often treated as fungi, although taxonomically, are not considered to be 'true fungi', and are instead placed in the phylum Oomycota. Within this phylum is the Family *Saprolegniaceae*, which includes *Achlya*, *Aphanomyces* and *Saprolegnia*, with some species of these genera being pathogens of crustaceans, fish and plants (Phillips et al. 2008). However, to minimise confusion with existing literature, *A. astaci* and other members of the Oomycota will be referred to as fungi in this document. *Aphanomyces astaci* should not be confused with *A. invadens*, which is endemic to Australia and is the causative agent of epizootic ulcerative syndrome (EUS) in finfish.

Aphanomyces astaci is a branching, non-septate fungus that produces spores under favourable conditions. It has a parasitic life-cycle and is adapted to living in or near chitin (Unestam 1969a). There are at least 5 different strains of *A. astaci* (Groups A to E, see Grandjean et al. (2014); Kozubíková et al. (2011a); Makkonen et al. (2011); Makkonen et al. (2012a)), several of which differ markedly in their pathogenicity in susceptible species of crayfish (Makkonen et al. 2012b; Makkonen et al. 2014). Studies have shown that sporulation of *A. astaci* in sub-clinically infected North American crayfish carriers normally occurs at low levels but increases during moulting (Svoboda et al. 2013).

In susceptible species of crayfish, sporulation markedly increases during the terminal stages of disease (Strand et al. 2012), peaking in the first 48-72 hours after the death of the host (Makkonen et al. 2013). More spores are produced at 18°C than at 4°C (Strand et al. 2012), but spores are able to survive at 2°C. Despite sporulation occurring at a wide range of temperatures, the optimal temperature for infection is around 18°C, and is generally not infective above 25°C (29.5°C in some strains). Spore survival also varies depending on water chemistry (see section 1.6.3).

The lifecycle is direct and motile biflagellate zoospores measuring 8 to 15µm that emerge from spores are the infective stage. These attach to the cuticle and other surfaces of new hosts within the water body before penetrating the cuticle and colonising host tissues (Cerenius & Söderhäll 1984a)(Figure 1).

Figure 1 Generalised lifecycle of *Aphanomyces astaci*



Note: Sporangia with primary spores (1) are released as a spore ball (2) from which motile biflagellate zoospores (3) are released into the water. Zoospores will either encyst (4) and repeat zoospore emergence in the absence of a suitable host, or locate a suitable host with chemotaxis (5) and encyst on the host surface (6). An infection spike develops and penetrates the host cuticle (7) and non-septate hyphae ramify (8-9) within the host exoskeleton. In resistant species, the host immune response usually restricts the infection to focal melanised areas within the cuticle of the exoskeleton, while in susceptible species the infection rapidly proceeds through the exoskeleton into the body cavity and nerve system, leading to host death. The death of the host triggers the production of new spore producing hyphae/sporangia (10). Adapted with permission from (Vrålstad et al. 2011).

1.2 Susceptible species

All species of freshwater crayfish should be considered as susceptible to infection with *A. astaci.* (OIE, 2018d). However, the outcome of infection differs depending on species. American crayfish, including red swamp crawfish (*Procambarus clarkii*) and signal crayfish (*Pacifastacus leniusculus*) are tolerant of infection with *A. astaci*. They can remain carriers for life, and may exhibit little or no signs of infection, but can die from crayfish plague following stressors that compromise their immune system (Unestam 1969c; Unestam 1972; Unestam 1975; Roy 1993), or if they are exposed to high levels of infection (Aydin et al. 2014).

Crayfish endemic to Australia, New Guinea, Japan and Europe are highly susceptible to crayfish plague (Table 1). Australia has over 135 species of freshwater crayfish, many of which are already threatened by land use changes or translocation of native crayfish into areas outside their natural range (Coughran & Furse 2012). Of the Australian commercial aquaculture species, red claw (*Cherax quadricarinatus*) and yabby (*Cherax destructor*) are susceptible to the disease (OIE 2014b; Unestam 1975), however, there are no published reports of the susceptibility of marron (*Cherax tenuimanus*). It is assumed, on the basis of experimental work (Unestam 1975) and the epidemiology of the disease agent, that all species of freshwater crayfish in Australia are likely to be highly susceptible to infection by *A. astaci*.

Aphanomyces astaci is known to cause epizootic mortalities only in freshwater crayfish, but other decapod crustaceans can be infected and act as subclinical carriers, including the catadromous Chinese mitten crab (*Eriocheir sinensis*), river crab (*Potamon potamios*) and an

Asian freshwater shrimp (*Macrobrachium dayanum*) (Benisch (1940); Schrimpf et al. (2014); Svoboda et al. (2014a); Svoboda et al. (2014b); Tilmans et al. (2014)) (Table 1 and Table 2).

Table 1 Susceptibility of freshwater crayfishes and other crustaceans to crayfish plague according to published literature

Species Name			
Scientific	**Common**	**Disease severity**	**Region of origin**
Non-Australian species			
Cambarus fasciatus	Etowah crayfish	Resistant carrier [13]	North America
Cambarus manningi	Green saddle crayfish	Resistant carrier [13]	North America
Pacifastacus leniusculus	Signal crayfish	Resistant carrier [1,2,5]	North America
Procambrus alleni	Florida crayfish	Resistant carrier [14]	North America
Procambarus clarkii	Louisiana swamp crayfish	Resistant carrier [2]	North America
Procambarus enoplostenum		Resistant carrier [14]	North America
Procambarus llamasi	---	Resistant carrier [13]	North and Central America
Procambarus simulans	Southern plains crayfish	Resistant carrier [13]	North America
Procambarus vazquezae		Resistant carrier [14]	Central America
Orconectes immunis	Calico crayfish	Resistant carrier [11]	North America
Orconectes luteus	Golden crayfish	Resistant carrier [13]	North America
Orconectes neglectus	Ringed crayfish	Resistant carrier [13]	North America
Orconectes ozarkae	Ozark crayfish	Resistant carrier [13]	North America
Astacus astacus	Noble crayfish	Overt disease [1,2,5]	North west Europe
Austropotamobius pallipes	Whiteclaw crayfish	Overt disease [2]	West and south west Europe
Austropotamobius torrentium	Stone crayfish	Overt disease [4,6]	Mountains in south-west Europe
Astacus leptodactylus	Slender clawed or Turkish crayfish	Overt disease [2]	Eastern Europe, Middle East
Cambaroides japonicus	Zarigani	Overt disease [2]	Japan
Cherax papuanus	Orange zebra crayfish	Overt disease [5]	Papua-New Guinea
Eriocheir sinensis	Chinese mitten crab	Resistant carrier [6,7,8]	Asia
Potamon potamios	River crab	Resistant carrier [7]	Mediterranean
Macrobrachium dayanum	Freshwater shrimp	Resistant carrier [9]	Asia
Australian species			
Smooth crayfish			
Cherax destructor	Yabby	Overt disease [5]	Australia-wide
Cherax quinquecarinatus	Gilgie	Overt disease [5]	Western Australia
Cherax quadricarinatus	Redclaw	Overt disease [3]	Queensland, Northern Territory
Geocherax gracilis	Otway cray	Overt disease [5]	Victoria, Tasmania
Spiny crayfish			
Astacopsis gouldi	Giant crayfish	Overt disease [5]	Tasmania

Species Name			
Scientific	**Common**	**Disease severity**	**Region of origin**
Astacopsis fluviatilis	Freshwater crayfish	Overt disease [5]	Tasmania
Euastacus kershawi	Gippsland crayfish	Overt disease [1, 5]	Victoria
Euastacus clydensis	Sydney crayfish	Overt disease [5]	New South Wales
Euastacus crassus	Alpine spiny crayfish	Overt disease [12]	New South Wales, Victoria

Note: Only a few Australian species have been experimentally challenged with crayfish plague but all are assumed susceptible to infection. The North American species are resistant but can succumb to the disease under certain conditions. Other northern hemisphere decapod crustaceans including the Chinese mitten crab, river crab and a species of shrimp are known to be potential carriers. References: **1** Unestam (1972), **2** Unestam (1969c), **3** Roy (1993), **4** Schikora (1906), **5** Unestam (1975), **6** Benisch (1940), **7** Svoboda et al. (2014b), **8** Tilmans et al. (2014), **9** Svoboda et al. (2014a), **10** Keller et al. (2014), **11** Schrimpf et al. (2013a), **12** Svoboda et al. (2017), **13** Panteleit et al. (2017). **14** Mrugala et al. (2015).

The susceptibility of many other freshwater decapod crustaceans to infection with *A. astaci* is unknown. Consequently, it is uncertain whether wild populations of Australian freshwater crabs and shrimp would develop clinical disease, or act as subclinical carriers following infection with *A. astaci*. All non-decapod crustaceans tested to date (mysids, copepods, amphipods, isopods, and carids) are resistant to infection, as are rotifers (Table 2). Figure 2 shows the phylogenetic relationship and susceptibility to A. astaci infection of six crustacean families that have been the subject of experimental A. astaci infection trials.

There is no evidence that A. astaci has any zoonotic potential. However, susceptible species of crayfish infected with A. astaci can rapidly become clinically diseased, often within a few days of exposure to infective zoospores, depending on water temperature (Alderman et al. 1987; Makkonen et al. 2014). Meat quality and food safety issues may arise with the development of secondary bacterial infections in such acutely-infected crayfish, and movement of *A. astaci*-infected crayfish (live or dead) is also a recognised risk factor for spread of infection. Because of this diseased crayfish are not generally recommended for human consumption (but see options for emergency harvesting of crayfish as part of an eradication or control program; Section 2.6).

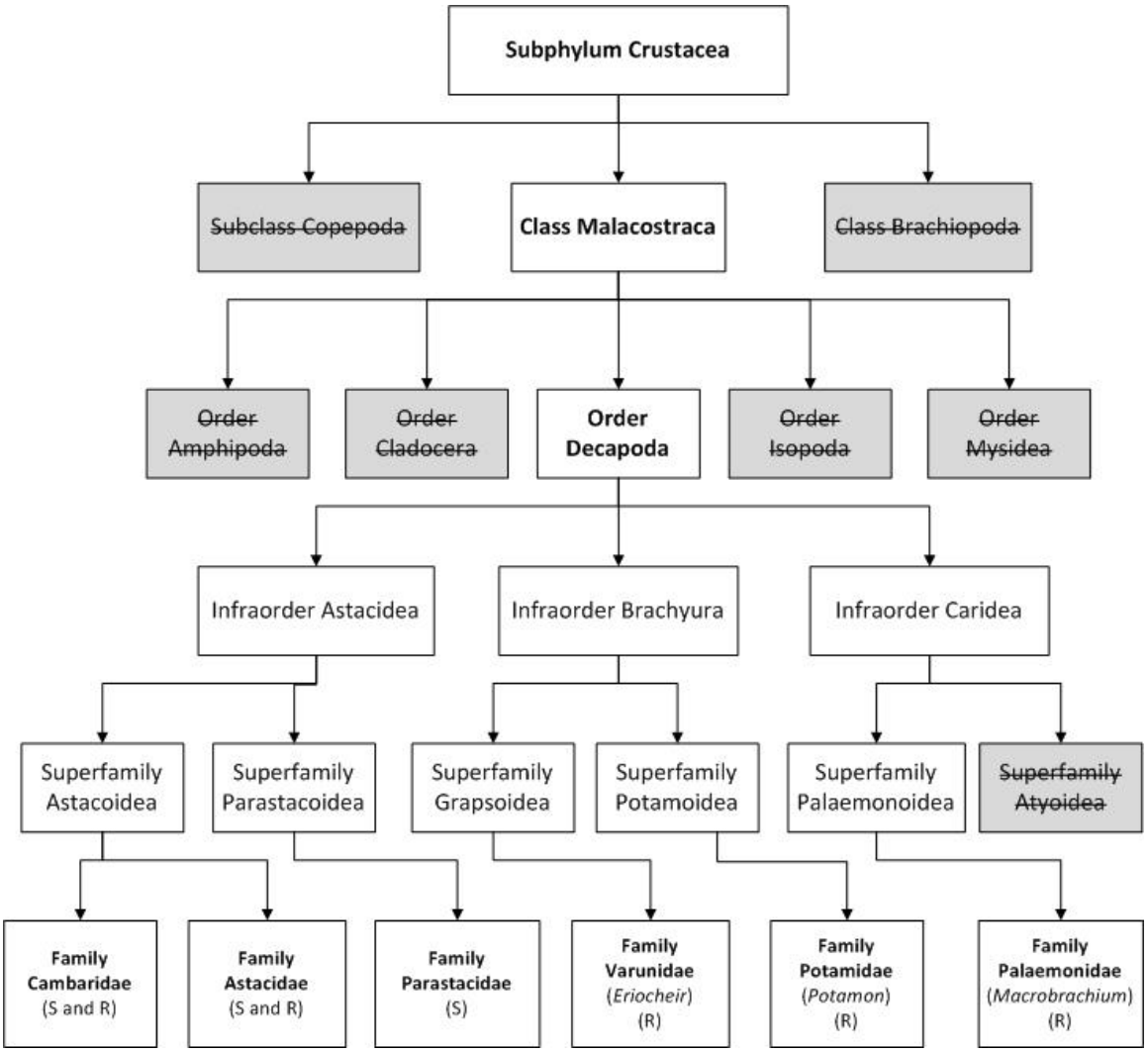
Table 2 Aquatic animals resistant to *A. astaci* infection in experimental studies

Species name		
Scientific	**Common**	**Taxonomic group**
Mysis relicta [1, 3]	mysids	Order: Mysidacea
Daphnia hyalina [2]	daphnia	Class: Branchiopoda
Leptodora hyalina [2]	-	Class: Branchiopoda
Chydorus sphaericus [2]	-	Class: Branchiopoda
Bytotrephes longimanus [2]	-	Class: Branchiopoda
Cyclops strenuous [2]	copepod	Class: Copepoda
Mesocyclops leuckarti [2]	copepod	Class: Copepoda
Asplanchna priodonta [2]	rotifer	Phylum: Rotifera
Bosmina sp. [2]	water flea	Order: Cladocera
Pallaseopsis quadrispinosa [3]	amphipod	Order: Amphipoda
Asellus aquaticus [3]	water louse	Order: Isopoda

Species name		
Scientific	**Common**	**Taxonomic group**
Neocaridina davidi [4]	shrimp	Order:Decapoda Infraorder: Caridea

Note: References **1** Unestam (1972), **2** Unestam (1969c), **3** Svoboda et al. (2014b), **4** Svoboda et al. (2014a).

Figure 2 Diagrammatic representation of the taxonomic relationship of freshwater crayfish to other crustaceans investigated as possible hosts for *A. astaci*



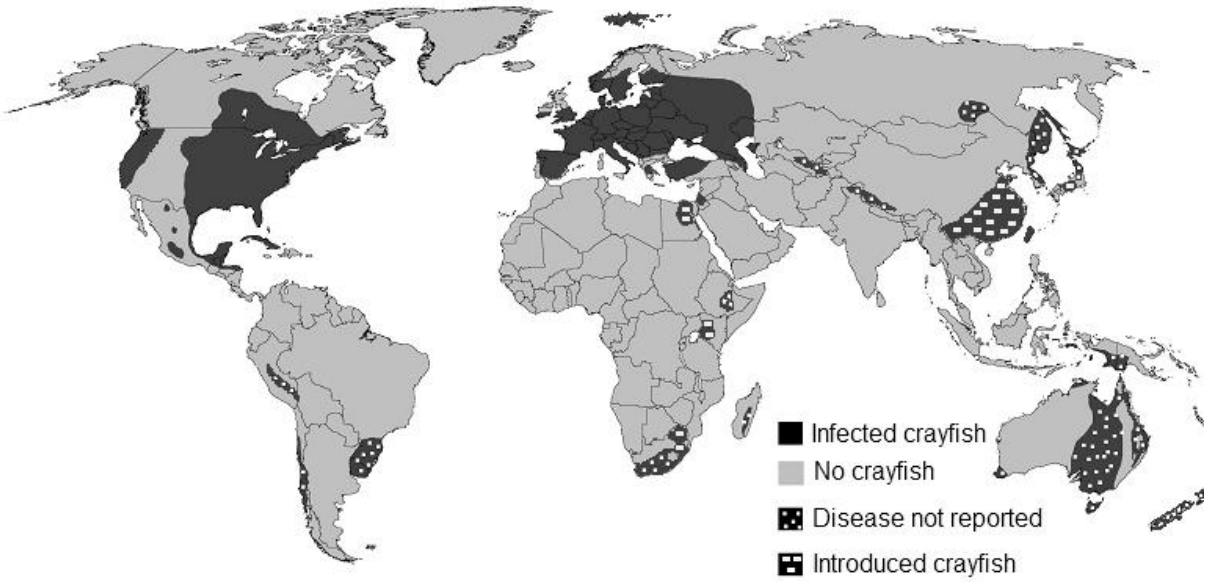
Note: Non susceptible hosts indicated by grey-shaded text box and strikethrough text. Infection has only been demonstrated in decapod crustaceans, namely freshwater crayfish, the Chinese mitten crab (Family Varunidae), the river crab (Family Potamidae) and an Asian freshwater shrimp (Family Palaemonidae). Susceptible hosts indicated by 'S'. Taxa containing resistant hosts that may act as subclinical carriers are indicated by 'R'. Crustacean classification follows that of Martin & Davis (2001).

1.3 World distribution

Figure 3 illustrates the known geographical distribution of *A. astaci*. The disease agent occurs naturally in wild and cultured crayfish populations in North America. These hosts were introduced into Europe on multiple occasions in the 19th, 20th and 21st centuries (Holdich et al. 2009), resulting in the establishment of *A. astaci* in over 30 European countries.

Aphanomyces astaci was reported from a closed system in Israel in 2013, as subclinical infection of farmed *Procambarus alleni*. Outbreaks of crayfish plague have also been reported in cultured crayfish in Taiwan (in 2013) and wild crayfish in Japan (in 2014) (OIE 2014b; OIE 2014c). The disease has never been reported in Australia or New Zealand, despite targeted and passive surveillance.

Figure 3 Global distribution of *Aphanomyces astaci*



Note: The glogabl distribution of *A. astaci* is shown by the dark grey shading with no stippling. The light grey areas indicate areas in which crayfish do not occur naturally. In some areas where the disease has not yet been reported (for example, Africa and China), non-native crayfish species from North America have been introduced (shown by the dark grey shading with large rectangular stippling), and have become established (crayfish distribution data from Holdich et al. (2009) and Liu et al. (2011)). Note, this map may be inaccurate in relation to the 'No crayfish' zone marked on Australia's eastern states. Multiple *Euastacus* species are reported to occur in this zone (Shull et al. 2008).

Until recently, outbreaks of crayfish plague were only known to occur in the cooler temperate climates of Europe with optimal disease transmission around 18°C. However, red swamp crawfish (*Procambarus clarkii*) and other species of North American crayfish have been introduced and become established in many countries outside Europe, including Egypt, Kenya, South Africa, Madagascar, Japan, China and Taiwan (Jones et al. 2009; Kawai & Kobayashi 2005; Li et al. 2012; Liu et al. 2011; OIE 2014b; Yue et al. 2010)(Figure 3). *Aphanomyces astaci* strains that infect *P. clarkii* can sporulate at water temperatures up to 29.5°C (OIE 2018d). These translocations have resulted in the introduction and spread of *A. astaci* into Africa and Asia, either via contaminated water or through movements of live or dead infected crayfish (Rezinciuc et al. 2014).

In late 2013, crayfish plague was detected for the first time in farmed redclaw crayfish (*Cherax quadricarinatus*) in Taiwan (OIE 2014b). The disease was found in diseased crayfish in five farms, four of which experienced 100% mortality, with the remaining farm experiencing 5% mortality and 20% morbidity (OIE 2014b). While the *A. astaci* strain involved was not reported, the 2013 outbreak in Taiwan is the first reported case of Australian native crayfish being naturally infected by *A. astaci* and demonstrates the high susceptibility of this species to the pathogen. The high mortality rate during the outbreak in Taiwan suggests that a similar outbreak in Australia could have devastating consequences for both wild and cultured crayfish.

Given that some strains of *A. astaci* can transmit at water temperatures up to 29.5°C (OIE 2018d), the introduction of crayfish plague to Australia could potentially impact large geographical areas.

The outbreaks in Taiwan were followed in early 2014 by detection of *A. astaci* in wild crayfish in one or more zones in Japan, although little other information is available about this incident at this time (OIE 2014c). More recently, there has been a recorded range extension of crayfish plague in Greece, representing the southern most European range (Perdikaris et al. 2018).

1.4 Diagnosis of infection with *Aphanomyces astaci*

Crayfish plague must be suspected whenever there is a significant mortality event in crayfish, particularly if other aquatic animals remain unaffected. Diagnosis is based on a combination of clinical signs, histopathology, laboratory culture of the disease agent and/or identification of the disease agent using molecular methods. Methodology for fungus isolation and diagnostic techniques can be found in Appendix C and;

- Office International des Epizooties (OIE) Manual of Diagnostic Tests for Aquatic Animals (OIE 2018d)
- Crayfish plague (*Aphanomyces astaci*) Australian and New Zealand Standard Diagnostic Procedure (ANZSDP) (Buller 2008)

The OIE (OIE 2018d) has provided the following case definitions for crayfish plague:

- Suspect case: whenever "any extensive mortality solely of the highly susceptible species of freshwater crayfish where all other aspects of the flora and fauna, particularly other aquatic crustaceans, are normal and healthy". In the case of North American crayfish species, the OIE (OIE 2018d) states that "any population of North American crayfish is generally to be regarded as potentially infected with *A. astaci*."
- Confirmed case: "Confirmation of presence of *A. astaci* by PCR or qPCR and sequencing", and that, "(1) where a crayfish mortality meets the definition of a suspect case and (2) PCR results indicate the presence of high levels of template DNA (in case of real-time PCR, this corresponds to Ct values ≤ 30), and (3) if the investigated suspect case is not the first case of detection of *A. astaci* in the country or region, the PCR result alone may be considered sufficient as a confirmation". In the case of North American crayfish species, the OIE (OIE 2018d) states it is sufficient to "(confirm the) presence of *A. astaci* by PCR or qPCR and sequencing."

1.4.1 Field methods: clinical signs and gross pathology

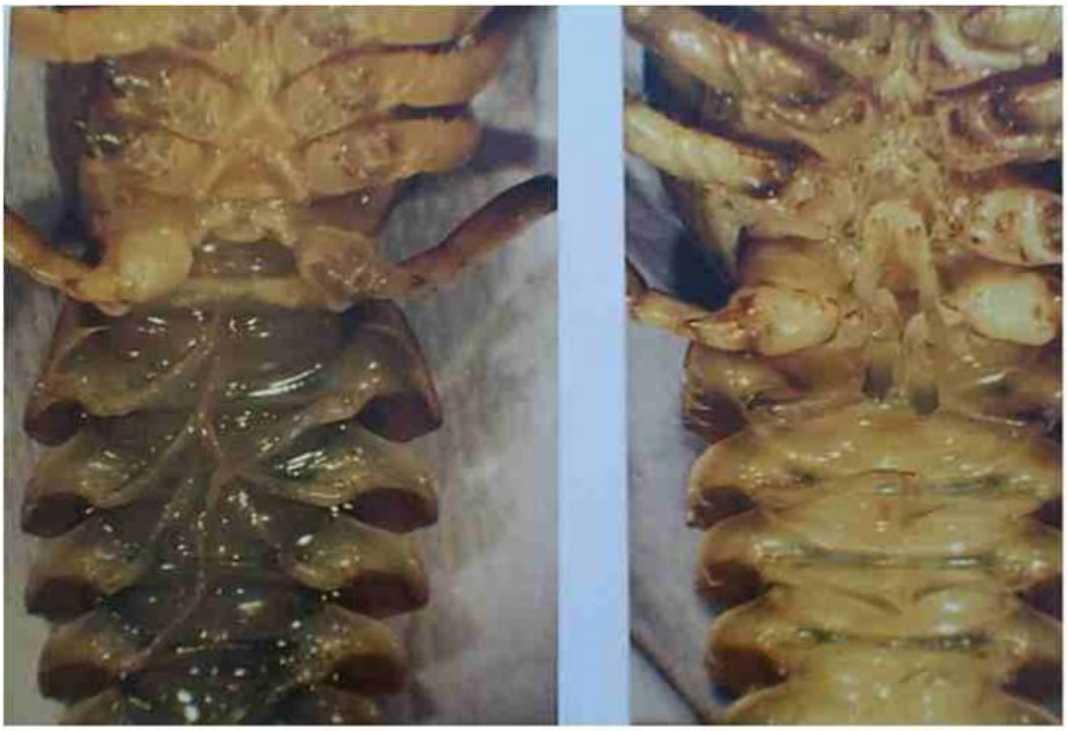
Gross clinical signs of crayfish plague are highly variable depending on the host species, challenge severity and water temperature (OIE 2018d). In acute outbreaks crayfish may die without grossly visible lesions, when high zoospore numbers and a high density of susceptible crayfish results in large-scale mortality within days of infection. Affected crayfish may be observed away from shelter during daylight, exhibiting unusual gait or activity, however, these clinical signs are not pathognomonic for this disease. Infected crayfish may exhibit some of the following signs (Alderman et al. 1990; Alderman et al. 1987; Alderman et al. 1984; Buller 2008; Nybelin 1931; 1936; Nylund & Westman 1995b; Oidtmann et al. 1999; Schäperclaus 1927; 1935; Unestam 1972; Unestam & Weiss 1970):

- high mortality
- easy to catch and lethargic when close to death
- appear to have poor limb coordination
- lie on their dorsal surface ('belly up')
- constant leg movements
- loss of appendages
- leave shelter during daylight hours
- move from water to land during daylight hours
- walking with stiffly stretched legs ('walking on stilts')
- paralysis
- have lost or have a weak tail 'flip' or 'flick' response
- may have brown, yellowish or dull grey patches on the base of legs or underside of the abdomen
- fine, white fungal growth visible at affected sites on legs and abdomen soon before, or after death

Other clinical signs of the disease in susceptible species of crayfish may include the presence of opaque whitish flesh between the abdominal segments (Figure 4). In resistant species such as North American crayfish, infected individuals may exhibit focal brown melanised spots in the cuticle of the carapace, walking legs or abdomen (Figure 5).

The presence of large numbers of dead crayfish, even in crayfish plague-affected watersheds, is not on its own sufficient for diagnosis. The general condition of other aquatic fauna must be assessed. Mortality or disappearance of crayfish and other aquatic invertebrates, even though fish survive, may indicate pollution (for example, insecticides such as cypermethrin have been associated with initial misdiagnoses, see OIE (2014d)). Thus, in all cases, laboratory tests are necessary to confirm any presumptive diagnosis based on the above clinical signs.

Figure 4 Crayfish plague in a susceptible species of crayfish



Note: Note areas of melanisation at the base of the legs and whitening of the abdominal segments in photograph B. Melanin deposits may not always be obvious in infected crayfish. Crayfish plague can only be diagnosed by the use of laboratory techniques, and the appearance or behaviour of crayfish can be used only as a guide to possible presence of the disease. (Photographs © British Crown Copyright, Courtesy Dr DJ Alderman).

Figure 5 A crayfish plague resistant signal crayfish (*Pacifastacus leniusculus*)

Note: The signal crayfish (Pacifastacus leniusculus) exhibiting focal melanised spots on **a** the upper carapaceand **b** abdominal segment, due to infection by *A. astaci.* With permission from Vrålstad et al. (2011).

1.4.2 Laboratory methods

The State/Territory Chief Veterinary Officer (CVO) must be notified immediately of any suspected incidents of crayfish plague. Preliminary identification of *A. astaci* may be undertaken by some State/Territory diagnostic laboratories. State/Territory governments will arrange for samples to be sent to the Commonwealth Scientific and Industrial Research Organisation (CSIRO) Australian Animal Health Laboratory (AAHL) at Geelong, Victoria, for confirmatory diagnosis upon suspicion of crayfish plague.

Sample submission

In the case of a suspected outbreak of crayfish plague in a population of highly susceptible crayfish species, the sample of crayfish selected for investigation for the presence of *A. astaci* should ideally consist of (Buller 2008; OIE 2018d):

a) live crayfish showing signs of disease
b) live crayfish appearing to be still healthy
c) dead crayfish collected within 12 hours of their death

In the first instance, samples should be submitted to the relevant State/Territory diagnostic 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), and transport of recently dead or moribund crayfish cannot be arranged quickly, crayfish tissue (soft abdominal cuticle, telson and uropods) may be fixed in ethanol (minimum 70%). However, fixation may reduce test sensitivity. The crayfish:ethanol ratio should ideally be 1:10 (1 part crayfish tissue, 10 parts ethanol) (OIE 2018d). It is recommended that multiple crayfish (5 to 10) be tested, to compensate for variations in sample quality and invasion site of the pathogen (OIE 2018d).

Live crayfish should be transported using polystyrene containers equipped with small holes to allow aeration, or an equivalent container. The container should provide insulation against major temperature differences outside the container. In periods of hot weather, freezer packs should be used to avoid temperatures deleterious to the animals. These can be attached at the inside bottom of the transport container. The crayfish must however be protected from direct contact with freezer packs. This can be achieved using, for instance, cardboard or several layers of newspaper (OIE 2018d).

Live crayfish should be transported in a moist atmosphere, for example using moistened wood shavings/wood, wool, newspaper or grass/hay. Unless transport water is sufficiently oxygenated, live crayfish should not be transported in water, as they may suffocate from lack of oxygen. The time between sampling of live animals and delivery to the investigating laboratory should not exceed 24 hours (OIE 2018d).

Dead crayfish or ethanol-fixed crayfish tissues should be packed according to IATA-approved protocols (that is, UN3373: Packaging requirements for the transport of biological and infectious substances). Ensure all samples are clearly labelled with the animal identification. Samples can then be “triple-packed” by sealing individual crayfish in double zip-locked bags (or equivalent leak proof primary receptacles), placing in Bio-bottles or Bio-pouches, and transporting chilled on ice in a small esky or other suitable container. Preferably, do not freeze samples. Also ensure

that the appropriate specimen submission form is placed in a watertight bag within the esky (contact your State/Territory veterinary diagnostic laboratory for details; see Appendix D). Secure the lid of the container with tape, and wipe the outside of the container with a disinfectant such as sodium hypochlorite (100 mg L^{-1} available chlorine) to reduce the potential for contamination.

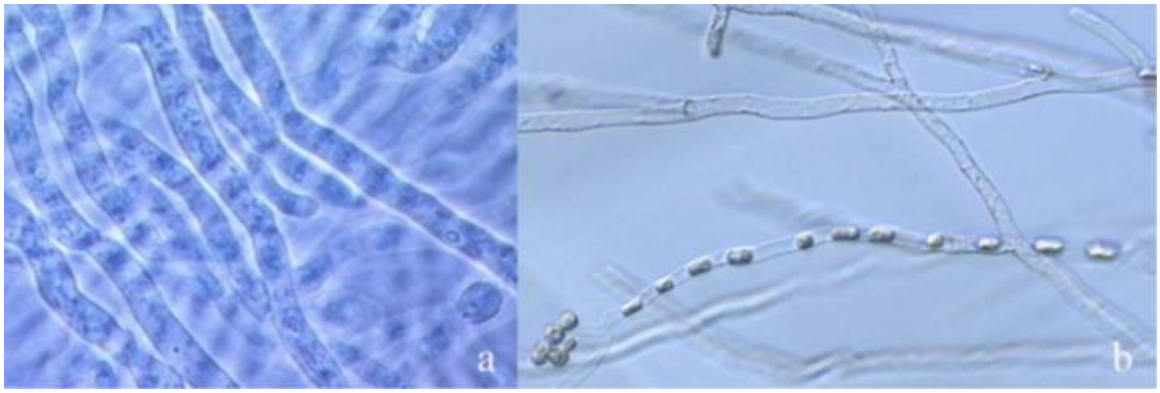
Samples may be frozen, as a last resort, where alcohol for fixation is unavailable. Frozen samples will destroy morphological features used for identification and will only allow for possible DNA identification using PCR.

Microscopy

- **Wet preparations of fresh tissue**

Small biopsies of tissue from appropriate collection sites (the soft cuticle of the abdomen, connective tissue, the limb joints, gills, nerve cord, gut, the eye, the telson near the anus, and particularly areas that show melanisation) are placed into a few drops of sterile/filtered water on a glass slide and flattened under a coverslip. Non-septate, branching hyphae of *A. astaci* approximately 5 to 10 µm in width may be seen in areas of infected tissues when viewed using a light microscope at low to medium power (40× to 200×magnification, OIE (2014d)) (Figure 6).

Figure 6 Wet mount preparations of *A. astaci*



Note: **a** hyphae and **b** sporangia with an emerging spore ball. Reproduced with permission from Vrålstad et al. (2011).

- **Histopathology**

Biopsies of affected tissues can be fixed in 10% neutral buffered formalin and examined for *A. astaci* using histology. Hyphae are 5 to 10 µm in diameter, aseptate and branching. Because the hyphae of *A. astaci* are thin-walled they are rarely detected using the standard haematoxylin and eosin stain. A combined Grocott silver stain with haematoxylin and eosin used as a counter stain can improve the visualisation of the hyphae in the crayfish tissue with the hyphae staining black (Buller, 2008) (Appendix C).

Hyphae are seen on and beneath the exoskeleton, particularly in the soft cuticle of the joints, membranes and abdominal segments where the organism is able to penetrate the exoskeleton. Growth of hyphae tends to be restricted to the area of cuticle penetration but can also grow along the ventral nerve cord and brain ganglion. Occasionally, hyphae are seen in the eye but rarely in other organs and they do not invade the musculature until late in the infection. The tissue around the area of infection becomes necrotic and yellow-brown in colour. Hyphae in the cuticle close to the epidermis and in the layer adjacent to the epicuticle can be surrounded by

deposits of melanin and haemocytes. In the later stages of the disease, zoosporangia and zoospores form on the exoskeleton (Nybelin 1936; Schäperclaus 1935; Unestam & Weiss 1970).

Culture methods

Confirmation of suspected crayfish plague can be achieved by culturing for the presence of A. astaci with identity confirmed by sporulation test and molecular methods. Fungal culture is carried out on isolation medium (IM) at 16°C to 20°C for 15 days (Alderman & Polglase 1986). During this time overgrowth with bacterial flora and other fungi can be problematic. For details of fungal culture, please refer to Appendix C.

Molecular Techniques

A variety of molecular diagnostic techniques have been developed for detecting *A. astaci*. These include various tests based on the polymerase chain reaction (PCR), quantitative PCR (qPCR) and sequencing of PCR products (Buller, 2008; Kozubíková et al. 2011b; Oidtmann et al. 2006; OIE 2018d; Tuffs & Oidtmann 2011; Vrålstad et al. 2009). The following information was obtained from the OIE Manual for Crayfish Plague (OIE 2018d). Any differences with the information published in the Australian and NZ Standard Diagnostic Procedure (ANZSDP) for Crayfish Plague (Buller, 2008) are noted where they occur. For both Buller (2008) and OIE (2014d) these diagnostic techniques are updated as required. Please refer to the relevant websites for the most recent methods.

- **DNA extraction**

The soft abdominal cuticle, telson and uropods are the preferred sample tissues for DNA extraction. Any superficial contamination should first be removed by thoroughly wiping the area with a wet (using autoclaved H_2O) clean disposable paper towel. The sample is then excised using flame sterilised equipment and 30 to 50 mg of the tissue sample ground in liquid nitrogen to a fine powder using a pestle and mortar (alternative grinding techniques may be used, but should be compared with the liquid nitrogen method before routine use).

For carrier identification, 30 to 50 mg tissue from each of soft abdominal cuticle, and telson and uropods are sampled and processed separately. DNA is extracted from the ground cuticle using a proteinase K-based DNA extraction method (for example, DNeasy tissue kit; Qiagen, Hilden, Germany; protocol for insect tissue) following the manufacturer's instructions (Oidtmann etal. 2006) or using a CTAB (cetyltrimethylammonium bromide-based)-based assay (Vrålstad et al. 2009). Negative controls and an environmental PCR control left open during pipetting of sample DNA should be run alongside the samples.

- **PCR**

Several PCR assays have been developed targeting the internal transcribed spacer (ITS) region of the nuclear ribosomal gene cluster within the *A. astaci* genome. The assay described by the OIE (OIE 2018d) uses primers BO42 and BO640 to amplify a 569 bp product of A. astaci DNA (OIE 2018d).

The same primers are referred to as 42F and 640R in the ANZSDP assay used to amplify a 569 base pair product of *A. astaci* DNA extracted from culture material and from soft cuticle infected with the fungus (Buller 2008). However, Buller (2008) notes the OIE primer pair generates a product that is too large for amplification from formalin-fixed, paraffin-embedded tissue. For the latter, primers 525F and 640R were developed to successfully amplify DNA from

paraffin-embedded sections, producing a short amplicon of 115 base pair, but the specificity of this primer pair is lower as it also was shown to produce weak product from *A. invadans* and *A. frigidophilus* (see Buller (2008)). Owing to the repeated discovery of new *Aphanomyces* strains, sequencing is required to confirm diagnosis (OIE 2018d).

- **Sequencing**

Following agarose gel electrophoresis, PCR amplicons of the expected size are purified from the gel and both DNA strands are sequenced using the primers used in the initial amplification. The consensus sequence is generated using sequence analysis software and compared with published sequences using an alignment search tool such as BLAST. If 100% identity between the submitted sequence and the published sequences is found, then *A. astaci* is confirmed. If the sequence is not 100% identical, further PCR testing and sequencing should be performed using the primers ITS-1 and ITS-4 (White et al. 1990), which generate an amplicon of 757 base pairs. Primers ITS-1 and ITS-4 provide sequence data in the same region, but expanded at both ends relative to the sequence generated by primers BO42 and BO640. This expanded sequence should confirm the identity of the pathogen to the species level.

- **Quantitative PCR**

See the quantitative PCR assay published by the OIE (2014b) (OIE 2018d; Vrålstad et al. 2009).

1.4.3 Confirmation of infection

For the purposes of this manual, confirmation of crayfish plague caused by infection with *A. astaci* requires either:

- generation of a positive result from PCR or qPCR followed by verification of the PCR or qPCR amplicon via sequencing, or,
- successful culture of *A. astaci* and confirmation of the identity of the isolate(s) by morphological criteria and PCR/qPCR followed by sequencing.

1.4.4 Differential diagnosis

White patches of abdominal muscle on limb bases, mid abdomen and perianal region may be caused by the microsporidian parasite *Thelohania contejeani* (Schäperclaus, 1927; Polglase & Alderman, 1984), although muscle infected with *Thelohania* spp. is a more vivid white than that infected with *A. astaci*. Lesions caused by fungi such as *Fusarium solani* or brown, melanotic spots from previous injuries or other infections must be differentiated from crayfish plague, but rarely cause the high and rapid mortality of crayfish plague (Schäperclaus, 1927). Sudden death of freshwater crayfish can also result from environmental disturbances or toxicity (OIE 2018d). Some bacterial infections and exposure to pesticides can cause losses of large numbers of crayfish in a pond, but these causes of mortality are likely to also affect other crustaceans in the pond.

1.5 Resistance and immunity

Crayfish native to North America are regarded as resistant to infection with *A. astaci*. However, most other freshwater crayfish species from around the world are believed to be highly susceptible (Table 1). Besides freshwater crayfish, only a few other decapod crustaceans are known to be susceptible to infection, with these being carriers apparently resistant to disease

(Table 2, Figure 2). In contrast, once highly susceptible species are infected, there is often 100% mortality with little or no evidence of acquired immunity (Unestam & Weiss 1970).

For many decades, there were no reports of development of resistance to *A. astaci* in European species of crayfish (Svärdson 1992; Unestam 1973; Westman 1991). However several more recent studies have demonstrated increased resistance in some populations of European crayfish (Makkonen et al. 2012b) and evidence of latent, subclinical *A. astaci* infections in European crayfish including noble crayfish (Astacus astacus), and narrow clawed crayfish (*A. leptodactylus*) (Jussila et al. 2011b; Kokko et al. 2012; Svoboda et al. 2012; Viljamaa-Dirks et al. 2011; Martin- Torrijos et al. 2017; Jussila et al. 2017).

Naive and highly susceptible crayfish species exposed to *A. astaci* usually die within several days or weeks post-infection (Unestam, 1969a; Unestam, 1973), with time to death depending on variables such as the host species, strain of *A. astaci*, infective dose and water temperature (Makkonen et al. 2012b; Makkonen et al. 2014).Introduction of *A. astaci* into a new environment outside of North America is usually followed by complete or near-complete eradication of native crayfish in the watershed downstream of the site of infection (Alderman 1993), with some examples of infection spreading at a rate of around 15 km per year (Parvulescu et al. 2012).

North American freshwater crayfish species and Chinese mitten crabs are known carriers, although prevalence estimates vary greatly, ranging from anywhere between 0% and 100%, but typically between 20% and 80% depending on host species and locality (Filipova et al. 2013; Kozubíková et al. 2009; Kozubíková et al. 2011b; Parvulescu et al. 2012; Schrimpf et al. 2013a; Schrimpf et al. 2013b; Schrimpf et al. 2014; Tilmans et al. 2014). In carrier animals, *A. astaci* is present in black or brown lesions in the carapace or soft cuticle (Figure 5) that become visible 3 to 4 months after moulting (Nylund & Westman 1983; Svärdson et al. 1991; Unestam 1969c; 1972; Unestam & Söderhäll 1977).

European crayfish species may also present with brown, melanised spots. This was seen in Turkey prior to a large-scale mortality event (Baran & Soylu 1989; Rahe & Soylu 1989; Skurdal & Taugbøl 1992) and may have been the result of epidemiological factors such as low zoospore numbers and low water temperatures, resulting in a prolonged period between infection and death (Alderman et al. 1987). Conversely it may demonstrate some degree of innate resistance to infection (Unestam 1969c).

The immune response of invertebrates such as freshwater crayfish is different to that of vertebrate animals but does involve both humoral and cellular defence mechanisms. The major host response to fungal infection involves melanisation of the fungal hyphae (Unestam & Weiss, 1970). Species susceptibility to crayfish plague appears to be the result of differences in the immune response and the amount of chitinase or proteinase inhibitors present in the cuticle, rather than any structural differences between species (Cerenius et al. 2003; Nyhlén & Unestam 1975; Unestam & Söderhäll 1977). Phenolic substances appear to be produced more rapidly around *A. astaci* hyphae in resistant species preventing further spread into the underlying muscle and nerve cord (Unestam & Söderhäll, 1977,; Unestam & Weiss,1970) (refer to section 1.6.4)

No vaccine is available to control crayfish plague. There is no evidence that vaccines offer long-term protection in crustaceans, and vaccination of natural populations of crayfish is also considered logistically impossible (OIE 2018d).

1.6 Epidemiology

The pattern and severity of crayfish plague outbreaks is dependent on several factors.

Host factors such as the innate susceptibility of the species, the presence of stress factors and damage to the exoskeleton are important in determining the severity of disease within individual animals and populations (Cerenius et al. 1988).

Factors relating to the pathogen, such as the substrain of the fungus, and environmental factors such as water temperature, influence the number of zoospores produced and, therefore, the potential for the disease to spread to other susceptible crayfish (Makkonen et al. 2012b; Makkonen et al. 2014; Strand et al. 2012).

Other factors such as stocking density are also important in determining the pattern of an outbreak in a crayfish population.

1.6.1 Incubation period

Lifecycle stages of *A. astaci* present in the environment include both spores and zoospores. *Aphanomyces astaci* spores can survive for up to 2 weeks in distilled water at 20°C and at least 2 months at 2°C (Unestam 1966). Zoospores emerging from spores can swim for up to 3-5 days at 20°C (Unestam 1966) but are capable of encystment and re-emergence up to 3 times, extending the period of their infective viability (Cerenius & Söderhäll 1984b; 1985).

Survival of zoospores is reduced at higher water temperatures. The proportion of motile zoospores was almost 100% at temperatures ranging from 4°C to 18°C, reduced to around 60% at 20°C and about 20% at 25°C in most *A. astaci* strains, except for a strain from *Procambarus clarkii*, in which 80% of the zoospores were still motile at 25°C, but no motile spores were found at 27°C (Diéguez-Uribeondo et al. 1995).

1.6.2 Persistence of the pathogen

Outbreaks of crayfish plague often occur when *A. astaci* is introduced to naive populations of susceptible crayfish. Common sources of infection include:

- introduction of infected, live or dead crayfish to a water body
- introduction of decapod carrier species to a water body
- introduction of water containing spores or zoospores from infected water bodies
- transfer of water droplets, spores and zoospores on or inside vectors such as fish, birds, terrestrial animals and fomites such as boats, nets, boots, crayfish traps and so on

Animal reservoirs

- **Crayfish**

Infected crayfish (both alive and dead) are the main source of *A. astaci* zoospores in the environment. Frequently the introduction of resistant carriers with no or few visible lesions is responsible for transfer of the disease to susceptible crayfish that co-inhabit the same water body (Alderman et al. 1990; Holdich et al. 2009; Parvulescu et al. 2012; Vorburger & Ribi 1999). On other occasions, when crayfish and zoospore densities are low, a long-term, low-grade

pattern of mortality may occur because there are fewer crayfish to act as reservoirs of infection (Alderman 2002; Alderman et al. 1990; Diéguez-Uribeondo & Söderhäll 1999; Fürst 1995).

- **Other crustaceans**

While *A. astaci* does not infect non-decapod crustaceans (Table 2, Figure 2), other decapod crustaceans, such as crabs and shrimp can be infected with no clinical signs of disease, and therefore act as lifelong carriers of infection (Table 1, Figure 2). In areas where species such as the Chinese mitten crab (*Eriocheir sinensis*), river crab (*Potamon potamios*) or freshwater shrimp (*Macrobrachium dayanum*) occur in the environment, these species can potentially act as reservoirs of infection (Benisch 1940; Svoboda et al. 2014a; Svoboda et al. 2014b; Tilmans et al. 2014) or vectors (Schrimpf et al. 2014). Indeed, between 60% and 75% of Chinese mitten crabs sampled from the River Rhine were positive for *A. astaci* infection (Schrimpf et al. 2013a; Schrimpf et al. 2014). Infected *E. sinensis* can transmit *A. astaci* horizontally to noble crayfish (*Astacus astacus*) by cohabitation (Schrimpf et al. 2014).

- **Other animals**

Consumption of infected crayfish by mammalian predators such as otters, mink or muskrats, and avian predators such as water birds, have sometimes been linked to the spread of crayfish plague in Europe, but scientific studies have found that zoospores are unlikely to survive the temperatures of the gastrointestinal tract of mammals or birds (Oidtmann et al. 2002). The movement of fish is of greater concern in the spread of crayfish plague (Alderman 1993; Alderman et al. 1987; Oidtmann et al. 2002; OIE 2018d) because zoospores remain viable in fish mucus, intestinal tracts and faeces. The cleaning and gutting of fish from other water bodies is another potential source of infection (Häll & Unestam, 1980).

Environmental reservoirs

- **Water**

Under ideal conditions, even small amounts of water can transfer enough zoospores to infect a new water body. Zoospores can rapidly spread downstream in river currents (Parvulescu et al. 2012), while movement upstream is slower and occurs by the movement of infected crayfish or other carriers such as Chinese mitten crabs (Schrimpf et al. 2014; Svoboda et al. 2014b) or fish (Oidtmann et al. 2002). Weirs, waterfalls or large tracts of water containing no crayfish, may slow the spread of the disease via carrier crayfish, however spread may still occur in such environments if other reservoir species such as freshwater crabs and shrimp are present (Schrimpf et al. 2014).

- **Fomites**

Spores and zoospores of *A. astaci* can also be spread by contaminated equipment (for example, nets, traps, boots, clothing) that has not been adequately disinfected (Alderman etal. 1987; Nylund et al. 1993; OIE 2018d; Reynolds 1988).

Inactivation of the pathogen

Aphanomyces astaci does not survive at –5°C and below for more than 24 hours in culture, although –20°C for greatr than 48 hours may be required to kill the pathogen inside infected crayfish tissues (Oidtmann et al. 2002). Sporangial formation and discharge occurs down to 4°C (OIE 2018d). *Aphanomyces astaci* is also killed by a short exposure to temperatures of 60°C and above, and the pathogen does not remain viable in crayfish tissues that have been subject to

normal cooking procedures (for example,. boiling for greater than 1 minute) (Oidtmann et al. 2002).

Sodium hypochlorite and iodophors are effective for disinfection of contaminated equipment (Alderman & Polgase 1985; OIE 2018d), as are peracetic acid (Jussila et al. 2011a), VirkonS, Proxitane5:14 (Jussila et al. 2014) and some fungicides including dichloraphen sodium, hexachlorophene and clotrimazole (Alderman & Polglase 1984). Equipment must be cleaned prior to disinfection, since organic matter decreases the effectiveness of iodophors (Alderman & Polgase 1985; Jussila et al. 2014).

Thorough drying of equipment (>24 hours) is also effective as *A. astaci* is not resistant to desiccation (OIE 2018d). For more information on inactivation of *A. astaci*, readers are referred to section 2.2.9 of this document and in the AQUAVETPLAN operational procedures manual – Decontamination (DAFF 2008).

1.6.3 Modes of transmission

Horizontal spread

Horizontal transmission of *A. astaci* from host to host occurs through the release of spores and zoospores from an infected animal, movement of the zoospores through the water, and attachment to a new host (Figure 1). The zoospores of *A. astaci* swim actively in the water column and display positive chemotaxis towards crayfish (Cerenius & Söderhäll 1984a). Schrimpf et al. (2014) found that infected Chinese mitten crabs (*E. sinensis*) can also transmit *A. astaci* horizontally to noble crayfish (Astacus astacus) by cohabitation. Methods of horizontal transmission include:

- translocation of infected crayfish
- water on wet boats, pumps, nets, traps, waders, fishing gear or other equipment
- sediment containing encysted zoospores, including contaminated items such as damp, muddy rubber boots
- vector-to-crayfish spread by other crustaceans, fish, terrestrial animals and humans

For the most virulent strains of *A. astaci*, a dose as little as 1 zoospore per millilitre of water can infect susceptible crayfish and cause mortality on average within 13.5 days at 18°C (Makkonen et al. 2014). Higher dose rates significantly reduce the time to mortality (Alderman et al. 1987; Makkonen et al. 2014). Minimum infective doses are higher for the less virulent strains, but also vary significantly depending on the strain or species (and stressors) of the target host (Makkonen et al. 2012b).

Spores of cold water strains may remain viable in the water for at least 2 months at 2°C (Unestam 1966), at least 2 weeks at 15°C (Svoboda et al. 2013) and for up to 3 to 5 days at 20°C (Unestam 1966), but are not infective at water temperatures above 25°C (Unestam 1969b). However, warm water strains of *A. astaci* that infect red swamp crawfish (*Procambarus clarkii*) can sporulate at water temperatures up to 29.5°C (OIE 2018d).

Strand et al. (2012) found that sub-clinically infected American signal crayfish in the carrier state shed around 2700 spores per crayfish per week in the absence of death or moulting events, with significantly more spores being shed at 18°C compared to 4°C. Strand et al. (2012) also

recorded a threefold increase in sporulation of A. astaci during moulting and the terminal stages of disease in the resistant signal crayfish. In contrast, Makkonen et al. (2013) studied sporulation in the susceptible European noble crayfish (*Astacus astacus*) and found sporulation peaked at an average of 67 times pre-mortality background levels in the first 48 to 72 hours after the death of the host before declining to pre-mortality levels by 108 hours post-death. This indicates that over 20 times the number of spores may be released by susceptible hosts compared to resistant hosts during the terminal stages of infection.

Vertical spread

Makkonen et al. (2010) found that berried female signal crayfish (*Pacifastacus leniusculus*) infected with *A. astaci* had eggs which were PCR positive for *A. astaci*, suggesting there was potential to spread the pathogen to juvenile crayfish, presumably via the surface of eggs. While "true" vertical transmission of the pathogen inside the egg was not demonstrated these findings demonstrate another potential infection pathway and the need to use egg disinfection treatments (such as iodophors) to prevent spread of *A. astaci* via crayfish eggs sourced from regions where *A. astaci* is known to be endemic.

1.6.4 Factors influencing transmission and expression of disease

North American species of crayfish appear to have a host-parasite balance which ensures continuity of both species without major population crashes. Overt disease in North American species, resulting in crayfish mortality, only occurs following stressful events such as overcrowding or unseasonal weather (Diéguez-Uribeondo & Söderhäll 1993; Smith & Söderhäll 1986). This sporadic pattern of disease outbreaks is typical of a long term co-existence of the host and parasite (Fürst 1995; Unestam 1973). Geographical location rather than taxonomic affinity is important in determining susceptibility to crayfish plague. For example, while North American *Pacifastacus leniusculus* is in the same family as European crayfish and is resistant to the disease, Japanese *Cambaroides japonicus* are phylogenetically related to crayfish from the eastern regions of the United States but are susceptible to the disease (Unestam, 1972) (Figure 2).

Stress factors that predispose resistant crayfish to crayfish plague include suboptimal water quality, high stocking density and intra- and interspecies aggression (Table 3). The presence of abrasions on the exoskeleton also increases the likelihood of A. astaci gaining entry into the crayfish cuticle. This is more likely to occur immediately after the moult, when the exoskeleton is still soft.

Table 3 Factors predisposing crayfish to acute crayfish plague infection

Predisposing factor	References
Host	
Susceptible species	Unestam (1969c); Unestam & Weiss (1970)
Recent moult	Smith & Söderhäll (1986)
Damaged exoskeleton	Unestam & Weiss (1970)
High stocking density	Fürst (1995)
Stress	Smith & Söderhäll (1986); Cerenius et al. (1988)
Starvation	Smith & Söderhäll (1986)
Handling	Smith & Söderhäll (1986)

Predisposing factor	References
Presence of other diseases	Smith & Söderhäll (1986)
Pathogen	
Strain suitable to environment	Diéguez-Uribeondo et al. (1995)
Number of zoospores produced	Unestam (1969a); Unestam (1969c); Unestam & Weiss (1970)
Environment	
Salinity suitable to *A. astaci* (<20 ppt)	Unestam (1969a); Lilley & Inglis (1997)
Temperature suitable to *A. astaci*	Unestam (1969a); Cerenius et al. (1988); Diéguez-Uribeondo et al. (1995)
Poor water quality parameters causing stress to crayfish	Svärdson et al. (1991); Diéguez-Uribeondo & Söderhäll (1993)

Host factors

- **Host species**

Many studies have highlighted the difference between the susceptibility to *A. astaci* of crayfish from Northern America and species from other parts of the world, including Australia (Table 1). Fewer spores are needed to cause disease in susceptible species and there is less evidence of an effective host immune response to the fungal infection (Unestam 1969c; Unestam 1975; Unestam & Weiss 1970). Although North American species of crayfish are frequently infected with *A. astaci*, it does not usually cause overt disease (Nyhlén & Unestam 1975; Unestam & Söderhäll 1977).

In resistant North American crayfish, most invading zoospores are rapidly encapsulated by melanin (Figure 5) and killed. The formation of melanin is a result of activation of the prophenoloxidase (proPO) system. The proPO enzyme is produced in the cuticle and haemocytes following exposure to carbohydrates (the β-1, 3-glucans) in the fungal cell walls (Söderhäll 1981; Söderhäll & Cerenius 1998; Unestam 1972; Unestam & Söderhäll 1977). The glucans activate a serine protease which in turn induces a Ca++-dependant phenoloxidase attachment to the fungal hyphae (Smith & Söderhäll 1986; Söderhäll 1981; Sritunyalucksana & Söderhäll 2000).

However, in susceptible crayfish species melanisation is slower, allowing more zoospores to survive and proliferate. Immunological studies show that resistant North American crayfish continuously produce high levels of proPO transcripts, priming one of their key defensive mechanisms to combat *A. astaci* infection. In contrast, susceptible crayfish species tend to have lower proPO transcript levels, correlating with systemic *A. astaci* infection through uncontrolled hyphal growth (Cerenius et al. 2003; Phillips et al. 2008). Unlike refractory species however, proPO levels in susceptible crayfish can be increased by immunostimulants (Cerenius et al. 2003).

- **Stage of moult**

Crayfish appear to be more susceptible to crayfish plague at the time of moulting (Smith & Söderhäll 1986). The physiological events during moulting and/or the increased ease of injury of the soft exoskeleton during this period may pre-dispose crayfish to the disease.

- **Physical damage to the exoskeleton**

Damage to the exoskeleton is likely to increase the chances of infection by *A. astaci* (Smith & Söderhäll 1986; Unestam & Weiss 1970). Injury and damage is more likely to occur during handling, moulting or fighting, particularly at higher stocking densities in aquaculture (Vorburger & Ribi 1999).

Environmental factors

- **Water temperature**

Aphanomyces astaci substrains found on introduced *Pacifastacus leniusculus* in Europe are inherently cool/temperate water pathogens. Crayfish are readily infected between 2°C and 20°C, whereas at 25°C not all animals become infected (Alderman et al. 1987; Smith & Söderhäll 1986; Unestam 1969a). Zoospore production and duration of infectivity are both increased at 13°C compared to 20°C (Cerenius et al. 1988). At temperatures below 10°C, infected crayfish take longer to die and there are more gross signs such as limb autotomy (limb loss) and melanisation (Alderman et al. 1987). At higher temperatures and high challenge, gross muscle necrosis is often the only disease sign. In a study using Australian red claw and strains of *A. astaci* from the United States, *A. astaci* was more pathogenic at 14°C than at 20°C (Roy 1993).

- **Salinity**

Seawater and brackish water inhibits the release of zoospores from sporangia and zoospore motility (Unestam 1969a). Lilley & Inglis (1997) found that mycelial growth and sporulation of one strain of *A. astaci* was inactivated at 10 ppt salt (as NaCl), while 20 ppt salt was required to deactivate a second strain.

Pathogen factors

- **Characteristics of pathogen substrain**

There are at least five strains of *A. astaci*, each having variable growth, sporulation and zoospore characteristics, and different temperature tolerances (Diéguez-Uribeondo & Söderhäll 1993; 1999; Huang et al. 1994; Makkonen et al. 2012a; Viljamaa-Dirks et al. 2013). Whole-genome analysis (RAPD-PCR) and microsatellite arrays demonstrate genetic differences among the different strains (Grandjean et al. 2014; Huang et al. 1994), with evidence suggesting that each original host species harbours its own strain of *A. astaci* (Grandjean et al. 2014).

The first characterised strains consisted of isolates from noble crayfish in Sweden and throughout Europe. These "Group A" *A. astaci* strains are less virulent to European crayfish and are assumed to represent the first genotype of *A. astaci* introduced to Europe approximately 150 years ago (Makkonen et al. 2012a; Viljamaa-Dirks et al. 2013).

A second group of isolates "Group B" was originally obtained from signal crayfish sampled from Sweden and the USA, and from noble crayfish from Sweden. This group has since spread throughout many European countries (Grandjean et al. 2014; Vrålstad et al. 2014).

"Group C" is represented by a single isolate from signal crayfish imported into Sweden from Canada. The "Group D" strain found in red swamp crayfish *Procambarus clarkii* in southern and central Europe (Rezinciuc et al. 2014) grows better at higher water temperatures, can sporulate at water temperatures up to 29.5°C (OIE 2018d), and has greater zoospore motility between 18°C to 25°C than other substrains (Diéguez-Uribeondo & Söderhäll 1993). A Group D strain was recently detected in the parthenogenetic marbled crayfish (or Marmorkrebs, *Procambarus fallax* forma *virginalis*) in Germany (Keller et al. 2014).

The most recently recognised strain "Group E", was isolated from the introduced spiny cheek crayfish (*Orconectes limosus*) in Europe (Diéguez-Uribeondo etal. 1995; Kozubíková et al. 2011a).

The general characteristics of the strains described to date are summarised in Table 4.

Table 4 Summary of the main characteristics of 5 different strains of *Aphanomyces astaci*

Group	Genotype	Original host	Date	Virulence	Distribution
A	As	Unknown	1850s	Lowered in some substrains	USA, Europe
B	Ps1	*Pacifastacus leniusculus*	1960s	Virulent	Lake Tahoe USA, Sweden, Europe
C	Ps2	*Pacifastacus leniusculus*	1970s	Virulent	Pitt Lake, Canada, Sweden
D	Pc	*Procambarus clarkii*	1970s	Remains virulent at higher water temperatures	USA, Spain, Germany
E	Or	*Orconectes limosus*	1890s	Virulent	Czech Republic

Note: the approximate date of introduction into Europe and present distribution is included.

- **Repeated zoospore emergence**

Zoospores of *A. astaci* can encyst and re-emerge as zoospores up to three times (Cerenius & Söderhäll 1985), effectively increasing zoospore survival time and the chance of contacting a susceptible crayfish host. This is thought to be a specific adaptation to their parasitic lifestyle, although the process is temperature dependent. Zoospores are unlikely to survive for more than one or two weeks at 14°C (Cerenius & Söderhäll 1985; Svoboda et al. 2013), but they may survive for up to eight weeks at 2°C (Unestam 1966; 1969a).

- **Zoospore density**

The density of both zoospores and crayfish influence infection and mortality rates, resulting in variable patterns of disease transmission in susceptible populations (Alderman etal. 1987; Diéguez-Uribeondo & Söderhäll 1993; Makkonen et al. 2014; Unestam 1969a; Unestam & Weiss 1970). Where zoospore and susceptible crayfish density is high, and water temperature optimal for *A. astaci* (12°C to 17°C), large scale mortalities can occur within days, and entire populations may be eliminated rapidly.

1.7 Impact

Crayfish plague is believed to have first been introduced into Europe from North America in the 1860s, following the translocation of American freshwater crayfish into rivers in Italy (Edgerton et al. 2004). Introduction of *A. astaci* was quickly followed by very high (>90%) mortalities and even local extirpation of native crayfish populations in many areas of Europe (Oidtmann et al. 2006). In many cases the first sign of a problem was the presence of large numbers of dead crayfish in a river or lake.

Due to the highly pathogenic nature of crayfish plague in susceptible crayfish species, it is widely recognised as a key threatening process in several European crayfish species assessed as threatened or endangered (Holdich et al. 2009). High mortalities during outbreaks of crayfish plague in farmed redclaw crayfish (*Cherax quadricarinatus*) in Taiwan (OIE 2014b) confirm that

a similar outbreak in Australia would likely have devastating consequences for both wild and cultured crayfish. Once introduced into water bodies in the wild, A. astaci has proven virtually impossible to eradicate.

While *A. astaci* does not directly affect biota other than crayfish, epizootics of crayfish plague can have adverse ecosystem effects due to the recognised role of crayfish as keystone species in aquatic food webs (Holdich 2002; Holdich et al. 2009). Crayfish can exert top-down effects on freshwater community structures by acting as higher-order predators (Momot 1995). Crayfish can also exert bottom-up effects as detritivores, playing an important role in the degradation and recycling of organic matter.

When crayfish populations are decimated by crayfish plague, sedimentation of organic material can increase (Vrålstad et al. 2011). This can have negative effects on water quality and result in changes to aquatic vegetation. There are also likely to be additional direct and indirect ecological effects caused by the loss of crayfish from freshwater systems, reflecting their complex functional role at multiple trophic levels, including loss of food sources for predatory fishes and birds (Dorn & Wojdak 2004).

Socio-economic impacts of crayfish plague outbreaks are also significant, as the disease causes high mortalities in farmed and wild populations of susceptible crayfish. In value terms, Australia's freshwater crayfish aquaculture production in 2016-2017 was $4.44 million, with a total volume of 149.1 tonnes (ABARES, 2017). Marron (*Cherax tenimanus*) and Redclaw (*C. quadricarinatus*) farming contribed almost equal amounts ($1.75 and $1.70 million respectively), with a smaller contribution from yabbies (*C. destructor*) at almost $1 million (ABARES, 2017).

Around 80% of the total production value in marron farming was generated in the Western Australian sector, which also accounted for over one third of freshwater crayfish production in volume terms (51.7 tonnes). The economic impact on Australian freshwater crayfish aquaculture production as a result of a crayfish plague incursion would therefore depend on where the pathogen established, and which industry sub-sectors were ultimately affected.

Besides the obvious threats to crayfish aquaculture industries, decline or even potential extirpation of wild crayfish populations can also drastically impact recreational and commercial fishing activity for freshwater crayfish, imparting significant socioeconomic harm to communities in many parts of the world, including Australia, where crayfish can have cultural and food source significance for indigenous communities (Geddes and Jones 1997).

Finally, given that crayfish plague is listed by the OIE as an internationally notifiable disease (OIE 2018a; 2018d), translocation of the disease into Australia or zones within Australia could have significant implications for trade of crustacean products.

2 Principles of control and eradication

2.1 Introduction

While a minimal infectious dose has not been definitively established, a single zoospore or drop of infected water could potentially start an outbreak of crayfish plague, and once introduced into the wild, crayfish plague has never been eradicated from a region (OIE 2018d). Bearing this in mind, this section provides background information to enable the choice of the most appropriate response option following detection of crayfish plague in Australia.

Control measures, sometimes backed by specific legislation such as the Prohibition of Keeping of Live Fish (Crayfish) Order 1996 (England and Wales), are in place in several European countries. Despite legislation and extensive public awareness campaigns (Nylund & Westman 1995a; Oidtmann et al. 1999; Westman & Westman 1992), spread of the disease by human activity continues to be the major cause of new outbreaks.

Eradication of the disease from a water body requires the complete removal of crayfish and other potential reservoirs of infection. This is difficult to achieve, particularly when plague-resistant species are present in a watershed (Vorburger & Ribi 1999). Populations of susceptible crayfish can recover naturally following a crayfish plague outbreak if no infected crayfish or zoospores are present and if juveniles or adults migrate from uninfected areas. However, it is often more than a decade before stocks reach harvestable numbers, and outbreaks of crayfish plague frequently recur in recovered stocks. Furthermore, in Europe the full magnitude of the threat posed by spread of crayfish plague via ornamental crayfish introduced through the aquarium fish industry is only now being fully realised (Holdich et al. 2009; Keller et al. 2014; Mrugala et al. 2015; Schrimpf et al. 2013a).

Australia also recognises the threat posed by the ornamental crayfish trade. Live ornamental crayfish are not permitted for import, although there have been several recent detections of imported ornamental species. Under Commonwealth biosecurity legislation, only cooked freshwater crayfish are permitted to be imported into Australia for human consumption.

Control measures that have been used in Europe include:

- prohibition of movement of live or dead crayfish between water bodies
- crayfish are only to be transported in new boxes
- drying and/or disinfection of boats and equipment before transfer to a second water body and between crayfish catching seasons
- bans on the importation of second hand harvesting or handling equipment
- boiling of crayfish at their place of capture or by the first purchaser
- bans on catching crayfish in infected waters
- bans on movements of water (via tankers etc.) into different water bodies
- bans on the importation of live or uncooked crayfish.

(CEFAS 2000; Cueller & Coll 1983; Oidtmann et al. 1999; Skurdal & Taugbøl 1992; Taugbøl et al. 1993; Westman 1992).

Rapid diagnosis and effective action after a sudden mass death of crayfish is important in management of a potential outbreak of crayfish plague. Two problems that have been identified overseas during outbreaks are: that disinfection methods in the field are expensive or unsuitable, and that instructions for crayfish farms are often inappropriate or unclear (Alderman 2002).

Australia has three advantages that may help in eradication of the disease.

- Firstly, much of Australia is a dry environment, which offers considerable advantages for zoning or compartmentalisation during an emergency disease response.
- Secondly, its hot climate may potentially reduce the growth rate of mycelia and spore production of temperate strains of *A. astaci*.
- Thirdly, there are no established populations of resistant North American crayfish.

Nevertheless, eradication of the disease from rivers or water bodies in close proximity to disease outbreaks will be extremely difficult. In Australia, there are three main rearing systems in which freshwater crayfish occur (see the AQUAVETPLAN Enterprise Manual, (DA 2015)). The methods of control or eradication will in part be determined by the type of system affected by crayfish plague.

2.1.1 Open systems

If an outbreak occurs in an open system (a river, creek, or lake), eradication will be extremely difficult. Destruction and removal of all crayfish and other potential decapod crustacean reservoir hosts from the water body by chemical treatment may be the only viable means of eradicating the infective agent, but the indiscriminate destruction of other aquatic animals would likely render this option highly unpalatable to the broader public. Strong community engagement programs would be required to raise public awareness of the importance of an eradication plan, and to inform the public of their potential role in preventing the spread of the pathogen (see section 2.5). A precedent for such large-scale removal of fish has been observed in Norway, where eradication of the salmon ectoparasite *Gyrodactylus salaris* was successful in smaller river systems using rotenone to destroy fish (Johnsen & Jenser, 1991).

2.1.2 Semi-closed systems

In semi-closed systems the movement of crayfish can be controlled and there is partial control of the distribution and flow of water. Many crayfish aquaculture facilities are semi-closed systems with fenced ponds or dams but runoff may enter waterways or ponds.

2.1.3 Closed systems

In closed systems both crayfish and water movements can be controlled. If outbreaks occur in closed systems such as recirculating aquaculture facilities with no runoff into nearby waterways, eradication may be easier to achieve with less ecological damage.

The basic principles of eradication and other response options are described in the AQUAVETPLAN Enterprise Manual (DA 2015) and the AQUAVETPLAN Control Centres Management Manual (DAFF 2001).

2.2 Methods to prevent spread and eliminate pathogens

2.2.1 Quarantine and movement controls

Prevention of movement of infected crayfish and the pathogen is the first priority in the event of an outbreak or suspected outbreak. Affected farms, water bodies and rivers must be identified and quarantined without delay.

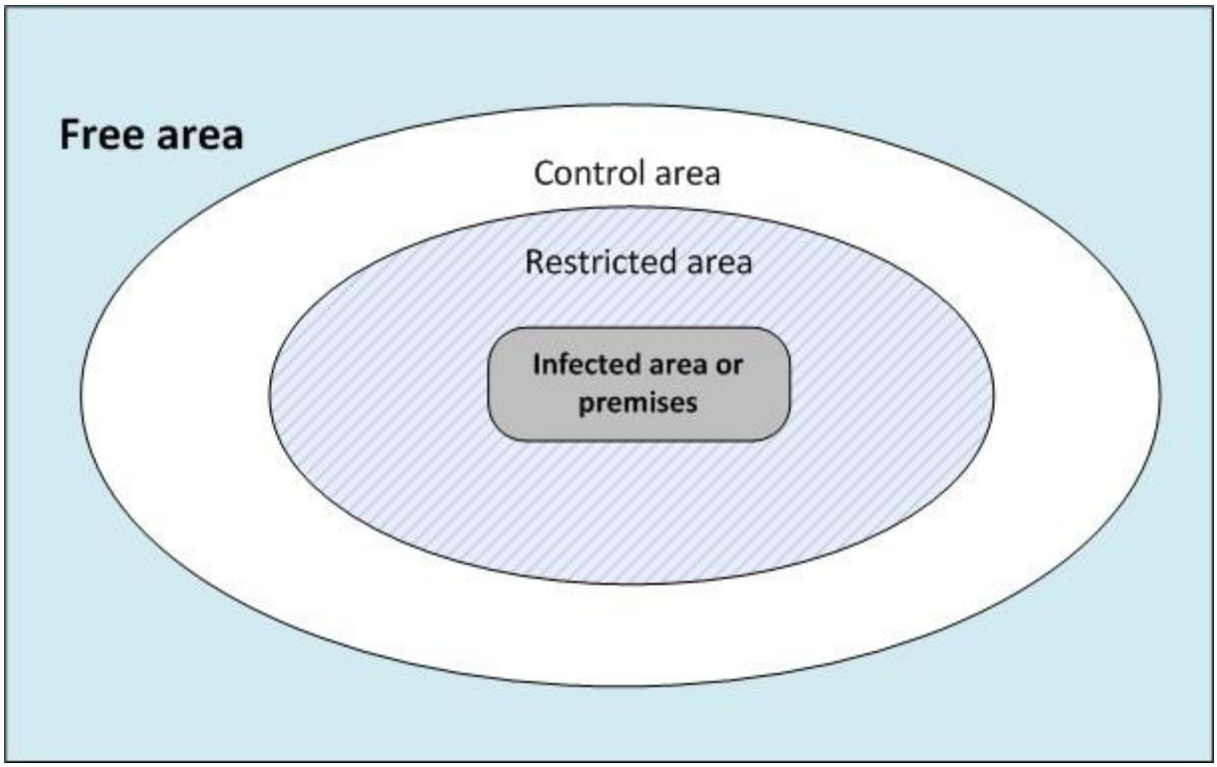
The following quarantine and movement restrictions should be implemented immediately upon suspicion of crayfish plague.

Establishment of quarantine areas

Establishment of specified areas (see AQUAVETPLAN Enterprise Manual Section A for more details), 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 *A. astaci* remains unassessed).

Figure 7 Establishment of specified areas to control *A. astaci*



In the declaration of quarantine areas under state or territory fisheries or livestock legislation, the following factors need to be considered:

- location of affected facilities (for example, crayfish farms) or affected wild crayfish populations
- location of other facilities and operations in the area (for example, crayfish farms, processors, fish markets, commercial and recreational fishing) and their practices
- the level of biosecurity routinely practised by the facilities (for example, whether there is movement of vehicles, equipment or personnel between farms, and the biosecurity precautions taken)
- the level of biosecurity practised by commercial and recreational fishers targeting wild crayfish
- the potential use of crayfish as bait by recreational fishers
- where the crayfish farms or processing facilities discharge their water
- distribution of wild crayfish populations in the area
- predominant water movement (for example, river flow) with respect to affected wild or farmed crayfish in the area
- presence of potential reservoir hosts or vectors (for example, other crustaceans, fish, scavenging birds, water rats)
- natural or manmade barriers to spread of the disease (for example, waterfalls, dams, weirs, areas without populations of suitable hosts).

The following practices must be considered when implementing response options:

- harvesting (both wild and farmed) and transportation of live crayfish to processing plants or live holding facilities
- movement of live crayfish for non-harvest purposes (for example, translocation of live crayfish between farms, from the wild to farms, or to different areas in the wild as bait etc.)
- movement of potential vectors (for example, reservoir hosts including finfish, mammalian and avian predators and scavengers, farm staff) between regions (wild and farmed)
- discharge of effluent from crayfish farms and processing plants
- movement of commercial and recreational fishers and their equipment (for example, nets, waders, vehicles)
- disposal of dead crayfish.

Movement controls

Movement controls include:

- bans on the movement of live or dead crayfish into, within, or out of infected and restricted areas
- restrictions on, or suspension of recreational and/or commercial fishing, and the movement of finfish within the declared area

- restrictions or bans on movement of people, vehicles or equipment within and between farms, dams or river systems containing crayfish within the declared area
- bans on use of high risk products (e.g. crayfish or parts thereof) as bait.

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.

2.2.2 Zoning and compartmentalisation

Zoning

If crayfish plague were to become endemic in specific regions of Australia, a zoning policy specific for crayfish plague may be necessary to protect non-infected areas and to prevent further spread of infection. Zones would be based on the distribution of *A. astaci* and any vector species present, the geographical and hydrological characteristics of affected water bodies, and predictions of the most likely method of spread of infection.

Zoning may rely on the identification of biogeographic barriers. A corresponding surveillance and monitoring program for *A. astaci* would be required to support the zoning policy. Principles of zoning for infected and non-infected zones in Australia are outlined in the AQUAPLAN Zoning Policy Guidelines and in the OIE Aquatic Animal Health Code (OIE 2018a).

Compartmentalisation

A compartment means 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. The key is that it must have in place a biosecurity management system the meets guidelines provided in Chapters 4.1 and 4.2 of the OIE Aquatic Animal Health Code (OIE 2018a), and that these systems have been documented by the competent authority (that is, the veterinary authority of the jurisdiction).

Disease management in aquatic environments

The establishment of Disease Management Area (DMA) boundaries during an Emergency Aquatic Animal Disease event present particular difficulties requiring detailed consideration beyond that normally required for terrestrial animal disease control. Water movement through and around farms, or within and between streams, rivers or other water bodies represent a substantial risk for spread of disease through transfer of infectious pathogens in the water

column, movement of infected material (particularly on suspended organic and inorganic matter), and any infected wild organisms.

For example, although a crayfish plague infected area may be established around an individual land-based farm, water bodies adjacent to the infected area and in the same catchment should also be considered for monitoring and control measures. The establishment of DMA boundaries around farms or wild fisheries on rivers may need to include comparatively large areas upstream and/or downstream taking into consideration local currents, natural or artificial barriers (dams, weirs, waterfalls) and the normal range of susceptible wild species.

Establishment of the relevant DMA boundaries must take into account dispersal of water discharged from any infected semi-closed aquaculture systems (for example, farms, hatcheries), and how this enters adjacent waterways. Similarly, outbreaks in semi-open systems (crayfish farms) require the consideration of all connected waterways and distribution of wild host or vector populations. Spread of infectious material through scavenging by other species also needs to be considered.

As a result, the geography, water flow, distance between crayfish farms and the range of susceptible species will define where DMA boundaries are placed, rather than individual property boundaries typically associated with terrestrial disease responses.

Establishment of DMA boundaries and their classification must also take into account potential mechanisms for disease transgression across established boundaries. In most circumstances it is advisable to overestimate the size of DMAs and reduce their area as the response takes effect. In most cases in the initial response, DMA boundaries will need to include the entire catchment area in freshwater systems.

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, and defining restricted and control areas. The information gathered from tracing will assist in determining the most appropriate response action. The immediate steps required are to trace-back all contacts with infected crayfish (or other infected hosts), premises and sites (to establish the origin of the outbreak) and to trace-forward all contacts with infected crayfish, premises and sites (to establish the current location and potential spread of infection).

The following items must be traced;

- movements of crayfish (or other infected hosts)
- movement of crayfish products
- movement of infected water/effluent
- movement of humans, and any other animals (both aquatic and terrestrial) that may act as vectors of the disease
- movement of vehicles, and any other equipment (waders, traps, nets etc.) that may act as fomites

- the location of other potentially infected farms/premises/areas

2.2.4 Surveillance

Surveillance is necessary to:

- define the extent (geographical and host range) of infection with *A. astaci*
- detect new outbreaks
- establish restricted, control and disease-free areas to which quarantine and movement restrictions are applied during an attempted eradication response
- establish infected and uninfected zones/compartments for an *A. astaci* containment and control response
- monitor the progress and success of an eradication strategy for *A. astaci*
- provide evidence to support declarations of disease freedom for trade purposes

Sensitive and specific diagnostic techniques have been developed which can be used to screen environmental samples and large numbers of crayfish for presence of *A. astaci* (see section 1.4.2). Surveillance programmes may be designed for detection of infection, or detection of clinical disease. For the former, molecular diagnostic techniques such as qPCR can be used to screen environmental samples for the presence of *A. astaci* spores. For the latter, techniques such as wet mounts, histology and/or culture of the pathogen can (and should) be used, but these are likely to be less sensitive compared to the molecular diagnostic techniques.

In all cases, confirmation of crayfish plague caused by infection with *A. astaci* requires either generation of a positive result from either PCR or qPCR followed by verification of the amplicon sequencing, and/or successful culture of *A. astaci* and confirmation of the identity of the isolate(s) by morphological criteria and PCR/qPCR and verification of the amplicon via sequencing.See section 1.4 for information on how the OIE (2014d) defines a confirmed case of crayfish plague.

Detailed information on general requirements for surveillance for recognition of freedom from infection is provided in the OIE Manual of Diagnostic Tests for Aquatic Animals (OIE 2018d). The manual also provides information on targeted surveillance for A. astaci. Online epidemiological calculators are available to assist with developing a surveillance plan (for example, Epitools).

2.2.5 Treatment of infected host species

There are no known treatments that can successfully treat crayfish infected with *A. astaci*. However, horizontal infection from crayfish to crayfish may be halted by treating water with >25 mM of $MgCl_2$ (Rantamäki et al. 1992), or >10 mg L^{-1} peracetic acid, or filtration to <5 µm (Jussila et al. 2011a).

Sodium hypochlorite, peracetic acid, iodophors, VirkonS and a variety of fungicides are effective for preventing sporulation and/or mycelial growth (Alderman & Polgase 1985; Jussila et al. 2011a; Jussila et al. 2014; OIE 2018d). See sections 1.6.2 and 2.2.9 for more information regarding inactivation of *A. astaci*.

Any chemicals used on crayfish must be approved for that use by the Australian Pesticide and Veterinary Medicines Authority (APVMA) (Appendix B). In addition, any chemical that is used

directly or indirectly for the control of an animal disease is governed in its use by relevant 'control of use' legislation in each state and territory. The relevant state or territory authority (in most cases this is the Chief Veterinary Officer within the relevant state department of primary industry or agriculture) should also be consulted for advice prior to the use of the chemical.

2.2.6 Treatment of host products and by-products

Crayfish from a declared area that are not clinically diseased may be suitable for human consumption but must be rendered free of harbouring viable mycelia or zoospores. Boiling at 100°C for one minute or cooking at 90°C for at least 10 minutes kills all mycelia and spores and therefore ensures that crayfish are no longer infectious (CEFAS 2000; Oidtmann et al. 2002; OIE 2018a), as does freezing at –20°C for 72 hours (Oidtmann et al. 2002; OIE 2018a).

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

2.2.7 Destruction of hosts

An important method of preventing further spread of crayfish plague is to ensure that crayfish and zoospores are not moved from infected premises. Destruction as applied to terrestrial animals in response to outbreaks of exotic disease where the slaughter of animals provides a buffer zone around infected sites is appropriate for crayfish plague.

Treatment of material from euthanased crayfish by boiling for one minute or cooking at 90°C for at least 10 minutes will kill the host and deactivate all mycelia and spores, ensuring that crayfish are no longer infectious (CEFAS 2000; Oidtmann et al. 2002;), as does freezing to –20°C for 72 hours (Oidtmann et al. 2002).

Methods suitable for the euthanasia of crayfish and the disposal of dead animals are outlined in detail in the AQUAVETPLAN Operational Procedures Manuals: Destruction (DAFF 2009a), and Disposal (DAFF 2009b).

2.2.8 Disposal of hosts

Destroyed crayfish must be disposed of by a method such as burning or burial followed by liming (Cueller & Coll 1983). Additional potential means of disposal are outlined in the AQUAVETPLAN Operational Procedures Manuals: Destruction (DAFF 2009a), and Disposal (DAFF 2009b), and section 2.2.6.

2.2.9 Decontamination

There are several methods of decontamination that are used for items such as boats, boots, nets, lamps, tools, baskets and containers.

Desiccation

Mycelia and spores are both killed by drying for 48 hours (Smith & Söderhäll 1986). Drying surfaces may be the most effective and easy method of preventing spread of the disease on crayfish handling equipment and boats in some circumstances.

High temperature

30°C for 30 hours or 37°C for 12 hours is sufficient to kill zoospores and mycelia (Oidtmann et al. 2002; Smith & Söderhäll 1986) but temperatures of 50°C are preferred as some strains may survive the lower temperatures (K. Söderhäll, pers. comm.). Cooking at 90°C for >10 minutes or boiling at 100°C for >1 minute are also proven methods of killing *A. astaci* (see OIE (2014a)).

Chemical disinfection

Several chemicals successfully kill mycelia and zoospores. The most widely used and readily available is sodium hypochlorite, but iodophors, hydrogen peroxide and peracetic acid in hydrogen peroxide can also be used (Jussila et al. 2014; Lilley & Inglis 1997) and are registered for use by the Australian Pesticides and Veterinary Medicines Authority (APVMA).

Gross contamination such as mud on boots and equipment must always be removed prior to disinfection.

- **Chlorine**

Sodium hypochlorite and many of the solid chemicals used to provide free available chlorine in swimming pools and spas in Australia such as calcium hypochlorite are suitable sources of chlorine. Chlorination of water used to clean equipment and effluent from infected premises or processing facilities renders it free of *A. astaci* prior to release into the environment. A dose rate of 100 mg L^{-1} of free available chlorine for at least 30 seconds is required for this purpose (Alderman & Polgase 1985). Liquid chlorine sources rapidly lose their strength hence the need to measure free chlorine prior to and during application. It should be noted that chlorine based disinfectants damage rubber products such as vehicle tyres and gumboots.

- **Iodine**

A dose of 100 mg L^{-1} available iodine supplied as iodophors for at least 32 minutes is an effective disinfectant for crayfish plague. The time necessary is dependent on the brand of iodophor (Alderman & Polgase 1985). Iodophors are less corrosive than chlorine-based disinfectants but are not suitable for all items as they leave residual stains (Alderman & Polgase 1985).

- **Peracetic acid and hydrogen peroxide**

Treating surfaces with 100 mg L^{-1} of Proxitane0510 (5% peracetic acid in hydrogen peroxide, a commonly used disinfectant in the food industry), for 5 minutes was an effective disinfectant (Lilley & Inglis 1997). Jussila et al. (2014) found that Proxitane 5:14 (a solution of 15% hydrogen peroxide and 15% peracetic acid) was effective at concentrations of 10 mg L-1 and 30 mg L^{-1}. Peracetic acid deactived *A. astaci* spores on clean and dirty cotton fabric, respectively. Jussila et al. (2011a) showed that *A. astaci* sporulation was prevented by constant exposure to 10 mg L^{-1} peracetic acid without mortality of crayfish, however they considered such a high concentration of peracetic acid in rearing water would likely cause mortality to any fish that may be present, and disruption to microbial growth in biofilters.

- **VirkonS**

As little as 120 mg L^{-1} VirkonS (21.4% solution of potassium peroximonosulphate (Oxone) and 1.5% sodium chlorine) was effective for deactivating *A. astaci* spores in-vitro, however doses of 1000 mg L^{-1} and 3000 mg L^{-1} VirkonS were required to deactivate all spores on clean and dirty cotton fabric materials, respectively (Jussila et al. 2014).

- **Not recommended**

Some chemicals may have a place in the control of crayfish plague but cannot be recommended as disinfectants on the basis of current knowledge. Formaldehyde, (solutions of 37% formaldehyde gas in water are commonly called formalin) inhibited hyphal growth, spore formation and germination of *A. astaci* at 80 mg L^{-1} formaldehyde (Häll & Unestam 1980). Formalin might be a useful disinfectant at higher concentrations but there are no other published reports of its efficacy against *A. astaci*.

Malachite green was a commonly used fungicide in aquaculture, however, its use is no longer condoned as it is a potential carcinogen and bioaccumulates in animal tissues (Treves-Brown 2000). After one hour, *A. astaci* mycelia and zoospores in 2 mg L^{-1} malachite green are no longer viable (Häll & Unestam 1980; Lilley & Inglis 1997).

Magnesium chloride is another chemical that was investigated for its effects on *A. astaci*. Low concentrations (20 mM) decreased mycelial growth and prevented sporulation. At higher levels (25 to 100 mM) it prevented transmission of the disease to susceptible crayfish (Rantamäki et al. 1992).

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

- the source and location of infection
- the type of enterprise (for example, farm, processing plant, hatchery, grow out ponds, water source etc)
- the construction materials of the buildings/structures on the site
- the 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 indicator.

2.2.10 Vaccination

No vaccine is available to control crayfish plague. Currently, there is no evidence that vaccines offer long-term protection in crustaceans (OIE 2018d). However, ProPO levels in susceptible crayfish can be increased by immunostimulants (Cerenius et al. 2003).

2.2.11 Vector control

Controlling the spread of crayfish plague between watercourses is difficult because zoospores can be spread by carrier crayfish walking to new locations, or translocated on equipment, motor vehicles, water or by other animals such as fish, water birds or rats. Potentially any water-borne entity, including detritus, could carry zoospores on its surface and spread disease.

The potential role of 4WD vehicles in the physical translocation of crustaceans between water bodies should also be considered; a recent study demonstrated that the red swamp crayfish *Procambarus clarkii* could be successfully transported over long-distances in mud adhering to off-road vehicles (Banha et al. 2014). Furthermore, other decapod crustaceans besides crayfish, such as crabs and shrimp can be subclinical carriers of infection (Table 1, Figure 2). It is therefore likely that other freshwater decapod crustaceans in Australia, besides crayfish, could act as vectors and/or reservoirs of infection, as has been found in Europe (Benisch 1940; Schrimpf et al. 2014; Svoboda et al. 2014a; Svoboda et al. 2014b; Tilmans et al. 2014).

Understanding possible vectors of infection is important in selecting appropriate policies and restrictions during an outbreak. The local freshwater decapod crustacean biodiversity will be an important component in the management of the disease response, and special consideration should be given for the inclusion of freshwater crustacean specialists in the emergency response team, to advise on potential reservoir host biology that could be important in an epidemiological context (distribution ranges, life-history details, movement ecology, etc.).

2.3 Environmental considerations

Effluent from processing plants and aquaculture farms that have handled potentially infected animals or been infected with *A astaci* must be contained and disinfected (see section 2.2.9), to reduce the likelihood of spread of crayfish plague.

The potential negative environmental impact of water treatment during an outbreak must be considered and strategies implemented to reduce their impact. Chlorine and iodine-based disinfectants can be rendered harmless prior to discharge by the addition of sodium thiosulphate. Burial sites for crayfish that have died or been destroyed during an outbreak must be selected to prevent runoff and seepage that could pose a threat to the environment.

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

2.4 Sentinel animals and restocking measures

Restocking should only occur after *A. astaci* has been eliminated from the water body. This may involve setting of traps to verify that no crayfish remain following the outbreak, and the use of sentinel animals (disease-free, susceptible crayfish held in cages at different locations in the water body).

It can be difficult to determine that *A. astaci* is no longer present, as sentinel crayfish may not be exposed to zoospores if there are low population densities of infected crayfish and zoospores in a water body. Experience in Europe suggests that restocking should not occur within three months of elimination of the last infected crayfish, and that sentinels would need to be used for two years to demonstrate that the watershed contains no *A. astaci*.

Environmental monitoring for *A. astaci* using filtration methods followed by *A. astaci* specific quantitative real-time (qPCR) methods is also recommended for screening water bodies for the absence of *A. astaci* prior to restocking (Strand et al. 2014).

2.5 Public awareness

Community engagement programs aimed at increasing public awareness of crayfish plague are an important component of an emergency disease response. This would include educational material informing members of the local community about their particular role in limiting further spread of the disease.

In Europe, posters, pamphlets and television campaigns have been used to promote public awareness, as part of the broader emergency disease response to outbreaks of crayfish plague. Additional social media platforms including Facebook® and Twitter® are also effective means of providing real-time information updates to affected local communities, and should be considered as part of the overall communication strategy in any emergency disease response.

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

- crayfish plague is not infective for humans;
- eating crayfish that may have been exposed to crayfish plague is not considered a health risk.

2.6 Feasbility of control or eradication of *A. astaci* in Australia

The feasibility of eradicating or controlling an outbreak of crayfish plague in Australia depends upon the nature and location of the outbreak and the management strategy that is adopted. Essentially, as outlined in section 2.1, there are two response options:

- Eradication - eradication (complete elimination) of *A. astaci* from Australia (highest level of control measure and cost)
- Containment and control through zoning or compartmentalisation - limiting the pathogen to areas with endemic infection, prevention of further spread and protection of uninfected areas

A third option, control and mitigation of disease, that is, - the implementation of management practices that decrease the incidence and severity of clinical outbreaks- , is not favoured for the following reasons:

- mitigation of the disease in wild populations is not considered possible (and indeed they may be severely affected)
- mortality rates in farmed crayfish populations are likely to be so high that eradication of clinical disease is preferred over mitigation

Emergency harvest of crayfish for human consumption may be an option in both eradication and control responses if measures, approved by organisations responsible for environmental and consumer safety, are used to prevent the spread of *A. astaci*. Emergency harvesting is most likely

to be used for healthy crayfish of marketable size from aquaculture facilities within declared areas.

Emergency harvesting carries an increased risk of further spreading infection and can potentially jeopardise the success of an eradication strategy. Strict control measures are necessary to reduce this risk during emergency harvests, including:

- on-site processing to prevent spread of zoospores in water
- product removed from harvested sites or processing plants must be cooked or otherwise treated to prevent the spread of viable *A. astaci* mycelia and zoospores
- disinfection of all equipment/personnel involved in harvesting, slaughter and processing prior to moving off site
- quarantine restrictions and procedures apply to the infected site, including personnel, equipment and vehicles
- treatment of slaughter/processing effluent (including holding water and waste offal) to inactivate all *A. astaci* mycelia and zoospores

2.6.1 Response option 1: eradication

Eradication of crayfish plague is the complete removal of *A. astaci*. This is achieved by destroying all susceptible crustacea, crayfish, and other potential decapod crustacean hosts, in the designated restricted area (and possibly in the control area) to prevent spread of the pathogen to disease-free crayfish populations.

In the absence of susceptible hosts, the disease agent cannot survive for more than a few months, even at low temperatures (<4°C). Concurrent surveillance of crayfish populations in control and disease-free areas must be undertaken as part of the eradication response. Detection of *A. astaci* 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).

Unexposed crayfish

In an eradication response, unexposed crayfish in disease-free areas would not be subject to destruction notices, and commercial farms could continue normal operations provided that future exposure to infection can be prevented.

Strict farm hygiene practices and transportation protocols are necessary to ensure that there is no transfer of infection to unexposed crayfish populations via crayfish, other vectors, water, equipment or any husbandry practices. Unexposed farmed crayfish in control areas may be allowed to grow-out, although emergency de-stocking (with appropriate controls) should also be considered as part of the eradication response.

Exposed or potentially exposed crayfish

All live crayfish within a 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 crayfish stocks both within and beyond the restricted area. All crayfish must be removed from the water, destroyed and safely disposed of as soon as possible

to avoid further spread of infection to wild and farmed crayfish and the aquatic environment. Although such crayfish are safe for human consumption, their emergency harvest may jeopardise the success of an eradication strategy if it is not carefully controlled and the animals are not cooked immediately to ensure that product, water and equipment leaving the processing facility is free of viable *A. astaci*.

While the immediate destruction of all exposed crayfish within the restricted area is the preferred option, it is logistically challenging and unlikely to be successful in many cases, especially in open systems. Destruction of all exposed decapod crustaceans in open systems would be particularly difficult to achieve, and is likely not feasible.

Infected crayfish

Diseased and dead crayfish are the main source of *A. astaci* in the environment and they must be removed from the water body as a matter of urgency. Dead crayfish are not suitable for human consumption and destruction and disposal of dead, diseased and exposed crayfish is vital to the success of an eradication strategy, as moribund and dead crayfish are the main source of fungal zoospores.

Burial sites should be chosen carefully to ensure there is no contact with waterways or vectors (refer to AQUAVETPLAN Operational Procedures Manuals: Destruction (DAFF 2009a) and Disposal (DAFF 2009b)).

If there are any resistant carrier crayfish such as North American species present in a water body, they must be removed if eradication is to be successful. Eradication is unlikely to be successful or feasible if epidemiological investigations determine that infection is widespread, has no point source, is unable to be contained and is present or is potentially present in wild crayfish or other reservoir hosts in rivers.

The ability of *A. astaci* to spread rapidly downstream, infecting wild crayfish populations and other reservoir hosts (such as the migratory Chinese mitten crab in Europe), may make it impossible to eradicate in river systems. In Europe, eradication has been unsuccessful once infected carrier crayfish became established in rivers or lakes.

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

The implementation of a zoning or compartmentalisation program is of fundamental importance if containment and control is the chosen response option. While the establishment of infected and uninfected zones may be a suitable option for a containment and control response to crayfish plague, the response strategy may be more likely to succeed if the selected spatial management units are based on epidemiologically-defined compartments (see Article 4.1.1 in the OIE's Aquatic Animal Health Code (OIE 2018a) for a detailed discussion of the conceptual and practical distinctions between zones and compartments).

The use of control (or buffer) zones/compartments may also be considered as part of this disease response strategy; for example, de-stocking of crayfish in an established control zone/compartment could be used to increase confidence that uninfected zones/compartments are truly segregated from infected zones/compartments. Irrespective of whether zones or compartments are ultimately chosen as the DMAs, movement restrictions of potentially infected

crayfish, crayfish products, water, and equipment will be an important part of this response option.

The potential for animal vectors such as fish, other freshwater decapod crustaceans, water birds and water rats to spread *A. astaci* between water bodies will also need to be considered, because the primary aim of the disease control strategy is to prevent infection spreading to uninfected zones/compartments.

Ongoing, structured surveillance in control and uninfected zones/compartments will need to be implemented to prove freedom from disease. The feasibility of this option will to some extent depend on the spatial scale of the defined zones/compartments; smaller geographical zones within the same jurisdiction may offer an advantage from a local management perspective, while large epidemiological compartments crossing state or territory borders will require an integrated management strategy involving multiple local, state and federal government agencies.

Unexposed crayfish

Aquaculture and harvesting for human consumption can occur as normal in declared uninfected zones/compartments. Control measures are only required to prevent transmission of infection to unexposed crayfish in uninfected zones/compartments. Water and vehicles used to transport crayfish are known vectors in transmission of crayfish plague and this route of transmission must be managed. Immediate destruction is an option for unexposed crayfish populations located within a de-stocking control zone/compartment, as it will decrease the chance of infection spreading to unexposed crayfish stocks and help mitigate further propagation of the disease.

Exposed or potentially exposed crayfish

Potentially exposed crayfish within an infected zone/compartment are assumed to be infected. Immediate destruction remains an option for ensuring containment and control of crayfish plague. However, in a declared infected area, grow-out and slaughter is possible. Movement restrictions, in conjunction with decontamination protocols, on crayfish and crayfish products, processing equipment, people, vehicles and boats will be necessary to protect uninfected zones/compartments.

In particular, restrictions will need to be imposed on crayfish products released for human consumption to prevent spread to uninfected zones/compartments of Australia. Processing methods must ensure inactivation of all hyphae or zoospores in products (for example, if destined for domestic human consumption in zones/compartments free of *A. astaci*, the products must be cooked or otherwise processed to remove/inactivate viable fungal hyphae or zoospores).

Infected crayfish

Within declared infected zones/compartments, it must be assumed that crayfish plague will become endemic in both wild and farmed crayfish populations. The long-term commercial viability of existing freshwater crayfish aquaculture or fishery operations within declared infected zones/compartments cannot be guaranteed. The viability of local native and/or endemic freshwater crayfish populations (and other decapods crustaceans) would also be uncertain. Given the potential role of fish, fish products, vehicles and boats as disease vectors,

recreational freshwater fishing 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 crayfish plague 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

In most European countries, crayfish plague is endemic and has had a substantial detrimental effect on populations of native crayfish and both commercial and recreational fisheries. In addition, *A. astaci* has been introduced to aquaculture facilities growing European species of crayfish, resulting in considerable economic loss to business operators.

In some countries, native species have been largely replaced by the more plague-resistant and rapidly growing North American species of crayfish. Sometimes this has resulted in further spread of crayfish plague and habitat damage caused by the burrowing habits of these animals.

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

Export markets

Crayfish plague is listed as a reportable disease of aquatic animals by the World Organisation for Animal Health (OIE 2018a). Therefore, many countries have import conditions in place related to crayfish plague, such as requiring imports to be certified free of *A. astaci*. Access of Australian crayfish to export markets may be seriously affected by an incursion of crayfish plague in Australia.

The Department of Agriculture, Water and the Environment is responsible for the health certification of all exports and should be contacted for further information (mailto:export@agriculture.gov.au)

Domestic markets

The precautionary principle should be applied when considering the harvest of exposed or potentially exposed product for the domestic market from infected zones/compartments, due to the significant risk of spread of crayfish plague to uninfected zones/compartments.

Food safety requirements would need to be met and the spread of *A. astaci* to uninfected water or crayfish prevented. Decisions on the release of crayfish products to the domestic market will depend on the response strategy implemented.

3 Preferred Australian response options

3.1 Overall policy for crayfish plague

Crayfish plague is a highly contagious disease of freshwater crayfish that has the potential to cause almost 100% mortality in farmed and wild crayfish in Australia. The disease could devastate the natural ecology of freshwater habitats in affected areas because populations of native species of freshwater crayfish are likely to become seriously depleted. The freshwater crayfish aquaculture industry would also be seriously affected by the loss of overseas markets and increased costs from the implementation of extra disease control measures.

The methods used to control an outbreak of crayfish plague in Australia will depend upon the nature of the outbreak. Following epidemiological investigation, the Director of Fisheries and/or the Chief Veterinary Officer (CVO) of the State/Territory in which the outbreak occurs will select the most suitable control option.

There are two possible response options for crayfish plague in Australia:

- Option 1 - eradication of *A. astaci* from Australia
- Option 2 - containment and control via zoning/compartmentalisation with the aim of containing the pathogen within known endemic areas, thus preventing further spread of the disease to uninfected areas

Each of these response options involves the use of a combination of strategies such as:

- Quarantine and movement controls on crayfish, crayfish products, water and equipment in declared areas to prevent spread of infection
- Destruction of crayfish that may be infected with *A. astaci* as soon as possible to prevent further production and spread of fungal zoospores
- Decontamination of facilities, crayfish products, water and equipment to eliminate the pathogen
- Surveillance to determine the source and extent of infection and to provide proof of freedom from the disease; and
- Zoning/compartmentalisation to define and maintain infected and disease-free zones

Crayfish plague has the capacity to cause severe, long-term ecological damage to the freshwater environment and production losses and loss of market access in the crayfish farming industry. It will therefore be necessary to act immediately to control or eradicate the disease.

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

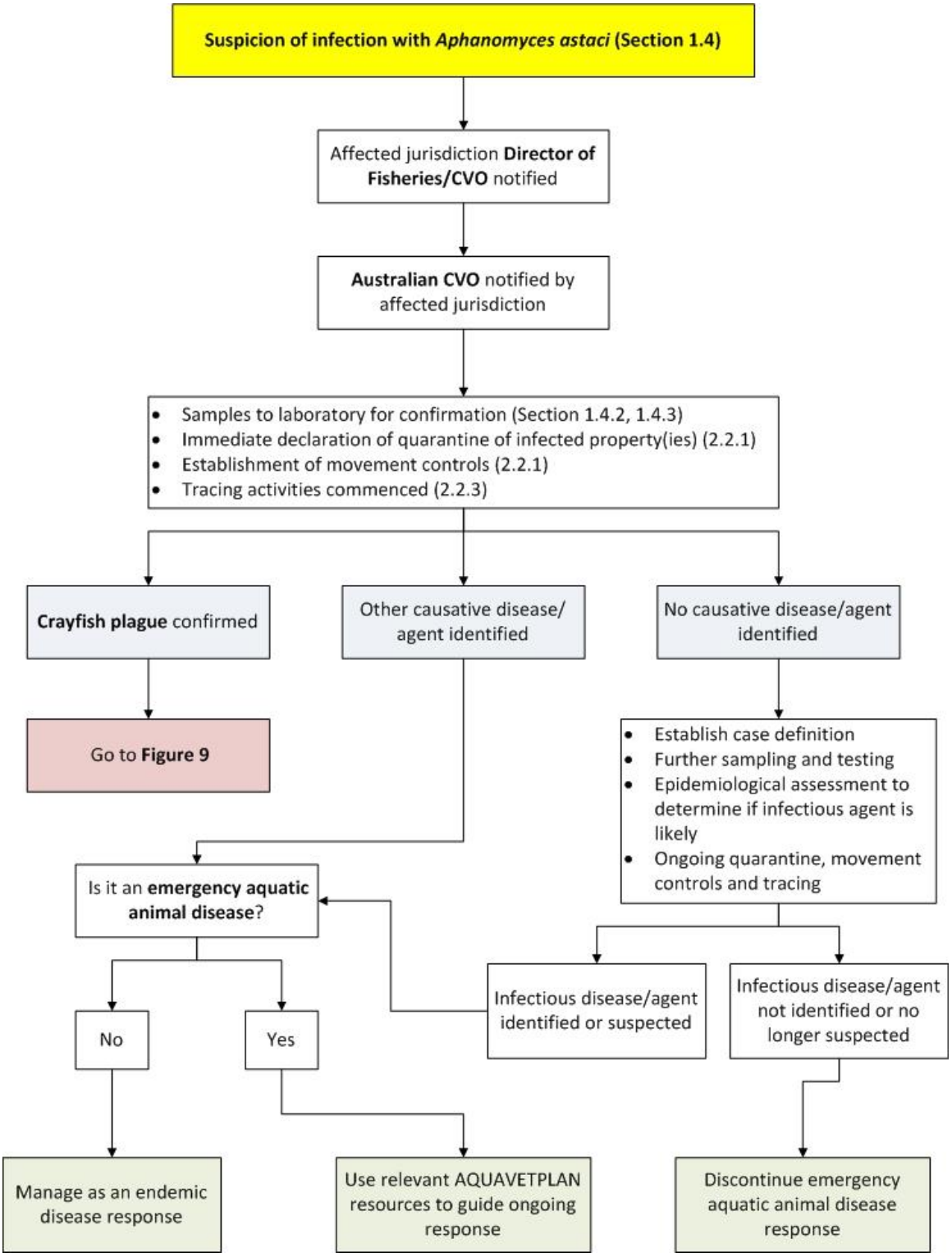
Directors of Fisheries and/or CVO's will implement the disease control measures as agreed in the EAD Response Plan and in accordance with relevant legislation. They will make ongoing decisions on follow up disease response measures in consultation with AqCCEAD. The detailed

response measures adopted will be determined using the principles of control and eradication (see section 2), epidemiological information about the outbreak and the financial feasibility of the option. For information on the responsibilities of the other state or territory disease control headquarters and local disease control centres, see the AQUAVETPLAN Control Centres Management Manual (DAFF 2001).

3.2 Response options

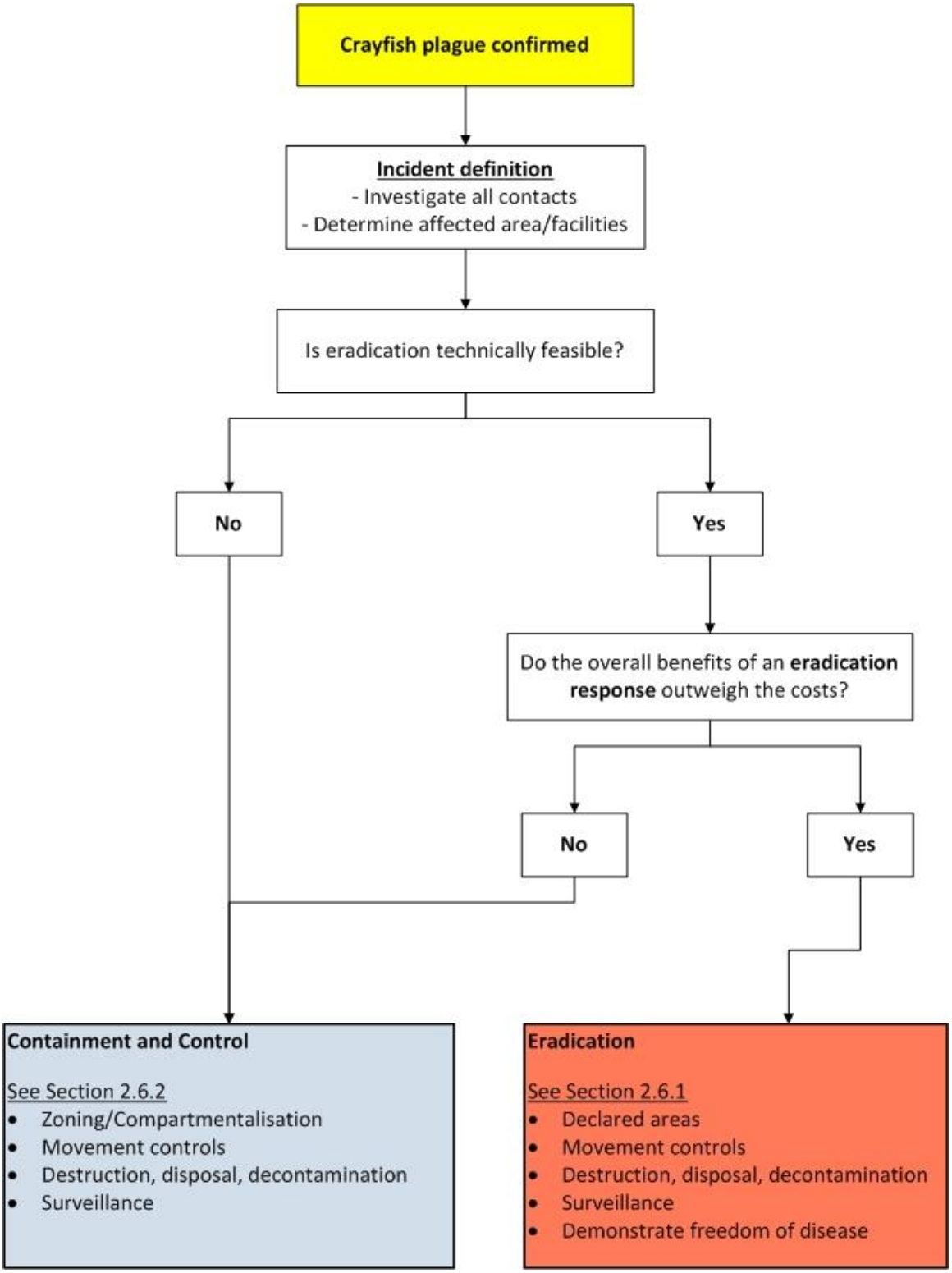
The circumstances surrounding an outbreak of crayfish plague will greatly influence selection of the most suitable response option. Figure 8 details the actions that should occur on initial suspicion of crayfish plague.

Figure 8 Decision flow chart for suspected infection with *A. astaci*



Note: **CVO** Chief veterinary officer.

Figure 9 Determining response to outbreak or confirmed infectious with *A. astaci*



3.2.1 Option 1: eradication

If an outbreak of crayfish plague occurred in Australia, there would be immediate and direct economic costs to government, the crayfish industry and recreational fishers as part of the initial response strategy. In addition to the loss of farmed and wild stock, the cost of control and eradication responses would not be insignificant. There would also be additional, indirect costs associated with environmental impacts on freshwater ecosystems if the disease became established in wild freshwater crayfish populations. Hence while an eradication response to crayfish plague has the highest short-term economic costs, the long-term environmental and economic benefits of a successful eradication program would most likely outweigh these short-term costs.

Eradication is likely to be successful in a closed system, particularly if epidemiological investigations determine an obvious point source of infection that has been or may be contained with minimal or no spread of the pathogen (for example, in a closed system such as an aquarium or fully recirculating system). In open or semi-open systems, the success of an eradication strategy would in part depend on the availability of resources for surveying and destocking wild crayfish and other reservoir hosts in the affected area.

Eradication is unlikely to be successful or feasible if tracing and surveillance activities determine that infection is widespread in commercial freshwater crayfish aquaculture operations, and/or the disease is present or potentially present in wild crayfish or other reservoir hosts. Eradication procedures include those outlined in sections 2.2 and 2.3 of this manual.

An eradication plan must include the following activities;

- quarantine and movement controls must be declared immediately and stringently enforced on crustaceans, crustacean products, water, and any vectors located in declared (for example, infected, restricted and controlled) areas.
  - restrictions must apply to movement out of the infected area of anything capable of transmitting *A. astaci* from infected to uninfected crustaceans, and to aquaculture facilities or processing plants
  - movement controls should be maintained until the agent is either eradicated or declared endemic
- all diseased and dead crustaceans must immediately be removed, destroyed and disposed of
- any exposed or potentially exposed crustaceans must immediately be removed, destroyed and disposed of
- any product from exposed or potentially exposed but clinically normal crustaceans must immediately be destroyed and disposed of
- all buildings, tanks, materials and equipment that may be contaminated including nets, boats and vehicles, must be decontaminated
- all infected crustaceans, wastes, effluent and equipment that cannot be decontaminated effectively must immediately be disposed of safely
- effluent must be treated

- restocking with sentinel crayfish can occur only after the site has been thoroughly decontaminated

3.2.2 Option 2: containment and control

If the disease became established in wild crayfish, eradication would likely be impossible. In this case, containment and control is the preferred response option. The aim is to maintain uninfected zones/compartments free of crayfish plague.

Restrictions on the movement of crayfish and crayfish products, fomites, potential vectors such as birds and ongoing surveillance and monitoring programs will be necessary to support a zoning/compartmentalisation program. The strategies outlined in sections 2.2, 2.3 and 2.4 of this manual will be implemented.

3.3 Criteria for proof of freedom

Proof of freedom from crayfish plague, which would be important for trade, can be demonstrated at the aquaculture establishment, zone and country level. Criteria for proof of freedom at each level are given in the OIE Aquatic Animal Health Code (OIE 2018a).

3.4 Funding and compensation

There are currently no national cost-sharing agreements in place for emergency responses to crayfish plague. 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.

Many countries have import conditions in place related to crayfish plague, such as requiring imports to be certified free of crayfish plague. The Department of Agriculture, Water and the Environmentand Water Resources is responsible for the health certification of all exports and should be contacted for further information (export@agriculture.gov.au).

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

OIE Aquatic Code

The objective of the OIE Aquatic Animal Health Code (OIE 2018a) is to prevent the spread of aquatic animal diseases, while facilitating international trade in aquatic animals and aquatic animal products. This annually updated volume is a reference document for use by veterinary departments, import and export services, epidemiologists and all those involved in international trade of aquatic animals and their products.

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

Chapter 9.1 of the OIE code is relevant to this manual.

OIE Aquatic Manual

The purpose of the OIE Manual of Diagnostic Tests for Aquatic Animals (OIE 2018d) is to contribute to the international harmonisation of methods for the surveillance and control of the most important aquatic animal diseases. Standards are described for laboratory diagnostic tests and the production and control of biological products (principally vaccines) for veterinary use across the globe.

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

Chapter 2.2.1 of the OIE manual is relevant to this manual:

Further information

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

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.

Minor use permit system

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

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

Emergency use permits

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

For further details or permit application forms, visit the APVMA website (www.apvma.gov.au).

Appendix C: Culture methods for diagnosis of *Aphanomyces astaci*

See also section 1.4.2 of this document for more details on sampling, wet preparations and molecular identification.

Sampling

Moribund animals are preferred, but if they are not available, specimens for examination should be transported to the laboratory within 12 hours of death of the animal. The animals should be chilled but the temperature during transportation should not go below 4°C as freezing will destroy the pathogen. Animals may be kept at an appropriate temperature by wrapping in paper and placing in a plastic bag, which is then put on ice in a small esky.

Culture

Fungal culture is carried out on isolation medium (IM) at 16°C to 20°C for 15 days (Alderman & Polglase 1986). The medium consists of:

- 1.0 g of yeast extract,
- 5.0 g of glucose,
- 10 mg oxolinic acid,
- 12.0 g agar in 1000 mL of natural river water
- 1.0 g of penicillin G is filter sterilised and added after autoclaving the other ingredients

(Alderman & Polglase 1986)

An excised piece of abdominal cuticle 1 to 2 mm in size is placed into the middle of an IM agar plate. Placing the piece of tissue within a sterile stainless steel washer on the plate will assist in allowing *A. astaci* to grow through the agar and away from the contaminating bacterial growth. Incubate the plate at 16°C to 20°C for 15 days.

Fungi are normally differentiated based on the morphology of sexual reproductive stages, but because these stages are absent in *A. astaci* general morphology of the oomycetes is usually sufficient to arrive at a differential diagnosis of crayfish plague especially if the mortalities are associated with a rapid onset of high mortality.

Identification

Aphanomyces astaci grows as a colourless colony within the agar with no aerial hyphae visible. Some superficial growth may be seen at an incubation temperature of 7°C (Alderman & Polglase 1986).

Hyphae may be examined in lactophenol blue wet preparation under low power using a light microscope. *Aphanomyces astaci* hyphae are typically non-septate and 7 to 9 µm in width but may range from 5 to 10 µm (Alderman & Polglase 1986).

Actively growing cultures can be tested for the production of zoospores:

1) Use a coverslip to take a thin slice from the growing edge of the fungal colony
2) Place into an empty sterile petri dish
3) Add sufficient tap water to cover the slice of agar
4) Leave overnight at 20°C.
5) After 18 hours of incubation, examine the slice of the colony under an inverted microscope

Individual primary spores discharge through the tip of the hyphae. Released spores then round up and encyst to form a mulberry-like cluster. All species of *Aphanomyces* have spore clusters with a similar morphology. The process of release to encystment can take 2 to 5 minutes (Alderman & Polglase 1986).

Wet preparations and histopathology

Muscle or soft tissue of moribund crayfish can be examined in a wet preparation for hyphae. Melanized areas of cuticle may indicate foci of infection:

6) Thinly smear small pieces of tissue onto a glass slide
7) Allow to dry and stain with Diff-Quick or Giemsa stain
8) Examine under a light microscope

Histology slides of muscle or cuticle can be stained and examined for the presence of distinctive aseptate, wide hyphae. Appropriate stains are haematoxylin and eosin or Grocott's modification of Gomori stain (personal communication, DJ Alderman). The Grocott Gomori stain has the advantage of clearly distinguishing the black stained hyphae against a green background if fast green FCF is used (Drury & Wallington 1980).

Molecular identification

Molecular diagnostic tests have been developed to detect *A. astaci* by polymerase chain reaction (PCR), quantitative PCR (qPCR), and sequencing of PCR products (Kozubíková et al. 2011b; Oidtmann et al. 2006; OIE 2018d; Tuffs & Oidtmann 2011; Vrålstad et al. 2009).

The Australian and NZ Standard Diagnostic Procedure (ANZSDP) for Crayfish Plague is described by Buller (2008). Some of the details of the various molecular tests are presented in section 1.4.2 of this document.

Confirmation of infection with *A. astaci*

See also section 1.4.3 of this document.

For the purposes of this manual, confirmation of crayfish plague caused by infection with *A. astaci* requires either generation of a positive result from either PCR or qPCR followed by verification of the amplicon via sequencing, and/or successful culture of *A. astaci* and confirmation of the identity of the isolate(s) by morphological criteria and PCR/qPCR and verification of the amplicon via sequencing.

Appendix D: Contact details for state and territory veterinary diagnostic laboratories

Table 5 Contact details for state and territory veterinary diagnostic laboratories

Jurisdiction	Laboratory Address	Contact numbers	Email
National	CSIRO Australian Centre for Disease Preparedness Laboratory Specimen reception 5 5 Portarlington Road East Geelong VIC 3219	Phone: (03) 5227 5000 Emergency Animal Disease hotline: 1800 675 888	AFDLsubmissions@csiro.au
Queensland	Biosecurity Sciences Laboratory Block 12, Health and Food Sciences Precinct 39 Kessels Road Coopers Plains QLD 4108	For submission enquires: (07) 3708 8762 For more information 13 25 23	bslclo@daf.qld.gov.au
New South Wales	State Veterinary Diagnostic Laboratory Elizabeth Macarthur Agricultural Institute (EMAI) 'Camden Park', Woodbridge Road Menangle NSW 2568	(02) 4640 6327 Toll free: 1800 675 623	laboratory.services@dpi.nsw.gov.au
Victoria	Veterinary Diagnostic Services AgriBio Specimen Reception 5 Ring Road, La Trobe University. Bundoora VIC 3083	Phone: (03) 9032 7515 Fax: (03) 9032 7604	vet.diagnostics@depi.vic.gov.au
South Australia	Vetlab Gribbles 33 Flemington Street Glenside SA 5065	(08) 8202 3300	Glenside.enquiries@gribbles.com.au

Jurisdiction	Laboratory Address	Contact numbers	Email
Tasmania	Animal Health Laboratory – Specimen Reception Mt Pleasant Laboratory 165 Westbury Road Prospect TAS 7250	Phone: (03) 6777 2111 Fax: (03) 6344 3085	specimenreception@dpipwe.tas.gov.au
Western Australia	Department of Primary Industries and Regional Development Diagnostic Laboratory Services (DDLS) 3 Baron-Hay Court South Perth WA 6151	Phone: (08) 9368 3351 Emergency Animal Disease Hotline: 1800 675 888	DDLS@dpird.wa.gov.au
Northern Territory	Berrimah Veterinary Laboratory (BVL) Berrimah Farm Department of Primary Industry and Resources 29 Makagon Road Berrimah NT 0828	Phone: (08) 8999 2249 Fax: (08) 8999 2024	BVL@nt.gov.au

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