

# FINAL REPORT



## **Aquatic Animal Health Subprogram: Crayfish plague - disease strategy manual**

**Frances Stephens**

**March 2004**



Australian Government  
Department of Agriculture,  
Fisheries and Forestry



Australian Government  
Fisheries Research and  
Development Corporation



Author: Dr Frances Stephens

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**2002/641 Aquatic Animal Health Subprogram: crayfish plague - disease strategy manual**

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**OBJECTIVES**

- 1 Develop a draft crayfish plague disease strategy manual for management of a disease incursion.
- 2 Peer review of draft manual.
- 3 Raise industry awareness of the disease crayfish plague.
- 4 Communicate and develop disease management policies with stakeholder groups.

**NON-TECHNICAL SUMMARY**

**OUTCOMES ACHIEVED:** The production of the crayfish plague disease strategy manual provides background information on crayfish plague, its epidemiology and possible control strategies for Australia. It is a resource for the use of members of the public, government employees, aquaculturists and organisations with an interest in freshwater crayfish or the ecology and biodiversity of Australia's freshwater habitats. It was completed after extensive consultation with aquatic health specialists and the aquaculture industry and enhances Australia's ability to quickly and effectively manage an outbreak of crayfish plague.

Crayfish plague is a serious disease of freshwater crayfish in Europe. The infectious agent, *Aphanomyces astaci*, is an Oomycete that produces hyphae and zoospores and is similar to a fungus. Studies have shown that Australian freshwater crayfish are susceptible to the disease but the disease has never been diagnosed in Australia.

The production of a crayfish plague disease strategy manual was prioritised for funding as part of the Emergency Management Planning Program of the Federal Budget Initiative 'Building a National Approach to Animal and Plant Health'. The project was administered by the Aquatic Animal Health Subprogram of the Fisheries Research and Development Corporation. The manual was developed with extensive consultation with key industry and government groups, including public meetings in Brisbane, Melbourne, Perth and Adelaide. The draft manual was circulated for comment and endorsement to stakeholders following peer review by scientists in Europe with long-term research and practical experience of crayfish plague.

The manual has three sections. The first is a literature review of known information about the disease agent and the epidemiology of the disease. The second part is a summary of the control options that are available during outbreaks of crayfish plague and the third section outlines the preferred eradication/control options for Australia.

The manual has been prepared for formal endorsement by Australia's Aquatic Animal Health Committee and Primary Industries Standing Committee.

**KEYWORDS:** *Aphanomyces astaci*, crayfish plague, Oomycete, freshwater crayfish, control, marron, yabby, redclaw, disease strategy manual.

## Acknowledgements

Dr David Alderman provided substantial insight into the effects of crayfish plague in Britain. This information was useful in formulating eradication and control strategies that would be both effective and workable in Australia. The extensive comments provided on the final draft by Andrew Cameron, Mehdi Doroudi, Preston Suijdendorp, Marty Deveney and Rachel Bowater helped to make the manual more readable, accurate and usable.

## Background

Crayfish plague is caused by *Aphanomyces astaci* and is reportable to the Office International des Epizooties (OIE). It is widespread in Europe and North America but exotic to Australia and as such is listed as a notifiable disease by all Australian states and territories. Studies undertaken overseas demonstrated that many species of Australian freshwater crayfish were highly susceptible to the disease and therefore the Oomycete is considered to have the potential to cause widespread mortality in native crayfish fauna.

In Europe, the fungus was introduced to the habitat of local freshwater crayfish via the introduction of the North American crayfish which is a carrier of the disease. In many regions the native populations were destroyed, and in the absence of competition, the disease-resistant North American species replaced the native crayfish population. The fungus is spread in water by motile zoospores that infect other crayfish. The disease can also be spread by people, contaminated fishing equipment, fish and other animals.

Once established it is almost impossible to control the spread of the disease. In countries where the disease occurs, outbreaks have been associated with 100 % mortality and the subsequent alterations in ecology of affected waters may have repercussions within the fishing industry. Introduction of crayfish plague into Australia would greatly impact the yabby, red claw and marron industries. The result would be serious economic, ecological and aesthetic damage to the freshwater crayfish industry, the recreational industry, and the environment.

## Need

Keeping Australia free of crayfish plague relies on quarantine restrictions recommended by the OIE which prohibits the importation of live crayfish from countries where the disease is either reported or its presence cannot be ruled out. However, in Europe attempts to protect native stocks from crayfish plague by banning the importation of crayfish have been unsuccessful. Although quarantine protocols have so far excluded the disease agent from Australia, it is vital that an eradication and control strategy is in place in the event of a diagnosis of the disease.

This manual seeks to provide the Australian freshwater crayfish industry with an efficient

and successful eradication and control strategy for crayfish plague that can be immediately applied should a disease incursion occur. The crayfish plague disease strategy manual forms part of the AQUAVETPLAN requirements for management of disease incursions as identified under the Emergency Response Management Plan for aquatic diseases.

## **Objectives**

- 1        Develop a draft crayfish plague disease strategy manual for management of a disease incursion.
- 2        Peer review of draft manual.
- 3        Raise industry awareness of the disease crayfish plague.
- 4        Communicate and develop disease management policies with stakeholder groups.

## **Methods**

The disease strategy manual for crayfish plague was written using the manual format of the disease strategy for Furunculosis and other manuals in the AUSVETPLAN and AQUAVETPLAN Emergency Response Management Plan as a guide. A literature review of crayfish plague was undertaken. This included published journal articles, reports from organizations, searches of websites, and communication with personnel with experience in researching epidemiology of the disease or its control. The information about the disease and the disease agent is presented in the first part of the disease strategy manual.

Possible sources of introduction of the disease into Australia were compiled from the literature review as was epidemiological information such as routes of transmission. This information and a list of possible control strategies are presented in Section 2 of the disease strategy manual.

Industry groups and stakeholders were invited to complete a questionnaire and to attend a public meeting to discuss the disease and control options for Australia. Consultative meetings were held with stakeholders in Victoria, South Australia and Queensland. Groups and individuals that had expressed interest in the crayfish plague disease strategy manual were invited to the meetings in their state several weeks prior to the meeting date. The draft manual was submitted for peer review prior to submission of the draft manual to stakeholders in Australia for comment. Professor Kenneth Söderhäll, Dr Lage Cerenius (Sweden) and Dr David Alderman (UK) reviewed sections 1 and 2 of the manual. Minor changes were then made to the manual in line with their comments. Section 3 was completed and the manual was then submitted to 32 stakeholders in Australia for comment. The stakeholders are listed in Appendix 3.

## Results/Discussion

Key people from grower organisations and Fisheries Departments attended the meetings. The meetings were positively received, especially by members of crayfish growers groups, and generated useful discussion. Aquaculturists appeared to be particularly pleased that focus had been placed on communicating with their industry groups and in developing a policy for a major potential disease problem for their industry. The stakeholders that attended the meetings are shown in Appendix 3. The disease strategy manual that resulted from this process is shown in Attachment 4.

## Benefits

The main beneficiaries of the production of the crayfish plague disease strategy manual are freshwater crayfish aquaculture groups and government departments that are responsible the implementation of management of exotic diseases. Those with an interest in biodiversity and ecology of Australian freshwater habitats will also benefit from the manual.

## Further Development

There are no further activities planned to further disseminate the crayfish plague disease strategy manual. The manual has been prepared for formal endorsement by Australia's Aquatic Animal Health Committee and Primary Industries Standing Committee.

## Planned Outcomes

There main outcomes of preparation of the disease strategy manual are:

1. Increased awareness by members of freshwater crayfish aquaculture groups, government departments and diagnostic laboratories of the risk faced by Australian freshwater crayfish and the aquatic environment should the fungus *Aphanomyces astaci* enter Australia.
2. The availability of a resource for regulatory organizations in formulating an action plan following detection of crayfish plague in Australia. Implementation of a crayfish plague management plan outlined in the disease strategy manual will help to ensure that a disease outbreak is contained as quickly and efficiently as possible with minimal damage to the industry.
3. The availability of a ready reference for information about crayfish plague for use by members of the public, students, and animal health professionals.



## Conclusion

The crayfish plague disease strategy manual has been produced and all of the objectives of the project have been achieved during the course of its production.

## References

Nil

## Appendix 1

No intellectual property arose from the project.

## Appendix 2

Staff who have been involved in the project include Ms Nicky Buller and Dr Frances Stephens.

## Appendix 3

Stakeholders who attended the stakeholder meetings in the early stages of the project to discuss the control and eradication strategies for crayfish plague are outlined below.

State or territory	No. of	
Victoria	4	2 from state government departments, 2 from yabby aquaculture industry
South Australia	4	2 from state government departments, 2 from yabby and marron aquaculture
Western Australia	8	4 from 3 state government departments, 4 from the marron aquaculture industry <sup>1</sup> from the Aquaculture Council of Western
Queensland	9	2 from state government department, 7 from red claw aquaculture industry

The draft report was sent to the stakeholders listed below. A summary of endorsements and comments that were received on the draft report are outlined in the table below.

Person/representative of stakeholder group	Reply received
Peter Appleford Victorian Dept. of Natural Resources and the Environment	Yes, referred to Medhi Doroudi for comment

Andrew Cameron, CVO's Unit Victorian Dept. of Natural Resources and the Environment	Yes, comprehensive suggestions
Mehdi Doroudi CVO's Unit, Victorian Dept. of Natural Resources and the Environment	Yes, comprehensive suggestions
Greg Williams Australian Freshwater Crayfish Growers Assoc. Victoria	No
Graeme Creed Native Fish Australia, Victoria	No
Mark Crane, CSIRO Animal Health Laboratory, Victoria	No
Ray Page/Patrick Washington VR Fish, Victoria	No
Dr Robert Jones Veterinary Practitioner, Victoria	No
Bill Keast Queensland Crayfish Farmers Association Inc.	Yes
Tiina Hawkesford Dept of Primary Industries Queensland	No
Marty Deveney/Colin Johnston Aquaculture South Australia	Yes, comprehensive comment
Rod Andrewartha CVO, Dept of Primary Industries, Water and Environment, Tasmania	No
Peter Hughes Native fish Australia	No
Margaret Grose NSW Aquaculture Assoc Inc-North Coast branch	Yes
Dick Callinan, NSW Fisheries	No
Bruce Christie CVO NSW Agriculture	No
Graham Pike Recfish Australia	No
Kevin Doyle Australian Veterinary Association	No
Pierre Howitz Edith Cowan University	No

Brian Jones Dept of Fisheries Western Australia	Yes
Peter Buckman CVO, Western Australia	Yes, comprehensive comments from Preston Suijdendorp
Tom Rose Dept of Environment Western Australia	No
Cathy Shilton/Ian Roscoe Dept. of Business, Industry and Resource Development, Northern Territory	Yes, endorsement
John Luckens Australian Freshwater Crayfish Growers Association (South Australia) Inc.	No
Mary Nenke Yabby Processor, Western Australia	No
Robert Heard Biosecurity Australia	Yes, endorsement
Robin Vandegraaff CVO, South Australia	No
Ruth Reuter Veterinary Pathologist ex Idexx Laboratory South Australia	Yes, A few comments and endorsement
Dan Sampey Marron Growers Association of Western Australia	Verbal comments and endorsement only
Simon Bennison/ Dan Machin Aquaculture Council of Western Australia	No
Rachel Bowater Queensland dept of Primary Industries	Yes
David Carter Australian Dept of the Environment and Heritage	No

## **Appendix 4: DISEASE STRATEGY MANUAL FOR CRAYFISH PLAGUE**

Draft manual endorsement by Australia's Aquatic Animal Health Committee (AAHC) and Primary Industries Standing Committee (PISC).

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# 1 Nature of the disease

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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 adequately prepared to manage a disease outbreak because an incursion of the disease could devastate the freshwater crayfish aquaculture industry as well as wild populations of freshwater crayfish in Australia.

## 1.1 Aetiology

The aetiological agent of crayfish plague is the oomycete, *Aphanomyces astaci* Schikora (see reference section, Schikora 1906).

Oomycetes (commonly called water moulds) are not considered to be 'true fungi' taxonomically, but have been placed in the phylum Oomycota. Within this phylum is the Family *Saprolegniaceae*, which consists of *Achlya*, *Aphanomyces* and *Saprolegnia* with some species being pathogens of crustaceans, fish and plants.

*A. astaci* is a branching, non-septate fungus that produces spores under conditions that are favourable for each substrain. The spores can survive in fresh water for a variable time depending on water temperature and chemistry. Motile zoospores measuring 8-15µm emerge from spores and attach to new hosts within the water body.

## 1.2 Susceptible species

Freshwater crayfish species from Australia, New Guinea, Japan and Europe are highly susceptible to crayfish plague, whereas species from North America are more resistant but can die from the disease following stress that affects their immune system (Roy 1993; Unestam 1969b; 1972; 1975) (Table 1). Of the commercial aquaculture species, red claw (*Cherax quadricarinatus*) and yabbies (*Cherax destructor*) have been tested and are susceptible to the disease, however, there are no published reports of the susceptibility of marron (*Cherax tenuimanus*). It would be safe to assume, on the basis of experimental work, that all species of freshwater crayfish in Australia may be highly susceptible to the disease.

The disease has not been reported in aquatic animals except freshwater crayfish and the Chinese mitten crab *Eriocheir chinensis* (see Tables 1 and 2) but it should be noted that the susceptibility of many freshwater decapods to infection with *A. astaci* is unknown. Consequently, the likelihood of animals such as freshwater crabs and shrimp in Australia becoming carriers or developing clinical disease following infection with *A. astaci* in the wild is unknown.

**Table 1. Susceptibility of species of freshwater crayfish to crayfish plague. Only a few Australian species of crayfish have been experimentally challenged with crayfish plague but it is assumed that they are all susceptible to infection. The North American species can succumb to overt disease following stressful conditions such as overcrowding and inclement weather. The crab, *Eriocheir chinensis*, was also susceptible to infection in an experimental study by Benisch in 1940.**

Species name		Disease severity	Region of origin
Scientific	Common		
Non-Australian species			
<i>Pacifasticus leniusculus</i>	Signal crayfish	Resistant carrier <sup>1,2,5</sup>	North America
<i>Procambarus clarkii</i>	Louisiana swamp crayfish	Resistant carrier <sup>2</sup>	North America
<i>Orconectes limosus</i>	Mud crayfish	Resistant carrier <sup>2</sup>	North America
<i>Astacus astacus</i>	Noble crayfish	Overt disease <sup>1,2,5</sup>	North west Europe
<i>Austropotamobius pallipes</i>	Whiteclaw crayfish	Overt disease <sup>2</sup>	West and south west Europe
<i>Austropotamobius torrentium</i>	Stone crayfish	Overt disease <sup>4,6</sup>	Mountains in south-west Europe
<i>Astacus leptodactylus</i>	Slender clawed or Turkish crayfish	Overt disease <sup>2</sup>	Eastern Europe, Middle East
<i>Camaroides japonicus</i>		Overt disease <sup>2</sup>	Japan
<i>Cherax papuanus</i>		Overt disease <sup>5</sup>	Papua-New Guinea
Australian species			
Smooth crayfish			
<i>Cherax destructor</i>	Yabby	Overt disease <sup>5</sup>	Australia-wide
<i>Cherax quinquecarinatus</i>	Gilgie	Overt disease <sup>5</sup>	Western Australia
<i>Cherax quadricarinatus</i>	Redclaw	Overt disease <sup>3</sup>	Queensland, Northern Territory
Spiny crayfish			
<i>Astacopsis gouldi</i>	Giant crayfish	Overt disease <sup>5</sup>	Tasmania
<i>Astacopsis fluviatilis</i>		Overt disease <sup>5</sup>	Tasmania
<i>Euastacus kershawi</i>		Overt disease <sup>1</sup>	Victoria
<i>Euastacus clydensis</i>		Overt disease <sup>5</sup>	New South Wales

References: <sup>1</sup> Unestam, 1972 <sup>2</sup> Unestam, 1969b <sup>3</sup> Roy 1993 <sup>4</sup> Schikora, 1906, 1916 <sup>5</sup> Vorburger and Ribi, 1999



**Table 2** Species not found to be susceptible to *A. astaci* in experimental studies.

Species name		
Scientific name	Common name	References
<i>Mysis relicta</i> <sup>1</sup>	Mysis	Subphylum: Crustacea, Order: Mysidacea
<i>Daphnia hyalina</i> <sup>2</sup>	Daphnia	Subphylum: Crustacea, Class: Branchiopoda
<i>Leptodora hyalina</i> <sup>2</sup>		Subphylum: Crustacea, Class: Branchiopoda
<i>Chydorus sphaericus</i> <sup>2</sup>		Subphylum: Crustacea, Class: Branchiopoda
<i>Bytotrephes longimanus</i> <sup>2</sup>		Subphylum: Crustacea, Class: Branchiopoda
<i>Cyclops strenuous</i> <sup>2</sup>	Copepod	Subphylum: Crustacea, Class: Copepoda
<i>Mesocyclops leuckarti</i> <sup>2</sup>	Copepod	Subphylum: Crustacea, Class: Copepoda
<i>Asplanchna priodonta</i> <sup>2</sup>	Rotifer	Phylum: Rotifera
<i>Bosmina</i> sp. <sup>2</sup>		

References: <sup>1</sup>Unestam, 1972 <sup>2</sup>Unestam, 1969b

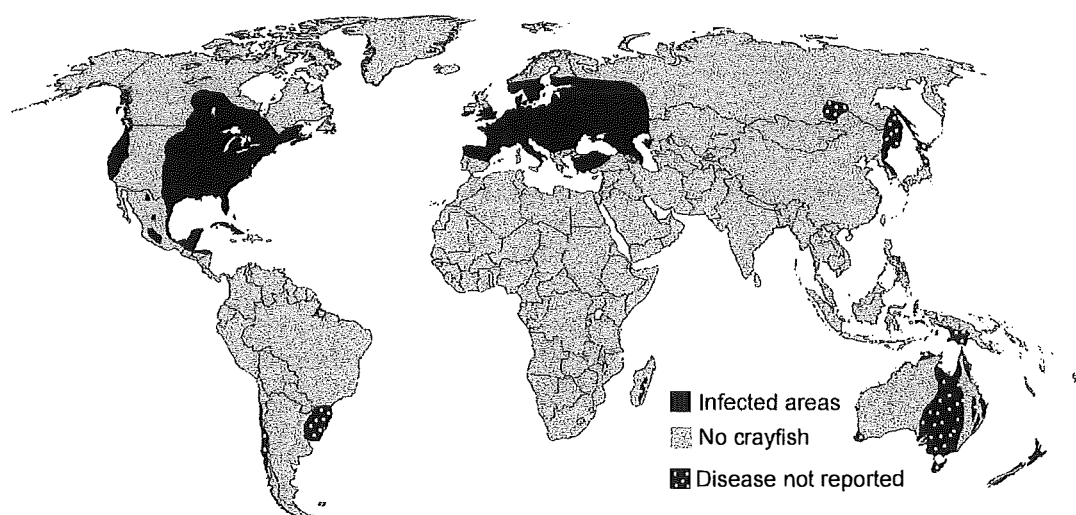
Figure 4 in Appendix 1 shows the phylogenetic relationship of various susceptible and resistant species of aquatic invertebrates that have been tested for susceptibility to crayfish plague. Figure 5 in Appendix 1 illustrates the original geographical distribution of the 3 families of freshwater crayfish.

### 1.3 World distribution and occurrence in Australia

Crayfish plague is a serious disease of freshwater crayfish in Europe and is endemic in North America, although it rarely causes overt disease in species of freshwater crayfish of North American origin except when they are stressed (Smith & Söderhäll 1986). Early reports of crayfish deaths suggest that crayfish plague was present in Europe by 1875 and there were further introductions to Europe in the 1970's with North American crayfish (Nylund, Kirjavainen, Tulonen & Westman 1993). The earliest report is from Italy, but the disease appears to have spread to the rest of Europe after 1975 from outbreaks in France and Germany. It has now been reported in Norway, Finland, Sweden, Russia, Germany, France, Switzerland, Spain, Greece, Turkey, the United Kingdom and Ireland. Losses of native European freshwater crayfish species have been catastrophic in infected waterways and considerable resources have been allocated in several countries including Finland and the United Kingdom in an attempt to eradicate or control the disease.

The advent of molecular techniques has confirmed earlier suspicions that *Aphanomyces astaci* entered Europe from North America. Four major strains of *A. astaci* were found. Three strains have been identified on *Pacifastacus leniusculus*. One strain appeared to have been introduced to Europe in the nineteenth century, a second strain was introduced to Sweden from the US after 1970 and a third strain was found on *Pacifastacus leniusculus* from Canada (Diéguez-Urbeondo &

Söderhäll 1999; Huang, Cerenius & Söderhäll 1994). A fourth strain, found on *Procambarus clarkii* in Spain and the US (Diéguez-Urbeondo & Söderhäll 1993), is adapted to warmer water temperatures. The origin of outbreaks in Europe can now be traced using these molecular tools (Lilley, Cerenius & Söderhäll 1997; Oidtmann, Cerenius, Schmid, Hoffman & Söderhäll 1999).



**Figure 1. Global distribution of crayfish plague. The grey areas indicate areas in which crayfish do not occur naturally. In some of these areas, non-native species have been introduced.**

Crayfish plague has never been reported in Australia nor found during passive surveillance. To date, no outbreaks of crayfish plague have been reported in red claw in Europe, Ecuador or the US despite the export of this species (Romero 1997; Westman & Westman 1992). Likewise, although *Pacifastacus leniusculus* has been introduced to Japan, and *Procambarus clarkii* to Kenya, South America, China, Japan, Taiwan and the Philippines (Huner 2002; Lewis 2002) there have been no reports of crayfish plague in these countries.

## 1.4 Diagnostic criteria

Crayfish plague must be suspected whenever there is a mortality event in which there are large numbers of dead crayfish but other aquatic animals remain unaffected. Diagnosis is based on clinical signs, histopathology and laboratory culture of the disease agent. Methodology for diagnosis and isolation techniques is based on culture and characterisation of the fungus and can be found in;

- Office International des Epizoties (OIE) Diagnostic Manual for Aquatic Animal Diseases, 3<sup>rd</sup> edition, 2003<sup>1</sup>
- OIE International Aquatic Animal Health Code, 5<sup>th</sup> edition, 2003<sup>1</sup>
- Crayfish plague (*Aphanomyces astaci*) Australian and New Zealand Standard Diagnostic Methods (ANZDT) (draft).
- Molecular diagnostic tests to detect Epizootic Ulcerative Syndrome (*Aphanomyces invadans*), and crayfish plague (*Aphanomyces astaci*) (ANZDT) draft.

#### 1.4.1 Clinical signs

Clinical signs of disease are often subtle, especially in acute outbreaks when environmental conditions, high zoospore numbers and a high density of susceptible crayfish results in large-scale mortality within days of infection. The behaviour of affected animals provides a clue to a diagnosis of crayfish plague as affected freshwater crayfish often demonstrate unusual gait or activity, however, the clinical signs are not specific for this disease. Infected crayfish may have some of the following characteristics. Laboratory tests and experiments to demonstrate that infected crayfish transmit the disease to uninfected crayfish are necessary to confirm the diagnosis:

- 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

References: Oidtmann *et al.* 1999; Nylund & Westman 1995; Alderman *et al.* 1990, 1987, 1984; Unestam 1972, Unestam & Weiss 1970; Nybelin 1936, 1931; Schäperclaus 1935, 1927.

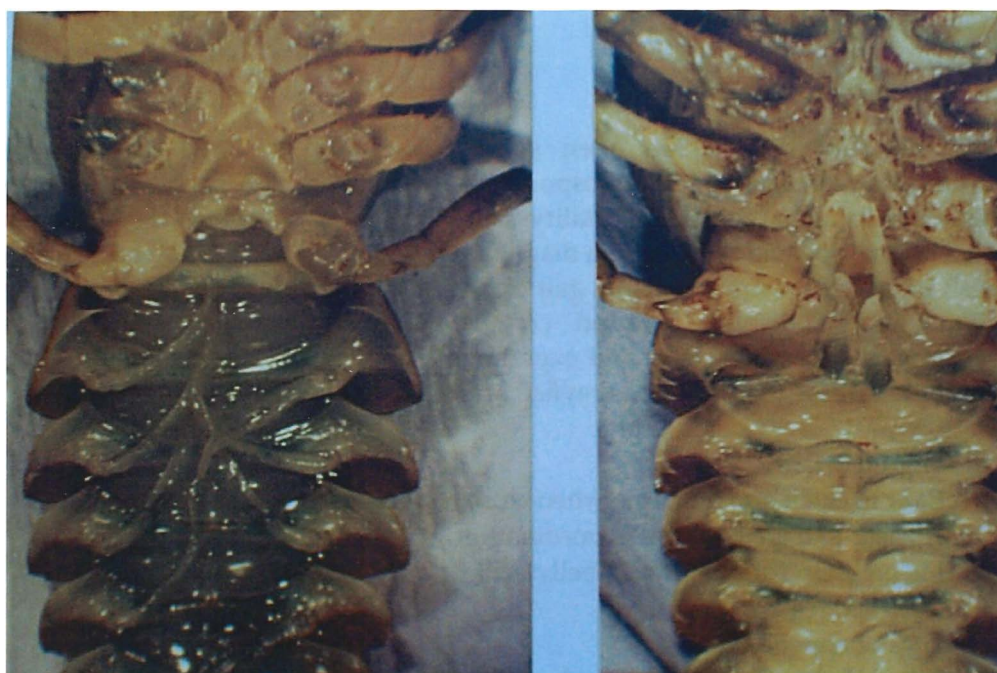
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<sup>1</sup> [http://www.oie.int/eng/publicat/en\\_enligne.htm](http://www.oie.int/eng/publicat/en_enligne.htm)

### 1.4.2 Pathology

#### Gross lesions

Gross pathological signs are not specific for crayfish plague and further tests must be undertaken to confirm the presence of *A. astaci*. Clinical signs vary greatly, ranging from no obvious signs or lesions to the presence of visible hyphae protruding from soft parts of the exoskeleton. Other signs of the disease are the presence of opaque whitish flesh between abdominal segments or brown melanised spots on the walking legs and abdomen.



A Normal Crayfish

B Infected Crayfish

**Figure 2. Crayfish plague in a susceptible species of crayfish. 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)

#### Microscopic lesions

*Wet preparations of fresh tissue.* *Aphanomyces astaci* hyphae may be seen in excised soft cuticle from affected areas of the infected tissues viewed using a light microscope.

*Histopathology.* *Aphanomyces astaci* hyphae are 5–10µm in diameter, aseptate and branching. The hyphae stain black against a green background using a Grocott-Gomori stain. 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 skeleton. 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. However, growth can be sparse and may not be seen on

histological examination. 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).

### 1.4.3 Laboratory tests

The State/Territory Chief Veterinary Officer (CVO) must be notified immediately of any suspected incidents of crayfish plague. Preliminary identification of *Aphanomyces 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.

### Submission of specimens

Samples should be submitted to the AAHL Fish Diseases Laboratory (AFDL) via the State/Territory diagnostic laboratory and the CVO. Freshly dead or dying freshwater crayfish showing signs of the disease should be submitted to the nearest state/territory diagnostic laboratory. Animals should be placed on ice, but not frozen, as freezing will kill the oomycete. Dead animals can be put into a plastic bag, and placed over ice in a small esky or other suitable container. Secure the lid of the container with tape, and wipe the outside of the container with a disinfectant such as sodium hypochlorite (100ppm available chlorine) so as to prevent transmission of the infection. The Oomycete is susceptible to drying, therefore transmission of viable spores is a low risk factor. Successful culture of the pathogen is more likely if samples are transported to the laboratory within 12 hours of death of the animal.

Sampling equipment may be available on-site, or may be obtained from State/Territory fisheries or agricultural officers (see **AQUAVETPLAN Enterprise Manual, Appendix 5** for contact details).

### Laboratory diagnosis

#### *Fungal culture*

Confirmation of suspected crayfish plague is achieved by culturing for the presence of *Aphanomyces astaci* with the identity confirmed by sporulation test. Culture of the fungus may take up to 15 days and overgrowth with bacterial flora and other fungi is also a problem. See Appendix 2.

#### *Molecular diagnosis*

Molecular diagnostic tests are being developed for *A. astaci* (Fisheries Research and Development Corporation (FRDC)-funded project 2001/621). These tests will offer a rapid detection method using fluorescent *in situ* hybridisation (FISH) that can be used on either tissue smears or histopathology slides, and provide a result within hours. A PCR test is also being developed that will detect the fungus from fresh or preserved tissues or culture.

#### 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 cotejeani* (Polglase & Alderman 1984; Schäperclaus 1927), although muscle infected with *Thelohania* spp. is a more vivid white than that infected with *Aphanomyces 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. Some bacterial infections and exposure to pesticides can cause losses of large numbers of crayfish in a pond, but are these causes of mortality are likely to also affect other crustaceans in the pond.

### 1.5 Resistance and immunity

Most freshwater crayfish species from around the world are believed to be susceptible to infection by *Aphanomyces astaci* (see Table 1). Once highly susceptible species are infected, there is often 100% mortality with little or no evidence of acquired immunity (Unestam & Weiss 1970).

Despite many decades of infection there are no reports of development of resistance to the disease in European species of crayfish (Svärdson 1992; Westman 1991; Unestam 1973). Susceptible animals die within several days or weeks of infection (Unestam 1969a; 1973), followed by complete eradication of crayfish from the watershed downstream of the site of infection. Although North American freshwater crayfish species are carriers of the disease, not every animal is infected. In carrier animals, the fungus is present in black or brown lesions in the soft cuticle and are most often seen 3-4 months after moulting (Nylund & Westman 1983; Svärdson *et al.* 1991; Unestam 1969b, 1972; Unestam & Söderhäll 1977).

European species sometimes have brown, melanised spots. This was seen in Turkey prior to large-scale mortality (Skurdal & Taugbøl 2002; Baran & Soylu 1989; Rahe & Soylu 1989;) and may have been the result of epidemiological factors such as zoospore number and water temperatures resulted in a prolonged period between infection and death (Alderman *et al.* 1987). Conversely it may demonstrate some degree of innate resistance (Unestam 1969b).

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 defence against fungal infection involves melanisation of the fungal hyphae (Unestam & Weiss 1970). 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  $\beta$ -1, 3-glucans, in the fungal cell walls (Söderhäll & Cerenius 1999; Söderhäll 1981; Unestam & Söderhäll 1977; Unestam 1972). The glucans activate a serine protease which in turn induces a  $\text{Ca}^{++}$ -dependant phenoloxidase attachment to the fungal hyphae (Sritunyalucksana & Söderhäll 2000; Smith & Söderhäll 1986; Söderhäll 1981).

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; Unestam & Söderhäll 1977; Nyhlén & Unestam 1975). Phenolic substances appear to be produced more rapidly around *Aphanomyces astaci* hyphae in resistant species preventing further spread into the underlying muscle and nerve cord (Unestam & Söderhäll 1977; Unestam & Weiss 1970).

## 1.6 Epidemiology

The pattern and severity of disease 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.* 1986). Factors relating to the fungus, such as the substrain of the fungus and water temperature, influence the number of zoospores produced and, therefore, the potential for the disease to spread to susceptible crayfish. Other factors such as stocking density are also important in determining the pattern of an outbreak in a crayfish population.

### 1.6.1 Sources of *Aphanomyces astaci*

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

- Introduction of infected, live or dead crayfish to a water system
- Introduction of water from infected water bodies
- Transfer of water droplets and zoospores from nearby water on vectors including boats, nets, boots, birds, fish and terrestrial animals

### 1.6.2 Reservoirs

#### Animal reservoirs

##### *Crayfish*

Infected and dead crayfish 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 (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; Fürst 1995; Diéguez-Uribeondo & Söderhäll 1993; Alderman, Holdich & Reeve 1990).

##### *Other animals*

Mammals such as otters, mink or muskrats and water birds have sometimes been blamed for spreading crayfish plague in Europe but scientific studies have found that zoospores do not survive the temperatures of the gastrointestinal tract of mammals or birds (Oidtmann *et al.* 2002). The movement of fish may be of greater concern in the spread of crayfish plague (Oidtmann *et al.* 2002; Alderman *et al.* 1987) because zoospores remain viable in fish mucus and fish intestinal tracts. 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 sufficient zoospores to infect a new water body. As few as 1.3 zoospores per millilitre of water can infect susceptible animals (Alderman *et al.* 1987). The zoospores rapidly spread downstream in the current in rivers. Movement upstream is slower and occurs by the movement of infected crayfish. Weirs, or large tracts of water that contain no crayfish, act as barriers to the progression of the disease via carrier crayfish.

### *Contaminated Equipment*

The majority of new outbreaks in countries where crayfish are harvested as a recreational or commercial pursuit are caused by human activity (Westman 1991) including translocation of contaminated water or crayfish from their site of origin and trapping using contaminated traps or nets that have not been adequately disinfected (Alderman *et al.* 1987; Nylund *et al.* 1993; Reynolds 1988).

## 1.6.3 Predisposing factors

North American species of crayfish appear to have a host-parasite balance which ensures continuity of both species without major population crashes. Overt disease resulting in crayfish mortality only occurs in North American species following stressful events such as overcrowding or unseasonal weather (Diéguez-Urbeondo & 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 phylogenetic origin is important in determining susceptibility to crayfish plague. For example, although *Pacifastacus leniusculus* is in the same Family as European crayfish it is resistant to the disease, whereas *Cambaroides japonicus* from Japan is susceptible to the disease although it is phylogenetically related to crayfish from the eastern regions of the United States (Unestam 1972).

Stress factors that pre-dispose 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 risk of *Aphanomyces astaci* gaining entry into the crayfish cuticle. This is more likely to occur immediately post-moult when the exoskeleton is still soft.

## Endogenous factors

### *Species*

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

### *Stage of moult*



Crayfish appear to be more susceptible to crayfish plague at the time of moulting (Smith & Söderhäll 1986). Although no scientific evidence has been presented to substantiate the influence of this factor, 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.

**Table 3. Factors predisposing crayfish to acute crayfish plague infection**

Host	Predisposing Factor	
	Pathogen	Environment
Susceptible species <sup>1,7</sup>	Strain suitable to environment <sup>4</sup>	Salinity suitable to <i>A. astaci</i> <sup>5</sup>
Recent moult <sup>2</sup>	Number of zoospores produced <sup>1,5,7</sup>	Suitable temperature for <i>A. astaci</i> <sup>3,4,5</sup>
Damaged exoskeleton <sup>1</sup>		Poor water quality parameters causing stress to crayfish <sup>8,10</sup>
High stocking density <sup>9</sup>		
Stress <sup>2,3</sup>		
Starvation <sup>2</sup>		
Handling <sup>2</sup>		
Presence of other diseases <sup>2</sup>		

References: <sup>1</sup> Unestam, 1970 <sup>2</sup> Smith and Söderhäll, 1986 <sup>3</sup> Cerenius et al., 1988 <sup>4</sup> Diéguez-Urbeondo et al., 1995 <sup>5</sup> Unestam, 1969a <sup>6</sup> Vorburger and Ribí, 1999 <sup>7</sup> Unestam, 1969b <sup>8</sup> Diéguez-Urbeondo et al., 1993 <sup>9</sup> Fürst, 1995 <sup>10</sup> Svärdson et al., 1991.

## Exogenous factors

### *Water temperature*

*Aphanomyces astaci* is a parasite adapted to living in or near chitin (Unestam 1969a). The substrains found on *Pacifasticus leniusculus* in Europe are inherently cool/temperate water pathogens. Crayfish are readily infected with the disease between 2- 20°C, whereas at 25°C not all animals become infected (Alderman *et al.* 1987; Smith & Söderhäll 1986; Unestam 1969a). At 13°C, zoospores production is greater and zoospores are more likely to be infective for longer than at 20°C (Cerenius *et al.* 1988). At temperatures below 10°C, infected crayfish took 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 (Roy 1993), *A. astaci* was more pathogenic at 14°C than at 20°C.

### *Characteristics of pathogen substrain.*

There are at least four strains of *Aphanomyces astaci*, each having different growth, sporulation and zoospore characteristics and different temperature tolerances (Diéguez-Urbeondo & Söderhäll 1993; 1999; Huang *et al.* 1994). The substrain found on *Procambarus clarkii* in Spain grows and sporulates better and has greater zoospore motility between 18 to 25°C than other substrains (Diéguez-Urbeondo & Söderhäll 1993). This appears to be an adaptation to the more temperate/subtropical climate of *P. clarkii*. It is probable that more strains with adaptations to different environmental factors will be identified in the future.

*Re-emergence of zoospores*

*Aphanomyces astaci* zoospores can encyst and re-emerge as zoospores up to three times (Cerenius & Söderhäll 1985). This is thought to be an adaptation to a parasitic lifestyle by increasing the survival time of zoospores and their chance of finding a crayfish host. This process is temperature dependent. At 14°C zoospores are unlikely to survive for more than one week (Cerenius & Söderhäll 1985), but survival may be longer at 2°C (Unestam 1969a). The pathogen survives for a limited time once infected crayfish have been removed from the water body but it is recommended that water bodies are not restocked with crayfish until three months after removal of the last crayfish (David Alderman, pers comm) .

*Zoospore density*

The density of both zoospores and crayfish influence the time taken for the disease to infect and kill individual animals and the rate of disease spread in a susceptible population (Diéguez-Urbeondo & Söderhäll 1993; Unestam 1969a; Unestam & Weiss 1970). Where zoospore density is high, water temperature optimal for *Aphanomyces astaci* and there are large numbers of susceptible crayfish, mortality occurs within days and entire populations may be rapidly eliminated. It is very difficult to be sure that crayfish plague is not present in a water shed if there is a low density of native crayfish and low zoospore numbers in which case large-scale rapid mortalities will not occur (David Alderman, pers comm.).

*Salinity.*

Seawater and brackish water inhibited the release of zoospores from sporangia and zoospore motility (Unestam 1969a). However, neither the critical level of salinity in parts per thousand nor the effects of concentrations of various mixtures of ions have been studied.

*Physical damage to the exoskeleton*

Damage to the exoskeleton is likely to increase the risk of infection by *Aphanomyces astaci* (Smith & Söderhäll 1986; Unestam & Weiss 1970). Injury and damage is more likely to occur during handling, moulting or fighting (Vorbürger & Ribi 1999).

**1.6.5 Modes of transmission**

The translocation of North American crayfish with benign infections is a major source of new disease epidemics in the United Kingdom. *Pacifastacus leniusculus* is almost always found in watersheds experiencing epidemics of crayfish plague for the first time in the United Kingdom (Alderman 1993). It should be noted that not all North American crayfish carry the fungus and uninfected North American and European crayfish co-exist in some parts of Europe. However, the disease has been impossible to contain in many European countries, especially where water bodies are separated by short distances and where human activity levels are high.

**Horizontal spread**

*Aphanomyces astaci* is transmitted horizontally in freshwater, causing acute disease in susceptible crayfish or chronic lesions of no clinical significance in resistant crayfish. Horizontal transmission can occur by:

- Translocation of infected crayfish
- Water on wet boats, pumps, nets, traps or other equipment
- Sediment containing encysted zoospores, including contaminated items such as damp, muddy rubber boots
- Vector-to-crayfish spread by fish, terrestrial animals and man

### Vertical spread

Vertical transmission, in which disease is spread from one generation to the next by infected eggs, is not a mode of transmission of crayfish plague.

#### 1.6.7 Manner and risk of introduction to Australia

The two epidemiological weaknesses of *Aphanomyces astaci*, are its obligate parasite status, whereby zoospores must find a new crayfish host within days as they have a finite survival period in the environment, and the susceptibility of mycelia and spores to boiling, drying, freezing and chemicals. These characteristics may help in preventing entry of the pathogen into Australia, however, the disease agent could still be imported illegally in:

- live crayfish from overseas
- uncooked or unfrozen crayfish products
- untreated water that may or may not contain fish, plants or invertebrates
- importation of used fishing or boating equipment from infected areas, particularly if the equipment is still damp.

The risk factors and probability of introduction of crayfish plague are being assessed by Biosecurity Australia as part of an import risk assessment. One factor that is likely to affect the likelihood of *Aphanomyces astaci* becoming established in Australia is water temperature. Some substrains of the fungus prefer temperatures of approximately 13-20°C and parts of Australia may be too warm for propagation of such strains. However, the substrain found in *Procambarus clarkii* in Spain (Diéguez-Urbeondo & Söderhäll 1999) is likely to infect Australian crayfish because this strain sporulates at higher temperatures.

## 2 Principles of control and eradication

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### 2.1 Introduction

Just one zoospore or one drop of water can start an outbreak of crayfish plague and crayfish plague has never been eradicated from a region. 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 1995; Oidtmann *et al.* 1999; Westman & Westman 1992), spread of the disease by human activity continues to be the major source of new outbreaks.

Eradication of the disease from an area or water body requires the complete removal of crayfish and thus the infectious agent. 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 new outbreaks of crayfish plague frequently occur in recovered stocks.

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 water in tankers or containers being emptied into a different water body
- Bans on the importation of live or uncooked crayfish

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

Rapid diagnosis and effective action after 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 two advantages that may help in eradication of the disease. The first is its dry environment and the second is its hot climate which may reduce the growth rate of mycelia and spore production. Nevertheless eradication of the disease from rivers or water bodies in close proximity will be extremely difficult.

In Australia, there are three main systems in which freshwater crayfish occur (see **AQUAVETPLAN Enterprise Manual, Section B 3.8**). The methods of control or eradication will be partially determined by which types of system are affected by crayfish plague:

#### *Open systems*

If the outbreak occurs in an open system (a river or creek) eradication will be extremely difficult. Methods such as removal of all crayfish from the water body by chemical means with consequential destruction of other aquatic animals may be the only viable means of eradicating the infective agent, but this option is likely to be very unpalatable to the public. A publicity campaign would be required to raise public awareness of the importance of eradication and to educate the public of the need to prevent spread of the pathogen. A precedent for such a large-scale removal of fish has been made in Norway, where rotenone was used to remove all fish from rivers in an attempt to eliminate the skin parasite *Gyrodactylus salaris* that had become established in local salmon populations. This was successful in smaller rivers.

#### *Semi-closed systems*

In semi-closed system 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-open systems with fenced ponds or dams but runoff may enter or leave waterways or ponds.

#### *Closed systems*

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

The **AQUAVETPLAN Enterprise Manual, Appendix 1** outlines the State/Territory legislation relating to disease control and eradication.

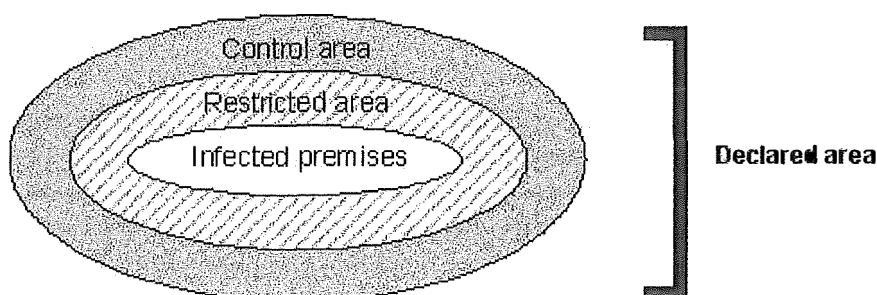
## **2.2 Methods to prevent spread and eliminate pathogens**

### **2.2.1 Quarantine and movement controls**

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

The quarantine and movement restrictions that should be implemented immediately upon suspicion of crayfish plague are:

- establishment of specified areas (Figure 3) (see **AQUAVETPLAN Enterprise Manual, Section A** for more details). The areas include:
  - a infected area or premises
  - a restricted area surrounding an infected premises or area
  - a control area which is a buffer between the restricted area and free areas
 Together these 3 areas form the declared area. The free area is the area outside the declared area and may include large areas of Australia in which *Aphanomyces astaci* does not occur or remains unassessed.



**Figure 3. Establishment of specified areas to control crayfish plague**

- Bans on the movement of live crayfish into, within, or out of infected and restricted areas
- Suspension of recreational fishing for crayfish 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

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

Zones may be gazetted. Zones are geographical regions that are delineated to decrease the spread of disease from an infected zone to a disease-free zone. The process of zoning is outlined in AQUAPLAN Zoning Policy Guidelines and in the OIE International Aquatic Animal Health Code. Zones are based on species distribution, the geographical characteristics of water bodies and landform and predictions of the most likely method of spread of disease. Zoning may not always be used in crayfish plague outbreaks.

### 2.2.2 Tracing

Tracing a disease outbreak is the process of retrospectively determining the method and pattern of disease spread. It is crucial to defining and updating the restricted area and requires investigations that determine:

- The initial source and location of infection
- The movement of infected crayfish and water

- The possible movement of vehicles, humans, animals and equipment that may act as vectors of the disease
- The presence of other potentially infected premises/areas

### 2.2.3 Surveillance

Surveillance is used to detect new disease outbreaks, to define the infected area for quarantine and movement restriction purposes and to monitor eradication and control programs. There are three methods that may be appropriate:

- A targeted survey of crayfish from different sites using laboratory testing to determine their disease status
- Observation of crayfish behaviour and mortality in various crayfish habitats around the region, state or nation
- Using healthy, susceptible crayfish as sentinels in water bodies with a high risk of having crayfish plague. If zoospores are present, susceptible crayfish are expected to develop overt disease.

### 2.2.4 Destruction and disposal of dead and infected crayfish

An important method of preventing further spread of crayfish plague is to ensure that crayfish and zoospores are not moved from infected premises. Control methods that are used for terrestrial animals in outbreaks of exotic disease where the slaughter of animals provides a buffer zone around infected sites are also appropriate for crayfish plague. Destroyed crayfish must be disposed of by a method such as burning or burial followed by liming (Cueller & Coll 1983). Methods suitable for the euthanasia of crayfish and the disposal of dead animals are outlined in more detail in the **AQUAVETPLAN Operational Procedures Manuals: Destruction and Disposal** provide further detail of suitable procedures.

### 2.2.5 Treatment of crayfish, crayfish products and by-products

Crayfish from a declared area may be suitable for human consumption but must be rendered free of any possibility of harbouring viable mycelia or zoospores. Boiling for one minute kills all mycelia and spores and therefore ensures that crayfish are no longer infectious (CEFAS 2000; Oidtmann *et al.* 2002), as does freezing to  $-20^{\circ}\text{C}$  for 24 to 72 hours (Oidtmann *et al.* 2002).

### 2.2.6 Decontamination of equipment and water

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

#### *Dessication*

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 temperature (K. Söderhäll, pers. comm.).

### 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 (Lilley & Inglis 1997). 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, as well as effluent from infected premises or processing facilities renders it free of *Aphanomyces astaci* prior to release into the environment. 100ppm of free available chlorine for 30 seconds is required for this purpose (Alderman & Polglase 1985). Liquid chlorine sources rapidly lose their strength hence the need to measure free chlorine prior to use. It should be noted that chlorine based disinfectants damage rubber products such as vehicles tyres and gumboots.
- Iodine. 100 ppm available iodine supplied as iodophors for up to 32 minutes is an effective disinfectant for crayfish plague. The time necessary is dependent on the brand of iodophor (Alderman & Polglase 1985). Iodophors are less corrosive than chlorine-based disinfectants but are not suitable for all items as they leave residual stains (Alderman & Polglase 1985).
- Treating surfaces with 100ppm of 5% peracetic acid in hydrogen peroxide, a commonly used disinfectant in the food industry, for 5 minutes was an effective disinfectant (Lilley & Inglis 1997).

Some chemicals may have a place in the control of crayfish plague but can not be recommended as disinfectants on the basis of current knowledge. Formaldehyde, commonly called formalin, inhibited hyphal growth, spore formation and germination of *Aphanomyces astaci* at 80mgL<sup>-1</sup> 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 is another such chemical. It 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, *Aphanomyces astaci* mycelia and zoospores in 2 mgL<sup>-1</sup> 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 decreased mycelial growth and prevented sporulation. At higher



levels it prevented transmission of the disease to susceptible crayfish (Rantamäki, Cerenius & Söderhäll 1992).

### **2.2.7 Effluent and environmental considerations**

Effluent from processing plants and aquaculture farms that have handled potentially infected animals or been infected with *Aphanomyces astaci* must be contained and disinfected to reduce the risk of spread of the disease. 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.

### **2.2.8 Vector controls**

Controlling the spread of crayfish plague between water courses 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 water birds or rats. Understanding possible vectors of infection is important in selecting appropriate policies and restrictions during an outbreak.

### **2.2.9 Sentinel and restocking measures**

Restocking should only occur once it has been ascertained that *Aphanomyces astaci* is no longer present in the water body. This may involve trapping to verify that no crayfish remain following the outbreak and the holding of disease-free, susceptible sentinel crayfish in cages at different locations in the water body.

It can be difficult to ascertain 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 ensure that a watershed does not contain *A. astaci* (David Alderman, pers comm).

Crayfish used for restocking must be disease-free. Sometimes restocking occurs naturally following outbreaks where all crayfish have been removed from a lake or downstream of a certain point. In such instances, uninfected crayfish migrate from streams, tributaries or across other barriers such as crayfish-free stretches of water or weirs that prevented the spread of zoospores during the outbreaks (Alderman 1993; Taugbøl & Skurdal 1993).

### **2.2.10 Public awareness**

Publicity campaigns directed at making the public aware of crayfish plague and education to prevent further spread of the disease are important in outbreaks of crayfish plague. In Europe, posters and pamphlets have been produced and awareness campaigns have been run on television in an attempt to reduce the spread of crayfish plague to uninfected crayfish populations.

## 2.3 Feasibility of control in Australia

The feasibility of 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 there are two control options for crayfish plague in Australia:

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

Emergency harvest of crayfish for human consumption may be an option in both eradication and control programs if harvesting poses no risk of spread of *A. astaci* and is approved by organisations responsible for environmental and consumer safety. Emergency harvesting is most likely to be used for crayfish of marketable size from aquaculture facilities within declared areas.

Emergency harvesting carries a high risk of further spreading infection and can jeopardise the success of an eradication strategy. Strict control measures are necessary to prevent further spread of infection including:

- Processing should be on-site to prevent spread of zoospores in water
- Product removed from harvested sites or processing plants must not contain any viable *A. astaci* mycelia and zoospores
- Disinfection of all equipment/personnel involved in harvesting, slaughter and processing
- 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 render *A. astaci* mycelia and zoospores non-viable

### 2.3.1 Eradication

Eradication of crayfish plague is the complete removal of *Aphanomyces astaci*. This is achieved by removing all crayfish and preventing spread of the fungus, thereby preventing infection of uninfected crayfish populations. Once there are no infected crayfish, the disease agent cannot survive for more than a few weeks.

#### *Unexposed crayfish*

Immediate destruction of all crayfish within a restricted area will decrease the chance of spread of infection to uninfected crayfish. This is the preferred option, however this option is not easy and is unlikely to be successful in many cases.

Uninfected crayfish may be emergency harvested for human consumption provided there is no risk of exposure to *A. astaci*. Strict hygiene practices are required at processing, and on-farm processing may be preferable, as this will prevent any potential infection during transport to off-site processing plants.

Unexposed crayfish will only be allowed to grow-out provided there is no risk of future infection. Strict farm hygiene practices and transportation protocols are necessary to ensure that there is no transfer of infection to non-infected crayfish populations via crayfish, water, equipment or any husbandry practices.

#### *Exposed or potentially exposed crayfish*

All live crayfish within a restricted area must be assumed to be infected. Therefore, grow-out is not an eradication option as it would increase the risk of spread of infection to other farms or wild crayfish stocks. All crayfish must be removed from the water, destroyed and disposed of safely 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 to ensure that product, water and equipment leaving the processing facility does not contain viable *A. astaci*.

#### *Infected crayfish*

Diseased and dead crayfish are the main source of *Aphanomyces astaci* in the environment and they must be removed from the water body as a matter of urgency. Destruction and disposal of all diseased and dead crayfish is vital to the success of an eradication strategy as 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 **AQUAVEPLAN Operational Procedures Manuals: Destruction and Disposal**).

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 in rivers. The ability of *Aphanomyces astaci* to spread rapidly downstream, infecting wild crayfish populations, 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.

### **2.3.2 Containment, control and zoning**

The implementation of a zoning program, and associated control measures to maintain uninfected zones, is necessary if this control option is chosen. Movement restrictions of potentially infected crayfish, crayfish products, water, and equipment would be an important part of this program. The potential for animal vectors such as water birds and rats to spread crayfish plague between water bodies would also need to be considered because the aim of this control strategy is to prevent infection spreading to uninfected zones. The feasibility of this option will depend on the location of the crayfish and the likely reservoirs of infection.

#### *Unexposed crayfish*

Aquaculture and harvesting for human consumption can occur as normal. Control measures are only required to prevent transmission of infection to unexposed crayfish in uninfected areas/zones. Thus, the method of harvest, equipment used

and the choice of location should ensure there is no exposure to infection. On-farm slaughter and processing may be preferable if the site is uninfected, as this will prevent any potential infection during transport to an off-site processing plant. Water used and vehicles used to transport crayfish is a vector in transmission of crayfish plague and this risk of transmission must be minimised.

Immediate destruction is an option for unexposed crayfish populations located within an infected zone or within a de-stocking area as it will decrease the chance of spread of infection to these crayfish stocks and prevent propagation of the disease.

#### *Exposed or potentially exposed crayfish*

Potentially infected crayfish must be treated as infected and immediate destruction remains an option for ensuring containment and control of crayfish plague.

In a declared area, grow-out and slaughter may be feasible without further spread of infection. However, final products must be processed to the degree required for the designated market (eg if destined for domestic human consumption in areas free of *Aphanomyces astaci*, the products must be processed so that they do not contain viable fungal hyphae or zoospores.

Grow-out of potentially exposed crayfish within infected zones is possible. Movement restrictions on crayfish and crayfish products, people, vehicles, boats and market access for final product will be necessary to protect uninfected zones. Restrictions will be necessary on crayfish products released for human consumption to prevent spread to uninfected zones of Australia. Processing methods must ensure that the final product does not contain viable hyphae or zoospores.

#### *Infected crayfish*

Infected, dead and dying crayfish with crayfish plague are handled in the same manner as they are during an eradication program. This is outlined in above in Section 2.3.1 of this manual.

### **2.3.3 Trade, industry and environmental considerations**

In most European countries, crayfish plague is endemic and has had a substantial detrimental effect on populations of native crayfish and professional crayfish harvesting industries. It has affected recreational fisheries in Sweden. In addition, *Aphanomyces 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 a 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 Office International des Épidémiologies (World Organisation for Animal Health) (OIE)<sup>2</sup>. Consequently, many countries require imports to be certified free from crayfish plague. For further information regarding export market requirements, contact the Australian Quarantine and Inspection Service (AQIS)<sup>3</sup>. Access of Australian crayfish to export markets is likely to be seriously affected by an incursion of crayfish plague in Australia.

**Domestic markets**

Public opinion and various government departments and agencies may determine the feasibility of releasing crayfish product from potentially infected animals on to the domestic market. Food safety requirements would need to be met and there must be no potential for spread of *A. astaci* to uninfected water or crayfish.

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<sup>2</sup> <http://www.oie.int>

<sup>3</sup> <http://www.affa.gov.au/quarantine.html>

## 3 Preferred control policy in Australia

### 3.1 Overall policy for crayfish plague

Crayfish plague is a highly contagious fungal 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 control options for crayfish plague in Australia:

- *Option 1 — eradication of *Aphanomyces astaci* from Australia; and*
- *Option 2 — containment, control and zoning with the aim of containing the fungus within known endemic areas, thus preventing further spread of the disease to uninfected areas*

Each of these control 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 *Aphanomyces 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 fungus*
- *Surveillance to determine the source and extent of infection and to provide proof of freedom from the disease; and*
- *Zoning 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 of the State/Territory in which the outbreak occurs will decide upon the appropriate response option in consultation with the aquatic Consultative Committee on Emergency Animal Diseases (CCEAD). The decision will be made after consideration of results of the epidemiological investigation (see Section 3.3.1). Whilst eradication may be the

preferred option, it may not be feasible, given the limited success of eradication and control policies in Europe.

For a description of the notification arrangements, order of procedures, management structures and roles of personnel following suspicion of the presence of *Aphanomyces astaci* in Australia, refer to the **AQUAVETPLAN Control Centre Management Manual**.

## **3.2 Control options**

### **3.2.1 Option 1 — Eradication**

If epidemiological investigations determine an obvious point source of infection that has been or may be contained with minimal or no spread of the fungus (eg in a closed system such as an aquarium or fully recirculating system), an eradication strategy may be successful and should be attempted. Eradication has the highest short-term economic costs, and if successful, the long-term economic and ecological benefits are likely to outweigh those short-term costs.

Eradication is unlikely to be successful or feasible if epidemiological investigations determine that infection is widespread, the source of infection is unable to be determined and/or the disease is present or potentially present in wild crayfish. Eradication procedures include those outlined in Section 2.2 and 2.3.1 of this manual

### **3.2.2 Option 2 — Containment, control and zoning**

If the disease became established in wild crayfish, eradication would be impossible. In this case, containment and prevention of further spread of disease is the preferred control option. The aim is to maintain uninfected areas/zones free of crayfish plague. Restrictions on the movement of crayfish and crayfish products and ongoing surveillance and monitoring programs will be necessary to support a zoning program. The strategies outlined in Section 2.2 and 2.3.2 of this manual will be implemented.

## **3.3 Strategy for control**

### **3.3.1 Epidemiological investigation**

Thorough epidemiological investigation and tracing is fundamental to the success of eradication or zoning programs and must be conducted immediately upon suspicion of an outbreak of crayfish plague to determine the actual and potential spread of infection. This knowledge is required to determine the most scientifically and economically feasible response option.

### **3.3.2 Quarantine and movement controls**

Quarantine and/or movement controls should be implemented on any material or equipment capable of transmitting infection. Restricted and Control areas (Section 2.2.1) should be established immediately and refined as further information becomes available from epidemiological investigations and hydrographical data.

See the **AQUAVETPLAN Enterprise Manual** for different enterprise systems and response options.

For eradication, quarantine and movement controls must be stringently enforced on crayfish, crayfish products, water, and any vectors located in declared areas. Movement controls should be maintained until the disease is either eradicated or declared endemic.

For containment, control and zoning, movement controls are essential to maintain uninfected areas/zones. Restrictions must apply to movement out of the infected area of anything capable of transmitting *Aphanomyces astaci* from infected to uninfected crayfish, watersheds, dams, ponds, aquaculture facilities or processing plants. If zoning is implemented, an active targeted surveillance program for *A. astaci* is necessary within the uninfected zone.

### **3.3.3 Destruction of clinically diseased crayfish**

Immediate removal, destruction and disposal of all diseased and dead crayfish is essential to the success of any response strategy. These crayfish are the main source of *Aphanomyces astaci* hyphae in the environment. Burial sites should be chosen carefully to ensure there is no contact with waterways or vectors. Refer to the **AQUAVETPLAN Destruction and Disposal Manuals** for more details.

### **3.3.4 Treatment of crayfish products**

Unexposed products may be marketed and disseminated without any risk of transmission of infection. However, products from exposed crayfish populations will require processing and/or may have a restricted market in order to maintain crayfish plague-free areas/zones.

The treatment of potentially infected crayfish products and by-products must take into account trade regulations, market requirements, food safety standards and potential spread of the pathogen via product (see Section 2.2.5 of this document). If destined for human consumption, harvested crayfish can be stored in a freezer until a definitive diagnosis is obtained and decisions are made regarding release of product. This will prevent the spread of infection and allow salvage of product for sale (provided the relevant authority approves release).

### **3.3.5 Disposal**

Immediate, safe disposal of all infected crayfish, wastes, effluent and equipment (that cannot be decontaminated) is necessary for the eradication of the fungus. See Section 2.2.4 of this document and the **AQUAVETPLAN Operational Procedures Manual** for details. Effluent must be treated to prevent spread of infection.

### **3.3.6 Decontamination**

See Section 2.2.6 and the **AQUAVETPLAN Operational Procedures Manuals: Decontamination and Disinfection** for further details.

## **Eradication**

All buildings, tanks, materials and equipment including nets, boats and vehicles that can be contaminated must be cleaned and disinfected. If disinfection cannot be achieved effectively and quickly, then contaminated materials, equipment and



buildings should be destroyed. At all stages of decontamination, steps must be taken to prevent the spread of infection via water, wastes or materials, especially into natural waterways.

### **Containment, control and zoning of disease**

Thorough cleaning and disinfection of water and equipment including nets, boats and vehicles that may move from an infected to a disease-free zone is important.

#### **3.3.7 Surveillance**

Active surveillance for the presence of *Aphanomyces astaci* in restricted and control areas should continue until crayfish plague is either declared eradicated or endemic. If a zoning program is implemented, targeted active surveillance for *A. astaci* outside the restricted and control areas is necessary to support the declaration of crayfish plague-free zones.

#### **3.3.8 Sentinel and restocking measures**

##### **Eradication**

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

##### **Containment, control and zoning**

Restocking with sentinel, caged crayfish is one method of ascertaining freedom from infection in areas such as watersheds where sparse populations of infected crayfish could still remain. Large-scale restocking with susceptible species should only occur once the water body is known to be free from the disease.

### **3.4 Economic effects**

If an outbreak of crayfish plague occurred in Australia, the cost to the crayfish industry and the environment would be enormous. Not only would there be loss of farmed and wild stock, but the cost of control and eradication campaigns would be substantial. The presence of crayfish plague in the environment would continue to be a source of infection causing new outbreaks if the disease could not be eradicated. Additionally the export of many commodities that may harbour *Aphanomyces astaci* hyphae or zoospores is likely to be impacted.

## Appendix 1 Overview of crayfish and the crayfishing industry

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Crayfish belong to two superfamilies. The Parastacoidea are found only in the southern hemisphere: Australia; New Guinea; South America; Madagascar. Crayfish from the northern hemisphere are members of the superfamily Astacoidea, of which there are two families. Crayfish from the eastern parts of the United States and from Japan are members of family Cambaridae, whereas European species and *Pacifastacus leniusculus* from the west of the United States are in the family Astacidae. Their phylogenetic relationship with some other freshwater invertebrate animals is shown in Figure 4.

In some regions, the natural range of freshwater crayfish is determined by environmental conditions. For example, in England and Wales they are restricted to lime-rich waters (Alderman *et al.* 1984) and in Norway, Sweden and Finland to more southern areas with milder winters (Taugbøl & Skurdal 1993). Freshwater crayfish are not naturally occurring in the African continent, and in Asia they are naturally occurring only in Turkey, Iran, Japan and a small area of mainland China (Figure 5). Translocation of crayfish from North America and Australia has extended the range of freshwater crayfish to various African and Asian countries (Holdich 2002; Huner 2002; Lewis 2002).

In Australia there are three major groups of crayfish. They are the small burrowers, the moderately sized burrowers and the smooth crayfish (Mills, Morrissy & Huner 1994). The species of interest to aquaculture are relatively large, fast growing, smooth crayfish with large tails including red claw, marron and yabbies.

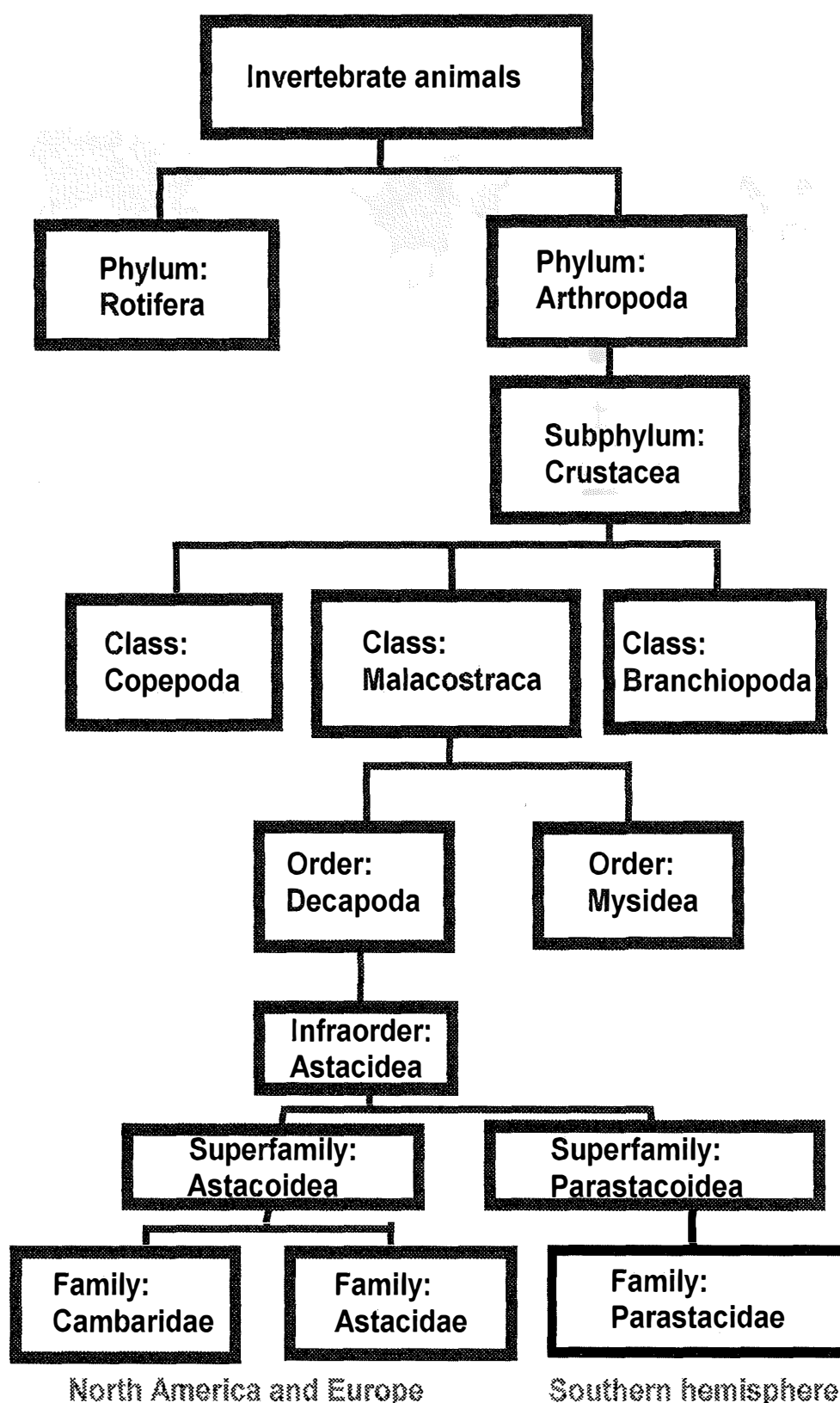
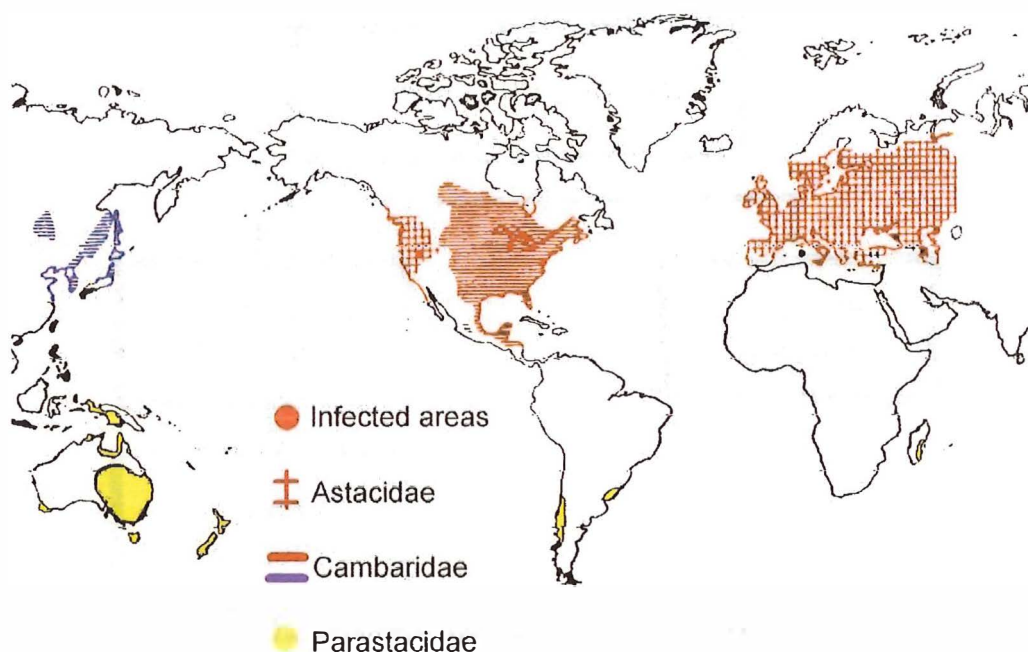


Figure 4. A diagram showing the relationship of freshwater crayfish to other species that have been investigated as possible hosts for crayfish plague. Freshwater crayfish, crabs, lobster, prawns and shrimp are all decapods (Order: Decapoda) however, only freshwater crayfish and the mitten crab *Eriocheir chinensis* (Benisch 1940) have been infected with crayfish plague in experimental studies.



**Figure 5.** The global distribution of crayfish families and areas where *Aphanomyces astaci* is known to occur. Members of the family Parastacidae are found in the southern hemisphere. Family Cambaridae is native to the United States and Japan and the Astacidae to Europe and western areas of the United States.

Crayfish are keystone species in the ecology of freshwater habitats as many species are herbivores and scavengers of decomposing plant and animal material, having a role in maintaining the balance of aquatic plants and detritus in water bodies (Holdich 2002; Marren 1986). Some countries where native stocks of crayfish have declined following crayfish plague outbreaks have been reports of weed overgrowth in rivers. They are also prey for carnivorous fish and. In some parts of Europe such as Sweden and the United Kingdom, native species have been largely replaced by North American species that are larger and grow more rapidly. For example, native species such as *Astacus astacus* have been displaced by non native species such as *Pacifastacus leniusculus* (Svårdson 1992; Svårdson *et al.* 1991; Vorburger & Ribi 1999). In some areas the burrowing activity of the introduced species has caused significant damage to the banks of rivers.

Crayfish have been a luxury food in many European countries for several centuries, with Sweden, Finland and to a lesser extent other countries such as the UK having a recreational capture industry. Today, Sweden, Finland and France import crayfish from other parts of Europe and the world. The European species *Astacus astacus* remains the most highly priced and sought after crayfish in Europe, followed by *Pacifastacus leniusculus* (Ackefors 1998; Ackefors & Lindqvist 1994).

Interest in the aquaculture of edible species of crayfish in Europe has increased as a result of the decline in many wild capture fisheries following crayfish plague outbreaks and habitat degradation caused by pollution, acidification, clearing and dredging, together with increases in the number of predators such as eels and other carnivorous fish (Skurdal & Taugbøl 2002; Westman & Westman 1992). This

has resulted in the introduction of plague resistant North American species and also some susceptible Australian species to several countries including Spain, Ecuador, Kenya, China and Brazil (Huner 2002; Lewis 2002; Romero 1997).

Following repeated outbreaks of crayfish plague in Europe, there have been deliberate attempts to replace native species with the more resistant North American species, most notably *Pasifastacus leniusculus* which has a good flavour, fast growth rate and early maturity (Ackefors 1998; Ackefors & Lindqvist 1994; Svärdson 1992). Once these crayfish become established it is impossible to eradicate crayfish plague if the animals are carriers of *A. astaci*.

## Appendix 2 Diagnosis of *Aphanomyces astaci*

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The following methods are used for the diagnosis and identification of *Aphanomyces astaci*. They are based on the methods recommended in the chapter on crayfish plague in the 2003 edition of the Office International des Epizooties (OIE) Diagnostic Manual of Aquatic Diseases.

### Examination and culture of specimens

#### 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 temperature should not go below 4 °C as freezing will destroy the fungus. 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 IM (Isolation Medium) at 16-20 °C for 15 days. 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 and Polglase 1986).

An excised piece of abdominal cuticle 1-2 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 the fungus to grow through the agar and away from the contaminating bacterial growth. Incubate the plate at 16-20 °C for 15 days.

Fungi are normally differentiated based on the morphology of sexual reproductive stages, but because these stages are absent in *Aphanomyces astaci* general morphology of the oomycetes is usually sufficient. It is unlikely that any other fungus would cause such 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 and Polglase 1986).

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

Actively growing cultures can be tested for the production of zoospores. Use a coverslip to take a thin slice from the growing edge of the fungal colony. Place into an empty sterile Petri dish. Add sufficient tap water to cover the slice of agar. Leave overnight at 20 °C. 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 2-5 minutes (Alderman and Polglase 1986).

### **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. Thinly smear small pieces of tissue onto a glass slide. Allow to dry and stain with Diff-Quick® or Giemsa stain. 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 and Wallington 1980).

### **Molecular Identification**

Molecular diagnostic tests are being developed to detect *A. astaci* by polymerase chain reaction (PCR) and fluorescent *in-situ* hybridisation (FISH). These tests will also differentiate *A. astaci* from *A. invadans*, the causative agent of epizootic ulcerative syndrome (EUS) in freshwater fishes. EUS is present in Australia. The development of molecular diagnostic tests is being done by Fisheries Research and Development Corporation project number 2001/621 due for completion in 2004 and will be reported as an Australian and New Zealand Standard Diagnostic Procedure (ANZSDP). A PCR-based diagnostic test has been developed in Europe (patent pending).

## Glossary

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AQUAVETPLAN	A series of documents that describe the Australian response to exotic aquatic animal diseases, linking policy, strategies, implementation, coordination and emergency-management plans.
AUSVETPLAN	A series of documents that describe the Australian response to exotic animal diseases, linking policy, strategies, implementation, coordination and emergency-management plans.
Control area <sup>a</sup>	A buffer between the restricted area and areas free of disease. Restrictions on this area will reduce the likelihood of the disease spreading further afield. As the extent of the outbreak is confirmed, the control area may be reduced or expanded in size. The shape of the area may be modified according to circumstances, eg water flows, catchment limits etc. In most cases, permits will be required to move animals and specified product out of the control area into the disease-free area.
Chronic infection	Clinically inapparent or low-grade infection that is transmissible and that may eventually lead to clinical disease.
Dangerous contact premises or area <sup>a</sup>	A premises or area that has had a possible contact with an infected premises or area. It may be direct contact involving movements of crayfish or indirect contact by nets or other equipment.
Declared area <sup>a</sup>	An area that has been subjected to a legal declaration and includes both a restricted area and a control area.
Decontamination	Includes all stages of cleaning and disinfection.
Disinfectant	An agent used to destroy microorganisms outside a living animal.
Disposal	Removal of crayfish carcasses and equipment by burial, burning or some other process so as to prevent the spread of disease.
crayfish by-products	Products of crayfish origin such as heads and crayfish meal destined for industrial use.
Crayfish products	Crayfish meat products and products of crayfish origin for human consumption or use in animal feeding.
Fomites	Inanimate objects (eg boots, clothing, equipment, vehicles, crate, packaging) that carry the exotic agent and spread the disease through mechanical transmission.
Free area <sup>a</sup>	An area known to be free of the disease agent.
Infected premises or area <sup>a</sup>	The area in which the disease has been confirmed. Definition of an 'infected area' is more likely to apply to an open system such as an oceanic lease.



Mitigation	Reduction in severity, eg mitigation of the impact of disease is to decrease the severity of the impact of the disease.
Movement control	Restrictions placed on movement of crayfish, people and things to prevent spread of disease.
PCR	A diagnostic technique involving the production of millions of copies of a specific target DNA segment <i>in vitro</i> .
Premises or area <sup>a</sup>	Production sites or water bodies that may range from an aquarium to a river.
Quarantine	Legal restrictions imposed on a place, crayfish, vehicle, or other things, limiting movement.
Restricted area <sup>a</sup>	The area around an infected premises (or area) that is subject to intense surveillance and movement controls. Movement of potential vectors of disease out of the area will, in general, be prohibited. Movement into the restricted area would only be by permit. Multiple restricted areas may exist within one control area.
Sentinel crayfish	Crayfish of known health status monitored for the purpose of detecting the presence of a specific exotic disease agent.
Surveillance	A systematic series of investigations of a given population of crayfish to detect the occurrence of disease for control purposes, and which may involve testing samples of a population.
Susceptible species	Crayfish that can be infected with the disease.
Suspect premises or area <sup>a</sup>	Where the emergency disease is suspected but not yet confirmed. The reason for the suspicion varies with the agent, however it may involve clinical signs or increased mortality.
Tracing	The process of locating animals, people or things that may be implicated in the spread of disease, so that appropriate action can be taken.
Vector	Transmission of infection from one host to another on another animal or inanimate object. A <i>biological</i> vector is one in which the infectious agent must develop or multiply before becoming infective to a recipient host. A <i>mechanical</i> vector is one that transmits an infectious agent from one host to another but is not part of the life cycle of the agent.
Zoning	The process of defining disease-free and infected zones.(See AUSVETPLAN definition)
Zoonotic disease	Disease transmissible from animals to humans.

<sup>a</sup>Due to the nature of the aquatic environment and of aquatic animal disease, these areas may be difficult to define and may need to be revised as further information is obtained about the nature of the agent and the extent of the disease.

## Abbreviations

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AAHL	Australian Animal Health Laboratory
AFDL	AAHL Fish Diseases Laboratory
AFHRL	Australian Fish Health Reference Laboratory
ANZSDT	Australian and New Zealand Standard Diagnostic Test
AQIS	Australian Quarantine and Inspection Service
CCEAD	Consultative Committee on Emergency Animal Diseases
CRC	Cooperative Research Centre
CSIRO	Commonwealth Scientific and Industrial Research Organisation
CVO	Chief Veterinary Officer
DPIWE	Tasmanian Department of Primary Industries, Water and Environment
EUS	Epizootic ulcerative syndrome
FISH	Fluorescent <i>in situ</i> hybridisation
FRDC	Fisheries Research and Development Corporation
IM	Isolation medium
OIE	Office International des Épizooties (World Organisation for Animal Health)
PCR	Polymerase Chain Reaction

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