

AUSTRALIAN AQUATIC VETERINARY EMERGENCY PLAN

AQUAVETPLAN

Disease Strategy

White spot disease

Version 1.0, 2005

AQUAVETPLAN is a series of technical response plans that describe the proposed Australian approach to aquatic animal disease incursions. The documents provide guidance based on sound analysis, linking policy, strategies, implementation, coordination and emergency-management plans.

Primary Industries Ministerial Council

This disease strategy forms part of:

AQUAVETPLAN Edition 2

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

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IMPORTANT NOTE: Important regulatory information is contained in the OIE *International Aquatic Animal Health Code (OIE 2004)* for white spot disease, which is updated annually and is available on the internet at the OIE website:

http://www.oie.int/eng/normes/fcode/fcode2004/en_acode.htm

Further details are given in Appendix 1 of this manual.

DISEASE WATCH HOTLINES

These telephone numbers connect callers to the relevant state or territory officer to report concerns about any potential emergency disease situation. Anyone suspecting an emergency disease outbreak should use this number for immediate advice and assistance.

New South Wales	1800 043 536	Northern Territory	1800 720 002
Queensland	07 3830 8550	Victoria	136 186
South Australia	1800 065 522	Western Australia	1800 815 507
Tasmania	1800 005 555		

Preface

This disease strategy for the control and eradication of white spot disease (WSD) is an integral part of the **Australian Aquatic Veterinary Emergency Plan, or AQUAVETPLAN (Edition 2)**.

The strategy sets out the disease control principles for use in an aquatic veterinary emergency incident caused by the suspicion or confirmation of WSD in Australia. The strategy was approved by:

- the National Aquatic Animal Health Technical Working Group of the Aquatic Animal Health Committee, at meeting 04 in May 2004;
- the Aquatic Animal Health Committee of the Primary Industries Standing Committee, at meeting 04 in June 2004; and
- the Primary Industries Standing Committee, at meeting 08 in March 2005.

White spot disease is listed by the OIE (World Organisation for Animal Health, formerly Office International des Epizooties) in the *International Aquatic Animal Health Code* (OIE 2004).¹

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

Disease strategies

Individual strategies for each disease

Operational procedures manuals

Disposal

Destruction

Enterprise Manual

Includes sections on:

- open systems
- semi-open systems
- semi-closed systems
- closed systems

Management manual

Control centres management

Aquatic Animal Diseases Significant to Australia: Identification Field Guide by Alistair Herfort, Department of Agriculture, Fisheries and Forestry, Canberra (Herfort 2004) is a source for some of the information about the aetiology, diagnosis and epidemiology of the disease and should be read in conjunction with this strategy.

This manual was drafted by Drs Chris Baldock, Iain East and Richard Callinan, with the assistance of Professor Tim Flegel and Mr Dan Fegan.

Scientific editing: Biotext Pty Ltd, Canberra.

¹See http://www.oie.int/eng/normes/fcode/a_index.htm (Accessed 11 May 2005).

This manual was adapted from similar manuals in AUSVETPLAN, the Australian emergency plan for terrestrial animal diseases, and from the AQUAVETPLAN *Enterprise Manual*. The format and content have been kept as similar as possible to those documents to enable animal health professionals trained in AUSVETPLAN procedures to work efficiently with this document in the event of an aquatic veterinary emergency. The work of the AUSVETPLAN writing teams and the permission to use the original AUSVETPLAN documents is gratefully acknowledged.

The text was amended at various stages of the consultation/approval process, and the policies expressed in this version do not necessarily reflect the views of all the members of the writing group. Contributions made by others not mentioned above are also gratefully acknowledged.

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

Government	Industry
Commonwealth of Australia	CSIRO Division of Livestock Industries
State of New South Wales	Tasmanian Salmonid Growers' Association
State of Queensland	Tuna Boat Operators Association
State of South Australia	Pearl Producers' Association
State of Tasmania	Australian Prawn Farmers Association
State of Victoria	Pet Industry Joint Advisory Council
State of Western Australia	RecFish Australia
Northern Territory	National Aquaculture Council
Australian Capital Territory	

The complete series of AQUAVETPLAN documents is available on the internet at: <http://www.affa.gov.au/AQUAVETPLAN> (Accessed 11 May 2005).

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1 Nature of the disease

White spot disease (WSD) is a highly contagious viral disease of penaeid prawns (family Penaeidae), characterised by the rapid onset of high levels of mortality in farmed prawn populations. Outbreaks are preceded by cessation of shrimp feeding followed within a few days by the appearance of moribund shrimp at the edge of ponds and then mass mortality. The causative virus also infects a wide range of other crustaceans, often without causing any clinical signs. WSD has exhibited pandemic behaviour in Asia and the Americas but does not currently occur in Australia.

1.1 Aetiology

The causative agent of WSD is white spot virus (WSV), also known as white spot syndrome virus (WSSV). WSV was first reported from WSD outbreaks in farmed *Penaeus japonicus* (kuruma prawn) in Japan in 1993, although the disease probably occurred in Taiwan and China in 1991 and 1992. Within a few years, morphologically similar viruses were described under various names from similar disease outbreaks in farmed prawns in China, Taiwan and Thailand (Flegel 2001). These viruses were grouped together into the white spot virus complex (Lightner 1996, Lo et al 1999) and are now considered by the International Committee on Taxonomy of Viruses to represent a new virus genus, called *Whispovirus*, within the family Nimaviridae.²

WSV is a large (80–120 × 250–380 nm), rod-shaped to elliptical double-stranded DNA virus with a trilaminar envelope and a unique, tail-like appendage (OIE 2003a). Using molecular techniques, it has been shown that WSV isolates from WSD outbreaks in both eastern and western hemispheres are identical or closely related (Flegel 2001). However, comparative studies (Wang Q et al 2000) have suggested slight differences in virulence between genotypes, raising the possibility that more virulent genotypes may be more likely to cause high levels of mortality (Walker et al 2002). Until the issue is clarified, it will be assumed in this document that WSV strains are closely related and that the virulence of all strains is similar.

1.2 Susceptible species

It should be noted that Pérez Farfante and Kensley (1997) proposed a revised taxonomic classification of penaeids in which five subgenera of *Penaeus* were raised to genera, namely *Farfantepenaeus*, *Fenneropenaeus*, *Litopenaeus*, *Marsupenaeus* and *Melicertus*. Given that this revision remains under discussion, the proposed genus names are not used in this manual, although they are used in many of the references cited.

²See http://www.ncbi.nlm.nih.gov/ICTVdb/Ictv/fs_nimav.htm (Accessed 11 May 2005).

All decapod crustaceans (order Decapoda), including prawns, lobsters and crabs from marine, brackish water or freshwater environments are considered susceptible to infection (OIE 2003a), but WSD has mainly been a problem in farmed penaeid prawns. Currently, three marine prawn species, *Penaeus monodon* (giant tiger prawn), *P. japonicus* and *P. merguensis* (banana prawn), and one freshwater species, *Macrobrachium rosenbergii* (giant freshwater prawn), are farmed commercially in Australia.

Although WSD has not been reported in wild crustacean populations, a number of species have expressed disease when experimentally infected with WSV by injection (Supamattaya et al 1998), bath exposure (Chen et al 2000) or ingestion. Experimental susceptibility through ingestion is probably of most relevance. Mortalities following ingestion of infective material have been demonstrated in the crayfish *Procambarus clarkii* (red swamp crayfish; Wang YC et al 1998), in the freshwater prawns *Macrobrachium idella* and *M. lamerrae* (Sahul Hameed et al 2000), in freshwater crabs *Paratelphusa hydrodomous* and *Paratelphusa pulvinata* (Sahul Hameed et al 2001), and in several European marine and freshwater crustacean species (Corbel et al 2001). Moreover, Richman et al (1997) reported mortalities due to WSV infection in captive North American freshwater crayfishes (*Procambarus clarkii* and *Orconectes punctimanus*). The route of infection in these studies was not recorded, but is likely to have been by ingestion. Relatively high mortalities following experimental feeding of infective material have also been recorded in postlarvae and juveniles of the giant freshwater prawn *Macrobrachium rosenbergii*, with lower mortalities in subadults and adults, suggesting greater tolerance of WSV infection with age (Pramod Kiran et al 2002).

Australia is home to one of the richest freshwater crayfish faunas in the world and, of these, three *Cherax* spp are important in semi-intensive aquaculture. *C. quadricarinatus* (Australian red claw crayfish) suffered very high mortalities when experimentally injected with WSV (Shi et al 2000), although it is possible that the species exposed was misidentified and was more likely to have been *Procambarus clarkii* (red swamp crayfish). To address this possibility, repeat transmission trials confirmed susceptibility to WSD of early stage *C. quadricarinatus* juveniles (B Edgerton, private consultant, pers comm, January 2002). High mortalities have also been recorded in *C. destructor* subsp *albidus* (yabby) experimentally injected with WSV. In the same study, over 50% of *C. destructor albidus* infected orally with WSV and subjected to a significant stress also died with WSD, while there were no deaths in animals exposed orally but not stressed. Results suggested that, when exposed via natural routes of infection, farmed or wild *C. destructor albidus* are likely to be less susceptible to WSD than are penaeid prawns in equivalent environments (B Edgerton, unpublished). The susceptibility to WSV of other Australian freshwater crayfish species has not been determined.

Although WSV infection is present in wild prawn populations in countries where WSD is endemic on farms, there is no evidence that the virus causes significant mortalities in these populations (Alliance Resource Consulting 1998). Factors which contribute to the absence of an observable impact include lower stress levels in the wild, lower levels of infection (Lo et al 1997) and lower host densities (Lotz and Soto 2002). The impact, if any, of WSV infection on other wild crustacean populations is unknown.

There have been no reports of WSV causing sickness in humans.

1.3 World distribution and occurrence in Australia

WSD is believed to have first occurred in Taiwan and China between 1991 and 1992 with subsequent spread via imported prawns from China to Japan, where it caused outbreaks in 1993 (Nakano et al 1994). WSV infection is now endemic in almost all prawn-producing countries in Asia and the Americas (Subasinghe et al 2001). Spread between countries is reported to be mainly through the importation of live animals and uncooked, harvested prawns (Nunan et al 1998, Durand et al 2000).

WSV has been officially reported from 14 countries in the Asia-Pacific region, namely Bangladesh, China, India, Indonesia, Japan, South Korea, Malaysia, Pakistan, Philippines, Singapore, Sri Lanka, Taiwan, Thailand and Vietnam (NACA 2002, OIE 2003b) and nine countries in the Americas, namely Colombia, Ecuador, Guatemala, Honduras, Mexico, Nicaragua, Panama, Peru and the United States.

Australia, New Zealand and the islands of the South Pacific are currently free of WSV (NACA 2002, OIE 2003b). However, positive polymerase chain reaction (PCR) test results for WSV were recorded for animals held in two aquaculture facilities in Darwin in November 2000. Both facilities were destocked and disinfected as a precautionary measure. Imported green prawns being used as feed at the two facilities also tested positive with the WSV PCR test.

1.4 Diagnostic criteria

Prawns affected with WSD often show no distinctive clinical signs or pathognomonic gross lesions. For presumptive diagnosis of suspected outbreaks in ponds, histopathological examination of haematoxylin and eosin-stained (H&E) tissue sections from moribund animals is sufficient. For a definitive diagnosis of WSD in prawns and for certification of WSV infection status of broodstock and postlarvae, PCR is recommended (OIE 2003a). Tissue culture has yet to be developed as a usable, routine diagnostic tool for crustacean pathogens such as WSV, and clinical chemistry has not yet become a routine diagnostic tool of crustacean pathologists.

1.4.1 Clinical signs and gross lesions

The clinical signs and gross lesions associated with WSD (Table 1) vary between outbreaks and do not provide a sufficient basis for a diagnosis.

WSD outbreaks can occur at any stage of grow-out and are typically associated with high and rapid mortality. The first evidence of a problem is often a sudden and dramatic increase in the number of moribund and dead prawns found at pond edges, with cumulative mortalities approaching 100% within 3-10 days. Acutely affected prawns show a rapid reduction in food consumption and become lethargic. The shell is often loose with white, initially circular, spots within the cuticle and/or an overall red body colouration. The intracuticular white spots can range from minute foci to discs up to 2 mm in diameter, which may coalesce (Lightner 1996). They are most easily observed by removing the cuticle over the cephalothorax, scraping away any attached tissue and holding the cuticle up to the light (OIE 2003a). The spots may be abnormal deposits of calcium salts by the cuticular epidermis (Lightner 1996) or result from disruption to transfer of exudate from epithelial cells to the cuticle (Wang YG et al 1999).

Despite usually being associated with massive mortalities, WSD outbreaks can be characterised by very low morbidity and mortality for the duration of grow-out (Flegel 1997, Tsai et al 1999). Outbreaks of this type begin to occur in an infected area one to two years after the initial WSV incursion and its associated massive losses. Flegel (2001) has suggested that infection and subsequent tolerance to the virus, probably acquired in the hatchery, allow most prawns, although infected, to survive grow-out provided ponds are well managed (see also Section 1.5.2).

It is important to appreciate that white spots on the carapace of prawns are not pathognomonic for WSD. They have also been attributed to environmental factors such as high alkalinity (OIE 2003a) or to bacterial shell disease, in both cases unassociated with significant mortalities (Goarant et al 2000, Wang YG et al 2000,). Conversely, moribund prawns with WSD may have few, if any, white spots. Instead, they may have a generalised pink to reddish-brown colouration of the entire cuticle (hence the alternative name 'red disease' for WSD), due to expansion of the cuticular chromatophores.

Table 1 Comparative features of clinical WSD and subclinical WSV infection

Sign	Clinical disease	Subclinical (latent or covert) infection
Age of prawns	Any stage of grow-out	All lifecycle stages
Anorexia	Yes	No
White spots	Often present	No
Red carapace	Often present	No
Time of death	3–4 days	Remain clinically normal if not stressed

WSD = white spot disease
 WSV = white spot virus

1.4.2 Histopathology

The histopathology of WSD in moribund prawns collected during outbreaks is distinctive and can be used for preliminary confirmation of an initial diagnosis. However, additional tests such as PCR, in situ DNA hybridisation, Western blot analysis and transmission electron microscopy are required for final confirmation (OIE 2003a).

Moribund prawns with WSD have systemic viral infection leading to necrosis of tissues of ectodermal and mesodermal origin. Infection and necrosis are most commonly seen in cuticular epithelial cells and connective tissue cells of the stomach, carapace and gills. Infection is also seen in the antennal gland epithelium, lymphoid organ sheath cells, haematopoietic tissues, and in fixed phagocytes of the heart. Infected cells typically have hypertrophied (enlarged) nuclei containing a single intranuclear inclusion. Inclusions are initially eosinophilic and (as an artefact of fixation in Davidson's fixative solution) are separated by a clear halo from the margined chromatin. These are known as Cowdry type A inclusions and are found in many viral infections in both vertebrates and invertebrates; they are intranuclear, eosinophilic, amorphous and surrounded by a clear halo beneath the nuclear membrane. Later, inclusions become lightly to deeply basophilic and fill the entire nucleus (Lightner 1996, OIE 2003a). This latter feature can be used to distinguish WSD from infection with infectious hypodermal and haematopoietic necrosis virus (IHHNV), in which only Cowdry type A inclusion bodies are typically present.

1.4.3 Laboratory tests

Wherever possible, laboratory procedures should comply with the *Manual of Diagnostic Tests for Aquatic Animals* (the OIE Aquatic Manual, OIE 2003a). The recommended minimum numbers of specimens to collect for diagnosis are 100 for the larval stages of most crustaceans; 50 for the postlarval stages; and 10 for juveniles and adults, with preference for individuals with signs and/or gross lesions. These numbers are a guide only, as fewer, good-quality specimens are more useful than a large number of poorly prepared ones (OIE 2003a).

There are two situations in which WSV infection requires detection: for confirmation of suspect clinical WSD and in screening to establish the infection status of asymptomatic populations.

Confirmation of suspect clinical WSD

For confirmation of a suspected outbreak, animals that are representative of those showing clinical signs and/or gross lesions should be sampled. Whole animals, lymph, gills and pleopods provide suitable specimens for examination. Although dead animals can sometimes provide useful diagnostic information (Mohan et al 2002), they are often unsuitable for examination because of the rapid onset of post-mortem changes. Several rapid laboratory methods are available to give a presumptive diagnosis, which can later be confirmed by histological examination and other methods if required.

Screening

For screening apparently healthy populations, the number of animals to be tested will depend on the required level of confidence in the findings. Whole larvae, postlarvae and juvenile animals, as well as haemolymph, gills or pleopods from juveniles to broodstock, provide suitable specimens for examination. PCR is the preferred test, with follow-up bioassay to confirm the presence of viable virus in PCR-positive samples combined with a WSV-specific confirmatory test if required.

Table 2 compares the suitability of the different methods for screening and diagnosis.

Table 2 Comparison of WSV screening and diagnostic methods

Method	WSV screening				Presumptive WSD diagnosis	Confirmatory WSD diagnosis
	Larvae	Post-larvae	Juveniles	Adults		
Gross signs	–	–	–	–	+	–
Rapid methods	–	–	–	–	+	+
Histopathology	–	–	–	–	++	++
PCR	+++	+++	+++	+++	+++	+++
Transmission EM	–	–	–	–	+++	+++
Antibody-based assays	?	?	+++	+++	+++	+++
In situ DNA hybridisation	?	?	+	+	+++	+++
Bioassay ^a	–	–	–	–	+	–

EM = electron microscopy; PCR = polymerase chain reaction, WSD = white spot disease, WSV = white spot virus.

– the method is currently unavailable or unsuitable

? the method is available but untested

+

the method has application in some situations, but cost, accuracy or other factors severely limit its application

++ the method is a standard method with good diagnostic sensitivity and specificity

+++ the method is the recommended method because of its availability, utility, and diagnostic specificity and sensitivity

^a Bioassay is likely to be used for confirmation of an initial diagnosis of WSD in Australia, but other methods may be used subsequently during an outbreak.

Source: modified from OIE (2003a)

Rapid methods for presumptive diagnosis

Two approaches are available. One employs unstained wet-mounts fixed with formalin and viewed by dark-field microscopy. The other employs fixed, stained tissue viewed with conventional microscopy. There are two variations of the latter approach.

Dark field method (Momoyama et al 1994)

From a moribund prawn suspected of having WSD, dissect out the stomach as a source of subcuticular tissue or peel off thin layers of subcuticular tissue from the cephalothorax and fix in a 10% formalin solution. Using fine forceps, spread thin pieces of the subcuticular tissue on a slide in a small volume of 10% formalin. Add a cover slip and remove excess solution by placing a filter paper at the edge of the cover slip. Using dark-field optics, focus the microscope on an area of the preparation where prawn pigment cells are poorly distributed. Specimens with WSD will show moderate to large numbers of refractile, hypertrophied nuclei.

Rapid staining method 1 (Lightner 1996)

From a moribund prawn suspected of having WSD, excise gills, appendages or stomach. Mince and then squash, dab or smear onto a slide. Fix the smear in methanol for 6 minutes or fix by carefully heating the slide. Flood the smear with an appropriate stain such as Giemsa or other blood smear stain. Stain for ~1 to 5 minutes. Cover-slip the preparation and examine with 10, 20 and 40× objectives. Specimens with WSD will display cells with hypertrophied nuclei with diagnostic inclusions. Normal cell nuclei are 4 to 10 µm in diameter and display chromatin threads and a nucleolus. Infected nuclei are hypertrophied and usually contain a single eosinophilic to bluish inclusion body (depending on the stain used). In

severely affected prawns, results comparable to those obtained with H&E histological methods can be obtained in approximately 10 minutes.

Rapid staining method 2 (OIE 2003a)

Fix a whole moribund prawn or gill filaments in Davidson's fixative solution overnight (see below for alternative rapid fixation method). After fixation, wash some of the gill filaments thoroughly with tap water to remove the fixative and then stain with Meyer's H&E. After staining and dehydration, when the tissue is in xylene, place a gill filament on a microscope slide in a drop of xylene and, using a fine pair of needles (a stereo microscope is helpful), break off several secondary filaments and then replace the main filament in xylene where it can be stored indefinitely in a sealed vial as a permanent reference. Being careful not to let the xylene dry, tease apart the secondary filaments on the microscope slide and remove any large fragments or particles that would thicken the mount unnecessarily. Finally, add a drop of mounting fluid and a cover slip. Use light pressure to flatten the mount as much as possible. This procedure may also be used with thin layers of subcuticular tissue. With WSD outbreaks, examination with a 40× objective of a light microscope will reveal moderate to large numbers of hypertrophied nuclei with basophilic central inclusions surrounded by marginated chromatin. It is important also to detect some nuclei with Cowdry type A inclusions characteristic of the early stage of WSV infection.

Alternatively, if very rapid results are required, the overnight fixation step above can be shortened to only 2 hours by changing the acetic acid portion in the Davidson's fixative solution to 50% concentrated HCl. For best results, this fixative should not be stored for more than a few days before use. After fixation, wash thoroughly to remove the fixative and check that the pH has returned to near neutral before staining. Do not fix for longer periods or above 25°C, as this may result in excessive tissue damage that will make interpretation difficult or impossible.

Histopathology

Moribund prawns should be fixed in Davidson's fixative solution and processed by standard techniques to produce H&E stained tissue sections (Bell and Lightner 1988, Lightner 1996). Examine the sections by light microscopy for the presence of moderate to large numbers of hypertrophied nuclei with eosinophilic to basophilic central inclusions surrounded by marginated chromatin in tissues of ectodermal and mesodermal origin. The best tissues for examination are the subcuticular tissues of the stomach, cephalothorax or gill (Wongteerasupaya et al 1995).

Polymerase chain reaction test

Although several different PCR protocols have been described for WSV, the OIE-recommended technique is the nested PCR test of Lo et al (1996) and Lo and Kou (1998). Details of the technique can be found in the original publications and in the OIE Aquatic Manual (OIE 2003a). Commercial PCR kits for the detection of WSV are also available from several suppliers.

Note: Eyes from prawns older than 10-day-old post-larvae must be excluded from tissue for analysis, as they are known to contain a PCR inhibitor.

Care should be taken with the interpretation of results obtained with PCR, particularly when the test has been conducted on clinically normal animals. Repeat tests on a known infected specimen have resulted in both positive and negative results in some instances (Lo et al 1997, Hsu et al 1999), which may have been due to the concentration of WSV in the samples being close to the limit of the assay's detection sensitivity. Furthermore, PCR-based assays cannot distinguish between live and dead virus.

Transmission electron microscopy

The most suitable tissues for examination by transmission electron microscopy are subcuticular tissues, gills or pereopods that have been prescreened by histology. For screening or surveillance of clinically normal prawns, the most suitable tissue is subcuticular tissue from the stomach. Full procedure descriptions are available in Lightner (1996). WSV virions are rod-shaped to elliptical with a trilaminar envelope and measure 80–120 × 250–380 nm (OIE 2003a).

Antibody-based assays

Both polyclonal and monoclonal antibodies to WSV have been developed, with summary descriptions of assays provided in the OIE Aquatic Manual (OIE 2003a). The polyclonal antibody-based assay has a sensitivity of 1 ng of WSV protein. Three relatively rapid methods based on monoclonal antibodies are available.

In situ DNA hybridisation

In situ DNA hybridisation uses 5 µm paraffin sections that are examined with a bright field microscope after preparation, with positive hybridisation appearing as a dark blue to black precipitate against the yellow to brown counterstain (OIE 2003a).

Bioassay

Bioassay will confirm the presence of a pathogenic virus, but does not identify the specific virus. Therefore, bioassay must be used in conjunction with laboratory tests to confirm the identity of the virus. Protocols for bioassays have been published by several authors (Rajendran et al 1999, Durand et al 2000).

The advantages and disadvantages of the commonly used laboratory tests are summarised in Table 3.

Transport of specimens

Specimens should initially be sent to the state or territory diagnostic laboratory. After obtaining the necessary clearance from the chief veterinary officer (CVO) of the state or territory of the disease outbreak and informing the CVO of Victoria, specimens will then be forwarded to the Australian Animal Health Laboratory (AAHL) Fish Diseases Laboratory (AFDL) in Geelong for disease testing.

The AFDL should be contacted directly to ensure that samples are collected correctly and sample collection techniques satisfy the requirements of the laboratory.

Table 3 Advantages and disadvantages of WSV tests

Diagnostic method	Advantages	Disadvantages
Rapid methods	Rapid diagnostic results; field friendly; detect multiple pathogens; inexpensive.	May not detect light infections.
Histopathology	Low probability of misdiagnosis in heavy infections.	May not detect light infections; not field friendly; needs at least 2 days preparation time.
PCR	Highly sensitive, capable of detecting very low pathogen levels; can be used to test all life stages; WSV-specific; rapid results.	Hypersensitive, prone to misdiagnosis; technically complex; relatively costly.
Transmission electron microscopy	Sensitive; useful in conjunction with virus purification.	Sophisticated equipment required; laborious and technically complex; expensive.
Antibody-based assays	Sensitive and specific.	Sophisticated equipment required; laborious and technically complex; expensive.
In situ hybridisation	Very sensitive; reliable; pathogen specific.	Histological preparation of tissue required; laborious.
Bioassay	Demonstrates presence of viable pathogen; useful in conjunction with a WSV-specific test.	Several days for result; not WSV-specific; relatively complex; expensive.

PCR = polymerase chain reaction; WSV = white spot virus

Source: adapted from Fegan and Clifford (2001)

1.4.4 Differential diagnosis

The clinical signs and gross lesions observed during outbreaks of WSD are nonspecific. Therefore, the diagnostician must consider any rapidly increasing mortality in a prawn pond as potentially being due to infection by an exotic virus, including WSV. To aid in differential diagnosis, key features of the two major exotic viral diseases known to be capable of causing massive mortalities in one or more of the penaeid species farmed in Australia (WSD and yellowhead disease) are compared in Table 4 with features of a major endemic viral disease in eastern Australia, peripheral neuropathy and retinopathy (PNR), which is associated with infection by gill-associated virus (GAV) (Callinan and Jiang 2003, Callinan et al 2003).

Two other viral diseases, although unlikely to cause outbreaks on Australian farms, are also described here because they are associated with mass mortalities. Taura syndrome, caused by Taura syndrome virus (TSV), has caused serious commercial losses only in juvenile to adult *P. vannamei* (Pacific white shrimp) in the Americas and more recently in Asia. Although a number of other penaeid species are susceptible to TSV infection, none is currently farmed in Australia (Lightner 1996, Flegel 2001). The susceptibility to TSV of the three prawn species farmed in Australia is unknown, and for this reason the differential diagnostic features of Taura syndrome, as it occurs in *P. vannamei*, are included in Table 4. Infectious hypodermal and haematopoietic necrosis (IHHN), caused by IHHN virus (IHHNV), causes severe mortalities in farmed, and possibly wild, *P. stylirostris* (Pacific blue shrimp; Lightner 1996, Pantoja et al 1999). Although *P. monodon* and *P. japonicus* are susceptible to infection, there are no reports of IHHNV-related disease in these species. *P. merguensis* is reportedly refractory to IHHNV infection (Lightner 1996).

Table 4 Differential diagnosis of virus-induced mortalities that may occur in Australian farmed prawns (*Penaeus monodon*, *P. japonicus*, *P. merguensis*)

	White spot disease	Yellowhead disease	PNR (GAV-related disease)	Taura syndrome (in <i>P. vannamei</i>)
Susceptible Australian farmed species	<i>P. monodon</i> , <i>P. japonicus</i> , <i>P. merguensis</i>	<i>P. monodon</i>	<i>P. monodon</i>	unknown
Stage of grow-out	All	Usually 7–10 wks post stocking	Usually >13 wks post stocking	Usually 2–6 wks post stocking
Mortality	High, rapidly increasing to 100% within a few days	High, rapidly increasing to 100% within a few days	Low to moderate, slowly increasing	Moderate in the peracute and acute phases
External appearance	Usually white spots embedded in cuticle or general red colouration	Often yellowish cephalothorax and general pale colouration	Often general red colouration and amputated appendages	Acute phase: general red colouration, especially tail fan
Organs showing virus-induced necrosis	Subcuticular epithelium, connective tissue, gills, lymphoid organ	Subcuticular epithelium, gills, lymphoid organ	Peripheral nerves, eyes	Subcuticular epithelium, connective tissue, gills
Inclusion body type	Intranuclear; eosinophilic (Cowdry type A) to basophilic	Intracytoplasmic; basophilic	Uncommon; intracytoplasmic; basophilic	Intracytoplasmic; initially eosinophilic, then basophilic

GAV = gill-associated virus; PNR = peripheral neuropathy and retinopathy.

Note: PNR is endemic in *P. monodon* in eastern Australia only. Taura syndrome features are as described for *P. vannamei*; susceptibility of Australian farmed species to Taura syndrome virus is unknown.

Massive mortalities in individual prawn ponds unrelated to disease events are rare, but can follow equipment failure or serious management errors (eg miscalculating chemical concentrations) as well as exposure to environmental toxins such as pesticides. However, such causes can usually be identified. Causes of more moderate mortalities, such as poor pond environmental conditions and subsequent bacterial infections in the prawns, can usually be identified by inspection of pond records and examination of representative moribund animals, using histopathology and microbiology if necessary.

Of particular note is the recent description of bacterial white spot syndrome in farmed prawns (Goarant et al 2000, Wang YG et al 2000), in which white spots macroscopically resembling those induced by WSV are visible in the cuticle. Exposure to high alkalinity has also been associated with formation of white spots unrelated to WSV infection or bacterial colonisation (OIE 2003a). Neither of these nonviral white spot conditions is associated with significant mortalities in affected prawns.

In summary, a provisional diagnosis of WSD is justified in the case of a disease outbreak in farmed prawns characterised by high and rapid mortalities, white spots and/or red body colouration on moribund animals, and demonstration using histopathology of eosinophilic to basophilic intranuclear inclusions in subcuticular epithelial cells. PCR and other tests can be used to confirm the diagnosis and rule out other possible aetiologies.

1.4.5 Treatment of infected animals

There is no effective treatment for WSV infection.

1.5 Resistance and immunity

Prawns possess immune systems that, although quite complex, are substantially different from vertebrate immune systems (Flegel 2001, Newman and Bullis 2001). It has been generally accepted that they have no true specific immunity (that is, no true antibodies) and substantially less haemocyte heterogeneity than vertebrates. Prawns possess both humoral and cellular responses, although these appear less specialised than vertebrate immune responses. Instead, there is an innate immunity comprising a diverse array of humoral factors that originate from and/or reside in the haemocytes and are released only during the immune response.

1.5.1 Responses to bacterial or fungal infections

The battery of prawn defences against invading bacteria or fungi includes rapid clotting, agglutination, phagocytosis, production of free oxygen species and production of bactericidins. There is an associated strong cellular response aimed at clearing the invading organisms from tissues and haemolymph, often via encapsulation and granuloma formation.

1.5.2 Responses to viral infections

The prawn response to viral infections contrasts sharply with its response to bacterial or fungal infections. In prawns, other crustaceans, and perhaps arthropods in general, there is a lack of inflammatory response to viral pathogens. As a result, the occurrence of single to multiple, persistent viral infections in an individual host is the general rule.

As discussed in Section 1.4.1, field and laboratory observations in recent decades have shown that epidemics caused by viruses such as WSV are characterised by initial, widespread, massive crop losses. These are followed, within approximately two years, by more sporadic crop losses coupled with widespread occurrence of persistently infected ponds with significantly reduced mortality. Also, the viruses carried by the persistently infected prawns remain lethal for naïve prawns in cohabitation tests.

To explain these observations, Flegel (2001) has proposed that prawns that have been exposed to a specific virus, such as WSV, during their early larval stages can tolerate subsequent persistent infection by that virus without developing clinical disease, provided they are not subjected to excessive stress. The theory proposes that tolerance to viral infection in crustaceans is the manifestation of a specific and active adaptive system for accommodation based on molecular binding at the host cell membrane. This binding induces specific memory for suppression of viral-triggered apoptosis and allows persistent, nonlethal infections.

However, recent findings call into question some aspects of this tolerance theory. Studies of WSV infection in *P. japonicus* (Venegas et al 2000) suggest that prawns may have a 'quasi-immune' protective response, which is activated by exposure to WSV and which enhances their ability to survive subsequent challenge. Wu et al (2002) have shown that *P. japonicus* developed significant resistance to

experimental lethal challenge by WSV. This resistance may have been associated with one or more humoral neutralising factors; it developed 3–4 weeks after exposure to WSV and persisted for a further month in prawns held at 24°C.

1.5.3 Vaccination

There are currently no vaccines available that protect prawns against WSV infection.

1.6 Epidemiology

Although WSV infection occurs in a wide variety of both wild and farmed crustaceans, WSD is essentially a disease of farmed penaeid prawns. The disease has exhibited pandemic behaviour in both Asia and the Americas, with substantial economic impacts on national industries. Its initial introduction into a country has consistently resulted in a severe epidemic featuring mass mortalities in farmed prawns, followed after 1–2 years by more sporadic events. Although the reasons for these patterns are not well understood, factors such as wide host and life stage range, host immune response and stressors are likely to contribute. The first two of these were discussed in previous sections, while stressors are briefly discussed here.

The role of stressors is more noticeable for sporadic events (D Fegan, Regional Technical Manager – Aquaculture, Alltech Biotechnology Corporation, Bangkok, pers comm, February 2002). Stressful events include handling of captive broodstock and severe pond water-quality fluctuations (Fegan and Clifford 2001, Flegel 2001). Low water temperature has also been associated with WSD outbreaks in latently infected populations of *P. vannamei* (Vidal et al 2001). However, the effect of temperature may differ between species, as Zhu and Lu (2001) reported that low temperatures enhanced survival in North American freshwater crayfish (*Procambarus clarkii*) infected with WSV.

To gain a better understanding of the dynamics of WSD outbreaks, Lotz and Soto (2002) simulated transmission of WSV within an individual pond using a Reed–Frost mathematical model. They concluded that there is likely to be a threshold density of susceptible prawns below which an outbreak of WSD will not occur. This, along with lower stress and infection levels (Lo et al 1997), may at least partly explain why WSD causes devastating outbreaks in farmed animals but not in wild populations.

1.6.1 Modes of transmission

Most studies of WSV transmission have focused on penaeid prawns. Infections have been found in all life stages. Prawns can acquire WSV infection by either vertical or horizontal routes of transmission.

Vertical transmission

Prawn larvae can become infected during spawning, although the precise route has not yet been identified. In their studies of WSV tissue tropisms, Lo et al (1997) were unable to find infected mature ova and suggested that infected ova were killed by the virus before maturation. Current evidence suggests that connective tissues in the gonads of parental broodstock may be a source of viral

contamination (Kou et al 1997, Lo et al 1997, Mohan et al 1997). Heavily infected postlarvae are strongly associated with crop failure due to WSD outbreaks during grow-out (Withyachumnarnkul 1999, Peng et al 2001).

Horizontal transmission

WSV can be transmitted horizontally via ingestion of infected tissue. Once an outbreak begins in a pond, rapid transmission is thought to mainly occur through cannibalism of sick and dead prawns (Wu et al 2001, Soto and Lotz 2001). This is supported by findings from feeding trials with penaeid prawns where ingestion of as little as 5% bodyweight of heavily infected tissue can result in transmission (Wang Q et al 1999). WSV may also be transmitted horizontally via water, but under pond conditions this route is probably less important (Soto and Lotz 2001, Fegan and Clifford 2001).

1.6.2 Reservoirs of virus

Broodstock and wild populations

Table 5 shows published test results for WSV for captured broodstock in Thailand, Japan, Taiwan and Panama. These results provide a guide to WSV infection prevalence in wild populations, but their accuracy is unknown because the effects of sampling bias (how well captured animals represent the source population) and measurement bias (how well laboratory tests represent virus infection) are unknown. However, it is thought that WSV is common and increasing in prevalence in wild prawns in countries where farms are affected by WSD (Lo and Kou 1999). Some studies have also found an association between season and prevalence of WSV infection in wild prawn populations (Lo et al 1997, Mushiaki et al 1998, Withyachumnarnkul et al 2003), although this may merely reflect seasonal variation in capture locations rather than an effect of season per se.

Infections in wild prawns are generally lighter than in farmed prawns. Using *in situ* hybridisation, Lo et al (1996) found that fewer cells were positive in wild, captured prawns than in farmed or experimentally infected prawns.

Table 5 Published prevalence estimates of WSV in wild prawns

Prawn sp	Prevalence (%)	Location	Reference
<i>Penaeus monodon</i>	83.3 ($n = 66$) ^b	Taiwan	Lo et al (1996)
	77.2 ($n = 88$) ^b	Taiwan	Lo et al (1997a)
	0–18.6% ($n = 24,338$) ^a monthly in broodstock over three years	Thailand	Withyachumnarnkul et al (2003)
<i>Penaeus japonicus</i>	9.2 ($n = 1269$) ^b	Japan	Mushiake et al (1998)
	20.3 ($n = 474$) ^b	Japan	Maeda et al (1998a)
	58.5 ($n = 159$) ^c	Taiwan	Lo and Kou (1998)
<i>Penaeus semisulcatus</i>	26.7 ($n = 15$) ^b	Taiwan	Wang YC et al (1998)
	6.3 ($n = 32$) ^b	Taiwan	Lo et al (1996)
<i>Penaeus penicillatus</i>	11.1 ($n = 27$) ^b	Taiwan	Lo et al (1996)
<i>Metapenaeus ensis</i>	33.3 ($n = 30$) ^a	Taiwan	Wang CS et al (1997)
<i>Penaeus vannamei</i>	2 ($n = 104$)	Panama	Nunan et al (2001)

n = number of prawns in study

^a 1-step PCR

^b 2-step PCR

^c not specified

Note: Detection was by PCR in all Asian studies and by dot-blot assay in the Panama study.

Infected hatcheries and farms

By far the major source of infection for rearing ponds is infected postlarvae from hatcheries presumably derived from captured brood stock. In a study in Thailand, Withyachumnarnkul (1999) showed that only 5% of intensive *P. monodon* ponds stocked with one-step PCR-positive postlarvae reached a profitable harvest, compared with 69% for ponds stocked with one-step PCR-negative postlarvae. Comparable results were obtained from a Taiwanese study (Peng et al 2001).

WSV can remain viable for 28 days in decaying prawn tail tissues (Prior and Browdy 2000), although Wang YG et al (2002) found that carcasses were infectious for only 6 days.

It is considered good practice to collect and dispose of moribund and dead prawns found at pond edges during an outbreak (Dr Pornlerd Chanratchakool, Shrimp Health Management Specialist, Aquatic Animal Health Research Institute, Bangkok, pers comm), but the extent to which similarly affected prawns remain out of reach on the pond bottom is unknown. It is possible that most moribund prawns, in response to severe virus-induced gill damage, congregate in the more highly oxygenated water at the surface and edges of the pond (D Fegan, Regional Technical Manager – Aquaculture, Alltech Biotechnology Corporation, Bangkok, pers comm, February 2002).

Other decapod crustaceans

Various wild decapod crustaceans, such as prawns (*Metapenaeus* spp), grass shrimp (*Acetes* spp), mud crabs (*Scylla serrata*) and blue swimmer crabs (*Portunus*

pelagicus), can carry WSV infection into prawn ponds when they enter via intake water or, in the case of some crab species, by migrating overland. Evidence from tank studies shows that crustacean carriers may infect prawns via water or after death when prawns ingest infected tissue (Supamattaya et al 1998, Kanchanaphum et al 1998, Fegan and Clifford 2001). While the actual risk of transmission of infection from non-prawn crustaceans to prawns in commercial ponds remains unclear, it probably depends in part on the prevalence of infection and virus load in these carriers.

Other carriers

Other carriers, such as copepods and insect larvae (Lo et al 1996, Liu et al 2000) may be sources of virus for farmed prawns, but the level of risk relative to the above sources appears to be small (Fegan and Clifford 2001).

It is routine practice in prawn hatcheries to feed prawn larvae with *Artemia* spp hatched from cysts. Currently, there is no conclusive evidence that commercially supplied *Artemia* spp are infected with WSV or that, even after exposure to the virus, they can transmit infection to prawns (Chang et al 2002, Hameed et al 2000).

Birds, especially predatory or scavenging birds such as terns (Sternidae) or gulls (Laridae), may mechanically transmit infection between ponds by releasing captured, moribund or dead prawns (Garza et al 1997, Fegan and Clifford 2001). Transmission via bird faeces may also occur, but there is no information on WSV survival in the avian alimentary tract.

Water

Purified WSV remained viable for 30 days in sterile seawater kept in dark conditions at temperatures up to 30°C (Maeda et al 1998b, Momoyama et al 1998) but Wang YG et al (2002) observed that cell-free WSV in seawater lost infectivity by 48 hours. This latter finding concurs with Flegel et al (1997), who suggested that WSV from outbreak ponds probably remains infectious for only 3–4 days.

Laboratory experiments have shown that WSV can be shed from infected crabs into water and thereby infect cohabiting prawns (Kanchanaphum et al 1998, Supamattaya et al 1998), but such transmissions have generally been done using relatively high virus titres or an unnaturally close proximity between the infected and uninfected animals. Laboratory studies indicate that cohabitation transmission of WSV infection between prawns is more than an order of magnitude lower than ingestion transmission (Soto and Lotz 2001, Wu et al 2001). In general, findings suggest that the risk from water as a source of WSV infection may be considerably lower than previously believed, except when heavily infected water, discharged into a shared water body during an outbreak, is pumped into an uninfected neighbouring farm (Fegan and Clifford 2001).

Sediment

There is no information on sediment as a source of WSV infection, but it is unlikely to be a significant source. Fegan and Clifford (2001) noted that successful crops of prawns have been raised in Asia in ponds containing decomposing prawn carcasses (and presumably the associated sediment) remaining after WSD outbreaks.

Farm equipment

Although there is no information on farm equipment as a source of WSV infection for prawns, it is possible that items of equipment, such as inadequately cleaned nets, may transfer infected animals or tissues between ponds.

1.6.3 Predisposing factors

Host factors

There is some evidence that susceptibility to WSV differs between prawn species and life stages. Lightner et al (1998) found more severe infections in *P. setiferus* (white shrimp) and *P. vannamei* postlarvae than in *P. aztecus* (brown shrimp) and *P. duorarum* (northern pink shrimp) postlarvae exposed to artificial challenge. WSV challenge of juveniles resulted in severe infections and 100% mortality in *P. setiferus* and *P. vannamei*, moderate infections and 27% mortality in *P. aztecus*, and no signs of infection and no deaths in *P. duorarum*. Yoganandhan et al (2003) reported infection, but no disease, in *P. monodon* larvae and early postlarvae exposed to WSV, while there was significant mortality in exposed late postlarvae and juveniles.

Furthermore, within species and life stages, differences in response to WSV infection may depend on whether or not the individual has had prior exposure to WSV or to other viruses (Venegas et al 2000, Flegel 2001, Tang et al 2003). See Section 1.5 for a summary of this issue.

Environmental factors

WSD outbreaks in latently infected populations often follow deterioration in the pond environment. Triggers for the expression of clinical disease in latently infected prawns may include rapid changes in variables such as water temperature and dissolved oxygen concentrations, hardness or salinity; these latter changes may act through osmotic stress allowing viral expression (Flegel et al 1997, Fegan and Clifford 2001).

Table 6 lists recommended ranges for important water quality variables in *P. monodon* rearing ponds. Prolonged exposure to values outside the optimal range for each variable or to rapid fluctuations (Fegan and Clifford 2001) can trigger WSD outbreaks in latently infected populations.

Table 6 Recommended ranges for key water quality variables for farmed *Penaeus monodon*

Variable	Optimal range	Comments
pH	7.5 to 8.3	Daily fluctuation < 0.5
Salinity	10 to 30 ppt	Daily fluctuation < 0.5 ppt
Dissolved oxygen	5 to 6 ppm	Not less than 4 ppm
Alkalinity	> 80 ppm (as CaCO ₃)	Dependent on pH fluctuation
Secchi disc reading	30 to 50 cm	
Hydrogen sulphide	< 0.03 ppm	More toxic at low pH
Un-ionised ammonia	< 0.1 ppm	More toxic at high pH and temperature

ppt = parts per thousand; ppm = parts per million

Source: Chanratchakool et al (1998)

1.6.4 Immunostimulants

The crustacean innate immune system recognises molecular patterns shared by large groups of pathogens, such as beta-glucans from fungi and lipopolysaccharides and peptidoglycans from bacteria. Several studies have shown that resistance of prawns to WSV can be enhanced by exposure to these compounds (Itami et al 1998, Chang et al 1999, Huang and Song 1999, Takahashi et al 2000). As their efficacy and methods of administration become better defined, immunostimulants may be used to improve the resistance of farmed crustaceans to WSV and other pathogens in an attempt to reduce the risk of disease outbreaks. However, any benefit they may confer is likely to be minimal in adverse environments or in the absence of appropriate disease prevention strategies (Newman and Bullis 2001).

1.6.5 Inactivation of the virus

The OIE disease card for WSD provides information, summarised in Table 7, on the susceptibility of WSV to physical and chemical agents.

Table 7 Inactivating agents

Physical agents	
Heat	50°C for 20 minutes, 70°C for 5 minutes
UV light	Inactivated by 9×10^5 $\mu\text{w s/cm}^2$ for 60 minutes
pH	Inactivated by pH 1 for 10 minutes, pH 3 for 1 hour, or pH 12 for 25 minutes at 25°C
Chemical agents	
Chlorine	Inactivated by sodium hypochlorite at 100 ppm for 10 minutes and 10 ppm for 30 minutes
Iodine	Inactivated by povidone iodine at 100 ppm for 10 minutes and 10 ppm for 30 minutes
Quaternary ammonia	Inactivated by benzalkonium chloride at 75 ppm for 10 minutes

UV = ultraviolet; WSV = white spot virus; $\mu\text{w s/cm}^2$ = microwatt seconds per square centimeter; ppm = parts per million

2 Principles of control and eradication

2.1 Introduction

Based on overseas experience, an outbreak of WSD in Australia is most likely to occur and be detected in farmed penaeid prawns, although the possibility of occurrence in other farmed crustaceans cannot be ruled out. It is unlikely that an outbreak of WSD will occur in wild crustacean populations, but WSV infection could be detected.

This section provides background information to enable the choice of the most appropriate control measures following either the occurrence of a WSD outbreak or detection of subclinical WSV. It focuses on the prawn farming industry, since most available information is derived from this sector, although the principles can be applied to other crustacean aquaculture enterprises or wild populations. In the following discussion, the term WSD will be used to refer to both an outbreak of the disease and to confirmed WSV infection.

The basic principles of eradication and other control responses are described in the AQUAVETPLAN **Enterprise Manual** and **Control Centres Management** manual. See the **Enterprise Manual** for state and territory legislation relating to disease control and eradication.

There are essentially three broad control options for WSD in Australia:

- *Eradication* – eradication of WSV from Australia (highest level of control measure and likely to be highest cost).
- *Containment, control and zoning* – containment of the virus to areas with endemic infection, prevention of further spread and protection of uninfected areas.
- *Control and mitigation of disease* – implementation of management practices that decrease the incidence and severity of clinical outbreaks (lowest level of control measure and likely to be lowest cost).

Within these overall options, the general principles for the control and eradication of WSV include:

- rapid detection and identification of infection;
- rapid definition of the nature and extent of the problem;
- rapid definition and implementation of control measures;
- prevention of viral spread, by controlling stock and water movement within and between farms or other infected sites; and
- maintenance of good management practices and high hygienic standards.

The most appropriate option will depend on:

- location and presence or absence of reservoirs of infection;
- chances of success of eradication;

- level of risk accepted for future spread of infection (eg from grow-out of infected populations);
- short-term costs of control and disruption to production;
- long-term costs of production with or without the presence of the pathogen; and
- long-term costs of control should the pathogen become endemic.

2.2 Methods to prevent spread and eliminate pathogens

Available methods for the control and eradication of WSV include the following.

2.2.1 Quarantine and movement controls

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

- establishment of specified areas (Figure 1) (see the AQUAVETPLAN **Enterprise Manual** for more details)
 - *declared area* – includes restricted area and control area
 - *restricted area* – an area around infected premises or areas
 - *control area* – a buffer between the restricted area and free areas
 - *free area* – non-infected area (this area is not considered a ‘declared area’ and may include large areas of Australia in which the presence or absence of WSV remains unassessed);
- bans on the movement of live and uncooked crustaceans out of infected areas;
- bans on the movement of live crustaceans into disease-free areas;
- restrictions or bans on releasing crustaceans and water into river systems or marine locations;
- restrictions or bans on the movement of crustaceans between different river systems, marine locations or farms; and
- restrictions or bans on the use and movement of equipment within and between river systems and between farms.

The following practices must be considered when implementing control strategies:

- live crustacean transportation between and within farm operations (including broodstock and postlarvae);
- crustacean harvesting and transportation to off-site processing plants;
- discharge of processing plant effluent;
- transportation of uncooked crustaceans and crustacean products;
- end use (particularly potential for use as bait) of uncooked, emergency-harvested crustaceans;
- disposal of dead crustaceans; and
- disposal of potentially infected water.

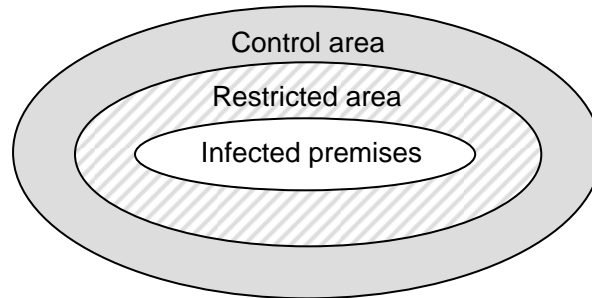


Figure 1 Establishment of specified areas to control WSD

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

Quarantine and farm types

Prawn farms can be classified, according to their dependence on external water supply during rearing, as open systems, partial recirculation systems, full recirculation systems or closed pond systems (Chanratchakool et al 1998). In reality, these systems are major groupings within a continuum, but for the purposes of this manual, open, partial recirculation and closed systems will be distinguished.

Farms using partial recirculation and full recirculation systems are common in Asia but not in Australia.

Open systems

Most Australian prawn farms employ an open system, whereby water is taken from and released to the source as necessary. Open system farms are not usually designed to be self-contained, and so preventing inflow or outflow of water may have adverse effects on farmed stocks. However, recent changes to farm management practices have led to the use of discharge/settlement ponds for treatment of effluent water. When present, settlement ponds provide an opportunity to hold and treat effluent water before its discharge into the general environment. Any empty ponds on the farm could also be used for the storage and treatment of effluent water.

Partial recirculation systems

Partial recirculation systems allow greater control over water intake and discharge than open systems, but there are differences between such farms in the extent to which input and output water can be contained. In addition to the production ponds, significant areas of the farm must be set aside to accommodate the inlet reservoir, effluent settlement areas and storage reservoir. Partial recirculation systems are often used in areas where there are occasional problems with water supply, such as disease agents or pollutants in inlet water. When water cannot be pumped onto the farm, effluent from the production ponds is first allowed to settle and may be treated before being mixed with the water reserve and subsequently reused in the ponds as necessary.

Closed systems

Closed systems include full recirculation systems and closed pond systems. Full recirculation systems, which predominate over partial recirculation systems where water supply problems are more severe and persistent, dedicate a relatively higher proportion of the farm area to water storage and treatment. Under this arrangement, the farm is filled with water at the start of the production cycle and the farm, but not the ponds within it, subsequently remains closed until harvest is completed.

A closed pond system is one where, from the start of the production cycle, all water is treated to remove agents of interest, the farm is closed to the introduction of additional water and animals during the production cycle, and ponds are operated with minimal or no water exchange. However, in many cases water must be added to ponds during rearing to compensate for losses due to evaporation or seepage.

Freshwater crustacean farms, such as those growing red claw crayfish or yabbies, operate as closed systems with either no water exchange (simple farm dam) or with water circulation to and from a reservoir on the property. In Queensland, licence conditions for red claw farmers require a closed water system. In these cases, spread of the disease through water movement is not a major threat, although the movement of water either into natural watercourses or overland may occur if the reservoir overflows.

Hatcheries

Hatchery systems also offer the potential for recirculation and/or the treatment of effluent water before its discharge.

Zoning

The principles of zoning for infected and non-infected zones in Australia are outlined in the **AQUAPLAN Zoning Policy Guidelines**.³ If WSV becomes endemic in specific regions of Australia, a zoning policy specific for WSV may be necessary to protect non-infected areas and to prevent further spread of infection. A corresponding surveillance and monitoring program for WSV would also be required to support zoning.

Zoning for WSD may be difficult. Several surveys have shown that prawns can carry WSV infections below the current level of detection available with a nested PCR test. Under stress, such as occurs during spawning in hatcheries, viral replication can occur, with infection reaching a level where it can be detected by PCR (Mushiaké et al 1999, Peng et al 2001). Thus, in the absence of stress, latently infected prawn populations may become established and could be very difficult to detect. Reservoirs of infection could become established in the environment in any of the 35 or more species of crustacean that are susceptible to WSV infection, and these reservoirs are unlikely to be successfully eradicated.

³ See <http://www.affa.gov.au/content/output.cfm?ObjectID=D2C48F86-BA1A-11A1-A2200060B0A00717> (Accessed 16 June 2005).

The major sources of infection for wild crustacean populations are thought to be infected tissue from moribund or dead prawns and heavily infected water released from diseased ponds (Fegan and Clifford 2001). Infected animals may disperse throughout the population's range, which may be extensive if the species is migratory, as in some species of prawns (Kailola et al 1993). Horizontal and vertical transmission of infection within wild prawn populations may occur but is probably much less important than that directly acquired from infected farms. It is when infected wild prawns are used as broodstock in hatcheries that they become important in WSV dissemination.

These factors make it very difficult to protect WSD-free zones. Currently, no zoning programs are established for WSD in any country where it is endemic. For several years, the Philippines remained WSV-free by strictly enforcing a ban on the importation of live penaeid broodstock and postlarvae. The eventual introduction of WSV into the Philippines is believed to have occurred as a result of illegal movement of infected postlarvae (Magbanua et al 2000).

Due to the current practices of sourcing broodstock prawns from the wild and the widespread distribution of postlarvae from a relatively small number of hatcheries, it may be simpler to certify disease-free farms rather than introduce full zones with accompanying movement restrictions. Zoning for crustacean species whose lifecycle has been closed in captivity (eg red claw crayfish) would be much simpler, because there is less demand to move livestock between farms.

Strategies for control

There are claims that WSV has been successfully eradicated from some Central American farms (Lawrence et al 2001, Boyd and Clay 2002) through the use of specific pathogen free (SPF) *P. vannamei* and *P. stylirostris* combined with closed farm management systems. Eradication of WSV from farms in Asia has not been attempted due to the industry's reliance on wild-caught broodstock. Research programs are under way in several countries, including Australia, to close the breeding cycle and produce SPF lines of *P. monodon*.

Currently, closed grow-out systems for prawns are uncommon in Australia; they are only being used experimentally, both in coastal and in inland saline sites. Conversion of a prawn farm from an open to a more self-contained system will generally involve substantial changes to farm layout to enable additional water storage and recirculation (Chanratchakool et al 1998).

Animal inputs and outputs can be controlled, although some movement restrictions could significantly interrupt farm management practices and production. Animal inputs to farms may be from off-farm or on-site hatcheries, or from growing stock obtained from other farms. Importantly, animals are also able to enter farm waterways and ponds from the adjacent aquatic environment, mainly via intake water. Red claw crayfish will move between ponds and thus all ponds on a farm should be considered to be part of the one system: if one pond contains infected animals, all ponds may contain infected animals. Boundary fences, a licence condition on red claw farms, will help prevent animal movement beyond the production ponds.

On prawn farms, wild crustaceans such as crabs can readily access the ponds. The use of small fences around each pond can prevent access by terrestrial crabs (Fegan and Clifford 2001).

Physical exclusion of WSV carriers from ponds by screening intake water is a valuable disease management strategy. Fegan and Clifford (2001) advocate screens in the inlet structures to a maximum of 500 µm, preferably 200–250 µm for initial filling. Bag net filters provide a much larger surface area than two-dimensional framed screens, and inserting one bag inside another is an economical way to decrease the effective mesh size.

Aerators, particularly the paddlewheel type, generate aerosols that may spread infection between ponds and possibly farms (Fegan and Clifford 2001).

2.2.2 Tracing

A critical step in determining the most appropriate control option is to conduct an investigation into the incident in order to determine all confirmed and potential locations of the virus. The presence or absence of predisposing factors should be examined when determining the duration of the outbreak and estimating the time and source of initial infection. It is possible that covert infection may be present for some time before clinical disease becomes apparent.

The information gathered from tracing will assist in determining the most appropriate response action. Immediate tracing steps are to trace-back all contacts with infected crustaceans, premises and sites (to establish the origin of the outbreak) and to trace-forward all contacts with infected crustaceans, premises and sites (to establish the current geographical distribution and potential for further spread of infection).

The following must be traced:

- live crustaceans – broodstock, postlarvae, live animals to restaurant trade etc;
- dead crustaceans – uncooked prawns intended for consumption or for bait use (cooked crustaceans need not be traced)
- effluent and waste products from processing and/or cooking;
- water – input and output;
- vehicles – crustacean transport vehicles, feed trucks, visitors' cars, boats;
- materials – nets, paddlewheels, pumps, tools and instruments; and
- personnel – farm workers, sales and feed representatives, tradespeople, veterinarians, scientists, technicians and visitors.

Neighbouring crustacean populations

Neighbouring crustacean farms and processing plants may become or may already be infected. Maps with the location of neighbouring crustacean farms, processing plants and waterways, and hydrographic data are necessary to monitor the potential spread of the pathogen. The location and abundance of susceptible crustacean species and potential vectors should also be considered, both upstream and downstream of the infected site. Further sources of infection may be identified if a number of facilities share common water.

For information on the location of farming establishments and wild crustacean populations at risk of infection, the relevant state or territory fisheries or agriculture agency can be contacted (see the **AQUAVETPLAN Enterprise Manual** for contact details).

2.2.3 Surveillance

Surveillance, by screening for clinical signs and by laboratory testing, is necessary to:

- define the extent of infection;
- detect new outbreaks;
- establish restricted and control areas to which quarantine and movement restrictions are applied;
- establish disease-free and infected areas/zones for a WSV zoning program; and
- monitor the progress and success of an eradication strategy.

2.2.4 Treatment of WSV-infected crustaceans

There is no effective treatment for WSV infection.

2.2.5 Destruction of crustaceans

Slaughter must be both hygienic and humane. There must be no spillage of infectious waste. Increased viral shedding may occur if crustaceans are stressed at slaughter, so methods that minimise stress should be used.

Methods for the destruction of crustaceans are described in the AQUAVETPLAN **Destruction Operational Procedures Manual**.⁴ Factors that will affect the choice of destruction method are:

- the ability to contain pond or tank water – all water must be treated before discharge;
- destination – human consumption or disposal;
- size and number of animals;
- desirability of removing most or all dead animals from the pond bottom before disinfecting the water;
- the need to prevent scavengers, particularly birds, from spreading infection during the destruction process;
- deadline for slaughter – depends on the risk of further spread posed by the particular infected population;
- slaughter facilities – site, equipment and methods available; and
- experience and availability of personnel.

In general, if farming practices that are routinely used in harvesting can be applied to stock destruction, these practices should be used. Farm staff will be familiar with them, and the necessary equipment may be available on site.

Depending on the circumstances of the outbreak or virus detection, grow-out or emergency harvesting may be possible.

⁴See <http://www.affa.gov.au/content/publications.cfm?ObjectID=D30314C9-CB66-4BE5-809CB7719F4C5906> (Accessed 16 June 2005).

2.2.6 Treatment of prawn products and byproducts

Emergency harvest and sale of clinically unaffected prawns may provide an attractive alternative to disposal because the farmer could recoup some financial losses through sale of the product. Trade regulations, market requirements, food safety standards and potential spread of the pathogen must be considered before this alternative is considered and the treatment/processing and destination of prawns is finalised.

WSV remains infectious for up to 28 days in decaying prawn tail tissues (Prior and Browdy 2000). In addition, the virus can survive well in prawns frozen for extended periods, and viable WSV has been recovered from commodity prawns purchased at retail supermarkets (Nunan et al 1998, Durand et al 2000). Therefore, prawns and prawn products should be processed before leaving the infected premises to prevent the spread of infection.

Studies on the heat stability of WSV in semipurified suspensions from three different studies are summarised in Table 8. Although Nakano et al (1998) found that the virus was inactivated after 1 minute at 60°C, Chang et al (1998) found live virus after 5 and 30 minutes at 55°C. In addition, WSV in prawn tissues may be more resistant to heating due to the protective effect of proteins. Because of this uncertainty, biological material potentially infected with WSV should be heat treated at 60°C or more for at least 20 minutes to ensure inactivation.

Table 8 Relationship between time and temperature for inactivation of WSV

Temperature (°C)	Time (minutes)								
	0.2	1	5	10	20	30	60	90	120
25	–	–	L	–	L	L	–	L	–
40	–	–	–	L	L	L	L	L	L
50	–	–	–	L	D	–	D	–	D
55	–	–	L	–	–	L	–	D	–
60	–	D	–	D	D	–	D	–	–
70	D	D	D	–	D	D	–	–	–
80	–	–	–	–	D	–	–	–	–

– = not done; L = live virus recovered after treatment; D = virus was dead; WSV = white spot virus.

Sources: Chang et al (1998), Nakano et al (1998) and Maeda et al (1998b)

Winkel (1998) recommended that prawns be cooked to a core temperature of 85°C to prevent blackspot and deterioration of prawn quality. A core temperature above 80°C was achieved and maintained for at least 75 seconds by heating 14 g, 22 g and 30 g *Penaeus monodon* for 3.5, 4.0 and 4.5 minutes respectively in boiling water. An examination of Table 8 suggests that Winkel's recommended cooking practices may be expected to inactivate WSV in infected prawns.

WSV is transmitted both vertically and horizontally in hatcheries. The virus is thought to contaminate the surface of fertilised eggs during spawning; there is no evidence that mature gametes are infected prior to fertilisation (Lo et al 1997b). Washing eggs with seawater alone is insufficient to remove WSV (Satoh et al 1999), and there is no reliable method for the disinfection of eggs to remove or inactivate the virus. However, WSV can in some cases be eliminated or its concentrations reduced through surface disinfection of eggs and/or recently hatched nauplii. A widely used method is presented in Chapter 1.1.5 of the OIE Aquatic Manual (OIE 2003a).

2.2.7 Disposal of animal products and byproducts

Disposal of dead and destroyed animals and infected material must be immediate to decrease infection pressure on the site. Diseased and dead prawns are the main source of WSV particles in the environment and, together with all other potential carrier crustaceans on the site, they should be removed as soon as possible and disposed of, together with other infectious waste, to prevent further dissemination of infection. Burial sites must be chosen carefully to ensure that there is no contact with waterways or vectors.

See the AQUAVETPLAN **Disposal Operational Procedures Manual**⁵ for details of disposal methods.

2.2.8 Decontamination

Due to differences in farming enterprises, disinfection protocols may need to be determined case by case in discussions between the farm manager and the state/territory CVO and/or director of fisheries. The protocol should take into consideration the factors outlined in Section 1.6, in particular:

- the source and location of infection;
- the type of enterprise (hatchery, grow-out ponds or processing plant);
- the construction materials of the buildings/structures on the site;
- the design of the site and its proximity to other waterways or buildings;
- options for removing and destroying infected animals before disinfecting water;
- options for preventing access to infected animals by scavenging birds;
- the protocol's environmental impact; and
- availability of approved, appropriate and effective disinfectants.

Under normal pond conditions, WSV survives in water for only 3–4 days (Flegel et al 1997). The recommended treatment for pond water is to add 30 ppm active chlorine and hold for 4 days before discharge.

Data are not available on the length of time that WSV can survive in mud or pond sludge. Following removal and safe disposal of dead prawns and other crustaceans, ponds, reservoirs, canals and drains should be thoroughly dried and the upper 10–15 cm of sludge should be removed. After drying for at least one month, all must then be treated with at least 0.5 kg/m² of slaked lime (Ca(OH)₂) before refilling and restocking.

Effective decontamination of equipment, materials, tanks and buildings requires thorough cleaning before disinfection.

Boots, nets and other equipment can be disinfected with a solution containing at least 30 ppm active chlorine.

⁵ See <http://www.affa.gov.au/content/publications.cfm?ObjectID=448A0116-62BC-44D7-9418A60DED71BCA5> (Accessed 16 June 2005).

Environmental considerations

Environmental considerations in the control of WSD include the following.

- Discharge of infected or potentially infected effluent into catchment areas or natural waterways will lead to further spread of infection and could lead to the establishment of reservoirs of infection in wild crustacean populations.
- The release of disinfectants could adversely affect the environment, especially if they are used in larger than normal quantities or concentrations, as is possible in a disease control operation. The local environmental protection agency may need to be consulted (see the AQUAVETPLAN **Enterprise Manual**).
- The destruction and disposal of infected carcasses/material will have an impact on the environment. This impact must be minimised while ensuring that there is no dissemination of infection.

2.2.9 Vaccination

Although recent studies of crustacean immunology suggest some capacity for acquired immunity, there are currently no vaccines for WSD, and vaccination is unlikely to be a practical option in the foreseeable future.

2.2.10 Vector control

Seabirds and wading birds are common on prawn farms. Dead or moribund prawns at the surface of open ponds typically attract many birds, so the ponds must be covered (eg using nets or similar) to prevent birds gaining access and thereby transmitting infection. Past experience has shown that the netting of sites is by far the most effective deterrent. A range of cheap netting, which is commonly used to protect orchards from birds, is commercially available and is quite suitable for this purpose.

Several other methods are available, including a range of pyrotechnics and automatic exploders that must be used in accordance with local laws and ordinances. Other techniques, such as recorded bird distress calls, are effective with some species, for some time.

As a last resort, firearms can be used as an alternative to noisemakers and, if necessary, to kill a limited number of birds to reinforce the fear instinct within a flock (Littauer 1990). In Australia, firearms may only be used by licensed shooters and may require further police permits. Extreme care must be taken with the use of live ammunition, and all staff should be briefed before it is used. In most jurisdictions, the killing of wild birds requires a permit from the local environmental protection or national parks agency (see the AQUAVETPLAN **Disposal Operational Procedures Manual**).

Where possible, contact between wild crustaceans and farmed prawns should also be prevented. Fences around pond perimeters, constructed from shadecloth-type netting (2 mm mesh size and 30–40 cm high), can prevent access by crabs and other crustaceans (Fegan and Clifford 2001).

2.2.11 Sentinel and restocking measures

Prawn species known to be susceptible to WSV infection and to WSD may be stocked as sentinel animals. Suitable species would include both *P. monodon* and *P. japonicus* because clinical disease has been observed after natural infection in both these species. This susceptibility, combined with the ready availability of these species in Australia, would make them the most suitable sentinel animal species.

The following time required before restocking will need to be assessed case by case. The period will depend on the number of sites with confirmed diagnoses, the features of the sites, the season and the extent of the outbreak. In Thailand, where WSV is endemic, best-practice sustainable prawn farming includes both fallowing to dry ponds and the use of lime to treat ponds before restocking (Chanratchakool et al 1998). A minimum of four weeks fallowing and drying before treatment with lime and refilling is suggested.

For eradication, restocked prawns must be free of infection. If areas are declared free of WSD, prawns introduced into those areas must also be free from infection.

2.2.12 Public awareness

A public awareness campaign must emphasise education, surveillance and cooperation from industry and the community in order to control potential outbreaks of WSV in Australia.

The campaign should emphasise that WSV is harmless to humans. The campaign should also stress that the use of potentially infected green prawns as aquaculture feed or bait could contribute to the spread of disease.

2.3 Feasibility of specific options for control in Australia

The feasibility of control of an outbreak of WSD in Australia depends both on the nature of the outbreak and on the control management strategy adopted. Essentially, as outlined in Section 2.1, there are three broad control options:

- *eradication* – eradication of WSV from Australia
- *containment, control and zoning* – containment of the virus to areas with endemic infection, prevention of further spread and protection of uninfected areas
- *control and mitigation of disease* – the implementation of management practices that decrease the incidence and severity of clinical outbreaks.

2.3.1 Eradication

Justification for attempting eradication is based on the following:

- Evidence suggests that WSV infection will not persist in wild populations in the absence of repeated inoculations from infected farms or processing plants.
- WSV has been successfully eradicated from farms in Central America, and those farms have subsequently produced profitable crops by using certified WSV-free domesticated stock and a completely closed culture system.
- Closed system farms in infected Australian zones could be stocked with PCR test-negative postlarvae derived from PCR test-negative wild-caught *P. monodon* broodstock collected from known free zones.

Eradication of WSV from an infected farm is possible only if the following measures are effectively implemented:

- entry or escape of susceptible, potentially infected wild crustaceans is prevented by perimeter fencing;
- all susceptible, potentially infected crustacean populations on the farm are eradicated;
- all water is disinfected before release; and
- the farm is completely dried out.

The farm could resume production, provided:

- a closed production system is implemented;
- individual ponds are fenced as described above;
- when reservoirs and ponds are re-filled, 250–500 µm filter screens (Fegan and Clifford 2001) are used to minimise the risk of entry of wild crustacean carriers;
- any crustacean carriers passing these barriers are eliminated by treating pond water with chemicals such as calcium hypochlorite or trichlorfon (Fegan and Clifford 2001) before ponds are filled; and
- ponds are stocked with tested WSV-free postlarvae derived from WSV-free broodstock.

Treated water must be held for at least 10 days to eliminate virus and allow chemical levels to drop to an acceptable level before pond stocking.

Eradication may not be feasible if epidemiological investigations determine that WSV infection is widespread across most or all Australian prawn-producing zones, has no controllable point source or is otherwise unable to be contained. This could be due to:

- the ability of WSV to spread widely and rapidly via translocation of infected hatchery-produced postlarvae and to establish reservoirs of infection in wild crustacean populations – it is likely that infections in wild populations can only be eradicated over time by eliminating the source of infection from farms;
- the pathogen's ability to produce latent/covert infections and the difficulty of detecting such infections;

- the lack of a full understanding of how the pathogen is transmitted and how it survives in the aquatic environment;
- the existing close contact between farmed and wild crustacean populations and water, and the relative lack of control over them, in Australian crustacean farming; and
- the possibility that WSV infection may become widespread under natural conditions in some wild Australian crustacean populations, distinct from those used for farming.

A further factor that might limit eradication is the current reliance of Australian *P. monodon* farmers on wild populations to provide broodstock. Depending on infection prevalence, the presence of WSV in wild populations would probably lead to its introduction to the prawn farming industry. By contrast, the lifecycle for farmed *P. japonicus* and *P. merguensis* in Australia is closed.

Unexposed prawns

Ponds holding young (pre-market sized) unexposed prawns may be allowed to grow out, provided that there has been no possibility of infection and there is no possibility of future infection. Older prawns that have had no possible exposure to infection may be harvested and sold.

Effective farm, transportation and processing hygiene protocols are necessary. On-farm processing and cooking may be preferable if the site is infected, to prevent any potential spread of infection during transport to off-site processing plants.

Immediate destruction of unexposed prawn populations in a declared area will decrease the chance of spread of infection. However, such action may be of doubtful benefit if infection has already spread to adjacent wild populations.

Exposed or potentially exposed, but clinically normal prawns

In an eradication campaign, exposed and potentially exposed but clinically normal farmed prawn populations should be treated as infected. Two options are available for these prawns:

- destruction and disposal in the same way as for clinically infected prawns
- emergency harvesting followed by on-site processing (for example, by cooking) to prevent the further spread of infection, and marketing if the prawns are of marketable size.

Both options will lead to the immediate destruction of these prawn populations, so both are very effective at decreasing the infectious load on a site and minimising the spread of infection.

Covertly infected prawns are safe for human consumption.

The techniques used in emergency harvesting of these prawns must ensure that there is no further spread of infection. Necessary measures include:

- disinfection of all equipment and personnel involved in harvesting, slaughter and processing;

- quarantine restrictions and procedures on the infected site, including for personnel, equipment and vehicles;
- on-site processing and cooking adequate to inactivate the virus; and
- holding, treatment and safe disposal of slaughter/processing effluent (including water and waste such as prawn heads and shells).

Clinically diseased prawns and other crustaceans

Immediate removal, destruction and disposal of all diseased and dead prawns are essential to the success of an eradication strategy. These prawns, along with infectious waste such as heads and shells, are the main source of WSV infection in the environment. Burial sites should be chosen carefully to ensure that there is no contact with waterways, groundwater or vectors.

2.3.2 Containment, control and zoning

There is no effective treatment available for WSV in infected animals. If eradication is not feasible, zoning and associated control measures to maintain uninfected zones will be necessary in the event of an outbreak.

A successful zoning strategy will rely on movement restrictions on exposed or potentially exposed prawns to prevent infection spreading to uninfected zones. The feasibility of zoning will depend on the farm management practices, the extent to which infection has already spread and the location, distribution and migratory behaviour of infected species (Kailola et al 1993).

The feasibility of containment, control and zoning can only be assessed at the time of an outbreak, taking into account movement restrictions required on prawns, people, vehicles and boats, and market access for the prawn products and byproducts.

In a declared area, normal or controlled grow-out and harvest may be feasible without further spread of infection, provided closed production systems and adequate processing are used.

Justification for attempting containment and control within a zone is based on the following:

- Infected tissue from moribund and dead prawns, together with heavily infected water discharged during outbreaks, is the main source of infection for wild populations adjacent to farms (Fegan and Clifford 2001).
- Provided appropriate disease control and health management measures are implemented (Chanratchakool et al 1998, Fegan and Clifford 2001), potentially infected and uninfected closed prawn farms can continue to operate, albeit at reduced profitability, in countries where WSV is endemic.
- Farms in infected Australian zones could be stocked with PCR test-negative postlarvae derived from PCR test-negative wild-caught *P. monodon* broodstock.

There are several containment, control and zoning options. The option chosen should prevent further exposure of crustacean populations within the zone and prevent the spread of infection beyond the zone.

Unexposed prawns

Provided there has been no possibility of infection with WSV, pre-market sized prawns may be allowed to grow out and be sold for human consumption.

Exposed or potentially exposed, but clinically normal prawns

In a containment, control and zoning strategy, grow-out of exposed or potentially exposed, but clinically normal prawns will be standard practice within infected zones. The likelihood of clinical disease will be minimised through appropriate farm management practices. Spread of the disease will be prevented through farms maintaining a high level of hygiene and biosecurity.

During grow-out, these prawns must be treated and handled as infected populations. Restrictions on movements of prawns, people, vehicles and boats, and on market access for products, may be necessary to protect WSV-free facilities or zones.

Within free zones, exposed or potentially exposed prawns will either be destroyed or harvested and processed on site (for example, by cooking) in the same way as in an eradication policy.

Clinically diseased prawns

In a containment, control and zoning strategy, clinically diseased prawns will be immediately destroyed and safely disposed of.

There is no vaccine or drug treatment available for WSD, and the virus persists in any prawns that survive infection. Clinically diseased prawns, along with infectious wastes, are considered to be the main source of WSV particles in the environment and are the most likely means for infection to spread to uninfected zones.

Water from infected ponds must be disinfected to destroy WSV and WSV carriers before release from the farm.

The ponds must be dried out and chemically treated to destroy WSV before refilling and restocking.

2.3.3 Control and mitigation of disease

Justification for attempting control and mitigation within a zone is based on the following:

- Infected tissue from moribund and dead prawns, together with heavily infected water discharged during outbreaks, is the main source of infection for wild populations adjacent to farms (Fegan and Clifford 2001).
- Provided appropriate disease control and health management measures (Chanratchakool et al 1998, Fegan and Clifford 2001) are implemented, potentially infected and infected partial recirculation and closed system prawn farms can continue to operate, albeit at reduced profitability, in zones where WSV is endemic.
- Farms in infected Australian zones could be stocked with PCR test-negative postlarvae derived from PCR test-negative wild-caught *P. monodon* broodstock.

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

- establishment of formal free and infected zones;
- the exclusive requirement for closed production systems; and
- in partial recirculation systems, the disinfection of all water to destroy WSV and WSV carriers before release off-farm.

2.3.4 Trade and industry considerations

In countries where WSV is endemic, the only industries that have been affected by the disease are the penaeid prawn farming industries. However, many other species of crustacean have been shown to be susceptible to WSV infection, and it is impossible to predict whether intensive aquaculture practices would result in clinical disease in other cultured or wild species.

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 disease control programs to be released and sold for human consumption.

Export markets

WSV is listed by the OIE (OIE 2003b). The presence of viable WSV in commodity shrimp has been described previously (Nunan et al 1998), and this finding may make it difficult to access markets in WSV-free countries.

However, WSV is endemic throughout most of south, east and southeast Asia, including in major export markets such as Japan, Hong Kong and China, as well as in the Americas. Most of our major trading partners accept product from WSV-endemic areas, so problems with market access are not expected. The loss of WSD-free status could, however, affect pricing because the Australian product could no longer be differentiated from competitors on the basis of disease freedom.

Some countries have regional requirements that differ within the country, for example in some states of the United States. Biosecurity Australia and the Australian Quarantine and Inspection Service should be consulted for detailed information about current export market requirements.

The presence of WSV in Australia is not expected to result in major impacts on market access for cooked prawns exported from Australia.

3 Policy and rationale

3.1 Overall policy

White spot disease (WSD) is a highly contagious disease of penaeid prawns that has the potential to cause high levels of mortality in farmed prawn populations, as well as significant control costs. It is endemic in both cultured and wild prawn populations in Asia and the Americas. Australia is currently free of WSD.

In an outbreak of WSD, or if the white spot virus (WSV) is detected, the choice of response option will be decided by the director of fisheries and/or the chief veterinary officer (CVO) of the state or territory in which the outbreak occurs, following initial epidemiological investigations.

There are three possible response options for WSD control in Australia:

- ☞ Option 1 – *eradication* with the aim of having Australia return to being free from WSV;
- ☞ Option 2 – *containment, control and zoning* of the virus to areas with endemic infection, prevention of further spread and protection of uninfected areas; and
- ☞ Option 3 – *control and mitigation* of disease if it is accepted that the virus will remain endemic in Australia.

All these response options involve the use of a combination of strategies, which may include:

- ☞ *quarantine and movement controls* on crustaceans and things in declared areas to prevent spread of infection;
- ☞ *destruction* of all clinically diseased or dead prawns as soon as possible, to prevent further virus shedding;
- ☞ *decontamination* of facilities, products and things to eliminate the virus from infected premises and to prevent spread of infection;
- ☞ *surveillance* to determine the source and extent of infection and to provide proof of freedom;
- ☞ *zoning* to define and maintain infected and disease-free zones; and
- ☞ *hygiene and biosecurity measures* aimed at mitigating the on-farm effects of WSD.

Eradication may not be feasible if epidemiological investigations determine that WSV infection is widespread across most or all Australian prawn producing zones, has no controllable source or is otherwise unable to be contained. Similarly, the feasibility of containment and zoning will depend on farm management practices, the extent to which infection has already spread, and the location, distribution and migratory behaviour of infected species. If infection is widespread, and there is evidence of widespread infection in wild broodstock populations, control and mitigation of the disease is likely to be the most appropriate option.

The director of fisheries and/or the CVO of the state or territory in which the outbreak occurs will decide the appropriate response option in consultation with the aquatic Consultative Committee on Emergency Animal Diseases (aqCCEAD). The nature of the response will be determined mainly by whether the outbreak is multifocal or localised, and the likelihood that containment and eradication can be achieved. The most appropriate strategy must be chosen after epidemiological investigations have been conducted (see Section 3.3.3), and the decision must be based on scientific effectiveness and financial feasibility. While eradication may be the preferred option, it may not be feasible, given the limited success of eradication and control policies in other countries.

For a description of the notification arrangements, order of procedures, management structures and roles of personnel following suspicion of the presence of WSD in Australia, see the AQUAVETPLAN **Control Centres Management** manual.

3.2 Overview of response options

As soon as adequate information becomes available, a decision will be made on the appropriate response, based on the reasoning shown in Figure 2.

Eradication will only be attempted if the infection appears to be limited to farmed prawns in one or a small number of facilities, and if eradication is deemed to be achievable. If infection occurs in a larger number of farms or extensively in wild prawns, one of two levels of control will be undertaken. The level of control chosen will depend primarily on the feasibility of zoning.

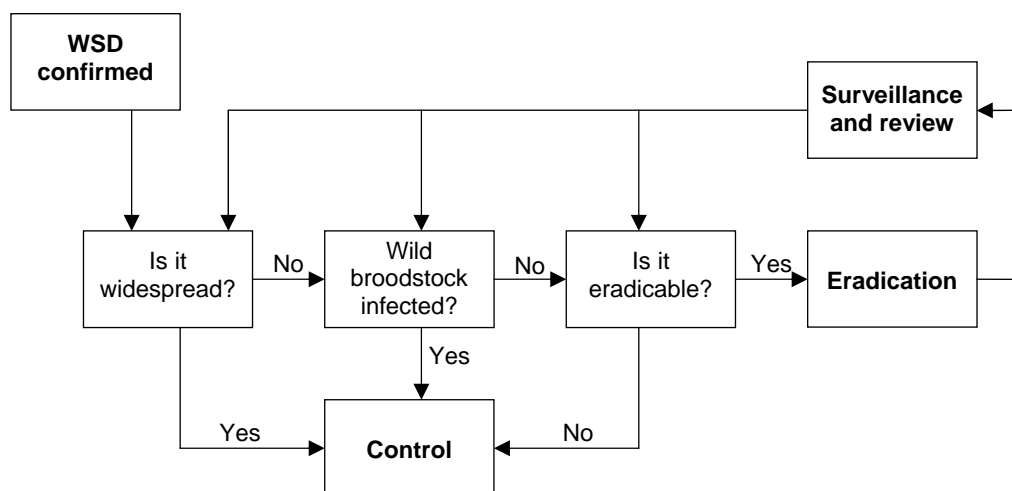


Figure 2 Decision flow chart

3.2.1 Option 1 — Eradication

If epidemiological investigations determine an obvious point source of infection that can be contained with minimal or no spread of the virus, an eradication strategy may be successful and will be attempted. Compared with the other response options, eradication may have the highest short-term costs.

Eradication is unlikely to be successful or feasible if epidemiological investigations determine that infection is widespread in farms, has no identifiable point source, is assessed as unable to be contained or is potentially widespread in wild prawns. However, the potential constraint on eradication posed by the presence of infection in wild prawns is equivocal, and judgment will need to be exercised based on whether or not a supply of uninfected broodstock is known to be available.

Eradication measures include:

- establishment of specified areas – restricted, control, free;
- quarantine and movement controls or restrictions on prawns, other crustaceans, water and any other vectors (including materials and equipment) in declared restricted and control areas to prevent the spread of infection;
- destruction and disposal of all clinically diseased prawns;
- on-farm processing (for example, by cooking) of exposed or potentially exposed, but clinically normal prawns to prevent the spread of infection;
- disinfection and safe disposal of processing effluent and waste (cooking water, prawn heads and shells);
- disinfection and safe disposal of pond water and decontamination of ponds, facilities, products, equipment, vehicles, boats etc to eliminate the virus from infected premises and to prevent spread;
- use of farm perimeter barriers to prevent entry or escape of potentially infected wild crustaceans;
- tracing and surveillance to determine the source and extent of infection and to provide proof of freedom from the disease; and
- a public awareness campaign to encourage cooperation from industry and the community.

3.2.2 Option 2 — Containment, control and zoning

If infection is widespread in wild prawn stocks or on numerous farms, eradication is likely to be impracticable. In this situation, containment and prevention of further spread and the protection of uninfected areas will be the preferred response. Containment, control and zoning will also apply outside affected farms when eradication is pursued.

As well as protecting uninfected regions, a zoning program will help the Australian prawn industry to maintain premium pricing in export markets. Restrictions on the movement of prawns and prawn products and a surveillance program will be necessary to support zoning.

Farms in infected zones will need to implement management practices to reduce the severity and impact of WSD outbreaks.

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

- zoning to define infected and disease-free areas;

- quarantine and movement controls or restrictions on prawns, water and any other vectors (including materials and equipment) within the infected zone and to free zones;
- eradication of outbreaks in the free zone where feasible;
- pond-level surveillance, with destruction and safe disposal of any clinically diseased prawns in the infected zone, followed by clean-up and disinfection;
- use of closed production systems;
- WSV testing of broodstock and postlarvae;
- emphasis on high standards of hygiene (including drying of ponds before restocking and disinfection of water before either use or release) and biosecurity (use of crustacean-proof land barriers and water filters and screening of incoming postlarvae for WSV);
- tracing and surveillance to determine the source and extent of infection; and
- a public awareness campaign to encourage cooperation from industry and the community.

3.2.3 Option 3 — Control and mitigation

If infection is widespread or present in the wild prawn population, it may not be appropriate to institute the controls described above, and an industry-based program to control and mitigate the effects of the disease may be appropriate. Zoning would not be used under this level of control, which would be similar to control measures in countries where WSD is endemic.

In a control and mitigation strategy, it will be the responsibility mainly of individual producers to manage the disease in their facilities using recommended measures to reduce the likelihood and severity of outbreaks. Producers may be encouraged to adopt current best practice through provision of enterprise-level standard operating procedures and quality assurance programs, leading to the eventual development of an accreditation scheme.

Measures for control and mitigation include:

- pond-level surveillance, with destruction and safe disposal of all clinically diseased prawns followed by clean-up and disinfection of affected ponds;
- use of closed or partial recirculation production systems, as appropriate;
- WSV testing of broodstock and postlarvae;
- emphasis on high standards of hygiene (including drying of ponds before restocking and disinfection of water before use or release) and biosecurity (including the use of crustacean-proof land barriers and water filters); and
- best-practice pond management methods to minimise stress and hence the risk of an outbreak during grow-out of covertly infected stock.

3.3 Strategies for control and eradication

Methods for the control and eradication of WSD are summarised in Table 9 and described in detail in Section 2.2.

Table 9 Summary of strategies used for each of the response options for WSD

Strategy	Control method		
	Eradication	Containment	Mitigation
Quarantine and movement controls	Yes	Yes	No
Declared restricted/control areas	Yes	No	No
Zoning	na	Yes	No
Movement controls within declared area or infected zone	Yes	Yes	na
Movement controls out of declared area or infected zone	Yes	Yes	na
Destruction of clinically diseased prawns	Yes	Yes	Yes
Destruction of unexposed prawns	Optional	No	No
Destruction or harvest with on-farm cooking of exposed or potentially exposed but clinically normal prawns, depending on size	Yes	In free zones only	No
On-farm processing (for example by cooking)	Yes	Optional	Optional
Disposal of infected prawns and wastes that cannot be cooked on farm	Yes	Yes	na
Decontamination	Required	Optional	Optional
Surveillance	Yes	Yes	Yes
Tracing	Yes	Optional	No
WSV screening of broodstock and postlarvae	Yes	Yes	Yes
Closed farming systems	na	Yes	Yes
Partial recirculation farming systems	na	No	Yes
Specific farm-level hygiene measures	Yes	Yes	Yes
Specific farm-level biosecurity measures	Yes	Yes	Yes

na = not applicable

3.3.1 Interim measures to minimise further spread

The initial phase of the response to suspicion of a WSD outbreak in farmed prawns will be one of containment while additional information is collected to support problem definition and a decision as to the appropriate response.

Movement controls and other measures will be implemented immediately on suspected infected premises or areas (see Section 2.2.1, and the AQUAVETPLAN **Enterprise Manual** for details).

Immediate measures may include:

- controls over the movement of live prawns and prawn products;
- water treatment and/or diversion; and
- isolation and/or destruction of suspected infected prawns.

3.3.2 Rapid confirmation of infection

The director of fisheries and/or the state/territory CVO must be notified immediately of a suspected incident of WSD.

Preliminary diagnosis of WSD and preliminary identification of WSV may be undertaken by some state/territory diagnostic laboratories. For definitive diagnosis, and immediately on suspicion of WSD, samples should be sent to the AFDL in Geelong.

For the purpose of initiating a response to a suspected disease outbreak, WSD will be deemed to be confirmed if:

- the history, signs and gross lesions are suggestive of WSD;
- typical histological lesions are present in tissue sections; and
- PCR testing returns a positive result for WSV.

Where one or more of the criteria are not met, additional testing will be required.

Once the response has begun, these criteria may be modified for confirming infected premises in the light of new information about the outbreak.

Submission of specimens

Samples should be submitted to the AFDL via a state/territory diagnostic laboratory and the CVO. It is recommended that the AFDL be contacted directly to ensure that samples are collected correctly and sample collection techniques satisfy the requirements of the laboratory. The CVO of Victoria must be informed before specimens from suspected WSD incidents are transported through Victoria.

Live prawns are preferred. A minimum of 100 representative larval to postlarval stage prawns or a minimum of 10 representative juvenile to adult prawns should be collected and submitted in a well-oxygenated, cooled container.

If it is not possible to transport live prawns to the laboratory, the following types of specimens, modified according to the populations at risk, must be collected and submitted. Where possible, prawns should be anaesthetised by a brief period of chilling (not freezing) before being injected with, or placed in, fixative.

Samples for PCR testing

For larvae and postlarvae, immerse live animals directly in a minimum of ten volumes of preservation medium (ethanol:glycerol:water 70:20:10). For live juvenile to adult prawns, dissect either gill tissue (2–3 mm³ pieces) or pleopods (the paired swimming legs on the ventral aspect of the abdomen) and immediately place into a minimum of ten volumes of preservation medium.

Samples for histopathology

For larvae and small postlarvae, immerse live animals directly into Davidson's fixative solution and fix for 12–24 hours. Transfer to 70% ethanol and transport at ambient temperature.

For larger postlarvae and very small juveniles, incise the cuticle with a needle before fixing as for smaller postlarvae.

For juvenile and adult animals, inject fixative (5–10% volume:weight), ensuring that the hepatopancreas is liberally injected first and that the whole specimen is thoroughly injected thereafter. If this is done properly, the whole body will turn red. Next, using a small pair of pointed scissors, the cuticle only should be cut along the mid-lateral side of the animal, starting at the sixth abdominal segment and moving up to the beginning of the cephalothorax, at which point the scissors should be angled in to meet the base of the rostrum. Then the whole prawn should be placed in 10× volume of Davidson’s fixative for 24 hours (up to 72 for larger prawns), after which it may be transferred to 70% ethanol. Precautions must be taken to avoid skin and eye contact with Davidson’s fixative solution.

Sampling equipment may be available on site, or may be obtained from state/territory fisheries or animal health officers (see the **AQUAVETPLAN Enterprise Manual** for contact details). Equipment for collecting sterile samples, reagents for sample preparation and facilities for chilled or frozen storage and transport of samples will be required.

3.3.3 Epidemiological investigations

Epidemiological investigations must be conducted immediately upon suspicion of an outbreak of WSD to determine the actual and potential spread of infection. Thorough epidemiological investigation, with tracing, is fundamental to the success of eradication or containment programs.

Investigations should include both clinical evaluation and laboratory screening of an appropriate sample of prawns. Sample sizes for surveillance should be calculated to at least meet the international standard described in the OIE Aquatic Code (OIE 2004).

Where the objective is to detect infection and not to measure prevalence, specimens may be pooled to reduce testing costs, provided there is no loss of sensitivity.

3.3.4 Quarantine and movement controls

Immediate quarantine and movement controls will be implemented where practicable on anything capable of transmitting infection. Once the most appropriate control strategy is determined, these controls may be modified. See the **AQUAVETPLAN Enterprise Manual** for details on movement controls for different enterprise systems.

For an eradication program, restricted and control areas will be declared. Quarantine and movement controls must be stringently enforced on prawns, water, materials, equipment and vectors in declared areas. Movement controls will be maintained until the disease is either eradicated or declared endemic.

For the other response options, movement controls will be essential to maintain free zones where these have been declared. Restrictions must apply to anything capable of transmitting WSV from infected to WSV-free prawn populations, aquatic systems and processing plants.

3.3.5 Zoning

Zoning for WSV will be based on OIE-determined principles as expanded in the AQUAVETPLAN **Zoning Policy Guidelines**⁶ and on the known distribution of WSV and infected host species, once these have been determined. At least initially, zoning should be limited to control (infected) and free (uninfected) zones, with effective controls on the movement of susceptible prawns and equipment between zones.

Where zoning is implemented, an active surveillance program for WSV will be necessary in free zones. State/territory legislation supports zoning, movement controls and surveillance activities.

3.3.6 Destruction of clinically diseased prawns

Immediate removal from ponds, destruction and safe disposal of all diseased and dead prawns are essential.

3.3.7 Management of other prawns

Unexposed prawns

Eradication

Unexposed prawns may be allowed to grow out, provided there is no likelihood of future infection. The water system, equipment and all handling procedures must preclude infection to ensure that the population remains unexposed throughout grow-out, harvesting and processing. Effective farm hygiene practices and transportation protocols are necessary to ensure that there is no transfer of infection to non-infected prawn populations via handling, equipment or husbandry practices.

Unexposed market-size prawns may be harvested and marketed through standard channels. The method of harvest, equipment used and location must not create a likelihood of exposure to infection. On-farm cooking is preferred.

Immediate destruction may be considered for unexposed prawn populations in an infected zone, particularly for young animals with a low unit value or to allow farm fallowing to begin immediately.

Containment, control and zoning; control and mitigation

In the other control strategies, grow-out and slaughter for human consumption of unexposed prawns is permitted. To prevent transmission of infection to unexposed prawns in free zones, the method of harvest, equipment used and choice of location must ensure that there is no exposure to infection.

⁶ See <http://www.affa.gov.au/content/output.cfm?ObjectID=D2C48F86-BA1A-11A1-A2200060B0A00717> (Accessed 16 June 2005).

Exposed or potentially exposed, but clinically normal prawns

Eradication

In facilities undergoing eradication, exposed or potentially exposed but clinically normal prawns should be regarded as infected. These prawns may be dealt with by:

- destruction and disposal in the same way as for clinically infected prawns; or
- emergency harvest, followed by appropriate on-site processing (for example by cooking – see Section 2.2.6) and marketing, if of marketable size.

Containment, control and zoning

In a containment, control and zoning strategy, grow-out of exposed or potentially exposed but clinically normal prawns will be standard practice in infected zones. During grow-out, these prawns must be treated as infected. Restrictions on movements of prawns, people, vehicles and boats and on market access for final product may be necessary to protect free facilities or zones. The risk of clinical disease and the spread of infection will be minimised through appropriate farm management.

In free zones, exposed or potentially exposed but clinically normal prawns will either be destroyed or harvested and processed on site in the same way as for an eradication policy.

Control and mitigation

In a control and mitigation strategy, grow-out of exposed or potentially exposed but clinically normal prawns will be standard practice. The likelihood of clinical disease and spread of infection will be minimised through appropriate farm management practices.

3.3.8 Disposal

Details of disposal methods are in the AQUAVETPLAN **Disposal Operational Procedures Manual**.⁷

Eradication

In an eradication strategy, the immediate, safe disposal of all infected prawns, wastes, effluent and equipment that cannot be decontaminated is required. If processing is undertaken on infected premises, effluent and any other waste will be treated before being safely discharged or disposed of.

Containment, control and zoning; containment and mitigation

In containment and mitigation strategies, the safe disposal of all infected dead prawns, wastes and effluent is important in decreasing the infectious load on infected sites.

⁷See <http://www.affa.gov.au/content/publications.cfm?ObjectID=448A0116-62BC-44D7-9418A60DED71BCA5> (Accessed 16 June 2005).

3.3.9 Decontamination

Eradication

In an eradication strategy, all buildings, tanks, ponds, materials and equipment (including nets, boats and vehicles) that may be contaminated must be cleaned and disinfected. Ponds must be decontaminated using the procedures outlined in Section 2.2.8. At all stages of decontamination, steps must be taken to prevent any spread of infection via water, wastes or materials, especially into natural waterways.

Containment, control and zoning; control and mitigation

In containment and mitigation strategies, good hygiene practices on infected sites will decrease the incidence of WSD outbreaks. Thorough cleaning and disinfection of buildings, tanks, materials and equipment (including nets, boats and vehicles) that may be contaminated, as well as thorough drying of empty ponds, is especially important after a clinical outbreak in order to decrease the infectious load on the site.

3.3.10 Surveillance

Surveillance will include both clinical surveillance for WSD and PCR screening for WSV. Where zoning is to be implemented, targeted (active) surveillance for WSV using random sample surveys will be necessary to support the declaration of WSV-free zones. Clinical surveillance will be used on farms in infected zones to allow early detection of new outbreaks and the application of contingency measures.

3.3.11 Tracing

In eradication or containment strategies, tracing will be undertaken as described in Section 2.2.2 for all infected facilities.

Tracing is not required for an infected facility in an endemic zone unless that facility is suspected as the source of an outbreak in another zone.

3.4 Social and economic effects

To date, Australia has remained free from WSD. Based on overseas experience, the initial occurrence of uncontrolled WSD in Australia is likely to result in major production losses in affected farms.

However, the overall impact on the Australian prawn industry is likely to be small relative to its total value. This is due mainly to likely differences in WSD's impact on the wild-caught and cultured prawn industries, and the large difference in the sizes of the two sectors.

Prawn aquaculture industries in countries where WSD is endemic have recovered to pre-infection production levels as they have learned to manage prawn aquaculture in the presence of the disease. The Australian industry could recover after a period of adjustment.

3.5 Criteria for proof of freedom

Wherever possible, proof of freedom should comply with the international standards that apply at the time, as described in the OIE Aquatic Code (OIE 2004). Proof of freedom after an outbreak has been eradicated is likely to rely both on clinical surveillance to show that no new outbreaks have occurred over the time period recommended by the current edition of the OIE Aquatic Manual (OIE 2003a) and on a random sample survey.

3.6 Funding and compensation

There are currently no cost-sharing arrangements in place for aquatic animal diseases.

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

OIE Aquatic Code

The objective of the *OIE International Aquatic Animal Health Code* (OIE 2004) is to prevent the spread of aquatic animal diseases, while facilitating international trade in fish and fish products. This annually updated volume is a reference document for use by veterinary departments, import/export services, epidemiologists and all those involved in international trade.

The current edition of the *OIE Aquatic Code* (7th edition) was published in 2004 and is available on the OIE website at:

http://www.oie.int/eng/normes/fcode/a_index.htm
(Accessed on 11 May 2005)

The following chapter is relevant to this manual:

Chapter 4.1.2 White spot disease

OIE Aquatic Manual

The purpose of the *OIE Manual of Diagnostic Tests for Aquatic Animals* (OIE 2003a) is to contribute to the international harmonisation of methods for the surveillance and control of the most important aquatic animal diseases. Standards are described for laboratory diagnostic tests and the production and control of biological products (principally vaccines) for veterinary use across the globe.

The current edition of the *OIE Aquatic Manual* (4th edition) was published in 2003 and is available on the OIE website at:

http://www.oie.int/eng/normes/fmanual/A_summry.htm
(Accessed on 11 May 2005)

The following chapter is relevant to this manual:

Chapter 4.1.2 White spot disease

OIE Disease Technical Cards

The purpose of the *OIE Disease Technical Cards* is to provide a summary of information relevant to the disease, its characteristics, diagnosis and control.

The current *Disease Technical Cards* are available on the OIE website at:

http://www.oie.int/aac/eng/cards/en_diseasecard.htm
(Accessed on 11 May 2005)

The following card is relevant to this manual:

White spot disease

Further information

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

http://www.oie.int/eng/normes/en_acode.htm
(Accessed on 11 May 2005)

Appendix 2 Scientific and common names of species mentioned in text

Scientific name	Common name
<i>Acetes</i> spp	grass shrimp
<i>Artemia</i> spp	brine shrimp
<i>Cherax destructor</i>	yabby
<i>Cherax quadricarinatus</i>	red claw crayfish
<i>Macrobrachium idella</i>	—
<i>Macrobrachium lamerrae</i>	—
<i>Macrobrachium rosenbergii</i>	giant freshwater prawn
<i>Metapenaeus ensis</i>	red endeavour prawn
<i>Orconectes punctimanus</i>	—
<i>Paratelphusa hydrodomous</i>	—
<i>Paratelphusa pulvinata</i>	—
<i>Penaeus aztecus</i>	brown shrimp
<i>Penaeus duorarum</i>	northern pink shrimp
<i>Penaeus japonicus</i>	kuruma prawn
<i>Penaeus monodon</i>	giant tiger prawn
<i>Penaeus penicillatus</i>	redtail prawn
<i>Penaeus semisulcatus</i>	grooved tiger prawn
<i>Penaeus setiferus</i>	white shrimp
<i>Penaeus stylirostris</i>	Pacific blue shrimp
<i>Penaeus vannamei</i>	Pacific white shrimp
<i>Penaeus merguensis</i>	banana prawn
<i>Portunus pelagicus</i>	blue swimmer crab, sand crab
<i>Procambarus clarkii</i>	red swamp crayfish
<i>Scylla serrata</i>	mud crab

Glossary

Aquatic Animal Health Committee	A committee comprising representatives of the Australian government, Australian state and territory governments, the major aquaculture, wild capture, aquarium and recreational fishing industries and a CSIRO representative. The committee provides advice to Primary Industries Ministerial Council on aquatic animal health matters, focusing on technical issues and regulatory policy. <i>See also</i> Primary Industries Ministerial Council
Australian Chief Veterinary Officer	The nominated senior veterinarian in the Australian Government Department of Agriculture, Fisheries and Forestry who manages international animal health commitments and the Australian Government's response to an animal disease outbreak. <i>See also</i> Chief veterinary officer
AQUAVETPLAN	<i>Australian Aquatic Veterinary Emergency Plan.</i> A series of technical response plans that describe the proposed Australian approach to an emergency aquatic animal disease incident. <i>See also</i> AUSVETPLAN
AUSVETPLAN	<i>Australian Veterinary Emergency Plan.</i> A series of technical response plans that describe the proposed Australian approach to an emergency animal disease incident. The documents provide guidance based on sound analysis, linking policy, strategies, implementation, coordination and emergency-management plans.
Chief veterinary officer (CVO)	The senior veterinarian of the animal health authority in each jurisdiction (national, state or territory) who has responsibility for animal disease control in that jurisdiction. <i>See also</i> Australian Chief Veterinary Officer
Control area	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 reduce 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 free area.
Covert infection	Clinically inapparent infection that is transmissible and that may eventually lead to clinical disease.

Dangerous contact animal	A susceptible animal that has been designated as being exposed to other infected animals or potentially infectious products following tracing and epidemiological investigation.
Dangerous contact premises or area	That which has had a direct, and possibly infectious, contact with an infected premises/area. The type of contact will depend on the agent involved in the outbreak but, for example, may involve animal movements or net/equipment movements.
Declared area	A defined tract of land or water that is subjected to disease control restrictions under emergency animal disease legislation. Types of declared areas include <i>restricted area, control area, infected premises, dangerous contact premises and suspect premises</i> .
Decontamination	Includes all stages of cleaning and disinfection.
Disease agent	A general term for a transmissible organism or other factor that causes an infectious disease.
Disinfectant	A chemical used to destroy disease agents outside a living animal.
Disinfection	The application, after thorough cleansing, of procedures intended to destroy the infectious or parasitic agents of animal diseases, including zoonoses; applies to premises, vehicles and other objects that may have been directly or indirectly contaminated.
Disposal	Sanitary removal of fish carcasses and things by burial, burning or some other process so as to prevent the spread of disease.
Emergency animal disease	A disease that is (a) exotic to Australia or (b) a variant of an endemic disease or (c) a serious infectious disease of unknown or uncertain cause or (d) a severe outbreak of a known endemic disease, and that is considered to be of national significance with serious social or trade implications. <i>See also</i> Endemic animal disease, Exotic animal disease
Endemic animal disease	A disease affecting animals (which may include humans) that is known to occur in Australia. <i>See also</i> Emergency animal disease, Exotic animal disease
Enterprise	<i>See</i> Risk enterprise
Epidemiological investigation	An investigation to identify and qualify the risk factors associated with the disease.

Exotic animal disease	A disease affecting animals (which may include humans) that does not normally occur in Australia. <i>See also</i> Emergency animal disease, Endemic animal disease
Fomites	Inanimate objects (eg boots, clothing, equipment, instruments, vehicles, crates, packaging) that can carry an infectious disease agent and may spread the disease through mechanical transmission.
Free area	An area known to be free of the disease agent.
Infected premises or area	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.
Local disease control centre	An emergency operations centre responsible for the command and control of field operations in a defined area.
Monitoring	Routine collection of data for assessing the health status of a population. <i>See also</i> Surveillance
Movement control	Restrictions placed on the movement of fish, people and other things to prevent the spread of disease.
Nested RT-PCR	A double-stage PCR process where the second round identifies a DNA sequence 'nested' within the initial sequence, thus increasing the specificity. <i>See</i> <i>Polymerase chain reaction (PCR)</i> and <i>Reverse transcriptase-PCR (RT-PCR)</i>
OIE Aquatic Code	OIE <i>International Aquatic Animal Health Code</i> (OIE 2004). Published on the internet at: http://www.oie.int/eng/normes/fcode/a_index.htm (Accessed 11 May 2005). <i>See</i> Appendix 1 for further details
OIE Aquatic Manual	OIE <i>Manual of Diagnostic Tests for Aquatic Animals</i> (OIE 2003a). Describes standards for laboratory diagnostic tests and the production and control of biological products (principally vaccines). The current edition is published on the internet at: http://www.oie.int/eng/normes/fmanual/A_summry.htm (Accessed 11 May 2005). <i>See</i> Appendix 1 for further details
Operational procedures	Detailed instructions for carrying out specific disease control activities, such as disposal, destruction, decontamination and valuation.
Owner	Person responsible for a premises (includes an agent of the owner, such as a manager or other controlling officer).
Pathognomonic	Characteristic of a particular disease.

Polymerase chain reaction (PCR)	A method of amplifying and analysing DNA sequences that can be used to detect the presence of virus DNA. See also <i>Reverse transcriptase-PCR (RT-PCR)</i> and <i>Nested RT-PCR</i>
Prawn byproducts	Products of prawn origin destined for industrial use (eg fishmeal).
Prawn products	Prawn meat products and products of prawn origin (eg eggs) for human consumption or use in animal feeding.
Premises or area	A production site, which may range from an aquarium to an aquaculture lease in the open ocean.
Prevalence	The proportion (or percentage) of animals in a particular population affected by a particular disease (or infection or positive antibody titre) at a given point in time.
Primary Industries Ministerial Council	The council of Australian national, state and territory and New Zealand ministers of agriculture that sets Australian and New Zealand agricultural policy (formerly the Agriculture and Resource Management Council of Australia and New Zealand).
Quarantine	Legal restrictions imposed on a place, prawns, vehicles, or other things, limiting movement.
Restricted area	The area around an infected premises (or area), likely to be subject to intense surveillance and movement controls. It is likely to be relatively small. It may include some dangerous contact premises (or area) and some suspect premises (or area), as well as enterprises that are not infected or under suspicion. 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.
Reverse transcriptase-PCR (RT-PCR)	A highly sensitive technique for the detection and quantitation of mRNA (messenger RNA) by reverse transcription to DNA followed by PCR. See <i>Polymerase chain reaction (PCR)</i> and <i>Nested RT-PCR</i>
Risk enterprise	A defined livestock or related enterprise, which is potentially a major source of infection for many other premises. Includes hatcheries, aquaculture farms, processing plants, packing sheds, fish markets, tourist angling premises, veterinary laboratories, road and rail freight depots and garbage depots.
Sensitivity	The proportion of affected individuals in the tested population that are correctly identified as positive by a diagnostic test (true positive rate). See also <i>Specificity</i>

Serotype	A subgroup of microorganisms identified by the antigens carried (as determined by a serology test).
Specificity	The proportion of nonaffected individuals in the tested population that are correctly identified as negative by a diagnostic test (true negative rate). <i>See also Sensitivity</i>
State or territory disease control headquarters	The emergency operations centre that directs the disease control operations to be undertaken in that state or territory.
Surveillance	A systematic series of investigations of a given population of fish to detect the occurrence of disease for control purposes, and which may involve testing samples of a population.
Susceptible animal	Animal that can be infected with a particular disease.
Suspect animal	Animal that may have been exposed to an emergency disease such that its quarantine and intensive surveillance, but not pre-emptive slaughter, is warranted. <i>or</i> Animal not known to have been exposed to a disease agent but showing clinical signs requiring differential diagnosis.
Suspect premises or area	Temporary classification of premises containing suspect fish. After rapid resolution of the status of the suspect animals contained on it, a suspect premises is reclassified either as an infected premises (and appropriate disease-control measures taken) or as free from disease. The reason for the suspicion varies with the agent; however, it may involve clinical signs or increased mortality.
Tracing	The process of locating animals, persons or other items that may be implicated in the spread of disease, so that appropriate action can be taken.
Vaccination	Inoculation of healthy individuals with weakened or attenuated strains of disease-causing agents to provide protection from disease.
Vaccine	Modified strains of disease-causing agents that, when inoculated, stimulate an immune response and provide protection from disease.
Vector	A living organism that transmits an infectious agent from one host to another. 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 essential to the lifecycle of the agent.
Zoning	The process of defining disease-free and infected areas.

Abbreviations

AAHL	Australian Animal Health Laboratory
AFDL	AAHL Fish Diseases Laboratory
AQUAVETPLAN	Australian Aquatic Veterinary Emergency Plan
aqCCEAD	Aquatic Consultative Committee on Emergency Animal Diseases
AUSVETPLAN	Australian Veterinary Emergency Plan
CCEAD	Consultative Committee on Emergency Animal Diseases
CSIRO	Commonwealth Scientific and Industrial Research Organisation
CVO	chief veterinary officer
DAFF	Department of Agriculture, Fisheries and Forestry (Australian Government)
DNA	deoxyribonucleic acid
GAV	gill-associated virus
H&E	haematoxylin and eosin
IHHN	infectious hypodermal and haematopoietic necrosis
IHHNV	IHHN virus
OIE	World Organisation for Animal Health (formerly Office International des Epizooties)
PCR	polymerase chain reaction
PNR	peripheral neuropathy and retinopathy
RNA	ribonucleic acid
RT-PCR	reverse transcriptase polymerase chain reaction
SPF	specific pathogen free
TSV	Taura syndrome virus
WSD	white spot disease
WSV	white spot virus

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