FINAL REPORT

Aquatic Animal Health Subprogram: development of the AQUAVETPLAN disease strategy manual for white spot disease of prawns

Chris Baldock, Iain East, Dick Callinan

March 2004

FRDC Project No. 2002/647

Australian Government
Department of Agriculture, Fisheries and Forestry

AusVet
ANIMAL HEALTH SERVICES

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OBJECTIVE:
To complete a disease strategy manual complete for white spot disease of prawns in accordance with the terms of reference provided by the FRDC Aquatic Animal Health Subprogram and to develop a consensus between governments and industry on a preferred control policy for this disease.

NON TECHNICAL SUMMARY:

OUTCOMES ACHIEVED TO DATE (BOXED)

The disease strategy manual for white spot disease (WSD) is now in draft form and will become part of the AQUAVETPLAN series of manuals, which, in their entirety, will enhance the capability of industry and government in Australia to quickly and effectively respond to aquatic animal disease emergency incidents. The disease strategy manual for WSD will specifically contribute to improved technical validity concerning the assumptions that underlie the development of strategies to combat WSD emergencies.

The major beneficiary will be aquaculture but maintenance of export markets and premium pricing for quality products will also benefit the wild capture industry. In the event of a WSD incident, Industry will also benefit through lower losses due to an effective and rapid response to the outbreak.

White spot disease (WSD) is caused by white spot virus (WSV), also known as white spot syndrome virus. It is a highly contagious disease of penaeid prawns 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. Since first being reported in the early 1990s, WSD has exhibited pandemic behaviour in Asia and the Americas. It is arguably the most serious disease affecting prawn cultivation and is one of the crustacean diseases listed by the World Organisation for Animal Health. WSV also infects a wide range of other crustaceans, often without causing any clinical signs. Although PCR signals consistent with WSV were detected in and around two aquaculture facilities in Darwin in November 2000, there was no disease outbreak, and signals did not reoccur. The source of infection was attributed to the use of imported green prawns as feed for cultivated crustaceans. Subsequently, an Australia-wide survey has

demonstrated freedom. Because of its potential devastating impact on Australia's farmed prawn industry, WSD is a high priority for Australia's emergency disease preparedness planning and a disease strategy manual was prepared as part of AQUAVETPLAN.

The manual follows the standard format for disease strategy manuals with three main sections: Nature of the disease; Principles of control and eradication; Preferred control strategies in Australia. The first section is a review of what is known about the disease and its causative agent, the second explores the options for control and eradication while the third offers the preferred option for Australia. The authors prepared a first draft based on a literature review and consultation with internationally recognised experts. This draft was peer reviewed by two international experts on WSD in May 2003 and comments incorporated. The next draft was circulated for comment in June to 32 technical experts and policy makers throughout Australia plus the Australian Prawn Farmers' Association and Queensland Crayfish Farmers' Association. These comments were incorporated into a final draft completed in September 2003.

Briefly, the preferred control strategies for Australia comprise three possible response options:

- Option 1 - *eradication* with the view to 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* where it is accepted that the virus will remain endemic in Australia.

The choice of response option will be decided by the Director of Fisheries and/or the CVO of the State/Territory in which the outbreak occurs, following initial epidemiological investigations.

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

- *quarantine and movement controls* on crustaceans, their products 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 from infection;
- *zoning* to define and maintain zones of different disease status; 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 point source or is otherwise unable to be contained. Similarly, the feasibility of zoning and containment 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 available wild broodstock populations control and mitigation of the disease is likely to be the most appropriate option.

KEYWORDS: **white spot disease, white spot virus, prawns, AQUAVETPLAN, disease strategy manual.**

Acknowledgements
We gratefully acknowledge the useful comments on different drafts of this manual which were received from a large number of people, both in government and industry

Background
White Spot Disease (WSD) was first recognised in 1992-1993 in north-east Asia and has spread throughout most prawn culture areas of the Indo-Pacific. The disease first appeared in farmed *Penaeus monodon* (black tiger prawn) in Thailand in 1994 where it surpassed yellow-head as the primary cause of stock losses. In 1995 WSV was observed in pond-reared *P. setiferus* in Texas. It has been claimed that it was introduced with raw and frozen prawns from Asia, which had been processed at nearby plants although this remains speculation and has not been conclusively demonstrated. Since that time, the disease has spread to Central and South America where it has been reported in Panama, Honduras, Nicaragua, Ecuador, Colombia, Peru and, most recently, Costa Rica.

Although mortalities due to WSD can occur at any stage in the culture cycle, most mortalities occur in young juvenile prawns weighing 3-5 gm. WSV causes mortalities in farmed populations of *P. monodon*, *P. japonicus*, *P. chinensis*, *P. indicus*, *P. merguiensis*, *P. setiferus*, *P. vannamei* and *P. stylirostris*. The wild penaeids, *Parapenaeopsis* spp., *P. semisulcatus*, *P. aztecus*, *P. duorarum* and *Metapenaeus* spp., as well as the caridean, *Macrobrachium* spp develop disease following experimental infection with WSV. Larvae of the freshwater shrimp, *Macrobrachium rosenbergii* may be infected experimentally and suffer some mortality while survivors can carry an infection without mortality as adults. The crabs, *Portunus pelagicus* and *Scylla serrata* are both susceptible to experimental infection as is the krill, *Acetes* sp. Mortalities occurred among *P. pelagicus*, but not for the other species. Although this study was conducted in Thailand, these species also exist in Australia. Resistance to WSV infection has not been reported for any penaeid species.

WSD has not been reported from Australia.

Need
Few major disease incidents have occurred in Australian aquaculture and fisheries, and as a result, State/Territory departments and industries have relatively little experience in incident management for emergency diseases.

The recent white spot virus incident in the Northern Territory and subsequent national survey demonstrating freedom has highlighted the need to have strategies in place to enable a swift and effective response to a suspect emergency disease incursion to rapidly contain an infectious disease agent.

Effective responses to emergency disease outbreaks require emergency disease planning at national, state/territory, district and industry/farm level and the involvement of animal health and fisheries authorities, emergency management organisations and the private sector. For the terrestrial animal sector, the basis for this planning is contained in the Australian Veterinary Emergency Plan, AUSVETPLAN, which is a series of technical response plans that describe the proposed Australian approach to an emergency disease incursion. The documents

provide guidance based on sound analysis, linking policy, strategies, implementation, coordination and emergency-management plans.

Based on the AUSVETPLAN model, the AQUAVETPLAN is currently being developed as a series of manuals and operational instruments which outline methods and protocols to manage emergency disease outbreaks in aquatic animals in Australia. Some manuals have been published (*AQUAVETPLAN Enterprise Manuals; AQUAVETPLAN Furunculosis Disease Strategy*; *Australian Aquatic Animal Disease Field Identification Guide, AQUAVETPLAN Control Centre Manual*; *AQUAVETPLAN Disposal* and *Destruction Manuals*). Following a detailed process of government and industry consultation, the development of a Disease Strategy Manual for White Spot Disease has been identified as a key need to improve Australia's preparedness in the event of emergency disease incursions.

Objective

To complete a disease strategy manual complete for white spot disease of prawns in accordance with the terms of reference provided by the FRDC Aquatic Animal Health Subprogram and to develop a consensus between governments and industry on a preferred control policy for this disease.

Methods

The Disease Strategy Plan was written by three primary writers: Richard Callinan (Section 1), Iain East (Section 2) and Chris Baldock (Section 3). In addition, Professor Tim Flegel and Mr Dan Fegan, internationally recognised authorities on WSV and WSD contributed by providing expert advice to the primary writers and commenting on the initial draft. The sections indicated for each writer indicate the leader for that particular section. All authors contributed to the writing of each section where required as well as addressing and incorporating comments.

The first two sections (Sections 1 and 2), plus some of the appendices, were written concurrently. Much of the work for these sections was undertaken by reviewing existing knowledge through literature searches and consultation with scientists around the world.

Section 3, focusing on the agreed control and eradication options, was written after the first two sections were completed to a reasonable draft stage. Section 3 of the manual was developed through of a stakeholder (government and industry) consultation to ensure consensus in the approach. Based on overseas experience, and in consultation with stakeholders, careful attention was given to options that are suitable for Australian conditions.

Results

The result from this project is an AQUAVETPLAN disease strategy manual for white spot disease of prawns which is at Appendix 3 in final draft form.

Briefly, the preferred WSD control strategies for Australia comprise three possible response options:

- Option 1 – *eradication* with the view to 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
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- *zoning* to define and maintain zones of different disease status; and
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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. Similarly, the feasibility of zoning and containment 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 available wild broodstock populations control and mitigation of the disease is likely to be the most appropriate option.

Benefits And Adoption

The major beneficiary will be aquaculture but maintenance of export markets and premium pricing for quality products will also benefit the wild capture industry. In the event of a WSD incident, Industry will also benefit through lower losses due to an effective and rapid response to the outbreak.

Further development

Like all disease contingency planning documents, this disease strategy manual will need to be regularly reviewed and updated to reflect changes in technology and understanding of the disease and its control.

Planned outcomes

The disease strategy manual for white spot disease (WSD) is now in draft form and will become part of the AQUAVETPLAN series of manuals, which, in their entirety, will enhance the capability of industry and government in Australia to quickly and effectively respond to aquatic animal disease emergency incidents. The disease strategy manual for WSD will specifically contribute to improved technical validity

concerning the assumptions that underlie the development of strategies to combat WSD emergencies.

Conclusion

The first version of the white spot disease strategy manual is now completed following an extensive drafting and consultation process.

References

The draft disease strategy manual at Appendix 3 includes an extensive list of references.

Appendix 1: Intellectual Property

The manual contains valuable information to guide the response to an outbreak of WSD in prawns should one occur in Australia.

Appendix 2: Staff

Researchers and writers -
Dr Chris Baldock (Project Leader)
Dr Iain East
Dr Richard (Dick) Callinan

Peer review of initial draft -
Prof Tim Flegel
Mr Dan Fegan

Appendix 3: Draft white spot disease strategy manual

Draft AQUAVETPLAN White Spot Disease Strategy Manual for endorsement by Primary Industries Standing Committee (through Aquatic Animal Health Committee and Primary Industries Health Committee).

AQUAVETPLAN
WSD MANUAL

DRAFT
3 March 2004

CONTENTS

1 NATURE OF THE DISEASE

White spot disease (WSD) is a highly contagious viral disease of penaeid prawns 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 presently 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* (*Marsupenaeus) japonicus* 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 (www.ncbi.nlm.nih.gov/ICTVdb/Ictv/fs_nimav.htm).

WSV is a large (80-120 x 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

All decapod crustaceans (Order Decapoda) including prawns, lobsters and crabs from marine, brackish water or freshwater environments are considered susceptible to infection (OIE 2003a). However, the disease has mainly been a problem in farmed penaeid (Family Penaeidae) prawns. Currently, three marine prawn species, *Penaeus monodon*, *P. japonicus* and *P. merguiensis*, and one freshwater species, *Macrobrachium rosenbergii* are farmed commercially in Australia.

It should be noted that Pérez Farfante and Kensley (1997) proposed a revised taxonomic classification of penaeids (i.e. Family Penaeidae within the Order Decapoda, Superclass Crustacea) 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 genera names are not used in this manual, although they are used in many of the references cited.

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 oral ingestion. Experimental susceptibility through ingestion is probably of most relevance. Mortalities following ingestion of infective material have been demonstrated in *P. clarkii* (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 *P. pulvinata* (Sahul Hameed *et al*. 2001) as well as in several European marine and freshwater crustacean species (Corbel *et al.* 2001). Moreover, Richman *et al.* (1997) reported mortalities due to WSV infection in the captive North American freshwater crayfishes, *P. 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 redclaw) 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 *P. clarkii* (red swamp crayfish). To address this possibility, repeat transmission trials confirmed susceptibility to WSD of early stage *C. quadricarinatus* juveniles (B. Edgerton, pers. comm.). High mortalities have also been recorded in *C. destructor* subsp. *albidus* experimentally injected with WSV. In the same study, over 50% of *C. destructor* subsp. *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* subsp. *albidus* are likely to be less susceptible to WSD than are penaied 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 Sotto 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 (OIE, 2003c; NACA 2002) 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 presently free of WSV (OIE, 2003c; NACA 2002). However, WSV was detected in and around two aquaculture facilities in Darwin, Northern Territory in November 2000 and promptly eliminated without disease being seen. The source of infection was imported green prawns. A subsequent survey demonstrated Australia's freedom (Animal Health Australia, 2002).

Australia has protected its disease free status by restricting importation of prawns and prawn products. The importation of live prawns into Australia is not generally permitted, and since 1996 the importation of green prawns has been allowed for human consumption only. In August 2000, Biosecurity Australia recommended a tightening of import restrictions to prevent entry of prawns that had been emergency harvested as a result of a disease incident and also to prevent entry of prawns less than 15g in weight. The second restriction was to prevent the importation of prawns that might originate from an early emergency harvest and because of their lower market value were more likely to be diverted into the fishing bait market. In February 2001, stricter interim measures including the requirement to test all imported green prawn consignments for WSV were introduced.

1.4 Diagnostic criteria

Prawns affected with WSD often do not show distinctive clinical signs, nor do they show 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 become a routinely used diagnostic tool by crustacean pathologists.

1.4.1 Clinical signs and gross lesions

The clinical signs and gross lesions associated with WSD (Table 2) vary between outbreaks and do not *per se* 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 to 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 intra-cuticular white spots can range from minute foci to discs up to 2 mm 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 (OIEa 2003) and may represent abnormal deposits of calcium salts by the cuticular epidermis (Lightner 1996) or 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, when present, 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 (Wang YG *et al*. 2000; Goarant *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 coloration (hence the alternative name "red disease" for WSD) of the entire cuticle, due to expansion of the cuticular chromatophores.

Table 2: Comparative features of clinical WSD and sub-clinical WSV infection.

Sign	Clinical disease	Sub-clinical (latent or covert) infection
Age of prawns	Any stage of grow-out	All life cycle 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

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 but additional tests such as PCR , i*n 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, hematopoietic 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 marginated chromatin. These known as Cowdry type A inclusions which are found in many viral infections in both vertebrates and invertebrates. They are intranuclear, eosinophilic, amorphous, 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 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* (OIE 2003a). The recommended minimum number 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 where WSV infection requires detection. One is the confirmation of suspect clinical WSD and the second is 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 be later 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 what level of confidence in the findings is required. Whole larvae, postlarvae and juvenile animals as well as haemolymph, gills or pleopods from juveniles to broodstock provide suitable specimens for examination. For screening apparently healthy populations, 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.

A comparison of the suitability of the different methods for screening and diagnosis is shown in Table 3 below.

Table 3: Comparison of WSV screening and diagnostic methods (modified from OIE 2003a)

Method	WSV screening				Presumptive WSD diagnosis	Confirmatory WSD diagnosis
	Larvae	PLs	Juveniles	Adults		
Gross signs	-	-	-	-	+	-
Rapid methods	-	-	-	-	+	+
Histopathology	-	-	-	-	++	++
PCR	+++	+++	+++	+++	+ ++	+++
Transmission EM	-	-	-	-	+++	+++
Antibody-based assays	?	?	+++	+++	+++	+++
in-situ DNA hybridisation	?	?	+	+	+++	+++
Bioassay *	-	-	-	-	+	-

PLs = postlarvae; LM = light microscope; EM = electron microscope; PCR = polymerase chain reaction.

* 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.

- \- the method is presently unavailable or unsuitable
- ? the method is available but untested
- \+ the method has application in some situations, but cost, accuracy, or other factors severely limits application of the method
- ++ the method is a standard method with good diagnostic sensitivity and specificity;
- +++ the method is the recommended method for reasons of availability, utility,

and diagnostic specificity and sensitivity

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 this second 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. Coverslip the preparation and examine with 10, 20 and 40x 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 upon 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. 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 as a permanent reference in a sealed vial. 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 40x 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, in the event that very rapid results are required, the overnight fixation step above can be shortened to only 2 h 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^{O}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 *Diagnostic Manual for Aquatic Animal Diseases* (OIE, 2003a). Commercial PCR kits for the detection of WSV are also available from several suppliers.

Note that eyes from prawns older than PL10 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 pereiopods that have been pre-screened 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 x 250-380 nm (OIE 2000a).

Antibody-based assays

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

In-situ DNA hybridisation

This method uses 5 μm paraffin sections which are examined with 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, Tang and Lightner 2000).

The advantages and disadvantages of the commonly used laboratory tests are summarised in Table 4 below.

Table 4: Advantages and disadvantages of WSV tests (after Fegan and Clifford 2001).

Diagnostic method	Advantages	Disadvantages
Rapid methods	Rapid diagnostic results; field friendly; detects 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 is 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.

1.4.4 Differential diagnosis

The clinical signs and gross lesions observed during outbreaks of WSD are non-specific. Therefore, the diagnostician must consider any rapidly increasing mortality event 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, WSD and yellowhead, known to be capable of causing massive mortalities in one or more of the penaeid species farmed in Australia are compared in Table 5 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 *et al*. 2003a, 2003b).

Two other viral diseases associated with severe mortalities, 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* 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 5, below. Infectious hypodermal and haematopoietic necrosis (IHHN), caused by IHHN virus (IHHNV), causes severe mortalities in farmed, and possibly wild, *P. stylirostris* (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. merguiensis* is reportedly refractory to IHHNV infection (Lightner 1996).

Table 5: Differential diagnosis of virus-induced mortalities which may occur in Australian farmed prawns (*P. monodon, P. japonicus, F.merguiensis*).

	White spot disease	**Yellowhead disease**	**PNR (GAV-related disease)**	**Taura syndrome (in *P. vannamei*)**
Susceptible Australian farmed species	*P. monodon, P. japonicus, P. merguiensis*	*P. monodon*	*P. monodon*	unknown
Stage of grow-out	All	Usually 7-10 wk post stocking	Usually >13 wk post stocking	Usually 2-6 wk 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 coloration	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	Intra-nuclear; eosinophilic (Cowdry type A) to basophilic	Intra-cytoplasmic; basophilic	Uncommon; intra-cytoplasmic; basophilic	Intra-cytoplasmic; initially eosinophilic then basophilic

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 TSV is unknown.

Massive mortalities in individual prawn ponds unrelated to disease events are rare, but can follow equipment failure or serious management errors (e.g. miscalculating chemical concentrations) as well as exposure to environmental toxicants such as pesticides. Generally, 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 be usually 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 in farmed prawns of bacterial white spot syndrome (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 2000a). Neither of these non-viral 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 coloration 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.

1.5 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, i.e., no true antibodies and substantially less haemocyte heterogeneity than vertebrates. Prawns possess both humoral and cellular responses, although they 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 that 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 which 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, non-lethal infections.

However, recent findings call into question some aspects of the above 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 three to four weeks after exposure to WSV and persisted for a further month in prawns held at 24^{O}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, pers. comm.). 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 *Penaeus 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 partially explain why WSD causes devastating outbreaks in farmed animals but not in wild populations.

1.6.1 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. Lo *et al*. (1997) in their studies of WSV tissue tropisms were

unable to find infected mature ova and suggested that infected ova were killed by the virus before maturation. Current evidence suggests connective tissues in 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% body weight 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

Broodstock and wild populations

Table 6 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 as the effects of sampling (how well captured animals represent the source population) and measurement (how well laboratory tests represent virus infection) bias 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; Mushiake *et al*. 1998; Withyachumnarkul *et al*. 2003) although this may merely reflect seasonal variation in capture locations rather than effect of season *per se*.

Table 6: Published prevalence estimates of WSV in wild prawns.

Prawn sp.	Prevalence (%) *	Location	Reference
P. monodon	83.3 (n = 66) [b]	Taiwan	Lo *et al.* (1996)
	77.2 (n = 88) [b]	Taiwan	Lo *et al.* (1997)
	0-18.6% (n = 24,338) [a] monthly in broodstock over three years	Thailand	Withyachumnarnkul *et al*. (2003)
P. japonicus	9.2 (n = 1269) [b]	Japan	Mushiake *et al.* (1998)
	20.3 (n = 474) [b]	Japan	Maeda *et al.* (1998a)
	58.5 (n = 159) [ns]	Taiwan	Lo and Kou (1998)
P. semisulcatus	26.7 (n = 15) [b]	Taiwan	Wang YC *et al.* (1998)
	6.3 (n = 32) [b]	Taiwan	Lo *et al.* (1996)
F. penicillatus	11.1 (n = 27) [b]	Taiwan	Lo *et al.* (1996)
M. ensis	33.3 (n = 30) [a]	Taiwan	Wang CS *et al.* (1997)
P. vannamei	2 (n = 104)	Panama	Nunan *et al.* (2001)

* Detection was by PCR in all Asian studies and by dot-blot assay in the Panama study.
n: number of prawns in study; a: 1-step PCR; b: 2-step PCR; ns: not specified.

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.

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 144 hr (6 days).
It is considered good practice to collect and dispose of moribund and dead prawns found at pond edges during an outbreak (Chanratchakool, pers. comm.), but the extent to which similarly affected prawns remain out of reach on the pond bottom is unknown. It is possible that the majority of moribund prawns, in response to severe virus-induced gill damage, congregate in the more highly oxygenated environments at the surface and edges of the pond (D. Fegan, pers. comm.).

Other decapod crustaceans

Various wild decapod crustaceans, such as prawns (*Metapenaeus* spp.), grass shrimp (*Acetes* spp.) and crabs (*Scylla serrata*, *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 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). However, the actual risk of transmission of infection from non-prawn crustaceans to prawns in commercial ponds remains unclear but 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 (Fegan and Clifford 2001; Garza *et al*. 1997). 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^{O}C (Momoyama *et al*. 1998; Maeda *et al*. 1998b) but Wang YG *et al*. (2002) observed that cell-free WSSV in sea water lost infectivity by 48 hr. This later 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 cohabitation transmission of WSV infection between prawns is over an order of magnitude lower than ingestion transmission (Soto and Lotz 2001, Wu *et al*. 2001). In general, findings suggests the risk from water itself 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 significant in this regard. Fegan and Clifford (2001) noted that successful crops of prawns have been raised in Asia in ponds containing dry, 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* and *P. vannamei* postlarvae than *P. aztecus* and *F. duorarum* 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 *F. 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 Resistance and Immunity 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 7 lists recommended ranges for important water quality variables in *P. monodon* rearing ponds. Prolonged exposure to values outside the optimum range for each variable, or to rapid fluctuations (Fegan and Clifford 2001) can trigger WSD outbreaks in latently infected populations.

Table 7: Recommended ranges for key water quality variables for farmed *P. monodon* (after Chanratchakool *et al*. 1998).

Variable	Optimum 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
Unionised ammonia	< 0.1 ppm	More toxic at high pH and temperature

1.6.4 Immunostimulants

The crustacean innate immune system recognizes 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 enhance 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).

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 sub-clinical 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 or the occurrence or confirmed WSV infection

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* - the implementation of management practices that decrease the incidence and severity of clinical outbreaks (lowest level of control measure and likely to be lowest cost).

The basic principles of eradication and other control responses are described in the AQUAVETPLAN **Enterprise Manual** and the AQUAVETPLAN **Control Centre Management Manual**.

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 (e.g. associated with 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.

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

2.2 Methods to prevent spread and eliminate pathogens

Available methods for the control and eradication of WSV include:

2.2.1 Quarantine and movement controls

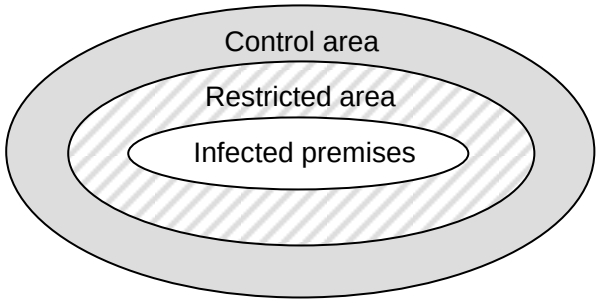
The quarantine and movement restrictions that should be implemented immediately upon suspicion of WSD are:

- establishment of specified areas (Figure 1) (see AQUAVETPLAN **Enterprise Manual**, Section A for more details)
  - *declared area* - includes restricted area and control area
  - *restricted area* - area around infected premises or area
  - *control area* - a buffer between the restricted area and free areas
  - *free area* - non-infected area (this area is not considered a 'declared area' and may include large areas of Australia in which the presence or absence of 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 or between marine locations and between different farm locations; 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 extent to which these are enforced will depend on the location of infection, the location and type of enterprises affected and the control response option chosen.

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 (Mushiake *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.

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 there are no zoning programs 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 life cycle has been closed in captivity e.g. redclaw crayfish would be much simpler due to less demand to move livestock between farms.

The principles of zoning for infected and non-infected zones in Australia are outlined in the AQUAPLAN **Zoning Policy Guidelines**. If WSV were to become 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.

Quarantine and farm type

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.

Open systems

Most Australian prawn farms employ an open system, whereby water is taken from and released to the source as necessary. These 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, these settlement ponds provide an opportunity to hold and treat effluent water prior to 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

Farms using partial recirculation and full recirculation systems are common in Asia but not in Australia. These systems allow greater control over water intake and discharge than the open system, however 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 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 subsebquently re-used in the ponds as necessary.

Closed systems

This category includes full recirculation systems and closed pond systems. Full recirculation systems, which predominate where water supply problems are more severe and persistent than for partial recirculation systems, 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 e.g. those growing redclaw 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 redclaw 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 prior to its discharge.

Strategies

There are claims that WSV has been successfully eradicated from some Central American farms (Boyd and Clay 2002; Lawrence *et al*. 2001) 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 reliance of the industry on wild-caught broodstock. Research programs are underway 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, only being used experimentally in both coastal and 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 growing stock from other farms. Importantly, animals are also able to enter farm waterways and ponds mainly via intake water from the adjacent aquatic environment. Aerators, particularly the paddle wheel type, generate aerosols which may spread infection between ponds and possibly farms (Fegan and Clifford 2001). Redclaw 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 then all ponds may contain infected animals. Boundary fences (a licence condition) on redclaw farms will help prevent movement beyond the production ponds.

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 500µm, preferably 200-250µm for initial filling. Bag net filters provide a much larger surface area than 2-dimensional framed screens, and inserting one bag inside another is an economical solution for decreasing the effective mesh size.

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).

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:

- crustaceans - broodstock, postlarvae, live animals to restaurant trade etc;
- crustacean products - uncooked prawns intended for consumption or for bait use, effluent and waste products from processing and/or cooking. Cooked crustaceans do not present a risk and need not be traced;
- water - input and output;
- vehicles - crustacean transport vehicles, feed trucks, visitors' cars, boats;
- materials - nets, paddle wheels, 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/Territory fisheries or agriculture agency can be contacted (see AQUAVETPLAN **Enterprise Manual**, Appendix 5 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 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, therefore the methods aimed at minimising stress should be used.

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

- the ability to contain pond or tank water. All water must be treated prior to discharge;
- destination - human consumption or disposal;
- size and number of animals;
- desirability of removing most or all dead animals from the pond bottom prior to disinfecting the water;
- 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 these practices and the necessary equipment may be available on site.

Studies on the heat stability of WSV in semi-purified 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^{O}C, Chang *et al.* (1998) found live virus after five and 30 minutes at 55 ^{O}C. In addition, WSSV in prawn tissues may be more resistant to heating due to the protective effect of proteins. Due to this uncertainty, it is concluded that heat treatment of biological material potentially infected with WSV should be at 60 ^{O}C or more for at least 20 minutes for inactivation.

Table 8: Relationship between time and temperature for inactivation of WSV (from Chang *et al*. (1998), Nakano *et al*. (1998) and Maeda *et al*. (1998b)).

Temperature	Time (minutes)								
(degrees C)	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 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*. 1997). 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 WSV. 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 Appendix 5.2.1 of the OIE *International Aquatic Animal Health Code* (OIE 2003b).

2.2.6 Treatment of prawn products and by-products

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

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 of time and viable WSV has been recovered from commodity prawns purchased at retail supermarkets (Nunan *et al*. 1998; Durand *et al*. 2000). Therefore, cooking of crustacean products and by-products is necessary to prevent the possibility of dissemination of infection through movement of product.

2.2.7 Disposal of prawns and prawn products

Disposal must be immediate to decrease infection pressure on the site. See the AQUAVETPLAN **Operational Procedures Manual - Disposal** for details. 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, 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 there is no contact with waterways or vectors.

2.2.8 Decontamination

Due to differences in farming enterprises, disinfection protocols may need to be determined on an individual basis involving 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 prior to disinfecting water;
- options for preventing access to infected animals by scavenging birds;
- current disinfection protocols;
- environmental impact of the disinfectant protocol; 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 prior to discharge (see the AQUAVETPLAN **Disinfection and Decontamination Manual**).

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. All must then be treated with slaked lime (Ca(OH)2) in the amount of at least 0.5 kg/m2 and left dry for at least one month before restocking.

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

2.2.9 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 use of disinfectants could impact on the environment, especially if used in larger than normal quantities or concentrations as is possible in a disease control situation. The local environmental protection agency may need to be consulted - see the AQUAVETPLAN **Enterprise Manual**.

The destruction and disposal of infected carcases/material will have an impact on the environment. This impact must be minimised while ensuring that there is no dissemination of infection.

2.2.10 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.

2.2.11 Vector control

Birds - Seabirds and wading birds are common on prawn farms. Dead or moribund prawns at the surface of open ponds typically attract many birds and thus the ponds must be covered (e.g. using nets or similar) to prevent birds from gaining access and 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. Live ammunition can be used as a last resort, firstly 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). Within Australia, firearms may only be used by a licensed shooter and may require further police permits. Extreme care must be exercised with the use of live ammunition and all staff should be briefed prior to its use. In most jurisdictions, the killing of wild birds requires a permit from the local Environment Protection or National Parks agency (see the AQUAVETPLAN **Disposal Manual**).

Fomites - effective hygiene is required, including disinfection of boots, nets and other equipment with a solution containing at least 30ppm active chlorine (see the AQUAVETPLAN **Disinfection and Decontamination Manual**).

Wild crustaceans - prevent contact, where possible, between wild crustaceans and farmed prawns. The use of fences, constructed from shade cloth type netting (2mm mesh size and 30-40 cm high) around pond perimeters can prevent access by crabs and other crustaceans (Fegan and Clifford 2001).

2.2.12 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 species for sentinel animals.

The fallowing time required before restocking will need to be assessed on an individual basis. The period will depend on the number of sites with confirmed diagnoses, the features of the sites (including season) and the extent of the outbreak. In Thailand, where WSV is endemic, sustainable prawn farming practices include both fallowing to dry ponds and the use of lime to treat ponds prior to re-stocking as best practice (Chanratchakool *et al*. 1998). A minimum of four weeks fallowing and drying prior to treatment with lime and re-filling the pond 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.

2.2.13 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. It should be emphasised that WSV is harmless to humans. Campaigns should 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 control in Australia

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

- *Eradication* - eradication of WSV from Australia (highest level of control measure and 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* - the implementation of management practices that decrease the incidence and severity of clinical outbreaks (lowest level of control measure and cost).

2.3.1 Eradication

There are several eradication options. The option chosen should ensure that there is no further exposure of WSV-free prawn or other crustacean populations to the virus and no further spread of infection.

Justification for attempting eradication within a zone is based upon the following:
Evidence suggests 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.

Unexposed prawns

Ponds holding young (pre-market sized) unexposed prawns may be allowed to grow out provided that there has been a very low risk of infection and that there is a very

low risk 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 infection during transport to off-site processing plants.

Immediate destruction of unexposed prawn populations located within a declared area or within a de-stocking 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, clinically normal prawns

Normal and controlled grow-out are not eradication options for exposed or potentially exposed, clinically normal farmed prawn populations. However, it is important to recognise that infection on one or more farms might be traced back to batches of postlarvae from one or more hatcheries. Other batches from these hatcheries, stocked onto other farms, could therefore be considered potentially exposed to WSV infection. Moreover, infection from any of the farms under consideration may already have spread to local wild crustacean populations and the original source of hatchery infection may not be identifiable.

These prawns are safe for human consumption. The techniques used in emergency harvesting of these prawns must ensure there is no further spread of infection. Control measures necessary to prevent further spread of infection include:

- disinfection of all equipment/personnel involved in harvesting, slaughter and processing;
- quarantine restrictions and procedures apply to the infected site, including personnel, equipment and vehicles;
- on-site processing and cooking;
- holding, treatment and safe disposal of slaughter/processing effluent (includes holding or cooking water and waste such as prawn heads and shells); and
- ensuring that the final product will not result in the spread of infection.

Immediate destruction of these prawn populations is an option for eradication as it is very effective at decreasing the infectious load on a site and minimising the spread of infection.

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/or shells, are the main source of WSV infection in the environment. Burial sites should be chosen carefully to ensure there is no contact with waterways, groundwater or vectors.

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 use of perimeter fencing around the farm;
- all susceptible, potentially infected crustacean populations on the farm are eradicated;

- all water is disinfected before release;
- the farm is completely dried out.

The farm could resume production, provided:
- a closed production system is implemented;
- individual ponds are fenced as described previously;
- when reservoirs and ponds are re-filled, 250-500µm filter screens (Fegan and Clifford 2001) are used to minimise the risk of entry of wild crustacean carriers;
- any crustacean carriers passing these barriers is eliminated by treating with chemicals such as calcium hypochlorite or trichlorfon (Fegan and Clifford 2001) prior to pond filling;
- treated water must be held for at least 10 days to eliminate virus before pond stocking;
- ponds are stocked with tested WSV-free postlarvae derived from WSV-free broodstock.

It is essential to recognise that 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 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 difficulty in detecting such infections;
- the lack of a full understanding of how the pathogen is transmitted and how it survives in the aquatic environment;
- the current reliance of Australian *P. monodon* farmers on wild populations to provide broodstock. The presence of WSV in such wild populations will, depending on infection prevalence, probably lead to its introduction to the prawn farming industry. By contrast, the life cycle for farmed *P. japonicus* and *P. merguiensis* in Australia is closed.
- the possibility that WSV infection may become widespread under natural conditions in some wild Australian crustacean populations, distinct from those used for farming. However, this may be of little consequence to crustacean aquaculture industries, provided infection is not transmitted to wild prawn populations used as sources of broodstock and closed farming systems are used; and
- the existing close contact between, and relative lack of control over farmed and wild crustacean populations and water in Australian crustacean farming.

2.3.2 Containment, control and zoning

There is no effective treatment available for WSV in infected animals. Implementation of zoning and associated control measures to maintain uninfected zones would be necessary in the event of a WSD outbreak.

A successful zoning strategy will rely on the implementation of movement restrictions on exposed or potentially exposed prawns that 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). This feasibility can only be assessed at the time of an outbreak, taking into account movement restrictions required on prawns, people, vehicles, boats and market access for the prawn products and by-products. If young prawns are allowed to grow-out within an infected zone, they should be considered to be infected.

In a declared area, normal or controlled grow-out and harvest may be feasible without further spread of infection. However, to prevent spread of infection to adjacent wild populations, closed production systems should be used and final products should be cooked prior to leaving the farm.

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

- infected tissue from moribund and dead prawns, together with heavily infected water discharged during outbreaks, are the main sources 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 infected 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 collected from known free zones or from known infected zones.

There are several containment, control and zoning options. The option chosen should ensure that there is no further exposure of crustacean populations within the zone and no spread of infection beyond the zone.

Unexposed prawns

Provided there has been no evidence of WSD, ponds holding pre-market sized prawns may be allowed to grow out and sold for human consumption.

Effective farm, transportation and processing hygiene protocols are necessary. On-farm processing and cooking is essential, if the site is potentially infected, to prevent spreading infection during transport to off-site processing plants.

Exposed or potentially exposed, clinically normal prawns

One option is to treat these groups in the same manner as unexposed prawns provided there has been no evidence of disease. Immediate destruction and safe disposal of the prawns is also an option as it is very effective at decreasing the infectious load on a site and minimising the spread of infection.

The choice of option will depend on the assessment of risk at the time. Where the outbreak is small and eradication appears feasible, it may be better to destroy and dispose of animals in these groups. However, for a large, disseminated outbreak, where the goal is some form of containment, the choice may be to treat these groups in the same manner as unexposed prawns.

Clinically diseased prawns and other crustaceans

These prawns, along with infectious wastes, are considered to be the main source of WSV particles in the environment and constitute the greatest risk for spreading the infection to uninfected zones.

The only real option for clinically diseased prawns is immediate destruction and safe disposal. There is no vaccine or drug treatment available and the virus persists in any prawns that survive infection.

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

2.3.3 Control and mitigation of disease

The principles of control and mitigation of disease are to reduce the impact of disease and to minimise the risk of WSV spreading to uninfected populations. Therefore all options listed in Section 2.3.2 for containment, control and zoning apply, except for the following:

- measures associated with zoning;
- the exclusive requirement for closed production systems;
- in the case of partial recirculation farming systems, all water must be disinfected so as to destroy WSV and WSV carriers before release off-farm.

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

- infected tissue from moribund and dead prawns, together with heavily infected water discharged during outbreaks, are the main sources 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 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 collected from known free zones or known infected zones.

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 Office International des Épizooties (OIE or World Organisation for Animal Health) (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 south-east Asia, as well as in the Americas, including major export markets such as Japan, Hong Kong and China. Most of our major trading partners accept product from WSV-endemic areas and thus major trade impacts are unlikely. Some countries however, have regional requirements that differ within the country, for example some states of the United States. Biosecurity Australia and AQIS should be consulted for detailed information regarding export market requirements applicable at the time.

Export of cooked prawns would not be affected by the presence of WSV in Australia.

3. PREFERRED CONTROL POLICIES IN AUSTRALIA

3.1 Overall policy for WSD

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 presently free of the disease.

The choice of response option will be decided by the Director of Fisheries and/or the CVO of the State/Territory in which the outbreak occurs, following initial epidemiological investigations.

There are three possible response options for WSD in Australia:

- Option 1 - *eradication* with the view to 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* where it is accepted that the virus will remain endemic in Australia.

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

- *quarantine and movement controls* on crustaceans, their products 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 zoning and containment 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 available 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/Territory in which the outbreak occurs will decide upon the appropriate response option in consultation with appropriate staff within his/her own department and other interested parties. The response will be determined mainly by whether or not the outbreak is multi-focal 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 and the decision must be based on scientific effectiveness and financial feasibility.

3.2 Problem definition

The initial phase of any 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.

The components of this phase include:

3.2.1 Rapid confirmation of infection.

The Director of Fisheries and/or the State/Territory Chief Veterinary Officer (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 Australian Animal Health Laboratory Fish Diseases Laboratory (AFDL) Commonwealth Scientific and Industrial Research Organisation (CSIRO) at Geelong, Victoria.

WSD can only be confirmed by laboratory examination. A presumptive diagnosis can be based on the nature of the outbreak and microscopic examination of squashes or impression smears of epithelial and connective tissues of the gills or stomach of moribund prawns, supported by histological examination of H&E stained tissue sections from moribund prawns. Confirmation of the presumptive diagnosis is achieved by testing undertaken at AFDL.

For the purpose of initiating a response to a suspected disease outbreak, WSD is deemed to be confirmed if the following conditions are met:

- the history, signs and gross lesions are suggestive of WSD; and
- 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. For example, if histology was not typical, a bioassay with follow-up laboratory tests might be undertaken.

Once the response has commenced, the 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 AFDL via a State/Territory diagnostic laboratory and the CVO. It is recommended that 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 also be informed before specimens from suspected WSD incidents are transported through Victoria.

AFDL has the following requirements for the submission of suspected WSD specimens:

- 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. Dead prawns are of little use due to the rapid autolysis that follows prawn deaths.
- If it is not possible to transport live prawns to the laboratory then the following types of specimens, modified according to the populations at risk, must be collected and submitted. Where possible, live 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 mm^3 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 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 shrimp starting at the 6^{th} 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 shrimp should be placed in 10 x volume of Davidson's fixative for 24 hours (up to 72 for larger shrimp) after which they 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 AQUAVETPLAN **Enterprise Manual**, Appendix 5 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.

3.2.2 Epidemiological investigations

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

Surveillance of prawn farms and wild prawns in the region must be undertaken immediately to determine the extent of the outbreak. Surveillance should comprise 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 existing at the time as described in the *International Aquatic Animal Health Code* (OIE 2003b). Where the objective is to detect infection and not measure prevalence, specimens may be pooled to reduce testing costs provided there is no loss of sensitivity.

3.2.3 Interim measures to reduce virus spread

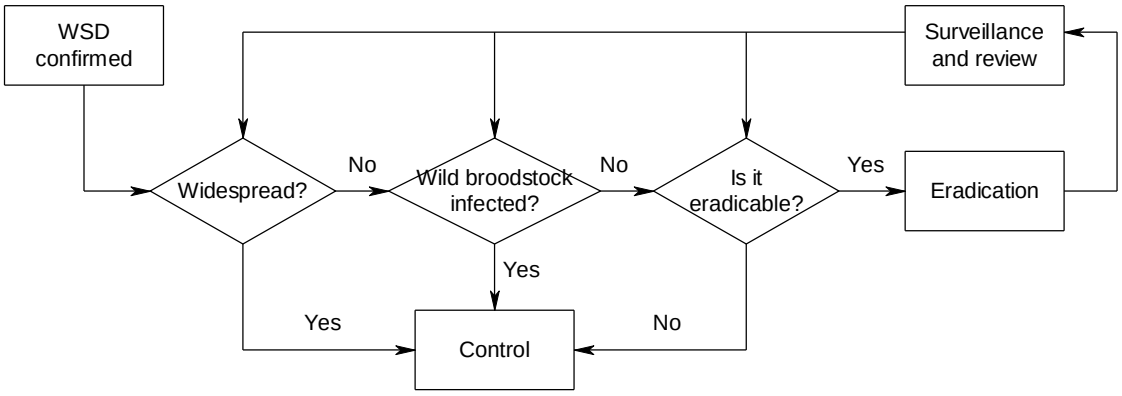
Movement controls and other measures should be implemented immediately on suspected infected premises or areas, pending confirmation of WSD and definition of

the extent of the outbreak (see section 3.4.1, and AQUAVETPLAN (2002) **Enterprise Manual** for details).

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.

3.2.4 Determination of the appropriate response
As soon as adequate information becomes available, a decision should be made as to the appropriate response, based on the flowchart shown in Figure 3. 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 chosen will depend primarily on the feasibility of zoning.

Figure 3: Decision flowchart to determine the preferred response to a WSD outbreak



3.3 Overview of response options
3.3.1 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 should 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, it is recognised that the potential constraint to eradication posed by the presence of infection in wild prawns is equivocal and judgement will need to be exercised based on whether or not a supply of uninfected broodstock is considered to be available.

Eradication strategies include the following:
- establishment of specified areas – *restricted*, *control*, *free*;
- quarantine and movement controls/restrictions on prawns, prawn products, other crustaceans, water and any other vectors (including fomites) located in declared (*restricted* and *control*) areas to prevent spread of infection;
- destruction and disposal of all prawns in ponds with clinical disease;

- on-farm processing and cooking of exposed or potentially exposed, clinically normal prawns with associated holding, treatment and safe disposal of effluent (includes holding of cooking water and waste such as prawn heads and shells).
- disinfection and safe disposal of pond water, decontamination of 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;
- a public awareness campaign to facilitate cooperation from industry and the community.

3.3.2 Containment, control and zoning

If infection is widespread in wild prawn stocks or on numerous farms, eradication is likely to be impracticable. In this case, containment and prevention of further spread is the preferred response option in order to protect and maintain uninfected areas. Containment, control and zoning would also apply outside the affected farm(s), even when eradication is pursued.

Though difficult, it may be possible to maintain uninfected zones free of WSD and the implementation of a zoning program would be advantageous to the Australian prawn industry to maintain market access, as well as providing protection for uninfected regions. Restrictions on the movement of prawns and prawn products and a surveillance program will be necessary to support zoning. Farms in infected zones would need to implement management practices to reduce the severity and impact of WSD outbreaks.

Control procedures are similar to those for eradication. However there would be an emphasis on managing the disease in individual facilities rather than eradication. Strategies used for control of WSD may include the following:

- zoning to define infected and disease-free areas; and
- quarantine and movement controls/restrictions on prawns, prawn products, water and any other vectors (including fomites) 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 followed by clean-up and disinfection in the infected zone;
- use of closed production systems;
- WSV testing of broodstock and postlarvae;
- emphasis on high standards of biosecurity (including drying of ponds before restocking; disinfection of water prior to both use and release; 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;
- a public awareness campaign to facilitate cooperation from industry and the community.

3.3.3 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. In this case, it would mainly be the responsibility of individual producers to manage the disease in their facility in accordance with recommended measures to reduce the likelihood and severity of outbreaks. Producers may be encouraged to adopt current best-practice measures through provision of enterprise-level standard operating procedures and quality assurance programs with the view to the eventual development of an accreditation program. No zoning is envisaged under this level of control which would be similar to that which applies in those countries where the disease is endemic.

Measures used would 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 prior to both use and release) and biosecurity (including the use of crustacean-proof land barriers and water filters);
- best practice pond management methods to minimise stress and hence risk of an outbreak occurring during grow-out of covertly infected stock.

3.4 Strategies for eradication and control

Strategies for the eradication and control of WSD are summarised in Table 9, and described in detail in sections 3.4.1 to 3.4.10.

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

Strategy	Eradication	Control	
		Containment	Mitigation
Quarantine and movement controls	Yes	Yes	No
Declared restricted/control areas	Yes	No	No
Zoning	N/A	Yes	No
Movement controls within declared area or infected zone	Yes	Yes	N/A
Movement controls out of declared area or infected zone	Yes	Yes	N/A
Destruction or harvest with on-farm cooking of clinical cases depending on size (equivalence with imports)	Yes	Yes	Yes
Destruction of unexposed prawns	Optional	No	No
Destruction or harvest with on-farm cooking of exposed or potentially exposed, clinically normal prawns depending on size (equivalence with imports)	Yes	Optional	No
On-farm processing and cooking	Yes	Optional	Optional
Treatment of exposed prawn products and by-products	Destroyed	Yes	Yes
Treatment of unexposed prawn products and by-products	Yes	No	No
Disposal of infected prawns and wastes which can not be cooked on farm	Yes	Yes	Yes
Decontamination	Required	Optional	Optional
Surveillance	Yes	Yes	Yes
Tracing	Yes	Optional	No
WSV screening of broodstock and postlarvae	Yes	Yes	Yes
Closed farming systems	N/A	Yes	Yes
Partial recirculation farming systems	N/A	No	Yes
Specific farm-level hygiene measures	Yes	Yes	Yes
Specific farm-level biosecurity measures	Yes	Yes	Yes

N/A – Not applicable

3.4.1 Quarantine and movement controls; declared restricted/control areas

Until the most appropriate control strategy is determined, quarantine and movement controls should be implemented on anything capable of transmitting infection where practicable. Once the most appropriate control strategy for the incident is determined, quarantine and movement controls can be altered accordingly. See the AQUAVETPLAN (2002) **Enterprise Manual** for details on movement controls for different enterprise systems and response options.

For eradication, restricted and control areas will be declared. Quarantine and movement controls must be stringently enforced on prawns, prawn products, water, fomites and any vectors located in declared areas capable of spreading the virus. Movement controls should be maintained until the disease is either eradicated or declared endemic.

For the other response options, movement controls are essential to maintain free zones where these have been declared. Restrictions must apply to anything capable

of transmitting WSV from infected to free prawn populations, aquatic systems and processing plants.

3.4.2 Zoning

Zoning for WSV should be based on the OIE determined principles as expanded in the AQUAVETPLAN **Zoning Policy Guidelines** and 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, prawn products and equipment between zones.

Where zoning is implemented, an active surveillance program for WSV is necessary in free zones, and State/Territory-based legislation is required to support zoning, movement controls and surveillance activities.

3.4.3 Destruction of clinically diseased prawns

Immediate removal, destruction and safe disposal of all diseased and dead prawns are essential to the success of any response strategy. These prawns, along with infectious wastes, are the main source of WSV in the environment. Diseased and dead prawns must be removed from tanks and ponds and destroyed, as a high priority. Burial sites should be chosen carefully to ensure there is no contact with waterways or vectors.

3.4.4 Destruction of unexposed prawns

Eradication

Unexposed prawns may be allowed to grow-out provided there is no risk of future infection. Water system, equipment and all handling procedures must bear no risk of infection to ensure 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 any husbandry practices.

Market size prawns without any apparent possible exposure to infection with WSV may be harvested and processed for human consumption. Effective hygiene practices are required at processing. The method of harvest, equipment used and location must also have no risk of exposure to infection. On-farm processing may be preferable, as this will prevent any potential infection during transport to off-site processing plants.

Immediate destruction should be considered for unexposed prawn populations located within an infected zone. This is particularly applicable to young animals that have a low unit value. Immediate destruction of such populations will decrease the chance of spread of infection to these and other prawn stocks thus helping prevent propagation of the disease.

Containment, control and zoning; Control and mitigation

Grow-out and slaughter for human consumption can occur as normal for both control options. Control measures are only required to prevent transmission of infection to unexposed prawns in free zones. Thus, the method of harvest, equipment used and the choice of location should ensure there is no exposure to infection.

3.4.5 Destruction of exposed or potentially exposed, clinically normal prawns

Eradication

In facilities undergoing eradication, exposed or potentially exposed, clinically normal prawns should be regarded as infected and destroyed. From a human health perspective, healthy covertly infected prawns are safe for human consumption. However emergency harvesting and processing of healthy exposed/potentially exposed prawns carries a high risk of further transfer of infection if taken off the infected farm.

Where it is feasible, emergency harvest with on-site cooking is an option as this should pose no greater infection dissemination risk than destruction and disposal.

Containment, control and zoning

Grow-out of exposed or potentially exposed, clinically normal prawns within infected zones under farm management practices involving a high level of hygiene and and biosecurity screening incoming postlarvae is possible. However, all-in-all-out management practices may need to be implemented. Destruction of these prawns will decrease the infectious load on a site and should minimise not only the spread of infection but also the incidence of outbreaks. However, if infection is endemic in the area, reinfection of the newly stocked prawn populations may occur via intake water or by crustacean reservoirs. Since de-stocking a hatchery has significant economic impact on not only hatchery operations but also the grow-out farms it supplies, the application of this option to hatcheries would depend on the infectious status of the local area.
If these prawns are allowed to grow-out, they must be treated and handled as infected populations. Restrictions on movements of prawns and prawn products, people, vehicles, boats and on market access for final product may be necessary to protect free facilities or zones.

Control and mitigation

Grow-out of exposed or potentially exposed, clinically normal prawns within infected zones under farm management practices involving a high level of hygiene and biosecurity and screening incoming postlarvae is possible.

3.4.6 Treatment of prawn products and by-products

The treatment of prawn products and by-products must take into account trade regulations, market requirements, food safety standards and potential spread of the pathogen via product. Harvested prawns, potentially for human consumption, may be stored safely in a freezer until a definitive diagnosis is obtained and decisions are made regarding release of product. This will prevent the spread of infection and allow salvage of product for sale (provided the relevant authority approves release). Another measure which can be used to prevent spread of virus from infected farms is on-farm processing and cooking of prawns with subsequent treatment of by-product and waste.

Eradication

All live prawns, products and by-products from facilities undergoing eradication should be destroyed and disposed of safely or, if of market size, cooked on farm and marketed.

Containment, control and zoning

Prawn product and by-products may be traded within an endemic zone without restrictions, but not from an infected zone into a free zone. Unexposed prawns may be marketed and disseminated without any risk of transmission of infection. However, products from exposed prawn populations will require processing and/or may have a restricted market in order to maintain WSV-free zones. Domestic market regulations (e.g. State/Territory legislation) and food safety standards must be considered when determining the required treatment of products and by-products.

Control and mitigation

Because free zones are not established under this control strategy, there will be fewer restrictions on the treatment and release of product onto the market.

3.4.7 Disposal

Eradication

Immediate, safe disposal of all infected prawns, wastes, effluent and equipment that cannot be decontaminated is necessary for the eradication of the virus. See the AQUAVETPLAN (2002) **Operational Procedures Manual** for details. If processing is undertaken on infected establishments, the effluent and any other waste and by-products will require treatment and safe discharge/disposal to prevent spread of infection.

Containment, control and zoning; Control and mitigation

Safe disposal of all infected dead prawns, wastes and effluent is important in decreasing the infectious load on a site. This will greatly assist in decreasing the incidence of WSD outbreaks.

3.4.8 Decontamination

Eradication

All buildings, tanks, materials and equipment including nets, boats and vehicles that may be contaminated must be cleaned and disinfected for successful eradication. 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 containment

The implementation of 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, so as to decrease the infectious load on the site.

3.4.9 Surveillance

Surveillance should comprise 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 would be necessary to support the declaration of WSV-free zones. Clinical surveillance should be used on farms in infected zones with the view to early detection of new outbreaks and consequent application of contingency measures.

3.4.10 Tracing

Tracing should be undertaken as described in section 2.2.2 for all infected facilities identified as part of an official control or eradication program. Tracing is not required for infected facilities in an endemic zone unless they are suspected as the source of an outbreak in another zone.

3.5 Social and economic effects

To date, Australia has remained free from WSD apart from a localised incident in Darwin where WSV was detected in two aquaculture facilities and promptly eliminated. Based on overseas experience, the occurrence of uncontrolled WSD in Australia is likely to devastate the national prawn aquaculture industry. However, the overall impact on the prawn industry as a whole is likely to be small relative to its total overall value in Australia (Alliance Resource Consulting 1998). This is due mainly to the likely differences in level of impact on the wild-caught and cultured prawn industries combined with the large differences in the size of these two sectors.

3.6 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 *International Aquatic Animal Health Code* (OIE 2003). Proof of a return to freedom following an outbreak that was eradicated is likely to rely on both clinical surveillance to show that no new outbreaks had occurred over a reasonable period of time and a random sample survey.

3.7 Funding and compensation

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

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