Australian Government

Department of Agriculture

AUSTRALIAN AQUATIC VETERINARY EMERGENCY PLAN

AQUAVETPLAN

Disease Strategy

White spot disease

Version 2, 2013

AQUAVETPLAN is a series of technical response manuals for aquatic animal disease incursions, based on sound analysis and linking policy, strategies, implementation, coordination and emergency-management plans.

Standing Council on Primary Industries

This disease strategy forms part of:

AQUAVETPLAN

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

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IMPORTANT NOTE: Regulatory information for white spot disease is contained in the World Organisation for Animal Health (OIE) Aquatic animal health code (OIE 2012a), which is updated annually and is available on the OIE website: www.oie.int/en/international-standard-setting/aquatic-code/access-online (see Appendix 1).

DISEASE WATCH HOTLINE

1800 675 888

The Disease Watch Hotline is a toll-free telephone number that connects callers to the relevant state or territory officer to report concerns about any potential emergency animal disease situation. Anyone suspecting an emergency disease outbreak should use this number to get immediate advice and assistance.

Preface

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

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

The Australian Government Department of Agriculture provides quarantine inspection for international passengers, cargo, mail, animals, plants, and animal or plant products arriving in Australia. The Department of Agriculture also inspects and certifies a range of agricultural products exported from Australia. Quarantine controls at Australia's borders minimise the risk of entry of exotic pests and diseases, thereby protecting Australia's favourable status for human, animal and plant health. Information on current import conditions can be found at the Department of Agriculture ICON website. [1]

This disease strategy sets out disease control principles for use in an aquatic veterinary emergency incident caused by the suspicion or confirmation of WSD in Australia. The strategy was scientifically reviewed by the Sub-Committee on Aquatic Animal Health before being endorsed by the Animal Health Committee of the Standing Council on Primary Industries in April 2013.

Detailed instructions for the field implementation of AQUAVETPLAN are contained in the disease strategies, operational procedures manuals and management manuals. Industry-specific information is given in the enterprise manual. The full list of AQUAVETPLAN manuals[2] that may need to be accessed during an aquatic animal disease emergency is shown below.

Disease strategies

Individual strategies for each disease

Operational procedures manuals

Destruction

Disposal

Decontamination

Management manual

Control centres management

1 www.aqis.gov.au/icon32/asp/homecontent.asp

2 The complete series of AQUAVETPLAN documents is available on the internet at www.daff.gov.au/animal-plant-health/aquatic.

Enterprise manual

Includes sections on:

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

This first edition of this manual was prepared by Dr Chris Baldock, Dr Iain East and Dr Richard Callinan, with the assistance of Professor Tim Flegel and Mr Dan Fegan in 2005. This revision was prepared by Dr Jeff Cowley and Dr Mark Crane, CSIRO Animal, Food and Health Sciences, and completed in August 2010. The authors were responsible for drafting the strategy, in consultation with a wide range of stakeholders from aquaculture, recreational fishing and government sectors throughout Australia. However, the text was amended at various stages of the consultation and endorsement process, and the policies expressed in this version do not necessarily reflect the views of the authors. Contributions made by others not mentioned here are also gratefully acknowledged.

The format of this manual has been adapted from similar manuals in AUSVETPLAN (the Australian Veterinary Emergency Plan for terrestrial animal diseases). A similar format and content have been used to enable personnel trained in AUSVETPLAN procedures to work efficiently with this document in the event of an aquatic animal disease emergency involving WSD. The work of the AUSVETPLAN writing teams and the permission to use the original AUSVETPLAN documents are gratefully acknowledged.

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This current version of the AQUAVETPLAN **Disease Strategy—White spot disease** has been reviewed and approved by the following representatives of government and industry:

Government

Department of Primary Industries, New South Wales

Department of Primary Industry and Fisheries, Northern Territory

Queensland Department of Agriculture, Fisheries and Forestry

Department of Primary Industries, Parks, Water and Environment, Tasmania

Department of Fisheries, Western Australia

Department of Environment and Primary Industries, Victoria

Department of Primary Industries and Regions of South Australia

Biosecurity Animal, Australian Government Department of Agriculture

Industry

Australian Prawn Farmers Association

National Aquatic Animal Health Industry Reference Group

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1 Nature of the disease

White spot disease (WSD) is a highly contagious viral disease of penaeid prawns (family Penaeidae). In farmed prawns, disease is characterised by the rapid onset of high mortalities. In a disease outbreak, prawns typically cease feeding a few days before moribund prawns appear at pond edges, followed within a day or two by mass mortalities. The causative virus, white spot syndrome virus (WSSV), can infect a wide range of crustaceans, often without causing clinical disease. During the 1990s, WSD spread rapidly throughout prawn-farming regions in Asia and became established in prawns farmed in the Americas. WSD caused extensive losses in farmed prawns and is causing damage to wild freshwater crayfish populations in the United States. A comprehensive survey found no evidence of WSSV in Australia in 2004 (East et al. 2004), and subsequent surveillance and testing has not detected presence of WSSV in Australia to date. Australia is one of the few countries in the world with a prawn-farming industry that is free of WSD.

1.1 Aetiology

In 1993, WSSV was first linked to WSD outbreaks in the kuruma prawn *Penaeus japonicus*, farmed in Japan. However, there is circumstantial evidence that WSSV was probably the cause of disease and mortalities in other prawn species being farmed in Taiwan and China in 1991 and 1992, respectively, from where it is suspected to have originated. Within a few years, viruses with characteristic WSSV morphology, but described under various other names, were associated with outbreaks of WSD in prawns being farmed in China, Taiwan and Thailand (Flegel 2001). Based on similarities in virus morphology, disease signs and pathology, the viruses were grouped collectively into the white spot virus complex (Lightner 1996; Lo et al. 1999) with WSSV being adopted as the generic virus name. Based on its unique genome structure, WSSV has since been classified by the International Committee on Taxonomy of Viruses in taxa (family *Nimaviridae*, genus *Whispovirus*; Lo et al. 2012) distinct from baculoviruses, which have general similarities in genome makeup and particle morphology.

Virions have a large (80–120 nm × 250–380 nm), elliptical or rod-shaped particle morphology and contain an approximately 300 kilobase pair (kbp), circular double-stranded DNA genome and a trilaminar envelope that sometimes can display a unique, tail-like appendage (OIE 2012b). Nucleotide sequence analysis of WSSV from crustaceans involved in WSD outbreaks and those with subclinical infections indicates that WSSV strains are largely identical, with variations present in the number of repeated DNA sequences (function unknown currently) (Flegel 2001). DNA sequence variation is 0.68% among completely sequenced WSSV strains originating from Thailand, China and Taiwan (Marks et al. 2004). WSSV strains from Thailand and China have nucleotide deletions of 1 kbp and 13 kbp, respectively. However, sequence variation among the three strains is primarily restricted between repeated DNA sequences. A second difference involves a genetically variable region of about 750 bp. Moreover, in a study of WSSV strains associated with WSD outbreaks in Thailand between 2000 and 2002, a repeated DNA sequence within ORF94 varied between 6 and 20 repeat copies (Wongteerasupaya et al. 2003). In India, the numbers of DNA repeat copies in this and two other ORFs has provided a means of genotyping WSSV strains accurately, thus providing a means of epidemiological tracing of infection origins and transmission (Pradeep et al. 2008).

Comparative bioassays in multiple prawn species suggest slight differences in virulence for different WSSV genotypes (Wang Q et al. 2000) and between strains originating from China and countries in the Americas (Laramore et al. 2009). As variants with increasing virulence emerge, the potential to cause devastating mortality events increases as well (Walker et al. 2002). However, as data on virulence determinants remain scant and as crustacean bioassays are the only reliable means of assessing virulence accurately, for the purposes of this document, any detection of WSSV assumes that the strain will have the potential for high virulence and acute disease in penaeid prawns.

1.2 Susceptible species

Penaeus will be used throughout this AQUAVETPLAN manual to describe species of the five recognised subgenera (*Farfantepenaeus*, *Fenneropenaeus*, *Litopenaeus*, *Marsupenaeus* and *Melicertus*), even though it has been suggested, based on genetic distinctions, that each of these subgenera could be elevated to a unique genus status (Pérez Farfante & Kensley 1997). Note that genus/subgenus names have been used interchangeably by authors of cited references.

All decapod crustaceans (order Decapoda), including prawns, lobsters and crabs from marine, brackish water or freshwater environments, are considered to be susceptible to WSSV infection (OIE 2012b). WSSV has been detected in wild prawns from Asia and the Americas. However, WSD outbreaks have mainly been reported from farmed prawns. In Australia, susceptible or potentially susceptible crustaceans include the major species of farmed marine prawns—*Penaeus monodon* (giant tiger prawn), *P. merguiensis* (banana prawn) and *P. japonicus* (kuruma prawn)—as well as several freshwater crustacean species—*Macrobrachium rosenbergii* (giant freshwater prawn), *Cherax quadricarinatus* (Australian red claw crayfish), *C. destructor albidus* (yabby) and *C. tenuimanus (*marron)—farmed commercially.

Although naturally occurring WSD has not been officially reported in wild (as opposed to farmed) crustaceans, several species can develop clinical disease following experimental infection by either injection (Supamattaya et al. 1998), exposure to contaminated water (Chen et al. 2000) or ingestion of WSSV-infected tissue (Sahul Hameed et al. 2003). Susceptibility to clinical disease and mortality through ingestion has been demonstrated in the crayfish *Procambarus clarkii* (red swamp crayfish; Wang et al. 1998), the freshwater prawn species *Macrobrachium idella* and *M. lamerrae* (Sahul Hameed et al. 2000), the freshwater crabs *Paratelphusa hydrodromous* and *P. pulvinata* (Sahul Hameed et al. 2001), several European marine and freshwater crustacean species (Corbel et al. 2001), and the freshwater crayfish species *Procambarus clarkii* and *Orconectes punctimanus* indigenous to North America (Richman et al. 1997). Ingestion of infected tissue can also cause relatively high mortality in *M. rosenbergii* postlarvae and juveniles, with lower mortality rates in subadults and adults, suggesting a greater tolerance to WSD with age (Pramod Kiran et al. 2002).

Australia is rich in freshwater crayfish fauna, and of the *Cherax* spp. cultured semi-intensively, high mortalities following WSSV injection can occur in *C. quadricarinatus* and *C. destructor albidus* (yabby) (Shi et al. 2000; B Edgerton, pers. comm.). In *C. destructor albidus*, however, ingestion of WSSV-infected tissue establishes a subclinical infection that requires stress to promote disease and mortality, suggesting this species might have higher resilience to WSD than penaeid prawns infected via the ingestion route (Edgerton 2004). The susceptibility of other Australian freshwater crayfish species to WSD remains unknown.

WSSV infection can be prevalent in wild prawns in regions where WSD is endemic in farmed prawns (Cavalli et al. 2010; Chapman et al. 2004; de la Peña et al. 2007; Withyachumnarnkul et al. 2003). Although declines in wild penaeid prawn populations have been attributed to other viral pathogens such as infectious hypodermal and haematopoietic necrosis virus (IHHNV) (McIlwain et al. 1997; Pantoja et al. 1999), there is no evidence of WSSV causing disease outbreaks in wild crustaceans (Alliance Resource Consulting 1998). Viral disease is likely a contributing factor to fluctuations in wild crustacean populations; however, it is often overlooked in fisheries research and stock assessment (Harvell et al. 2002, 2004) because of the difficulty in gathering convincing evidence. This may be influenced by the rapid onset of viral diseases, high predation pressures on wild populations reducing the likelihood of sampling affected individuals and inadequate surveillance sensitivity to detect low levels of infection.

Another reason for the lack of disease evidence in wild populations is the absence of the stress factors that are often associated with aquaculture environments, such as high stocking densities and resultant physiological pressures (Lotz & Soto 2002). Therefore, exposure to and infection with WSSV may not result in wild prawns or other wild crustaceans developing clinical signs of white spot disease (Lo et al. 1997a). Currently, no data exist on the impact, if any, of WSSV infection on other wild crustaceans. There is no evidence that WSSV can infect or cause disease in higher organisms, including humans.

1.3 World distribution

WSD is believed to have emerged in farmed prawns from Taiwan and China in 1991–92, from which it spread in 1993 to farmed *P. japonicus* in Japan via live prawn imports (Nakano et al. 1994). By the end of the 1990s, WSSV had become endemic throughout all countries in Asia and the Americas that had substantial prawn aquaculture industries (Subasinghe et al. 2001). The spread of WSSV between countries and regions has been linked primarily to translocations of live prawns for aquaculture or to imported uncooked prawns finding their way inadvertently into aquatic environments (Durand et al. 2000; Nunan et al. 1998).

The presence of WSSV has been reported officially by 14 countries in the Asia–Pacific region (Bangladesh, China, India, Indonesia, Japan, Malaysia, Pakistan, Philippines, Singapore, South Korea, Sri Lanka, Taiwan, Thailand and Vietnam) and 11 countries in the Americas (Argentina, Brazil, Colombia, Ecuador, Guatemala, Honduras, Mexico, Nicaragua, Panama, Peru and the United States) (Martorelli et al 2010; Muller et al. 2010; NACA 2002; OIE 2012b). WSSV-positive test results for wild-caught crustaceans are presented in Table 1.6.

WSD has been listed as a non-exotic pathogen in the European Union in EC Directive 2006/88 due to previous disease outbreaks in penaeid farms in Greece, Italy and Spain. However, the pathogen has not been reported in the wild fisheries (Stentiford & Lightner 2011).

Australia, New Zealand and South Pacific island countries are currently free of WSSV (East et al. 2004; NACA 2002; OIE 2012b). WSSV was detected by polymerase chain reaction (PCR) testing of imported green prawns being used as feed at two aquaculture facilities in Darwin in November 2000. Tests from potentially exposed stock were initially inconclusive, and subsequent tests were negative for the virus. Following precautionary destocking and thorough disinfection of these facilities, rigorous PCR screening of crustaceans near the facilities identified no evidence of endemic infection having established in the wild (Bernoth 2000, 2001, 2002; East et al. 2004).

Although WSSV and WSD are primarily reported from tropical regions, WSSV infection in areas with minimum temperatures as low as 4 °C has been recorded (Martorelli et al. 2010). Subclinical infection in cool water areas could exist and only be expressed when temperatures rise (see Section 1.6.5).

1.4 Diagnosis of infection with white spot syndrome virus

Prawns affected by WSD often show no distinctive gross signs or pathognomonic lesions. Examination of histological sections of cephalothorax tissues of moribund crustaceans stained with haematoxylin and eosin (H&E) can provide a presumptive diagnosis of WSSV infection when WSD is suspected. However, for definitive diagnosis, as well as for certification of the WSSV infection status of clinically normal crustaceans or prawn broodstock and postlarvae, PCR testing is recommended (OIE 2012b). No tissue culture systems are yet available for the routine culture, identification and diagnosis of any crustacean pathogen, including WSSV, and clinical chemistry methods of diagnosis are not used routinely by crustacean pathologists.

1.4.1 Field methods: clinical signs and gross pathology

Clinical signs and gross lesions associated with WSD (Table 1.1) can vary between outbreaks and, alone, are insufficient for diagnosis.

Table 1.1 Comparative features of clinical white spot disease and subclinical white spot syndrome virus infection

Sign	Clinical disease	Subclinical 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	2–4 days	Remain clinically normal if not stressed

WSD can occur at any stage of prawn grow-out, and is typified by rapid onset and high rates of mortality. The first evidence of disease is often a dramatic increase in moribund and dead prawns at pond edges, with cumulative mortalities reaching approximately 100% within 3–10 days. Farmed prawns that develop acute WSSV infection cease feeding suddenly and become lethargic. The shell often becomes loose and may be smattered with white, initially circular, spots within the cuticle, and/or a generalised reddish body discolouration may be evident. The intracuticular white spots can range from minute foci to discs up to 2 mm in diameter and become sufficiently dense in number to coalesce (Lightner 1996). The spots are most easily observed by removing the cuticle over the cephalothorax, scraping away any attached tissue and holding the cuticle up to light (OIE 2009). The spots have a similar chemical composition to the cuticle and have been suggested to arise as a result of either abnormal deposition of calcium salts by the cuticular epidermis (Lightner 1996) or disrupted transfer of exudate from epithelial cells to the cuticle (Wang YG et al. 1999).

Despite often being associated with mass mortality events, WSD can manifest as low-level morbidity and mortality during prawn grow-out (Flegel 1997; Tsai et al. 1999). Disease with these characteristics began to occur in affected regions one to two years after WSD first emerged in association with catastrophic mortalities. Flegel (2001, 2009) has suggested that prawns might adapt to tolerate WSSV infection better with time, and

proposed that infected seedstock generated in aquaculture hatcheries might survive grow-out well, provided that ponds are well managed to avoid increased stress (Section 1.5.2).

It is important to note that, although white spots on the carapace are a clinical feature of WSD, they are not pathognomonic. White spots on the cuticle can occur as a result of environmental factors such as high alkalinity (OIE 2012b) or bacterial shell disease, neither of which will result in significant mortalities (Goarant et al. 2000; Wang et al. 2002). Conversely, prawns with WSD that become moribund might display few, if any, white spots, but rather display a generalised pink to reddish-brown discolouration (the alternative name 'red disease' is sometimes used for WSD). The discolouration occurs because chromatophores become elevated in the cuticular epithelium.

1.4.2 Laboratory methods

Sample submission

In the first instance, clinical specimens should be sent to specialist regional aquatic animal veterinarians at state or territory diagnostic laboratories. If WSSV infection is diagnosed, and following any necessary state or territory regulatory clearances, the chief veterinary officer (CVO) of that state or territory should inform the CVO of Victoria that duplicate or additional specimens from the suspected WSSV incursion/WSD outbreak will be forwarded to the CSIRO Australian Animal Health Laboratory Fish Diseases Laboratory (AFDL), Geelong, for diagnostic confirmation.

The AFDL should be contacted directly to obtain information on what clinical material is required and how it should be collected and transported to satisfy AFDL requirements for confirming the preliminary diagnosis of WSSV infection.

Ideally, all laboratory procedures should comply with the *Manual of diagnostic tests for aquatic animals 2012* (Aquatic Manual; OIE 2012b). For most crustaceans, the recommended minimum number of specimens that should be collected for diagnosis is 100 for larval stages, 50 for postlarval stages and 10 for juvenile and adult stages, with preference for individuals with patent signs of disease and/or gross lesions. However, these numbers are a guide only, as fewer good-quality specimens are more useful than larger numbers of samples collected, preserved, stored or transported inappropriately.

There are two situations in which diagnosis of WSSV infection is required: (1) confirmation of suspected clinical WSD and (2) when the WSSV infection status of asymptomatic crustaceans needs to be established.

To confirm that WSSV infection is the cause of a suspected WSD outbreak, representative animals showing clinical signs and/or gross lesions should be sampled. Whole animals, haemolymph, gills and pleopods provide suitable specimens for examination. Dead crustaceans can provide useful diagnostic information (Mohan et al. 2002); however, unless samples are appropriately preserved (fixed with formalin/ethanol or refrigerated), they are often unusable because of the rapid onset of postmortem changes and associated tissue putrefaction/autolysis. Several rapid laboratory methods (see the following sections) are available to support a presumptive diagnosis, which can be confirmed subsequently by histological examination and molecular methods.

The number of individual samples required when screening overtly healthy crustaceans for WSSV will depend on the population size and level of confidence at which infection needs to be excluded (Lightner 1996). Suitable specimens for examination include whole larvae, postlarvae and juveniles, and gill or pleopod tissue and samples of haemolymph taken from juveniles through to broodstock. PCR is the preferred test for WSSV detection. When

necessary to confirm the presence of viable WSSV in a PCR-positive tissue sample, bioassays in a highly susceptible host species should use either fresh tissues or tissues stored appropriately at ultra-low temperatures.

Table 1.2 compares the suitability of the different methods for screening and diagnosis.

Table 1.2 Comparison of white spot syndrome virus screening and diagnostic methods

Method	Screening methods				Presumptive WSD diagnosis	Confirmatory WSD diagnosis
	Larvae	Postlarvae	Juveniles	Adults		
Gross signs	–	–	+	+	+	–
Bioassay[a]	–	–	–	–	+	++
Rapid microscopic methods	–	–	+	+	+	+
Histology	–	+	+	+	+++	+++
TEM	–	–	–	–	–	+++
Antibody-based assays	–	–	+	+	+++	++
In situ DNA hybridisation	–	–	+	+	+++	+++
PCR	–	++	+++	+++	+++	+++
Sequencing	–	–	–	–	–	+++

PCR = polymerase chain reaction; TEM = transmission electron microscopy; WSD = white spot disease

– the method is currently unavailable or unsuitable

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

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

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

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

Source: Modified from OIE (2012b)

Microscopy

There are two rapid histological approaches available for presumptive diagnosis of WSD. The first employs dark-field microscopy (Momoyama et al. 1994) to examine unstained wet-mounts fixed in formalin; the second employs light microscopy to examine fixed, stained tissue sections. For light microscopy, differing fixation and staining approaches can be used (method 1, Lightner 1996; method 2, OIE 2012b).

Dark-field microscopy

Select a moribund prawn with suspected WSD. Obtain subcuticular tissue by either dissecting out the stomach, or peeling thin layers of subcuticular tissue from the cephalothorax and fixing it in 10% formalin. Use fine forceps to spread thin pieces of the subcuticular tissue onto a microscope slide in a small volume of 10% formalin. Apply a cover slip and remove excess fixative using filter paper touched to the edge of the cover slip. Examine the tissue using a microscope fitted with dark-field optics. Upon focusing on tissue areas where pigment cells are poorly distributed, prawns infected with WSSV will display moderate-to-large numbers of refractile, hypertrophied nuclei.

Rapid staining method 1

Excise the stomach, gills or other appendages from a moribund prawn with suspected WSD. Mince the tissue and then squash, dab or smear onto a microscope slide. Fix the tissue smear in methanol for 6 minutes or by dehydrating the tissue by gently heating the slide. Flood the tissue smear with an appropriate stain such as Giemsa or another blood-smear stain, leave for 1–5 minutes, place a cover slip on the tissue and examine by light microscopy using 10×, 20× and 40× objectives. Normal cell nuclei will be 4–10 μm in diameter, and display chromatin threads and a nucleolus. Nuclei of WSSV-infected cells from specimens with WSD will be hypertrophied and usually contain a single eosinophilic to bluish-coloured inclusion body (depending on the stain used).

Using this method for prawns severely affected by WSD, diagnostic data comparable to that obtainable by H&E histology can be generated in approximately 10 minutes.

Rapid staining method 2

Fix a moribund prawn with suspected WSD in Davidson's fixative overnight. Either the entire prawn or dissected gill filaments can be used (see the next paragraph for an alternative, more rapid fixation method). Wash some gill filaments thoroughly with tap water to remove the fixative and stain in H&E. After staining and dehydrating in xylene, place a gill filament onto a microscope slide in a drop of xylene. Use a fine pair of needles to break off several secondary filaments (a stereo microscope can be helpful for this). The primary gill filament can be returned to the xylene and stored in a sealed vial indefinitely as a permanent reference. While under xylene on the slide, the secondary gill filaments can be teased apart to remove larger fragments that would unduly thicken the prepared mount. Once free of larger tissue pieces, mount the stained secondary gill filaments by adding a drop of mounting fluid and a cover slip, with light pressure applied to flatten the mount as much as practicable. Thin layers of subcuticular tissue can also be examined using this procedure. In prawns affected by WSD, examination by light microscopy using a 40× objective will reveal moderate to high numbers of cells with hypertrophied nuclei that contain basophilic inclusion bodies surrounded by marginated chromatin. The detection of some nuclei containing Cowdry type A inclusions characteristic of early-stage WSSV infection will provide additional acuity of diagnosis.

When an urgent diagnosis is required, the overnight fixation step can be shortened to 2 hours if the acetic acid component of the Davidson's fixative is replaced by 50% concentrated hydrochloric acid. Effective fixation requires this modified fixative to be prepared fresh or stored for no longer than a few days before use. After fixation, the tissue needs to be washed thoroughly to remove the fixative and the pH checked to ensure it has returned to near neutral before staining. Fixation for longer periods or at temperatures above 25 °C can damage the tissue excessively and compromise detection of cellular pathology.

Histopathology

For histology, soft cephalothorax tissues of moribund prawns should be preserved in Davidson's fixative, processed into paraffin blocks, and tissue sections stained with H&E using standard techniques (Bell & Lightner 1988; Lightner 1996). Examine tissue sections by light microscopy for the presence of moderate to high numbers of cells in tissues of ectodermal and mesodermal origin. These cells typically display hypertrophied nuclei with eosinophilic to basophilic central inclusions surrounded by marginated chromatin. Subcuticular tissues of the stomach, cephalothorax or gill are the most appropriate tissues for detecting histopathology characteristic of WSD (Wongteerasupaya et al. 1995).

Histopathology in moribund prawns affected by WSD is distinctive and provides a tentative preliminary diagnosis. However, transmission electron microscopy (TEM), and molecular tests such as PCR or in situ DNA hybridisation (both of which detect viral DNA), or immunohistochemical or western blot analyses that detect viral proteins, are required for confirmation (OIE 2012b).

In moribund prawns with WSD, systemic viral infection leads to necrosis in tissues of ectodermal and mesodermal origin. Viral particles and cellular necrosis occur most commonly in cuticular epithelial and connective tissues in the stomach, carapace and gills. Necrotic changes can also be seen in the antennal gland epithelium, lymphoid organ sheath cells and haematopoietic tissues, and in fixed phagocytes of the heart. Infected cells typically display hypertrophied nuclei containing a single intranuclear inclusion. In early stages of WSSV infection, nuclear inclusions are eosinophilic and (as an artefact of tissue preservation in Davidson's fixative) are separated by a clear halo from the marginated chromatin. Such eosinophilic or Cowdry type A intranuclear inclusions are characteristic of infections caused by many viruses in both vertebrates and invertebrates, and appear as amorphous structures surrounded by clear halos beneath the nuclear membrane. Later in infection, inclusions stain lightly to darkly basophilic and can enlarge to fill the entire nucleus (Lightner 1996; OIE 2012b). This feature can be used to distinguish infection caused by WSSV from that caused by IHHNV, in which only Cowdry type A inclusion bodies are formed.

Transmission electron microscopy

Tissues most suitable for detecting WSSV virions by TEM include subcuticular epithelium, gills or pereiopods preserved appropriately from crustaceans in which WSSV infection is predicted from WSD signs and/or histopathology. For TEM screening or surveillance of clinically normal crustaceans, stomach subcuticular tissue is recommended. Detailed procedures for TEM are available in Lightner (1996). WSSV virions are rod to elliptical in shape with a trilaminar envelope, 80–120 nm × 250–380 nm in dimension and often characterised by a tail-like protrusion from the envelope (OIE 2012b).

Culture methods

Primary cultures of prawn cells derived from lymphoid organ, hepatopancreas, ovary and haemocytes have been developed and shown to support the growth of WSSV. These have been used successfully to quantify virus infectivity in assays based on cytopathic effect and/or cell death to determine tissue culture infectivity dose (TCID50) end points, or on cell stains or virus-specific antibodies (Assavalapsakul et al. 2003; Jiang et al. 2006; Kasornchandra & Boonyaratpalin 1998; Maeda et al. 2004; Tapay et al. 1997; Uma et al. 2002; Wang CH et al. 2000). However, the lack of available, easily maintained continuous cells lines that support the replication of WSSV has largely precluded the routine use of cell culture methods for isolating WSSV and limits its diagnostic potential.

Molecular techniques

Polymerase chain reaction

Several PCR and real-time PCR protocols have been described for the specific and sensitive detection of WSSV DNA in clinical samples. However, the only test recommended by the World Organisation for Animal Health (OIE) is a nested PCR developed following the original isolation and cloning of viral DNA from WSSV associated with original outbreaks of WSD in South-East Asia (Lo et al. 1996; Lo & Kou 1998). Details on how to perform this test can be found in the original publications and in the OIE Aquatic Manual (OIE 2012b). Several commercial PCR and real-time PCR kits for detection of WSSV DNA are also available from several suppliers.

Note: The eyes and eyestalks of specimens older than 10-day-old postlarvae must be excluded from the tissue being analysed because they contain PCR inhibitors.

Care needs to be exercised when interpreting PCR data, particularly when clinically normal crustaceans are tested. Specimens with low-level infections can have WSSV DNA levels approaching the detection limit of the test. In such cases, positive or negative test data can be obtained for different aliquots of the same DNA sample. This is due to a non-uniform DNA template distribution in solution allowing DNA amounts in any given aliquot to slip below that required for reliable PCR amplification (Hsu et al. 1999; Lo et al. 1997a). Importantly, it needs to be reinforced that PCR is only capable of detecting WSSV DNA, and PCR data cannot discriminate between tissues containing infectious virus and non-infectious viral DNA remnants.

In situ DNA hybridisation

Detailed methods for preparing digoxigenin-labelled DNA probes, in situ DNA hybridisation and probe detection of WSSV DNA in histological tissue sections are provided in the OIE Aquatic Manual (OIE 2012b). The method uses paraffin-embedded tissue sections cut slightly thicker (5 μm) than those used routinely for histology. If standard digoxigenin-probe detection is used with colorimetric development reagents and Bismarck brown counterstain, a positive hybridisation signal will appear in bright-field microscopy as a dark-blue to black precipitate against a yellow to brown background.

Immunological assays

Both polyclonal and monoclonal antibodies have been produced to detect various WSSV proteins, and the OIE Aquatic Manual summarises antibody-based tests that can be used to diagnose WSSV infection (OIE 2012b). Using a dot-blot format, anti-WSSV polyclonal antibody is sensitive enough to detect about 1 ng WSSV protein. It is important to note that, even when using a combination of two monoclonal antibodies specific to different WSSV structural proteins (which can improve detection sensitivity twofold), these detection systems have a WSSV detection sensitivity in the order of 25 000-fold lower than that afforded by one-step PCR (Chaivisuthangkura et al. 2010). Immunoassay test-strip kits are also commercially available, which can provide relatively rapid pond-side detection of WSSV proteins in clinical samples. These tests are targeted more towards management to prevent farm mortalities in regions where WSD is endemic. Immunohistochemical methods are also available to detect virus proteins in histological tissue sections where histopathological characteristics of WSD are present.

Bioassays

Bioassays in a crustacean species that is highly susceptible to WSD are required to unequivocally confirm the presence of infectious WSSV in clinical samples. However, when used in isolation, a bioassay is insufficient for definitive WSSV diagnosis as the clinical sample might contain other pathogenic viruses. Therefore, other tests must be used in conjunction with a bioassay to confirm WSSV as the cause of morbidity and/or mortality events suspected to involve WSD. Bioassay protocols for WSSV have been published (Durand et al. 2000; McColl et al. 2004; Rajendran et al. 1999).

The advantages and disadvantages associated with commonly used laboratory tests for diagnosing WSSV infection and/or WSD are summarised in Table 1.3.

1.4.3 Confirmation of infection

To confirm cases of subclinical WSSV infection, where histopathological characteristics of WSD are present, WSSV DNA or proteins in histological tissue sections can be highlighted using in situ hybridisation (ISH) or immunohistochemistry (IHC) techniques. When

relatively fresh clinical material is available, infection can also be confirmed with PCR or bioassay in a susceptible prawn species, followed by any of the diagnostic methods for WSSV. Of the diagnostic methods available, PCR is preferred to confirm infection because of its speed, sensitivity and specificity.

Table 1.3 Advantages and disadvantages of white spot syndrome virus tests

Diagnostic method	Advantages	Disadvantages
Antibody-based assays	Quite sensitive and specific	Sophisticated equipment required; laborious and technically complex; expensive
Bioassay	Demonstrates presence of viable pathogen; useful in conjunction with a WSSV-specific test	Several days for result; not WSSV specific; relatively complex; expensive; requires confirmation by other methods
Histology	Low probability of misdiagnosis in high-level infections	Might not detect low-level infections; not field friendly; needs at least 24–36 hours of preparation time
In situ hybridisation	Very sensitive; reliable; pathogen specific	Histological preparation of tissues required; laborious
Polymerase chain reaction	Highly sensitive, capable of detecting very low pathogen levels; can be used to test all life stages; WSSV specific; rapid results	Hypersensitive; prone to misdiagnosis; technically complex; relatively costly; does not discriminate between presence of infectious virus and non-infectious nucleic acid fragments
Rapid methods	Rapid diagnostic results; field ready; detect multiple pathogens; inexpensive	May not detect low-level infection
Transmission electron microscopy	Sensitive; useful in conjunction with virus purification	Sophisticated equipment required; laborious and technically complex; expensive; may not detect low-level infection

WSSV = white spot syndrome virus
Source: Adapted from Fegan & Clifford (2001)

In a suspected outbreak of WSD that is affecting any species of crustacean, PCR should be used as the initial confirmatory test as it provides the most rapid turnaround required to make a presumptive diagnosis. The validity of the PCR-positive data should then be confirmed by histology and associated methods such as ISH or IHC. A definitive association can then be made between the presence of WSSV and observed histopathological tissue changes characteristic of WSD.

1.4.4 Differential diagnosis

Clinical signs and gross lesions associated with rapidly increasing numbers of dead and dying prawns in a pond should always prompt a diagnostician to include WSSV, in addition to other exotic viruses, on their differential diagnostic list. The information in Table 1.4 helps differentiate the two exotic viral diseases most capable of causing mass mortalities in

the penaeid species farmed in Australia—WSD and yellow head disease (YHD) caused by yellow head virus (YHV). Further information is provided to help differentiate WSD and YHD from major endemic viral diseases affecting *P. monodon* in eastern Australia, including gill-associated virus (GAV) associated with peripheral neuropathy and retinopathy (Callinan & Jiang 2003; Callinan et al. 2003), and mid-crop mortality syndrome.

Two other viruses are also of interest—Taura syndrome virus (TSV) and IHHNV. TSV and IHHNV have caused significant disease and mortalities in *Penaeus* spp. indigenous to the Americas. Although the risk for significant morbidity and mortality in currently farmed species of prawns in Australia is considered low, potential problems may arise because these susceptible South American penaeids are now being farmed extensively throughout South-East Asia. IHHNV has been reported from Australian-farmed prawns, but morbidity or mortality has not been reported from infected stocks.

Table 1.4 Differential diagnosis of virus-induced mortalities that might occur in Australian-farmed prawns[a]

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

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

a *Penaeus monodon*, *P. japonicus*, *P. merguiensis*

Note: PNR is endemic in *P. monodon* in eastern Australia only. Taura syndrome features are as described for *P. vannamei*.

Mass mortality events in farmed prawns unrelated to disease are rare, but can arise through equipment failures, serious errors in water-quality management or exposure to environmental toxins such as pesticides. The causes of such events are usually identified quite quickly, allowing staff to limit the effects. Moderate or protracted prawn mortalities may be caused, for example, by algal bloom crashes or poor pond environmental conditions leading to subsequent bacterial infections. Such occurrences can usually be identified by examining pond data records, or through the use of histology and/or microbiological methods to examine representative moribund prawns. A significant differential diagnosis when investigating a possible WSD event is bacterial white spot syndrome. Gross lesions include white spots in the prawn cuticle that closely resemble those induced by infection with WSSV (Goarant et al. 2000; Wang et al. 2002). Exposure of prawns to high alkalinity has also been linked to bacterial colonisation and the formation of white spots that are unrelated to WSSV infection (OIE 2012b). To differentiate non-viral causes of white spots from those caused by WSSV, a key feature to remember is that non–virally caused gross lesions are not typically associated with significant mortalities.

In summary, a provisional diagnosis of WSD is justified in cases when a disease outbreak in farmed prawns is characterised by:

- rapid onset of high mortality rates
- moribund prawns displaying white spots and/or red body discolouration
- histopathological changes in moribund prawns such as eosinophilic to basophilic intranuclear inclusions in subcuticular epithelial cells.

In any of the above circumstances, PCR or other molecular tests must be used to confirm any provisional diagnosis and discount alternative disease aetiologies.

1.4.5 Treatment of infected crustaceans

Many and varied therapeutic treatments have been trialled experimentally to combat WSSV infection in prawns, with variable levels of efficacy. There are currently no widely available commercial reagents with proven abilities to completely clear WSSV infections, or for prawn prophylaxis in the event of outbreaks of WSD.

1.5 Resistance and immunity

Prawns possess pathogen defence systems that, although quite complex, differ substantially from those present in vertebrates (Flegel 2001; Newman & Bullis 2001). It is generally accepted that any adaptive 'immune' response mechanisms are rudimentary compared with the humoral antibody and the cell-mediated response mechanisms of vertebrates, and—consistent with this—haemocyte heterogeneity in crustaceans is limited. Nonetheless, crustaceans have the capacity to mount substantial pathogen-defence responses based on innate systems involving:

- a diverse array of generalised humoral factors, including those that originate from and/or reside in haemocytes
- a specific intracellular RNA interference (RNAi) response based on recognition and specific cleavage of foreign double-stranded RNA that will destroy viral mRNA specifically, and thus inhibit virus replication (Hirono et al. 2011; Robalino et al. 2007; Su et al. 2008; Xu et al. 2007)
- limited adaptive memory response to native or recombinant viral proteins through mechanisms that are not well understood, but may possibly be similar to those characterised in terrestrial invertebrates (Johnson et al. 2008; Zhu et al. 2009).

1.5.1 Responses to bacterial or fungal infections

The battery of defence systems that prawns can mount against invading bacteria or fungi includes rapid haemolymph clotting, agglutination, phagocytosis, and production of free oxygen species and bactericidin. These occur concurrently with cellular responses to clear invading organisms—either in tissues or circulating in the haemolymph—via encapsulation and haemocyte granuloma formation.

1.5.2 Responses to viral infections

The responses used by prawns to defend themselves against viral pathogens differ markedly from those used in protection against bacterial or fungal pathogens. In prawns and other crustaceans, and perhaps arthropods in general, there is no inflammatory response to viral infection. As a result, persistent infection by one or more viruses can be common.

There is a general phenomenon that, when viruses like WSSV first emerge, disease epidemics ensue that are characterised by initial catastrophic and widespread crop losses (Section 1.4.1). Within a couple of years, however, the disease epidemiology shifts progressively to more sporadic crop losses and/or substantially reduced mortality in conjunction with the widespread occurrence of prawns with subclinical or chronic infections. However, the viruses carried in subclinically infected prawns can remain highly pathogenic and lethal for naive prawns, and also can manifest as an acute infection associated with disease when activated in response to various stress factors. Thus, prawns can carry lifelong subclinical viral infections that are transmitted to their progeny, and larvae that become infected in this manner tolerate infection without developing clinical disease, unless subjected to adverse or unnatural stress.

Flegel (2001, 2009) proposed that there may be a specific and active adaptive system, based on viruses binding to host cell membranes, capable of inducing a specific memory response to suppress virus-triggered cellular apoptosis and destruction; thus non-lethal infections may persist. However, there are data that question aspects of this tolerance theory. For example, *P. japonicus* (Venegas et al. 2000) appears capable of generating a 'quasi-immune' defence response after WSSV exposure, which can protect against mortality following subsequent challenge with WSSV. Wu et al. (2002) also showed that *P. japonicus* can develop resistance to WSSV challenge approximately 3–4 weeks after WSSV exposure. This resistance could persist for another month in prawns held at 24 °C, which suggests that one or more neutralising humoral factors might be involved.

1.5.3 Vaccination

Although there are a number of non-specific immune stimulants that are reported to protect farmed prawns against WSSV infection and WSD, there are currently no specific vaccines commercially available to protect farmed prawns against WSSV infection.

The innate pathogen defence system of crustaceans has the ability to recognise patterns in macromolecules shared by broad groups of pathogens, such as the beta-glucans of fungi and the lipopolysaccharides and peptidoglycans of bacteria. This defence system recognises various uncharacterised immunostimulants present in herbal extracts. After feeding prawns diets supplemented with various immunostimulants, WSSV challenge trials often demonstrated increased prawn resistance to acute WSSV infection and WSD (Chang et al. 1999; Citarasua et al. 2006; Heidarieh et al. 2010; Huang & Song 1999; Itami et al. 1998; Takahashi et al. 2000). As the efficacy and methods of administration of immunostimulants become better defined, they might be more commonly incorporated into farmed crustacean commercial feeds to improve resistance to, and reduce disease risks from, WSSV and other pathogens. However, any benefits conferred by immunostimulant additives in feed are

likely to be overwhelmed in adverse environmental situations or when other, more direct strategies for disease prevention are not used (Newman & Bullis 2001).

1.6 Epidemiology

Although WSSV can infect a wide variety of crustaceans, WSD is essentially restricted to farmed prawns. In the 1990s, the disease exhibited pandemic behaviour in Asia and caused substantial economic impacts following its emergence in the Americas. When first apparent in any particular region, WSD characteristically caused disease epidemics in farmed prawns. The epidemics featured mass mortalities for one to two years before disease events tended to become more sporadic. Although the reasons for this disease pattern are not well understood, the following are likely contributors:

- the broad host range of WSSV
- host or viral accommodation processes allowing persistence of subclinical infections
- the vertical transmission of subclinical infection to progeny
- stress-activation factors (factors other than stress have been covered earlier).

The role of stress in activating WSD appears to be linked to sporadic disease events rather than to widespread epidemics of mass mortality (D Fegan, pers. comm., February 2002). Stress can arise from many sources and includes handling during capture; broodstock transport and spawning; pond-water temperature, salinity and quality fluctuations; and prawn-rearing densities and biomass (Fegan & Clifford 2001; Flegel 2001). Rapid changes in water temperature can induce WSD, or affect disease severity and survival both in marine prawns and freshwater crayfish (Jiravanichpaisal et al. 2004; Rahman et al. 2007; Tendencia et al. 2010; Vidal et al. 2001; Zhu & Lu 2001).

To gain a better understanding of the dynamics of a WSD outbreak, Lotz and Soto (2002) simulated the transmission of WSSV within a prawn pond using a Reed–Frost mathematical model. This study concluded that there is likely to be a threshold density of susceptible prawns below which substantial disease and mortality will not occur. This, along with lower stress and lower infection prevalence and load levels might partly explain why catastrophic WSD has never been reported in wild prawn populations (Lo et al. 1997a).

1.6.1 Incubation period

WSD is a highly contagious disease of farmed penaeid prawns with a rapid onset; high levels of mortality (up to 100% on some farms) can occur within a few days of the disease becoming evident. Outbreaks can occur within 40–45 days of stocking WSSV PCR-positive stock into production ponds (Withyachumnarnkul 1999). In experimental situations (injection or bath with high viral loads), the incubation period is 4–7 days (Pratanpipat et al. 1996). At low temperatures (< 16 °C) or high temperatures (> 32 °C), the incubation period may be longer or expression of disease may not occur (Jiravanichpaisal et al. 2004; Vidal et al 2001).

1.6.2 Persistence of the pathogen

The OIE Aquatic Manual (OIE 2012b) provides information, summarised in Table 1.5, on protocols for chemical and physical treatment that effectively destroy WSSV infectivity.

Table 1.5 Agents and conditions that inactivate white spot syndrome virus

Physical agents	**Inactivation conditions**
Heat	50 °C for 20 min, 70 °C for 5 min
UV light	Inactivated by 9 × 105 µw s/cm^2 for 60 min
pH	Inactivated by pH 1 for 10 min, pH 3 for 60 min or pH 12 for 25 min at 25 °C
Chemical agents	**Inactivation conditions**
Chlorine	Inactivated by sodium hypochlorite at 100 ppm for 10 min and 10 ppm for 30 min
Iodine	Inactivated by povidone iodine at 100 ppm for 10 min and 10 ppm for 30 min
Quaternary ammonia	Inactivated by benzalkonium chloride at 75 ppm for 10 min

µw s/cm^2 = microwatt seconds per square centimetre; ppm = parts per million; UV = ultraviolet

In water

There are reports that WSSV particles kept in sterile sea water in the dark at temperatures of up to 30 °C can remain infectious for up to 30 days (Maeda et al. 1998a; Momoyama et al. 1998). However, infectivity during a far shorter period (48 hours in sea water) was reported in another study (Wang et al. 2002). WSSV released into pond water during outbreaks has been shown to probably remain infectious for only 3–4 days (Flegel et al. 1997), suggesting that persistence of potentially infective virus for 2–4 days is likely in pond-production systems.

Experimentally, WSSV shed into water from infected crabs causes infection in co-habiting prawns (Kanchanaphum et al. 1998; Supamattaya et al. 1998). However, these experiments co-located crabs and prawns unnaturally close together and generated relatively high levels of virus particles in the sea water. Other data indicate that WSSV infection transmission to naive prawns by co-habitation and exposure to free virus particles shed into sea water is about one order of magnitude lower than when infected tissue is ingested (Soto & Lotz 2001; Wu et al. 2001). Collectively, the data suggest that free virus particles shed into sea water pose considerably lower transmission risks than the risks through ingestion, except possibly in ponds during WSD outbreaks when prawn pond water is likely to be rich in virus particles (Fegan & Clifford 2001).

In sediment

There is no solid information on pond sediment as a source of WSSV infection. However, it seems unlikely to pose a significant infection risk because, in Asia, prawn crops have been reared successfully in ponds that contain decomposed prawn carcasses from crops destroyed by WSD. These ponds, therefore, presumably contained contaminated sediment (Fegan & Clifford 2001).

On-farm equipment

Although there is no hard evidence of contaminated farm equipment transmitting WSSV infection, it is likely that nets or other equipment moved between ponds could pose a

substantial risk of mechanically transporting infected material if not disinfected appropriately between uses.

1.6.3 Modes of transmission

Most studies of WSSV transmission have focused on penaeid prawns. WSSV infection has been documented in all life stages and can be transmitted both vertically and horizontally.

Vertical transmission

Prawn larvae can become infected during spawning, although the mechanism is presently unclear. In studies of WSSV tissue tropism, Lo et al. (1997a) were unable to find evidence of infection in mature ova and suggested that infection might kill the ova before they matured. Some evidence suggests that connective tissues in adult prawn gonads might be a source of WSSV (Kou et al. 1997; Lo et al. 1997a; Mohan et al. 1997). Prawns with substantial WSSV infections should be excluded from spawning, because postlarvae spawned from such broodstock become infected with high viral loads and such progeny are prone to crop failures due to WSD (Peng et al. 2001; Withyachumnarnkul 1999).

Horizontal transmission

WSSV can be transmitted horizontally via ingestion of infected tissue. Once WSD has established in a pond, WSSV can be transmitted rapidly, mainly through cannibalism of sick and dead prawns (Soto & Lotz 2001; Wu et al. 2001), but also by free virus particles shed into the water column (Fegan & Clifford 2001; Soto & Lotz 2001). Transmission via cannibalism is supported by feeding trials in which prawns that ingested as little as 5% of their bodyweight of tissue that was heavily infected with WSSV developed WSD and died (Wang Q et al. 1999).

1.6.4 Reservoirs of virus

Wild broodstock

The prevalence of WSSV in wild prawn broodstock captured in Thailand, Japan, Taiwan, the Philippines and Panama from 1996–2007 is shown in Table 1.6. The table provides only a rough guide because of unknowns such as how well the sample sets represent the source population and how closely the test data represent virus infection. Nevertheless, it gives an idea of infection prevalence that might be expected in wild prawn populations in prawn-farming regions in which WSSV remains endemic (Lo & Kou 1999). Evidence also shows seasonal differences in WSSV infection prevalence (de la Peña et al. 2007; Lo et al. 1997a; Mushiake et al. 1998; Withyachumnarkul et al. 2003). ISH tests provide evidence that WSSV infection loads in wild prawns are often lower than those in farmed prawns, as might be expected due to additional stress factors accompanying pond rearing (Lo et al. 1996). Rates of infection of wild prawns, particularly when prevalence may be very low due to seasonal conditions, may reduce the success of detection of infection in surveillance programs using a higher assumed infection rate.

Table 1.6 Published prevalence estimates of white spot syndrome virus in wild prawns

Prawn species	Prevalence (%)	Location	Reference
Metapenaeus ensis	33.3 (*n* = 30)[a]	Taiwan	Wang et al. (1997)
Palaemon macrodactylus	40	Argentina	Martorelli et al. 2010
Penaeus monodon	83.3 (*n* = 66)[b]	Taiwan	Lo et al. (1996)
Penaeus monodon	77.2 (*n* = 88)[b]	Taiwan	Lo et al. (1997a)
Penaeus monodon	0–18.6 (*n* = 24 338)[a] monthly in broodstock for 3 years	Thailand	Withyachumnarnkul et al. (2003)
Penaeus monodon	Wet season 10 (*n* = 713); dry season 0.3 (*n* = 714); (7 locations)[a]	Philippines	de la Peña et al. (2007)
Penaeus japonicus	9.2 (*n* = 1269)[b]	Japan	Mushiake et al.(1998)
Penaeus japonicus	20.3 (*n* = 474)[b]	Japan	Maeda et al.(1998b)
Penaeus japonicus	58.5 (*n* = 159)[c]	Taiwan	Lo & Kou (1998)
Penaeus semisulcatus	26.7 (*n* = 15)[b]	Taiwan	Wang et al. (1998)
Penaeus semisulcatus	6.3 (*n* = 32)[b]	Taiwan	Lo et al. (1996)
Penaeus penicillatus	11.1 (*n* = 27)[b]	Taiwan	Lo et al. (1996)
Penaeus vannamei	2 (*n* = 104)	Panama	Nunan et al. (2001)

n = number of prawns in study
a One-step PCR
b Two-step PCR
c PCR protocol not specified
Note: Detection was by PCR in all Asian studies, and by dot-blot assay in the Panamanian and Argentinian study.

Infections at hatcheries and farms

Postlarvae spawned in hatcheries from wild broodstock with pre-existing high-level infections are the major source of WSSV infection in farmed prawns. A Thai study examined WSSV loads in *P. monodon* postlarvae (Withyachumnarnkul 1999). The study found that only 5% of ponds stocked intensively with one-step PCR-positive postlarvae escaped WSD, as compared with the majority (69%) of ponds stocked with one-step PCR-negative postlarvae. A subsequent study in Taiwan had similar outcomes (Peng et al. 2001).

Prior and Browdy (2000) found that WSSV could remain infectious in decaying prawn tail tissue for up to 28 days. However, Wang et al. (2002) found that, in decaying carcasses, viruses remained infectious for only 6 days. Experimental differences—including examining tails compared with whole prawns, which would be expected to decompose faster—are the likely reasons for the inconsistent data. Importantly, both studies clearly indicate that dead and decaying prawn carcasses are a significant risk factor in transmitting WSSV.

Moribund prawns affected by WSD can congregate in the more highly oxygenated water present at the surface and edges of ponds in response to virus-induced gill dysfunction

(D Fegan, pers. comm., 2002). Therefore, it is considered good practice to collect and dispose of moribund and dead prawns found at pond edges during a WSD outbreak (Dr Pornlerd Chanratchakool, Shrimp Health Management Specialist, Aquatic Animal Health Research Institute, Bangkok, pers. comm.); however, this is unlikely to substantially affect harvest outcomes because diseased prawns will not be restricted to pond extremities.

Other decapod crustaceans

Various decapod crustaceans (*Metapenaeus* spp.), grass shrimp (*Acetes* spp.), mud crabs (*Scylla serrata*) and blue swimmer crabs (*Portunus* spp.) can carry subclinical WSSV infections and enter prawn ponds either via intake water or, in the case of some crab species, by migrating overland. There is evidence that crustaceans carrying WSSV can infect prawns via water or through ingestion of infected tissue (Fegan & Clifford 2001; Kanchanaphum et al. 1998; Supamattaya et al. 1998). However, molecular epidemiological studies have examined the different genotypes of WSSV isolated from prawn postlarvae and other crustaceans co-habiting the ponds. The results have shown that WSD most commonly originated from the virus strain pre-existing in the prawns or transmitted from infected prawns in neighbouring ponds (Hoa et al. 2005; Pradeep et al. 2008). The actual risks of WSSV transmission from non-prawn crustaceans to prawns reared in ponds appear to be quite low in general, but might depend on the virulence factors of the strain, the prevalence of infection and the infection load in the carriers.

Other carriers

Some evidence suggests that copepods and insect larvae (Liu et al. 2000; Lo et al. 1996) might be sources of WSSV infection in farmed prawns, but the risk they pose relative to other infection sources appears to be small (Fegan & Clifford 2001).

WSSV has also been detected by PCR in polychaete worms (*Marphysa* spp.) used in *P. monodon* hatcheries in India and, therefore, must be considered as a potential source of WSSV infection (Vijayan et al. 2005). Prawn larvae reared in hatcheries are routinely fed *Artemia* spp. hatched from cysts. Although WSSV has been detected in association with *Artemia* spp., there is no evidence that commercial cysts are a source of WSSV or that *Artemia* spp. exposed to the virus can transmit infection to prawns (Chang et al. 2002; Hameed et al. 2000).

Birds, especially predatory or scavenging birds such as terns (Sternidae) and gulls (Laridae), can transmit infection mechanically between ponds by collecting moribund or dead prawns from ponds affected by WSD and dropping these into unaffected ponds (Fegan & Clifford 2001; Garza et al. 1997). Although there were early suggestions that WSSV might also be transmitted by bird faeces, challenge experiments have indicated that WSSV infectivity does not survive passage through the avian alimentary system (Vanpatten et al. 2004).

1.6.5 Factors influencing transmission and expression of disease

Host factors

Different life stages of various prawn species appear to vary in their susceptibility to WSSV and disease. For example, in WSSV bioassays examining postlarvae, the white shrimp species *P. setiferus* and *P. vannamei* display greater susceptibility to adverse disease outcomes than do *P. aztecus* (brown shrimp) or *P. duorarum* (northern pink shrimp) (Lightner et al. 1998). Evidence also shows that *P. monodon* larvae and early postlarval stages are refractive to WSD, but that from the late postlarvae/juvenile stages onwards, the species becomes highly susceptible to acute infection and WSD, with high mortality rates (Yoganandhan et al. 2003). Moreover, within species and life stages, the outcome of

challenge by WSSV might depend on their general health and history of exposure to non-lethal infection with WSSV or other viruses (Section 1.5) (Flegel 2001; Tang et al. 2003; Venegas et al. 2000).

Environmental factors

In cohorts of prawns carrying subclinical WSSV infections, WSD outbreaks often appear to follow stress events induced by rapid changes in, or deterioration of, pond environmental conditions. Triggers of clinical WSD in such prawns include rapid changes in water temperature, dissolved oxygen concentration, water hardness and salinity, all of which ultimately result in osmotic stress (Fegan & Clifford 2001; Flegel et al. 1997).

Water temperature has a profound effect on disease expression. Average water temperatures of between 18 °C and 30 °C are conducive to WSD outbreaks (OIE 2012b). The virus can survive in temperatures as low as 4 °C (Martorelli et al. 2010) and can be expressed when temperatures rise to 15 °C (Guan et al. 2003). At 10 °C, no mortality was observed in *Procambarus clarkii*, and viral replication was reduced but not prevented (Du et al. 2008). High temperatures (> 32 °C) may be protective and reduce mortality (Lin et al. 2011; Vidal et al. 2001).

Table 1.7 lists the ranges of pond water-quality variables recommended as ideal for rearing *P. monodon*. Rapid fluctuations in or prolonged exposure to values outside the optimal range of each variable should be avoided when possible to mitigate stressors that might trigger WSD (Fegan & Clifford 2001).

Table 1.7 Recommended ranges for key water-quality variables for farmed *Penaeus monodon*

Variable	Optimal range	Comments
Alkalinity	>80 ppm (as $CaCO_3$)	Dependent on pH fluctuation
Dissolved oxygen	5 to 6 ppm	Not less than 4 ppm
Hydrogen sulfide	<0.03 ppm	More toxic at low pH
pH	7.5 to 8.3	Daily fluctuation <0.5
Salinity	10 to 30 ppt	Daily fluctuation <0.5 ppt
Secchi disc reading	30 to 50 cm	
Un-ionised ammonia	<0.1 ppm	More toxic at high pH and temperature

cm = centimetres; ppm = parts per million; ppt = parts per thousand
Source: Chanratchakool et al. (1998)

1.7 Impact of the disease

WSD is a highly contagious disease of farmed penaeid prawns. The disease has a rapid onset and is capable of causing mass mortalities (up to 100% of pond stocks) within a few days of the first evidence of disease. Following the initial outbreaks of WSD in China and Taiwan in 1992–93, it spread rapidly to other prawn-farming regions, including Japan and Thailand in 1993, the United States in 1995, Central and South America in 1999, and France and Iran in 2002. Currently, WSD is enzootic throughout East, South-East and South Asia, and in regions of North, South and Central America, where it causes serious economic losses and adds management costs to prawn-culture industries. Losses due to WSD before mitigation measures are in place can be devastating. At its peak in China, outbreaks of WSD reduced

total farmed prawn production by about 80%. In 1996, the value of prawn production decreased by more than US$500 million in Thailand alone (Hill 2010). At their peak in Ecuador in 1999 and 2000, WSD outbreaks resulted in (Hill 2010):

- more than 60% total production losses, which totalled more than US$800 million
- 50% production area losses
- more than 500 000 job losses
- declaration of a national emergency.

The widespread use of specific pathogen-free *P. vannamei* has alleviated the impact of WSD in most countries in which farmed prawn production has been seriously affected. However, WSSV remains by far the most problematic and widespread viral threat to prawn farming, and WSD generally remains the most serious threat to the global prawn-farming industry.

2 Principles of control and eradication

Based on experiences overseas, an outbreak of white spot disease (WSD) in Australia is most likely to occur and be detected in highly susceptible farmed prawn species such as *Penaeus monodon* or *P. merguiensis*. However, it cannot be discounted that disease may emerge in other crustaceans being farmed or reared by hobbyists. It is less likely that mass mortalities due to WSD could manifest and be detected in indigenous wild crustaceans. However, once introduced into populations of wild crustaceans, subclinical infections with white spot syndrome virus (WSSV) will likely persist and be detected in diagnostic surveys.

This section provides background information to inform appropriate control and eradication measures following:

- the occurrence of a WSD outbreak

  or

- the confirmed detection of subclinical WSSV infection in overtly healthy crustaceans.

This section will focus mainly on Australia's prawn-farming industry, since most available information comes from this sector. However, the disease management principles described can be applied to other crustacean aquaculture enterprises or to wild crustacean populations. In this section, WSD will be used to refer to both a disease outbreak occurrence and to the confirmed detection of WSSV.

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

2.1 Control options

The feasibility of containing an outbreak of WSD in Australia will depend on the species involved, the nature of the outbreak, the promptness of a diagnosis and the disease control strategy adopted. Essentially, three broad control options for WSD in Australia are available:

- *Eradication*—eradication of WSSV from Australia (highest level control measure and may be the most cost-effective in the long term).
- *Containment, control and zoning*—containment of WSSV to areas in which infection has become endemic, and prevention of further spread and protection of uninfected areas.
- *Control and mitigation of disease*—implementation of management practices that decrease the incidence and severity of clinical disease outbreaks (lowest level control measure and likely to be the least costly).

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

- rapid detection and confirmation of infection
- rapid identification of the nature and extent of the problem

- rapid selection and implementation of control measures
- prevention of virus spread by controlling movements of stock and water within and between farms and other sites considered susceptible to infection
- maintenance of appropriate disease management practices and high standards of hygiene.

The most appropriate option will depend on:

- geographical location, and the presence or absence of infection reservoirs
- chances of successful WSSV eradication
- level of risk accepted for future spread of infection (e.g. from commercial grow-out of seedstock derived from infected broodstock)
- short-term costs of control measures and disruption to production
- long-term costs to production in the presence or absence of WSSV
- long-term costs of control should WSSV become endemic.

2.1.1 Eradication

Attempting to eradicate WSSV is justified by:

- evidence suggesting that WSSV infection might not persist in wild prawn populations in the absence of repeated new inoculations from infected farms or processing plants
- WSSV being eradicated successfully from farms in Central America by using progeny of domesticated prawns certified to be specific pathogen-free (SPF) for WSSV in closed-culture systems.

In Australia, closed-farming systems operating in infected zones could be stocked with polymerase chain reaction (PCR)–negative postlarvae derived from either wild PCR-negative *P. monodon* captured from known WSSV-free zones or domesticated *P. monodon* broodstock certified to be SPF for WSSV.

Any attempt to eradicate WSSV infection from a farm in an infected zone will require consideration of the following measures:

- perimeter fencing to exclude entry or exit of potentially infected wild crustaceans
- destruction and safe disposal of all susceptible and potentially infected crustaceans on a farm
- disinfection of pond and reservoir water before discharge
- decontamination of bottom substrates of farm ponds and other water reservoirs by drying out and subsequent treatment with lime.

The farm could resume production, provided:

- a closed-production system is implemented
- individual ponds are fenced
- intake water is filtered through 250–500 μm screens (Fegan & Clifford 2001) to exclude potential wild crustacean carriers
- any crustaceans entering through the filter are killed by treating water with calcium hypochlorite or other effective chemicals (Fegan & Clifford 2001) before ponds are stocked

- ponds are stocked with PCR-negative postlarvae derived from WSSV-free broodstock.

Intake water should be held long enough (minimum 10 days) to eliminate WSSV and to allow disinfectant chemicals to reduce to an acceptable level before pond stocking. See Appendix 2 for information on approval for using drugs and chemicals in Australia.

Eradication is unlikely to be feasible if the WSSV incursion has no controllable point source and epidemiological investigations determine that infection is widespread across regions farming prawns, or if infection is otherwise unable to be contained because of:

- an inability to stop WSSV either spreading widely and rapidly via infected postlarvae produced at hatcheries, or infections establishing widely in wild crustacean reservoirs (it is likely that infections in wild populations might abate over time as farm sources of infection are eliminated)
- WSSV establishing subclinical infections at levels difficult to detect reliably
- the lack of an intimate understanding of WSSV transmission processes and how it maintains its long-term survival in aquatic invertebrates
- the proximity of prawn farms to waterways containing myriads of crustacean species, the movements of which cannot be controlled realistically
- the possibility that WSSV infection might become widespread in crustacean species in the wild that co-habit with wild crustaceans sourced for use in hatcheries or farms.

The establishment of widespread infection in wild crustaceans would likely frustrate and complicate efforts to eradicate WSSV infection from farmed prawns, particularly if infection becomes prevalent in regions where *P. monodon* broodstock is captured routinely for use in hatcheries. However, the potential solution to such a problem would be to use SPF-domesticated broodstock. Indeed, substantial successes in generating domesticated breeding lines of *P. monodon* have occurred during the past decade in Australia and elsewhere. Thus, the more widespread use of progeny of domesticated and virus-screened broodstock reared in facilities incorporating rigorous biosecurity measures would provide a robust means of avoiding WSSV introduction into farm ponds via infected postlarvae. If this could be achieved across the industry, the impacts of WSD on production could be curtailed substantially. The life cycle of *P. merguiensis* has been closed commercially in Australia for many years, and domesticated broodstock are in routine use at a large commercial enterprise. Therefore, WSSV-free breeding populations could be selected and maintained in biosecure facilities to avoid adverse impacts of WSD on the farming of this species. *P. japonicus* has also been domesticated successfully in Australia. However, it is farmed extensively, and there have been health issues caused by Mourilyan virus (MoV) (Cowley et al. 2005; Sellars et al. 2007) among pond-reared stocks selected for on-rearing as domesticated broodstock. Problems caused by MoV might be avoided if potential broodstock are reared in biosecure tanks rather than in non-biosecure commercial ponds.

Unexposed prawns

Ponds at an infected farm containing pre-market size juvenile prawns in which there is no evidence of exposure to WSSV can be on-reared to harvest size provided that the possibility of their being or becoming infected is acceptably low.

At sites where WSSV infections have occurred, effective farm, transportation and processing hygiene protocols will be necessary, with on-farm processing and cooking being preferable to prevent any potential for infection spread during prawn transport to off-site processing facilities.

Although the destruction of unexposed crustaceans being farmed in a declared area will decrease the chances of the infection spreading, any benefits of such action might be minimal if infections have become established in local wild crustaceans.

Exposed or potentially exposed clinically normal prawns

In any attempt to eradicate WSSV at a farm, clinically normal prawns that have or might have been exposed to infection need to be considered as potentially infected. Options available for such prawns are:

- destruction and disposal as undertaken with clinically diseased prawns
- prompt harvesting followed by on-site processing and cooking and, if a farm so desires, their sale though the normal systems.

The end result of both options is the prompt destruction of potentially infected prawns, which achieves the main goal—to decrease infectious loads at an affected site and thus reduce the risk of infection being spread. The systems used in harvesting the prawns must limit any possibility of further spread of infection and include:

- disinfection of all equipment and personnel involved in harvesting and processing
- instigation of quarantine procedures at the infected site that include personnel, equipment and vehicles
- on-site processing and cooking systems adequate to kill the virus
- holding, disinfection and safe disposal of all processed waste, including water and prawn heads or shells.

Clinically diseased prawns and other crustaceans

Immediate collection, destruction and disposal of all diseased and dead prawns will be essential to the success of any eradication strategy. These prawns, along with potentially infectious waste, will be the main means by which WSSV infection could spread. If prawns and other infected waste are buried, the sites used need to be chosen carefully to avoid any of the waste entering waterways and groundwater, or carriage by vectors.

2.1.2 Containment, control and zoning

No effective treatments are available for crustaceans that have become infected by WSSV. If virus eradication is deemed to be unfeasible following an outbreak of WSD, zoning and associated disease control measures should be implemented to mitigate virus spread to uninfected zones. The restricted movement of infected or potentially infected prawns will be paramount to the success of such measures. The feasibility of zoning will also depend on:

- the ability of farms, and the industry as a whole, to adjust management practices
- the extent to which infection has spread by the time quarantine measures are enforced
- the location, distribution and migratory behaviour of the crustacean species affected (Kailola et al. 1993).

The feasibility of containment, control and zoning in the event of an incursion of WSD will need to be assessed at that time. The implications of restrictions on movements of prawns, people, vehicles and boats, as well as on market access for products and byproducts of the crustacean species affected will require consideration.

In a declared infected area, controlled grow-out and harvesting might be feasible without risking further spread of infection, provided that closed-production systems and appropriate processing and waste-disinfection systems are used.

Justification for attempting to contain and control WSSV infection within a zone is based on knowledge that:

- tissue from moribund and dead prawns, and pond water laden with WSSV discharged during outbreaks, will be a source of infection in wild crustaceans in local waterways (Fegan & Clifford 2001)
- provided rigorous disease control and quarantine measures are implemented, prawn farms operating in a zone where WSSV has become endemic can continue to operate effectively, albeit at potentially reduced profitability (Chanratchakool et al. 1998; Fegan & Clifford 2001)
- farms in such zones could source postlarvae that are PCR-negative and derived from either wild or domesticated *P. monodon* broodstock determined to be SPF for WSSV.

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

Unexposed prawns

Provided there has been no exposure to WSSV infection, juvenile prawns may be on-reared to harvest size and processed for human consumption.

Exposed or potentially exposed clinically normal prawns

If containment, control and zoning strategies are implemented, prawns could be farmed within infected zones under heightened hygiene and biosecurity systems designed to limit the risk of exposure to WSSV from all potential sources.

However, from a quarantine perspective, any prawns being reared in a declared infected zone must be considered as potentially infected, and thus restrictions will be imposed on movements of prawns, people, vehicles and boats to prevent any potential for virus spread to uninfected zones.

All prawns, whether exposed or potentially exposed to WSSV during rearing, should be either destroyed or harvested and processed/cooked on-site, as in an eradication program.

Before release, water from infected ponds must be disinfected. Before pond refilling and restocking, the bottom substrate should be adequately dried and limed to destroy WSSV in residues.

2.1.3 Control and mitigation of disease

Justifications for attempting to control and mitigate WSD within a zone are based on knowledge that:

- infected tissue from moribund and dead prawns, together with heavily infected water discharged during outbreaks, is the main source of infection for wild crustaceans (Fegan & Clifford 2001)
- provided appropriate disease control and health management measures are implemented, potentially infected farms and infected farms employing partial water recirculation and closed-pond systems can continue to operate, albeit at reduced

profitability, in zones where WSSV is endemic (Chanratchakool et al. 1998; Fegan & Clifford 2001).

- farms in infected Australian zones could be stocked with PCR-negative postlarvae derived from SPF broodstock to enable continued farming in those areas.

All of the principles outlined for a containment, control and zoning strategy apply to the strategy of control and mitigate infection, except:

- the establishment of formal free and infected zones
- the exclusive requirement for closed-production systems
- the disinfection of all water to destroy WSSV and WSSV carriers before discharge (at farms using partial recirculation systems).

2.1.4 Trade and industry considerations

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

Domestic markets

A cautious approach is required for the harvest of exposed or potentially exposed product for the domestic market. WSSV has a broad host range and any waste released from uncooked prawns could present a risk if it were discarded in waterways containing susceptible hosts. Decisions regarding the release of exposed prawns or prawn products to the domestic market will depend on the response strategy implemented.

In countries where WSSV is endemic, the only industries that have been affected by WSD have been involved in farming marine and freshwater prawns. However, as many other crustacean species are susceptible to WSSV infection, any crustacean species, particularly intensively farmed species, are at risk.

National and international trade regulations, market requirements and food-safety standards must be considered as part of a control strategy. For example, permits might be required from the relevant authorities to allow crustacean products derived from disease zones to be released and sold for human consumption.

Export markets

WSSV is listed by the the World Organisation for Animal Health (OIE) as a notifiable disease (OIE 2012a). Potentially infectious WSSV commonly occurs in uncooked commodity prawns sold through retail outlets (Nunan et al. 1998). In some countries, including Australia, in which WSSV is exotic, import conditions such as requiring imports to be certified free of WSD and testing by quarantine organisations to reject prawn batches that test positive for WSSV are in place. In most regions of the world, with the exception of a few Pacific nations and Australia, WSSV is endemic. As most major trading partners accept product from regions in which WSSV is endemic, market-access restrictions seem unlikely should Australia lose its WSD-free status. It might, however, affect pricing because of Australian exporters no longer being able to differentiate their products from those of competitors on the basis of guaranteed freedom from WSSV.

In some countries such as the United States, import requirements can differ between states or regions. In Australia, the Australian Government Department of Agriculture is responsible for the health certification of all exports and should be consulted for detailed information about current export market requirements (export@daff.gov.au).

The presence of WSSV in Australia would be unlikely to impact adversely on market access for exported cooked prawns.

2.2 Farm types

All prawn farms operating in Australia are considered semi-closed systems, in that movement of stock is fully controlled and there is some control of water movement. Based on their dependence on an external water supply during rearing, prawn farms can be sub classified as (Chanratchakool et al. 1998):

- a flow-through pond system
- a partial recirculation pond system
- a full recirculation pond system
- a closed-pond system.

In reality, these systems represent broad groupings within a continuum—but for the purposes of this manual, open, partial recirculation and closed systems will be distinguished.

2.2.1 Flow-through systems

Most prawn farms in Australia use a flow-through system, where water is taken from, and released into, a supply source as necessary. Flow-through system farms are not usually designed to be self-contained, and so preventing inflows or outflows of water for any substantial period can adversely affect management decisions to maintain ideal pond water quality, and thus prawn health and growth rates. However, as environmental regulations for prawn farms in Australia preclude the discharge of untreated pond effluent water, farms must use settlement ponds designed and operated to meet these regulations. These waste-settlement ponds provide a resource for holding and disinfecting farm effluent water before discharge into natural environments. Similarly, any empty ponds on a farm could also be used to store and disinfect potentially infected effluent water.

2.2.2 Partial recirculation systems

Compared with flow-through systems, partial recirculation systems allow greater control over water intake and discharge because of their greater reliance on intake and effluent water reservoirs, and use of effluent settlement ponds. Partial recirculation systems are often used at sites where the intake water supply is more prone to quality fluctuations, which can include the variable existence of pollutants and pathogens. In instances when fresh water cannot be pumped onto the farm, once effluent water from ponds has settled in settlement ponds, it can be treated to make it suitable for mixing with the intake reservoir water for subsequent re-use in ponds.

2.2.3 Closed systems

Closed systems include full recirculation systems and closed-pond systems. Full recirculation systems are used in favour of partial recirculation systems at sites where problems with quality of intake water can be severe and persistent, thus requiring farms to dedicate substantial space to water storage and treatment. Often in such systems, farm reservoirs and ponds are filled opportunistically at the start of the production cycle at a time when water quality is good. This water resource is then managed intensively through to harvest with the farm potentially being 'closed' to any external water supplementation. Closed-pond systems thus require all water to be managed or treated by appropriate means that allow ponds to be maintained with minimal or zero water exchange.

In Australia, farms rearing freshwater crustaceans such as red claw crayfish or yabbies generally operate as closed systems with zero water exchange, or with water circulation to and from a reservoir. In Queensland, licensing conditions mandate the use of a closed-water system for farming red claw crayfish. In such closed systems, the spread of the disease beyond the farm through movements or discharge of water is thus not a major threat, unless heavy rainfall causes overflows from ponds or reservoirs into natural watercourses.

2.2.4 Hatcheries

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

2.3 Methods to prevent spread and eliminate pathogens

There are several methods to prevent the spread of and to eliminate pathogens, including quarantine and movement controls; tracing; surveillance; treatment, disinfection and destruction of infected crustaceans; decontamination; vaccination; and vector control. These methods are discussed in the following sections.

2.3.1 Quarantine and movement controls

Quarantine and movement restrictions should be implemented immediately upon suspicion of WSD. When declaring quarantine areas and movement controls, farm type must considered (see Section 2.2).

Establishment of quarantine areas

Specified quarantine areas (see **Enterprise Manual,** Section A for more details, and Figure 2.1) include:

- *declared area*—includes the restricted area and control area
- *infected premises or area*—the premises (e.g. farm) or area (e.g. fishing block or discrete geographical area in the wild) where the infection is present, and the immediate vicinity
- *restricted area*—area around infected premises or area
- *control area*—a buffer between the restricted area and free areas
- *free area*—non-infected area (this area is not considered a 'declared area' and may include large areas of Australia in which the presence or absence of WSD remains unassessed).

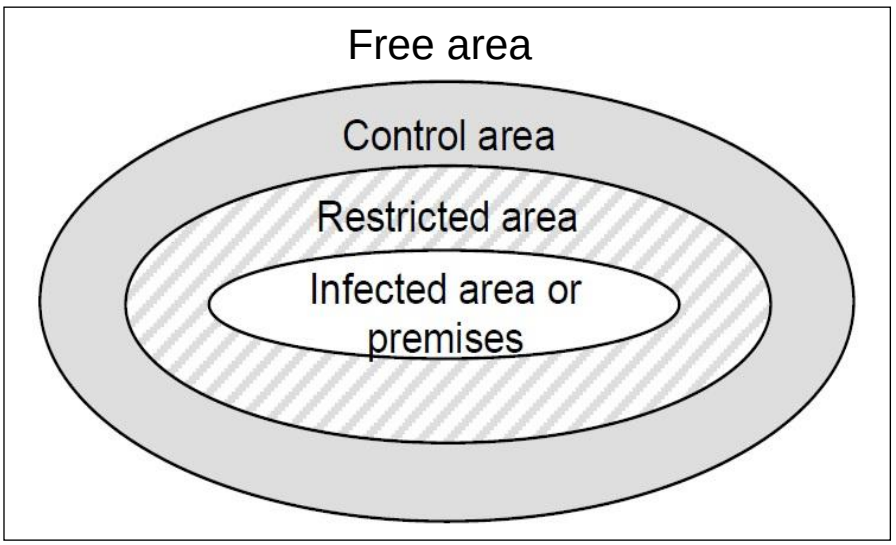

Figure 2.1 Establishment of specified areas to control white spot disease

Implementation of movement controls

Movement controls include:

- bans on the movement of live and uncooked crustaceans from 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 other aquatic environments
- restrictions or bans on the movement of crustaceans between different river systems, other aquatic environments or farms
- restrictions or bans on the use and movement of equipment within and between river systems, and between farms.

The following practices must be regulated when implementing control strategies:

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

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

The feasibility of restrictions and bans, and the extent to which these can be enforced, will depend on the location of infection or disease, the location and type of enterprises affected, and the control response option chosen.

Zoning

If WSD were to become endemic in regions of Australia, a zoning policy specific for WSD may be necessary to protect non-infected areas and to prevent further spread of the virus. Zones would be based on the distribution of susceptible species and of any vector species present (if appropriate), the geographical and hydrological characteristics of water bodies and landforms, and predictions of the most likely method of virus spread. Zoning may rely on the identification of biogeographical barriers. A corresponding surveillance and monitoring program for WSD would be required to support a zoning policy. Principles of

zoning for infected and non-infected zones in Australia are outlined in the AQUAPLAN *Zoning policy guidelines*[3] and in the OIE *Aquatic animal health code* (OIE 2012a).

Zoning for WSD might prove difficult, however, particularly as prawns can carry subclinical WSSV infections at levels undetectable by many diagnostic procedures. In this case, WSSV might be detected only when infection loads in prawns become sufficiently elevated as a result of stressors such as ablation and spawning in hatcheries (Mushiake et al. 1999; Peng et al. 2001). In the absence of adverse stress, prawns with subclinical WSSV infections could become established, and go undetected. Reservoirs of infection could also establish in natural watercourses in any of the many indigenous crustacean species susceptible to WSSV infection. If such circumstances occur, eradication would be impractical, if not impossible.

WSSV infection in wild crustaceans is most likely to arise from ingestion of infected tissues of moribund or dead prawns, or from water heavily contaminated with viral particles discharged from prawn ponds affected by WSD (Fegan & Clifford 2001). Once infected, carriers might disperse across their ecological range, which, for some migratory prawn species, could be quite extensive (Kailola et al. 1993). Horizontal and vertical transmission of infection within populations of wild prawns is probable, but likely to have less of an overall impact than infections acquired from WSD-affected farms. If infection establishes in wild populations of *P. monodon* or other penaeid species farmed locally, any wild broodstock used in hatcheries would need to be screened for WSSV to minimise infections becoming widely disseminated in commercial farms.

The varied transmission possibilities, the many susceptible hosts and the difficulty of disinfecting aquatic environments will make it difficult to protect WSD-free zones in the long term. Zoning programs for WSD have not been implemented successfully in any country in which WSSV has become endemic. Although the Philippines remained free of WSSV for several years by strictly enforcing a ban on the importation of live penaeid broodstock and postlarvae, it was ultimately introduced, purportedly by illegal imports of infected postlarvae (Magbanua et al. 2000).

Because of the continuing use in Australia of wild-captured broodstock and the widespread dissemination of postlarvae from a few hatcheries, it might be simpler to certify disease-free farms rather than impose zones accompanied by restrictions in prawn movements. Zoning for domesticated crustacean species such as red claw crayfish would be much simpler and afford more practical outcomes because of the lower requirement for movements of broodstock between farms and different locations. The widespread availability of domesticated WSSV-free *P. monodon* broodstock reared in biosecure facilities would provide similar benefits. Domesticated, selectively bred *P. monodon* became available in 2010 and may eventually replace the use of wild-caught broodstock.

Strategies for control

There have been claims of WSSV infection being eradicated successfully from some prawn farms in Central America (Boyd & Clay 2002; Lawrence et al. 2001) through the use of SPF *P. vannamei* and *P. stylirostris* in conjunction with heightened farm-management practices, including the use of closed-water systems. However, the virus remains endemic in wild prawns in this region. The impacts of WSD were massively reduced in the late 1990s and in the past decade in South-East Asia, because most countries shifted extensively to the culture of WSSV-free *P. vannamei* as an alternative to *P. monodon.* However, the complete eradication of WSSV from the prawn-farming industries in this region is viewed as

[3] www.daff.gov.au/animal-plant-health/aquatic/resources

impractical because of its omnipotent presence in wild crustaceans and the heavy reliance on wild-caught *P. monodon* broodstock (where *P. monodon* is still farmed). In other countries such as Australia, where *P. monodon* is still favoured for farming, impacts of disease are being addressed by closing its breeding cycle. This closure enables generation of high-health and fast-growing domesticated breeding populations that can be selected and monitored intensively to be SPF for viruses, including WSSV (Preston et al. 2009).

Prawn farms in Australia predominately employ flow-through systems. Thus, any pressures to convert to more self-contained 'closed' systems would invariably involve substantial capital costs to alter farm layouts to accommodate additional water storage and recirculation systems (Chanratchakool et al. 1998).

Restrictions could be placed on movements of live crustaceans as necessary, but these would invariably impact on current farm management practices and production systems. Controls could be placed on farms receiving and rearing seedstock from remote or on-site hatcheries, or on-rearing of juveniles obtained from other farms. Importantly, more stringent systems might need to be implemented to restrict inadvertent entry of wild crustaceans from neighbouring watercourses into farm waterways or ponds, particularly via intake water. In the case of red claw crayfish, which can move between ponds over land, all ponds at a farm might need to be considered as a single system, even if infection is localised initially to only one pond at a farm. Implementation of biosecurity conditions (either on-farm or licence conditions), including the use of boundary fencing, might be needed at red claw farms to help prevent infected crayfish from moving between production ponds.

At prawn farms, wild crustaceans such as local prawn species and crabs often co-habit in ponds with the species being farmed. Appropriate fencing around individual ponds would effectively prevent overland entry by crabs (Fegan & Clifford 2001).

As a useful disease exclusion strategy, small crustaceans potentially carrying WSSV can be excluded effectively by filtering pond intake water with fine (500 µm or preferably 200–250 µm) mesh screens (Fegan & Clifford 2001). Additionally, bag-net screens used for this purpose provide a much larger surface area than do framed vertical screens; inserting one bag inside another offers an economical means of further decreasing the effective mesh size.

Aerators, particularly the paddle-wheel type, generate aerosols that might spread infection between ponds and possibly farms (Fegan & Clifford 2001).

2.3.2 Tracing

Thorough investigation of any WSD incident to identify all potential dissemination sites of the virus is critical to determining the most appropriate control option. Any predisposing factors also must be identified to help determine when and how infection and/or disease arose. Considering the possibility that WSSV infection existed well before the appearance of clinical disease is also important.

Tracing the origin and spread of disease involves retrospectively identifying the method and pattern of disease spread. Tracing investigations are crucial for identifying all confirmed and potential locations of infection to define the boundaries of restricted and control areas, and in informing the most appropriate response actions. In the tracing process, there is an urgent need to identify all potential means of exposure to infected crustaceans, premises or sites before its discovery to help establish the source or origin of infection or disease. Similar tracing is also essential to demarcate the local geographical

boundaries of infection and identify any more remote locations to which the disease might have spread. Movements of the following must be traced:

- live crustaceans—for example, broodstock, postlarvae and stock sold to restaurants or aquarium shops
- dead crustaceans—uncooked prawns intended for consumption or for use as bait (if cooked, tracing is not required)
- effluent and waste products—from processing and/or cooking
- water—intake and outlet
- vehicles—crustacean transport vehicles, feed trucks, visitors' cars and boats
- materials—nets, paddle wheels, pumps, tools and instruments
- personnel—farm workers, sales and feed company representatives, trades people, veterinarians, scientists, technicians and visitors.

Neighbouring wild and farmed crustaceans

Wild and farmed crustaceans in close proximity to a farm or locality in which WSD or WSSV infection has been diagnosed might be at risk of becoming infected; they could also already be infected. Maps detailing the locations of neighbouring farms, hatcheries and processing plants, and natural waterways (including hydrographic data) are essential for assessing the potential spread of the pathogen. The local environment should be assessed to determine the types and relative abundance of susceptible crustacean species, both upstream and downstream of the infected site.

To obtain information on farm locations and the nature of wild crustacean populations at risk of infection, the relevant state or territory fisheries or agriculture agencies can be contacted (see AQUAVETPLAN **Enterprise Manual** for contact details).

2.3.3 Surveillance

Surveillance measures should include observations of crustaceans for evidence of clinical disease, as well as laboratory testing for WSSV infection, to:

- define the geographical distribution of the virus
- predict and/or detect new outbreaks
- establish restricted and control areas to which quarantine and movement restrictions can be applied
- establish disease-free and infected areas/zones to implement a WSSV zoning strategy
- monitor the progress and success of an eradication strategy.

Detailed information on general requirements for surveillance to establish freedom from infection at various prevalence thresholds is provided in the OIE *Manual of diagnostic tests for aquatic animals* (Aquatic Manual; OIE 2012b). The manual also provides specific information on surveillance for WSD.

2.3.4 Treatment of virus-infected crustaceans

There are no effective commercially available prophylactic or curative treatments for WSSV infection.

2.3.5 Disinfection of crustaceans and crustacean products

If prawns are sufficient in size, emergency harvesting, cooking and sale of clinically normal prawns infected with WSSV might provide an attractive option for farmers that reduces the financial burden of forced destruction of stock. However, before disinfection/processing methods and the destination of destroyed prawns are finalised, food-safety standards, trade and market requirements, and the potential risks of WSSV spread need to be assessed.

WSSV can potentially remain infectious for up to 28 days in decaying prawn tails (Prior & Browdy 2000), and for extended periods in frozen prawns destined for retail outlets (Durand et al. 2000; Nunan et al. 1998). Ideally, whole prawns and prawn products should be cooked to destroy virus infectivity before they leave an infected farm/location.

Data from three independent studies on the heat stability of semi-purified WSSV suspensions are summarised in Table 2.1. At 55 °C, the virus can remain infectious for 5–30 minutes (Nakano et al. 1998), but at temperatures in excess of 60 °C infectivity is destroyed rapidly—in less than 1 minute (Chang et al. 1998). However, these studies examined virus particles in suspension. When WSSV is present in tissue, host protein mass will help protect viral infectivity against thermal destruction. Because of this unknown, biological waste derived from crustaceans potentially infected with WSSV should be heated at 60 °C or more for at least 20 minutes to ensure that the virus is destroyed.

Table 2.1 Treatment times and temperatures needed to inactivate white spot syndrome virus infectivity

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

– = not done; D = virus infectivity destroyed; L = live virus recovered after heating
Sources: Chang et al. (1998), Maeda et al. (1998a), Nakano et al. (1998)

Heating whole prawns to a core temperature of 85 °C has been recommended to prevent black spot and deterioration of meat quality (Winkel 1998). In *P. monodon* that are about 14 grams, 22 grams and 30 grams in weight, core temperatures above 80 °C maintained for at least 75 seconds are achieved by cooking in boiling water for 3.5, 4.0 and 4.5 minutes, respectively. Based on the heat inactivation data in Table 2.1, therefore, cooking practices for prawn carcasses recommended by Winkel (1998) are expected to destroy WSSV infectivity.

In prawn hatcheries, WSSV can be transmitted to and among progeny by vertical and horizontal routes. As there is no solid evidence of mature gametes being infected by WSSV before fertilisation and spawning, vertical transmission is suspected to occur through virus particles becoming attached to the surface of fertilised eggs (Lo et al. 1997b). Washing eggs extensively in sea water can be beneficial to reduce viral loads and prevalence in progeny,

but alone it is generally insufficient to remove all contaminating WSSV (Satoh et al. 1999). There are no reliable, more robust methods of disinfecting eggs that retain good egg viability. However, in cases when WSSV infection loads in broodstock are very low, infection of progeny can be eliminated or loads reduced through more rigorous washing or disinfection of eggs and/or newly hatched nauplii. A widely used method of egg disinfection is detailed in Chapter 1.1.3 of the OIE Aquatic Manual (OIE 2012b).

Trade regulations, market requirements, food-safety standards and potential for spread of the pathogen must be considered when determining the treatment or processing strategy and final destination of potentially infected prawn products and byproducts.

2.3.6 Destruction of hosts

Any chemicals used to disinfect or destroy WSSV-infected crustaceans must be approved for that use by the Australian Pesticide and Veterinary Medicines Authority (Appendix 2). In addition, any chemical that is used directly or indirectly for the control of an animal disease is governed in its use by relevant 'control of use' legislation in each state and territory. The relevant state or territory authority (in most cases this is the veterinary registrar within the relevant state department of primary industry or agriculture) should also be consulted for advice before using the chemical.

Slaughter of diseased crustaceans should be both hygienic and humane, and avoid spillage or escape of infectious waste. As increased viral shedding might occur when crustaceans are stressed at slaughter, stress should be minimised and slaughter should occur promptly.

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

- the ability to contain pond or tank water—all water must be disinfected before discharge
- destination—human consumption or disposal
- size and number of animals
- desirability of removing most or all dead animals from the pond bottom before disinfecting the water
- the need to prevent scavengers, particularly birds, from spreading infection mechanically 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
- experience and availability of personnel.

In general, if farming practices routinely used for harvesting can be used to destroy stock, these practices should be used because farm staff are familiar with them and the necessary equipment is available on-site.

Depending on the circumstances in which WSSV is detected (laboratory detection of subclinical infection in crustaceans or an outbreak of WSD), either continued grow-out or emergency harvesting can be considered.

2.3.7 Disposal of hosts

Diseased and dead prawns are a primary source of infectious WSSV. Therefore, they and other possible infectious waste or sources of infection, such as potential carrier crustaceans, must be destroyed and disposed of promptly and appropriately to reduce risks of infection persisting at, or spreading from, a site. Burial sites for dead and destroyed prawns and other waste must be chosen carefully to mitigate any risk of infectious material entering waterways or being exposed to susceptible species.

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

2.3.8 Decontamination

Marked differences in crustacean farming enterprises mean that disinfection protocols must be determined on a case-by-case basis through discussions between farm managers and the state or territory chief veterinary officer (CVO) and/or Director of Fisheries. The protocol should consider factors outlined in Section 1.6, and in particular:

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

In typical pond-water conditions, WSSV particles will remain viable for at least 3–4 days, possibly longer (Flegel et al. 1997). The recommended disinfection protocol for pond water is to add active chlorine to a concentration of 30 parts per million (ppm) and to hold the chlorinated water for 4 days before discharge.

No definitive data are available on the length of time WSSV can remain viable in substrates and sludge on the pond bottom. Following the removal and appropriate decontamination and disposal of diseased and dead prawns and other crustaceans, and disinfection of pond water (and all other water reservoirs, canals and drains on the farm), bottom substrates should be dried thoroughly after water discharge. If pond sludge is removed, it should be disposed of appropriately. After drying and sun exposure for at least one month, pond substrates should be treated with a minimum of 0.5 kg/m^2 of slaked lime ($CaOH_2$). They should then be held for periods normally used to elevate pH before the ponds are refilled with water for restocking.

Equipment, materials, tanks and buildings that might be contaminated with WSSV also need to be disinfected appropriately and cleaned before re-use.

Boots, nets and other small equipment can be disinfected effectively by wiping thoroughly with or soaking for an appropriate time in a solution containing at least 30 ppm active chlorine.

See the AQUAVETPLAN **Operational Procedures Manual—Decontamination** for details of decontamination methods and their indicators.

2.3.9 Vaccination

There have been significant advances in the understanding of how crustaceans respond to and defend themselves against various pathogens, particularly highly destructive viruses such as WSSV. Many studies have shown that prawns possess mechanisms to mount an extremely effective and specific RNA-interference (RNAi) response to double-stranded (dsRNA) delivered via various methods. A number of studies have examined RNAi approaches to interfere specifically with WSSV replication. The studies have shown that long dsRNAs—and to a lesser degree, short-interfering dsRNAs (siRNAs)—delivered before challenge with virulent WSSV substantially or profoundly slow and/or curtail mortalities resulting from WSD (Krishnan et al. 2009; Robalino et al. 2004; Westerberg et al. 2005; Xu et al. 2007).

Moreover, many studies have examined the impacts of pre-exposure to subclinical infection by WSSV or other viruses, or pre-exposure to specific native WSSV proteins or recombinant WSSV proteins generated by various methodologies. Results clearly demonstrated that crustaceans possess some form of memory response that can protect against disease after subsequent challenge by virulent WSSV (Jha et al. 2006; Kim et al. 2007; Ning et al. 2009; Witteveldt et al. 2004; Xu et al. 2006). These dsRNA-based and protein-based protection or 'vaccination' approaches to viruses such as WSSV have shown tremendous potential experimentally. However, delivery methods have not been refined sufficiently for such approaches to become available commercially.

2.3.10 Vector control

Seabirds and wading birds occur commonly around prawn farms and will typically be attracted to dead or moribund prawns at pond edges. In an outbreak of WSD, therefore, access of birds to diseased prawns in affected ponds must be controlled. Past experience has shown that netting the 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. Pyrotechnics or automated exploders can also be used in accordance with local laws. Broadcasting of recorded bird distress calls can also provide an effective short-term deterrent with some species, but effectiveness usually diminishes over time.

Firearms can be used as an alternative to noisemakers and, if approved, killing a limited number of birds can reinforce fear instincts within flocks (Littauer 1990). In most regions of Australia, however, the use of firearms would be a last resort, would require shooters to be licensed, and would likely require further permits from state police departments and environmental protection and/or national parks agencies (see AQUAVETPLAN **Operational Procedures Manual—Disposal**). If live ammunition is used, extreme care must be exercised and all staff briefed beforehand in safety procedures.

Where possible, contact between wild crustaceans and infected farmed prawns should also be prevented. For crabs and other semiterrestrial crustaceans, access can be prevented by fencing pond perimeters. Shade-cloth–type netting (2 mm mesh size and 30–40 cm high) is effective for this purpose (Fegan & Clifford 2001).

2.4 Environmental considerations

Environmental considerations in the control of WSD include the following:

- Discharge of infectious or potentially infectious effluent into catchment areas or natural waterways will pose a serious risk of spreading infection more widely and could lead to populations of wild crustaceans becoming reservoirs of infection.

- The release of disinfectants could adversely affect aquatic fauna and flora, especially when used in quantities or concentrations higher than normal, as might be necessitated in a disease emergency situation. In such situations, state or territory environmental protection agencies should be consulted (see the AQUAVETPLAN **Enterprise Manual**).
- Any environmental impacts associated with the destruction and disposal of infected carcasses or material should be minimised, while ensuring measures are met to avoid infection being disseminated.

For details of decontamination methods, see the AQUAVETPLAN **Operational Procedures Manual—Decontamination**.

2.5 Sentinel animals and restocking measures

Prawn species known to be highly susceptible to WSSV infection and WSD, such as *P. monodon*, *P. merguiensis* or *P. japonicus*, may be obtained from virus-free locations and used as sentinel animals to assess the effectiveness of site decontamination before any large-scale restocking of individual prawn farms or prawn-farming regions affected by WSD.

Pond or site fallowing durations before restocking should be assessed on a species and case-by-case basis to minimise risks of the recurrence of WSD. The duration will depend on the season, the extent of the outbreak, the numbers of sites with confirmed diagnoses and the features of these sites. Where WSSV is endemic and has seriously affected farming of *P. monodon*, best practice for sustainable prawn farming has included both fallowing (pond dry-out for a minimum of 4 weeks) in conjunction with the application of lime to pond substrates to neutralise acidic pH and to help destroy viruses before restocking (Chanratchakool et al. 1998).

For any attempts to eradicate WSD, it is important that prawns restocked into ponds are free of infection. For areas declared free of WSD, this status can only be retained if introduced prawns originating from elsewhere are similarly free of infection. Using seedstock derived from broodstock that are SPF for WSSV infection and have been reared under strict biosecurity measures is also valuable to avoid reintroducing infections to individual farms, farm clusters or broader regions.

2.6 Public awareness

Public awareness campaigns should emphasise education, surveillance and cooperation at both industry and community levels. The goal is to broadly disseminate information to avoid practices that might exacerbate the likelihood of WSSV infections being spread inadvertently and WSD devastating wild and farmed crustacean stocks in Australia.

The importance of not using imported uncooked prawns as bait or as aquaculture feed because of the substantial risks of disseminating WSSV infection and WSD should be emphasised. Also, public awareness documents should emphasise that WSSV is harmless to humans.

3 Preferred Australian response options

3.1 Overall policy for white spot disease

Summary of policy

The cause of white spot disease (WSD)—white spot syndrome virus (WSSV)—is highly contagious in penaeid prawns and has the potential to cause mass mortalities and substantial financial losses at farms. Moreover, clean-up and control of any major outbreak of WSD would result in substantial human and financial costs to both industry and government. Although Australia is currently free of WSD, WSSV infection is endemic in wild and farmed crustacean species and, importantly, in prawns in Asia and the Americas.

In the event of WSD occurring, or WSSV being detected in indigenous crustaceans, and following initial epidemiological investigations (see Section 3.3.3), the appropriate response option will be decided by the Director of Fisheries and/or the chief veterinary officer (CVO) of the state or territory in which the outbreak/detection has occurred, in consultation with the Aquatic Consultative Committee on Emergency Animal Disease (Aquatic CCEAD).

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

- option 1—eradication with the aim of returning Australia to freedom from WSSV
- option 2—containment, control and zoning with the aim of placing restrictions in areas in which WSSV infection is endemic to prevent its further spread to uninfected areas
- option 3—control and mitigation with the aim of mitigating the impacts of WSD if it is accepted that the virus will remain endemic in Australia.

Each of these response options will involve the use of a combination of strategies, which might include:

- quarantine and movement controls on crustaceans within declared areas to prevent infection spreading
- prompt destruction of diseased crustaceans to prevent further shedding of virus
- decontamination of facilities, equipment, and vehicles or vessels to eliminate and prevent virus spreading
- surveillance to determine the source and distribution of infection, and freedom of infection
- zoning to define and assist in maintaining virus-free zones
- hygiene and biosecurity measures to mitigate on-farm impacts of WSD.

The nature of the response will be determined mainly by whether the outbreak is multifocal or localised, and the likelihood that containment and eradication can be achieved. The most appropriate strategy must be chosen after epidemiological investigations have been conducted, and the decision must be based on scientific effectiveness and financial feasibility. Although eradication might be the preferred option, it might not be feasible, given the limited success of eradication and control policies in other countries.

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

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

Directors of Fisheries and/or CVOs will implement the disease control measures as agreed in the EAD response plan and in accordance with relevant legislation. They will make ongoing decisions on follow-up disease response measures in consultation with the Aquatic CCEAD. The detailed response measures adopted will be determined using the principles of control and eradication (see Section 2), epidemiological information about the outbreak and the financial feasibility of the option.

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

3.2 Response options

The circumstances surrounding an outbreak of WSD will greatly influence selection of the most suitable response option. Figure 3.1 details the actions that should occur on initial suspicion of WSD.

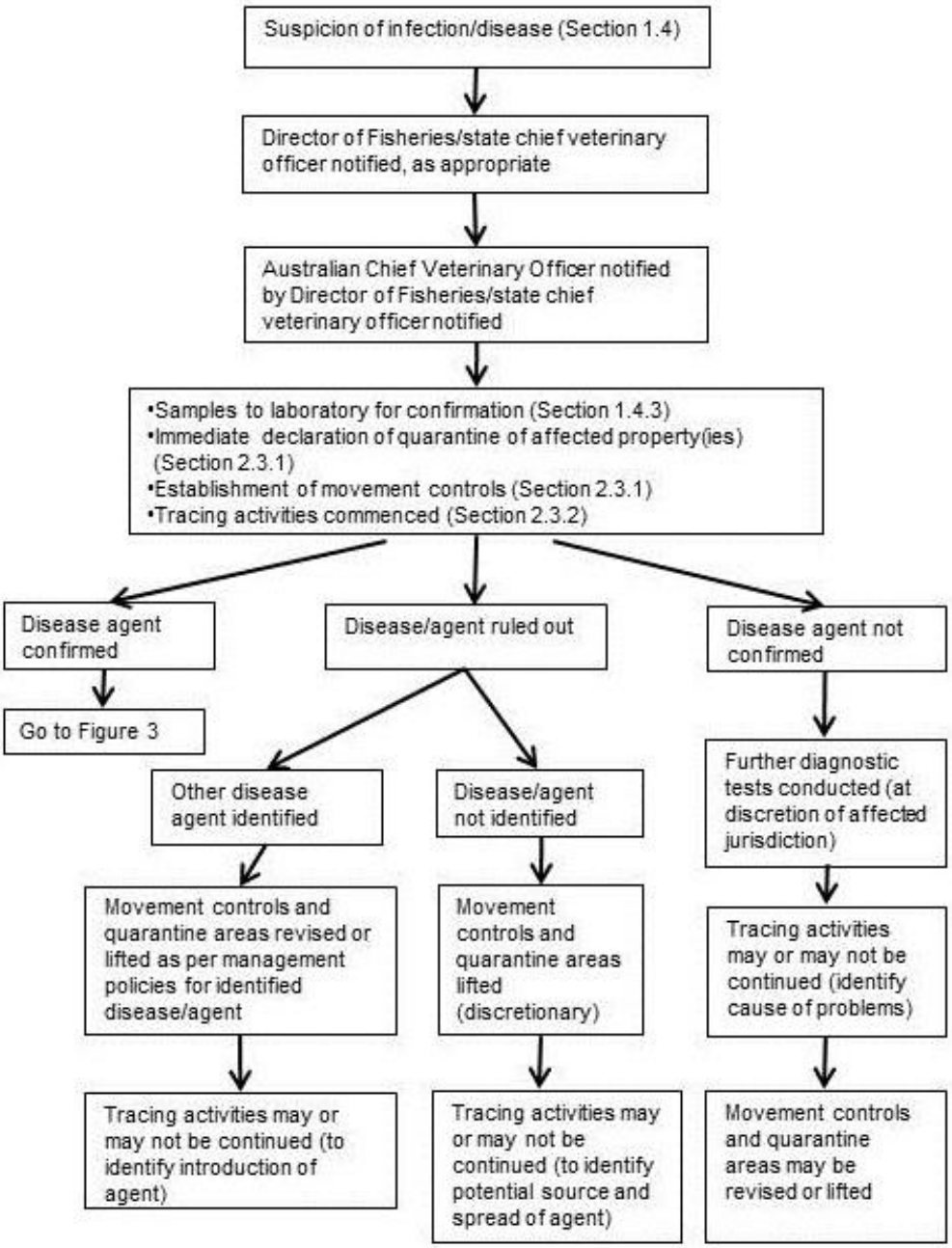

Figure 3.1 Decision matrix/flow chart

As soon as adequate information becomes available, a decision will be made on the appropriate response, based on the reasoning shown in Figure 3.2.

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

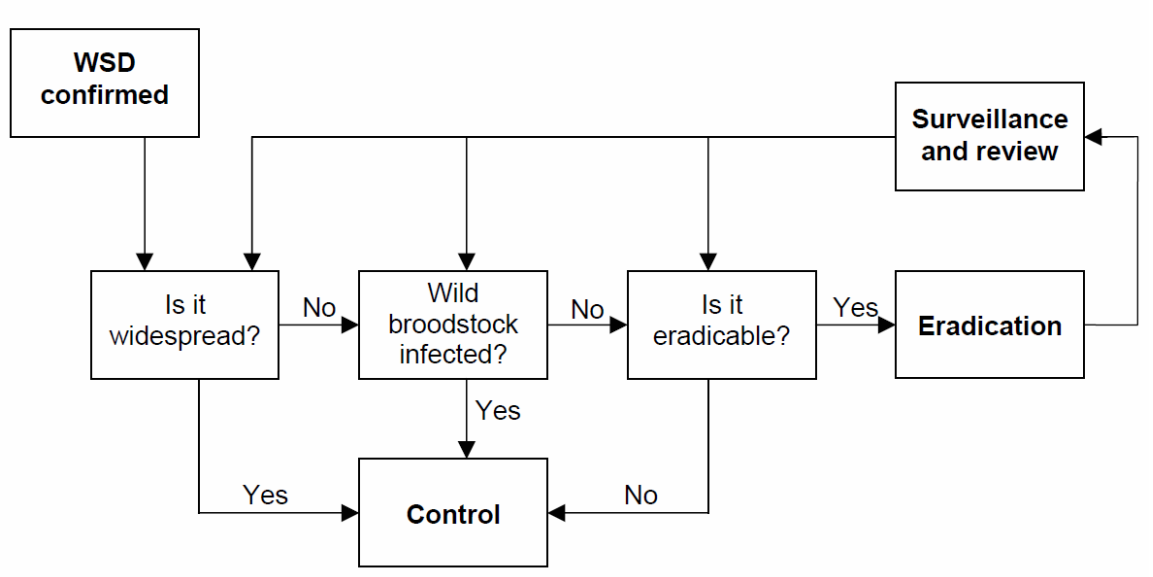

Figure 3.2 Decision flowchart

3.2.1 Option 1—Eradication

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

As stated earlier, eradication is unlikely to be successful or feasible if epidemiological investigations determine that infection in farms is widespread, has no identifiable point source, is assessed as unable to be contained, or is potentially widespread in wild prawns. However, the potential constraint on eradication posed by the presence of infection in wild prawns is equivocal, and judgement will need to be exercised based on whether a supply of uninfected broodstock is available.

Eradication measures include:

- establishment of specified zones—*restricted, control, free*
- quarantine and movement controls/restrictions on prawns and other crustaceans, water and any other potential vectors (including materials and equipment) in restricted or control zones, to prevent the spread of infection
- destruction and disposal of all clinically diseased prawns
- on-farm processing (e.g. by cooking) of exposed or potentially exposed, but clinically normal, prawns to prevent the spread of infection
- disinfection and safe disposal of processing effluent and waste (cooking water, prawn heads and shells)
- disinfection and safe disposal of pond water, and decontamination of ponds, facilities, products, equipment, vehicles, boats and so on 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
- control of scavenger access, particularly birds, to live and dead 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 encourage cooperation from industry and the community.

3.2.2 Option 2—Containment, control and zoning

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

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

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

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

- zoning/compartments to define infected and disease-free areas
- quarantine and movement controls/restrictions on prawns, water and any other potential vectors (including materials and equipment) within the infected zone and to free zones
- eradication of outbreaks in the free zone where feasible
- pond-level surveillance, with destruction and safe disposal of any clinically diseased prawns in the infected zone, followed by clean-up and disinfection
- use of closed-production systems
- testing of broodstock and postlarvae for WSSV
- compartmentalisation of selected facilities, such as hatcheries for production of specific pathogen-free (SPF) stock, as part of a control and mitigation strategy
- emphasis on high standards of hygiene (including drying ponds before restocking and disinfecting water before either use or release) and biosecurity (use of crustacean-proof land barriers and water filters, and screening of incoming postlarvae for WSSV)
- tracing and surveillance to determine the source and extent of infection
- a public awareness campaign to encourage cooperation from industry and the community.

3.2.3 Option 3—Control and mitigation of disease

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

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

Measures for control and mitigation include:

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

Compartmentalisation of selected facilities (such as hatcheries for production of SPF stock) may be a part of a control and mitigation strategy

3.3 Strategies for control and eradication

Methods for the control and eradication of WSD are summarised in Table 3.1 and described in detail in Section 2.3.

3.3.1 Interim measures to minimise further spread

The initial phase of the response to suspicion of a WSD outbreak in farmed prawns will be one of containment while additional information is collected to further define the extent of the problem and a decision is made on the appropriate response.

Movement controls and other measures will be implemented immediately on premises or areas suspected of being infected (see Section 2.3.1 and the AQUAVETPLAN **Enterprise Manual** for details).

Immediate measures might include:

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

3.3.2 Rapid confirmation of infection

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

Some state/territory diagnostic laboratories might perform some preliminary WSD diagnosis and WSSV detection. For definitive diagnosis, and upon immediate suspicion of WSD, samples should be sent to the CSIRO Australian Animal Health Laboratory Fish Diseases Laboratory (AFDL) in Geelong.

Table 3.1 Summary of strategies used for each of the response options for white spot disease

Strategy	Control method		
	Eradication	Containment	Mitigation
Quarantine and movement controls	Yes	Yes	No
Declared restricted and 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 of clinically diseased prawns	Yes	Yes	Yes
Destruction of unexposed prawns	Optional	No	No
Destruction or harvest with on-farm cooking of exposed or potentially exposed but clinically normal prawns, depending on size	Yes	In free zones only	No
On-farm processing (e.g. by cooking)	Yes	Optional	Optional
Disposal of infected prawns and wastes that cannot be cooked on-farm	Yes	Yes	n.a.
Decontamination	Required	Optional	Optional
Surveillance	Yes	Yes	Yes
Tracing	Yes	Optional	No
WSSV 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; WSSV = white spot syndrome virus

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

- the history, signs and gross lesions are indicative of WSD
- typical histological lesions are present in tissue sections
- polymerase chain reaction (PCR) testing returns a positive result for WSSV.

Where one or more of the criteria are not met, additional testing will be required. Once the response has begun, these criteria can be modified for confirming infected premises in the light of new information about the outbreak.

Submission of specimens

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

Live prawns are preferred. A minimum of 100 representative larval- to postlarval-stage prawns or a minimum of 10 representative juvenile- to adult-stage prawns should be collected and submitted to the local state/territory veterinary diagnostic laboratory in a well-oxygenated, cooled container.

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

Samples for PCR testing

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

Samples for histopathology

For larvae and small postlarvae, live animals can be immersed directly into Davidson's fixative solution and left 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 prawns, after chilling or anaesthesia, inject fixative (5–10% volume per weight), ensuring that the hepatopancreas is injected liberally first, and that the whole specimen is thoroughly injected thereafter. If this is done properly, the whole body will turn red. Next, using a small pair of pointed scissors, the cuticle only should be cut along the mid-lateral side of the animal, starting at the sixth abdominal segment and moving up to the beginning of the cephalothorax, at which point the scissors should be angled in to meet the base of the rostrum. Then the whole prawn should be placed in 10 volumes of Davidson's fixative for 24 hours (up to 72 hours for larger prawns), after which it should 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 can be obtained from state/territory fisheries or animal health officers (see the AQUAVETPLAN **Enterprise Manual** for contact details). Equipment for collecting sterile samples, reagents for sample preparation, and facilities for chilled or frozen storage and transport of samples will be required.

3.3.3 Epidemiological investigations

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

Investigations should include both clinical evaluation and laboratory screening of an appropriately sized sample of prawns. Sample sizes for surveillance should be calculated to at least meet the international standard described in the World Organisation for Animal Health (OIE) *Aquatic animal health code* (Aquatic Code; OIE 2012a).

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

3.3.4 Quarantine and movement controls

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

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

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

3.3.5 Zoning

Zoning for WSSV will be based on OIE-determined principles as expanded in the AQUAPLAN *Zoning policy guidelines*[4] and on the known distribution of WSSV and the infected host species, once these have been determined. At least initially, zoning should be limited to control (infected) and free (uninfected) zones, with effective controls on the movement of susceptible prawns and equipment between zones.

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

3.3.6 Destruction of clinically diseased prawns

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

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[4] www.daff.gov.au/animal-plant-health/aquatic/resources

3.3.7 Management of other prawns

Unexposed prawns

Eradication

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

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

Immediate destruction might be considered for unexposed prawn stocks in an infected zone, particularly for young animals of low unit value, to allow farm fallowing to begin immediately.

Containment, control and zoning; control and mitigation

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

Exposed or potentially exposed, but clinically normal prawns

Eradication

In facilities at which WSSV eradication is being attempted, exposed or potentially exposed prawns that remain clinically normal should be regarded as infected. These prawns might be dealt with by:

- destruction and disposal in the same way as that for diseased prawns
- harvesting, followed by appropriate on-site processing (e.g. by cooking—see Section 2.3.6) and sale, if of marketable size.

Containment, control and zoning

In a containment, control and zoning strategy, grow-out of exposed or potentially exposed prawns that remain clinically normal will be standard practice in infected zones. During grow-out, these prawns must be treated as infected. Restrictions on movements of prawns, people, vehicles and boats and on market destinations of products might be necessary to protect free facilities or zones. The risk of clinical disease and the spread of infection will be minimised through appropriately adjusted farm management practices.

In free zones, exposed or potentially exposed prawns that remain clinically normal will either be destroyed or harvested and processed on-site in the same way as that used in an eradication strategy.

Control and mitigation

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

3.3.8 Disposal

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

Eradication

In an eradication strategy, all infected prawns, wastes, effluent and equipment that cannot be decontaminated must be immediately and safely disposed of. If processing is undertaken on infected premises, effluent and any other waste will be treated before being discharged or disposed of safely. 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; containment and mitigation

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

3.3.9 Decontamination

Eradication

In an eradication strategy, all buildings, tanks, ponds, materials and equipment (including nets, boats, vehicles, etc.) that might be contaminated must be cleaned and disinfected. Ponds must be decontaminated using the procedures outlined in Section 2.3.8. At all stages of decontamination, steps must be taken to prevent any spread of infection via water, wastes or materials, especially into natural waterways.

Containment, control and zoning; control and mitigation

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

3.3.10 Surveillance

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

3.3.11 Tracing

In eradication or containment strategies, tracing will be undertaken for all infected facilities as described in Section 2.3.2, as part of an official control or eradication program.

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

3.4 Social and economic effects

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

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

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

3.5 Criteria for proof of freedom

Wherever possible, proof of freedom should comply with the international standards that apply at the time, as described in the OIE Aquatic Code (OIE 2012a). Proof of disease freedom following the resolution of an outbreak is likely to rely both on clinical surveillance to show that no new outbreaks have occurred over the period recommended by the current edition of the OIE *Manual of diagnostic tests for aquatic animals* and on a random-sample survey.

3.6 Funding and compensation

There are currently no national cost-sharing agreements in place for emergency responses to white spot disease. It is the responsibility of the users of this publication to seek advice in relation to any relevant funding or compensation arrangements within the relevant jurisdiction.

Appendix 1 Aquatic animal health code and manual of diagnostic tests for aquatic animals

Aquatic animal health code

The objective of the World Organisation for Animal Health (OIE) *Aquatic animal health code* (OIE 2012a) is to prevent the spread of aquatic animal diseases while facilitating international trade in fish and fish products. This annually updated volume is a reference document for use by veterinary departments, import/export services, epidemiologists and all involved in international trade.

The current edition of the OIE Aquatic Code is available at www.oie.int/en/international-standard-setting/aquatic-code/access-online.

Chapter 9.6 'White spot disease' in the 2012 edition is relevant to this manual.

Manual of diagnostic tests for aquatic animals

The purpose of the OIE *Manual of diagnostic tests for aquatic animals* (OIE 2012b) is to contribute to the international harmonisation of methods for the surveillance and control of the most important aquatic animal diseases. Standards are described for laboratory diagnostic tests and for the production and control of biological products (principally vaccines) for veterinary use across the globe.

The current edition of the OIE Aquatic Manual is available at www.oie.int/en/international-standard-setting/aquatic-manual/access-online.

Chapter 2.2.6 'White spot disease' in the 2012 edition is relevant to this manual.

Further information

Further information about the OIE Aquatic Code and OIE Aquatic Manual is available at the OIE website: www.oie.int/en/international-standard-setting.

Appendix 2 Approval of chemicals for use in Australia

The Australian Pesticides and Veterinary Medicines Authority (APVMA) evaluates, registers and regulates agricultural and veterinary chemicals. Before an antibiotic or vaccine can enter the Australian market, it must go through the APVMA's rigorous assessment process to ensure that it meets high standards of safety and effectiveness. (In addition, an import permit is required from the Department of Agriculture if a product containing biological material is to be sourced from overseas.)

Detailed data about the product and its proposed use pattern must be submitted to the APVMA with the application for registration or permits. Because the assessment process is so detailed, the evaluation may take some time to complete.

Minor use permit system

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

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

Emergency use permits

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

For further details or permit application forms, visit the APVMA website.[5]

[5] www.apvma.gov.au

Glossary

Animal Health Committee (AHC)	A committee whose members are the Australian and state and territory chief veterinary officers, the Director of the CSIRO Australian Animal Health Laboratory, and the Director of Environmental Biosecurity in the Australian Government Department of Sustainability, Environment, Water, Population and Communities. The committee provides advice to the Standing Council on Primary Industries on animal health matters, focusing on technical issues and regulatory policy (formerly called the Veterinary Committee). *See also* Standing Council on Primary Industries
AQUAVETPLAN	*A*ustralian A*qua*tic *Vet*erinary Emergency *Plan*. A series of manuals that outlines Australia's approach to national disease preparedness, and proposes the technical response and control strategies to be activated in a national aquatic animal disease emergency. The manuals provide guidance based on sound analysis that links policy, strategies, implementation, coordination and emergency management plans.
Australian Chief Veterinary Officer	The nominated senior veterinarian in the Australian Government Department of Agriculture who manages international animal health commitments and the Australian Government's response to an animal disease outbreak. *See also* Chief veterinary officer
AUSVETPLAN	*Aus*tralian *Vet*erinary Emergency *Plan*. A series of technical response plans that describes the proposed Australian approach to an emergency animal disease incident. The documents provide guidance based on sound analysis that links policy, strategies, implementation, coordination and emergency management plans.
Chief veterinary officer (CVO)	The senior veterinarian of the animal health authority in each jurisdiction (national, state or territory) who has responsibility for animal disease control in that jurisdiction. *See also* Australian Chief Veterinary Officer
Compartment	One or more aquaculture establishments under a common biosecurity management system containing an aquatic animal population with a distinct health status with respect to a specific disease or diseases for which required surveillance and control measures are applied and basic biosecurity conditions are met for the purpose of international trade. Such compartments must be clearly documented by the Competent Authority(ies) (OIE 2012a).
Competent authority	The veterinary authority or other governmental authority of a member of the OIE having the responsibility and competence for ensuring or supervising the implementation of aquatic animal health and welfare measures, international health certification and other standards and recommendations in the Aquatic Code in the whole territory (OIE 2012a).
Control	Reduction in morbidity and mortality from disease by measures intended to interfere with the unrestrained occurrence of disease.

Control area	A buffer between the restricted area and areas free from disease. Restrictions on this area will reduce the likelihood of the disease spreading further afield. As the extent of the outbreak is confirmed, the control area may decrease or increase in size. The shape of the area may be modified according to circumstances (e.g. water flows, catchment limits). In most cases, permits will be required to move animals and specified product out of the control area into the free area.
Dangerous contact premises or area	A premises that may or may not contain a susceptible animal(s), including those not showing clinical signs, but that, following a risk assessment, is considered highly likely to contain an infected animal(s) or contaminated animal products, wastes or things, which present an unacceptable risk to the response if the risk is not addressed.
Declared area	A defined tract of land or water that is subjected to disease control restrictions under emergency animal disease legislation. Types of declared areas include restricted area, control area, infected premises, dangerous contact premises and suspect premises.
Decontamination	Includes all stages of cleaning and disinfection to remove contamination.
Disease agent	A general term for a transmissible organism that causes an infectious disease.
Disinfectant	A chemical used to destroy disease agents outside a living animal.
Disinfection	The application, after thorough cleansing, of procedures intended to destroy the infectious or parasitic agents of animal diseases, including zoonoses; applies to premises, vehicles and different objects that may have been directly or indirectly contaminated.
Disposal	Sanitary removal of aquatic animal carcasses, aquatic animal products, materials and wastes by burial, burning or some other process to prevent the spread of disease.
Emergency animal disease	A disease that is (a) exotic to Australia or (b) a variant of an endemic disease or (c) a serious infectious disease of unknown or uncertain cause or (d) a severe outbreak of a known endemic disease, and that is considered to be of national significance with serious social and/or trade implications. *See also* Endemic animal disease, Exotic animal disease
Endemic animal disease	A disease affecting animals (which may include humans) that is known to occur in Australia. *See also* Emergency animal disease, Exotic animal disease
Enterprise	*See* Risk enterprise
Epidemiological investigation	An investigation to identify and qualify the risk factors associated with the disease.
Eradication	Elimination of a disease from a specified area.
Exotic animal disease	A disease affecting animals (which may include humans) that does not normally occur in Australia. *See also* Emergency animal disease, Endemic animal disease
Fish	Any aquatic animal within the finfish, mollusc and crustacean groups.
Fomite	Any inanimate object (e.g. boots, clothing, equipment, instruments, vehicles, crates, packaging) that can carry an infectious disease agent and may spread the disease through mechanical transmission.
Free area	An area known to be free from the disease agent.

Infected premises or area	A defined area (which may be all or part of a property) in which an emergency disease meeting the case definition exists or is believed to exist, or in which the causative agent of that emergency disease exists or is believed to exist. The term 'infected area' is more likely to apply to an open system, such as an oceanic lease.
Local disease control centre	An emergency operations centre responsible for the command and control of field operations in a defined area.
Monitoring	Routine collection of data for assessing the health status of a population. *See also* Surveillance
Movement control	Restrictions placed on the movement of fish, people and fomites to prevent the spread of disease.
OIE *Aquatic animal health code* (Aquatic Code)	Sets out standards for the improvement of aquatic animal health and welfare, and veterinary public health worldwide, including through standards for safe international trade in aquatic animals and their products. Published on the internet at www.oie.int/en/international-standard-setting/aquatic-code/access-online.
OIE *Manual of diagnostic tests for aquatic animals* (Aquatic Manual)	Provides a uniform approach to the detection of the diseases listed in the OIE Aquatic Code, so that the requirements for health certification in connection with disease prevention and control programs and with trade in aquatic animals and aquatic animal products can be met. The current edition is published on the internet at www.oie.int/en/international-standard-setting/aquatic-manual/access-online.
Operational procedures	Detailed instructions for carrying out specific disease control activities, such as disposal, destruction, decontamination and valuation.
Pathognomonic	Specific characteristic of a particular disease.
Polymerase chain reaction (PCR)	A method of amplifying targeted nucleic acid sequences to detectable levels that can be used to detect the presence of nucleic acid from a disease agent.
Prawn byproducts	Products of prawn origin destined for industrial use (e.g. fishmeal).
Prawn products	Prawn meat products and products of prawn origin (e.g. eggs) for human consumption or use in animal feeding.
Premises or area	A tract of land or sea including its buildings, or a separate farm or facility that is maintained by a single set of services and personnel. A production site which might range from an aquarium to an aquaculture lease in the open ocean.
Prevalence	The proportion (or percentage) of animals in a particular population affected by a particular disease (or infection) at a given point in time.
Quarantine	Legal restrictions imposed on a place by the serving of a notice limiting access or egress of specified animals, persons or things.
Restricted area	A relatively small declared area (compared with a control area) around an infected premises that is subject to intense surveillance and movement controls.
Risk enterprise	A defined livestock or related enterprise that is potentially a major source of infection for many other premises. Includes hatcheries, aquaculture farms, processing plants, packing sheds, fish markets, tourist angling premises, veterinary laboratories, road and rail freight depots, and garbage depots.

Sensitivity	The proportion of affected individuals in the tested population that are correctly identified as positive by a diagnostic test (true positive rate). *See also* Specificity
Specificity	The proportion of unaffected individuals in the tested population that are correctly identified as negative by a diagnostic test (true negative rate). *See also* Sensitivity
Standing Council on Primary Industries (SCoPI)	The council of Australian national, state and territory and New Zealand ministers of agriculture that sets Australian and New Zealand agricultural policy (formerly the Primary Industries Ministerial Council). *See also* Animal Health Committee
State or territory disease control headquarters	The emergency operations centre that directs the disease control operations to be undertaken in that state or territory.
Subclinical infection	Clinically unapparent infection that is transmissible and that might eventually lead to clinical disease.
Sub-Committee on Aquatic Animal Health (SCAAH)	Provides high-level scientific and technical advice to the AHC in supporting policy and program development on national aquatic animal health affecting the capture and recreational fishing industries; aquaculture industries; and the ornamental fish industry. SCAAH comprises representation from the Australian, state and Northern Territory and New Zealand governments, the CSIRO Australian Animal Health Laboratory and Australian universities. Other aquatic animal health experts from both government and non-government agencies—including specialists from academia, industry and the private sector—may also be invited to participate.
Surveillance	A systematic program of investigation designed to establish the presence, extent or absence of a disease, or of infection or contamination with the causative organism. It includes the examination of animals for clinical signs, antibodies or the causative organism. *See also* Monitoring
Tracing	The process of locating animals, persons or other items that may be implicated in the spread of disease, so that appropriate action can be taken.
Vaccination	Inoculation of healthy individuals with weakened or attenuated strains of disease-causing agents to provide protection from disease.
Vaccine	Modified or attenuated strains of disease-causing agents that, when inoculated, stimulate an immune response and provide protection from disease.
Vector	A living organism that transmits an infection from one host to another. A biological vector is one in which the infectious agent must develop or multiply before becoming infective to a recipient host. A mechanical vector is one that transmits an infectious agent from one host to another but is not essential to the life cycle of the agent. *See also* Fomite
Zoning	The process of defining, implementing and maintaining a disease-free or infected area in accordance with OIE guidelines, based on geopolitical and/or physical boundaries and surveillance, in order to facilitate disease control and/or trade.

Abbreviations

Aquatic CCEAD	Aquatic Consultative Committee on Emergency Animal Diseases
Aquatic Code	OIE *Aquatic animal health code*
Aquatic Manual	OIE *Manual of diagnostic tests for aquatic animals*
AQUAVETPLAN	Australian Aquatic Veterinary Emergency Plan
AUSVETPLAN	Australian Veterinary Emergency Plan
CSIRO	Commonwealth Scientific and Industrial Research Organisation
CVO	chief veterinary officer
DNA	deoxyribonucleic acid
H&E	haematoxylin and eosin
IHHNV	infectious hypodermal and haematopoietic necrosis virus
ISH	in situ hybridisation
OIE	World Organisation for Animal Health
ORF	open reading frame
PCR	polymerase chain reaction
RNA	ribonucleic acid
SPF	specific pathogen-free
WSD	white spot disease
WSSV	white spot syndrome virus

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