Australian Government
Department of Agriculture

Australian aquatic veterinary emergency plan (AQUAVETPLAN) for white spot disease

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Cataloguing data

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AQUAVETPLAN

AQUAVETPLAN is a series of manuals that outline Australia's approach to national disease preparedness and proposes the technical response and control strategies to be activated in a national aquatic animal disease emergency.

This strategy will be reviewed regularly. Forward suggestions and recommendations for amendments to:

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Being a guide only, outbreaks or suspected outbreaks must be assessed case by case and expert advice should be obtained to determine the most appropriate management plan in response to the risk.

NOTE: Important regulatory information for white spot disease is contained in the World Organisation for Animal Health Aquatic Animal Health Code, which is updated annually.

Disease watch hotline 1 800 675 888

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

Preface

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

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

The Department of Agriculture provides biosecurity inspection for international passengers, cargo, mail, animals, plants and animal or plant products arriving in Australia, and inspection and certification for a range of agricultural products exported from Australia. Biosecurity controls at Australia's borders minimise the risk of entry of exotic pests and diseases, and protect Australia's favourable human, animal and plant health status. Information on current import conditions can be found at the Department of Agriculture BICON website.

This strategy sets out the disease control principles for use in an aquatic veterinary emergency incident caused by the suspicion or confirmation of white spot disease in Australia. The strategy was scientifically reviewed by the Sub Committee for Aquatic Animal Health of the Animal Health Committee, before being endorsed by the Animal Health Committee of the National Biosecurity Committee in February 2020.

White spot disease is listed by the OIE in the Aquatic Animal Health Code. White spot disease is also listed on Australia's National List of Reportable Diseases of Aquatic Animals (Agriculture 2019).

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

- disease strategies
  - individual strategies for each disease
- operational procedures manuals
  - disposal
  - destruction
  - decontamination
- enterprise manual, including sections on
  - open systems
  - semi-open systems
  - semi-closed systems
  - closed systems
- management manuals

- control centre manual.

The Aquatic Animal Diseases Significant to Australia: Identification Field Guide 5th edition (Department of Agriculture 2019) is a source of information about the aetiology, diagnosis and epidemiology of infection with white spot disease and should be read in conjunction with this strategy.

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. The second revision was prepared by Dr Jeff Cowley and Dr Mark Crane, CSIRO Animal, Food and Health Sciences, and completed in August 2010. The third revision was prepared by Dr Ben Diggles and was completed in January 2020. 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 was adapted from similar manuals in AUSVETPLAN (the Australian veterinary emergency plan for terrestrial animal diseases) and from the AQUAVETPLAN enterprise manual. The format and content have been kept as similar as possible to these documents, so animal health professionals trained in AUSVETPLAN procedures can work efficiently with this document in the event of an aquatic veterinary emergency. The work of the AUSVETPLAN writing teams and the permission to use the original AUSVETPLAN documents are gratefully acknowledged.

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

- **Government**
  - CSIRO Australian Animal Health Laboratory
  - Department of Primary Industries, New South Wales
  - Department of Primary Industry and Fisheries, Northern Territory
  - Department of Agriculture and Fisheries, Queensland
  - Department of Primary Industries, Parks, Water and Environment, Tasmania
  - Department of Fisheries, Western Australia
  - Department of Economic Development, Jobs, Transport and Resources, Victoria
  - Department of Primary Industries and Regions, South Australia
  - Biosecurity Animal Division, Department of Agriculture, Australian Government
  - Department of the Environment, Australian Government
- **Industry**
  - Australian Prawn Farmers Association
  - Northern Prawn Fishery Industry Inc.

- Queensland Seafood Industry Association
- National Aquatic Animal Health Industry Reference Group

The complete series of AQUAVETPLAN documents is available on the Department of Agriculture website.

Contents

Tables

Figures

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 has caused extensive losses in farmed prawns and other decapod crustaceans including farmed and wild freshwater crayfish, with worldwide economic impacts exceeding US $15 billion since the emergence and initial spread of the disease, increasing at a rate of around US$1 billion annually (Stentiford et al. 2012). A comprehensive survey found no evidence of WSSV in Australia in 2004 (East et al. 2004), however in late November 2016 an outbreak of WSD occurred on prawn farms along the Logan River near Brisbane (DAF QLD 2017, Scott-Orr et al. 2017). Subsequent delimiting surveillance detected the presence of WSSV in wild prawns and crabs in the Logan River in December 2016 and in Northern Moreton Bay in March 2017 (Biosecurity QLD 2018a, 2018b). At the time of publication, an emergency response to contain and eradicate the disease is ongoing.

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 was 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. Recent analysis indicates a shared phylogenetic origin for the *Nimaviridae* and insect-associated double-stranded DNA viruses, and suggest that the high virulence of WSSV in penaeid prawns may be due to acquisition of unique envelope protein and nonstructural virulence-associated genes (Kawato et al. 2019).

WSSV virions have a large (80–120 nm × 250–380 nm), ovoid or ellipsoid to bacilliform particle morphology and contain a 281-312 kilobase pair (kbp), circular double-stranded DNA genome and a trilaminar envelope that sometimes can display a unique, tail-like appendage (OIE 2019). Nucleotide sequence analysis of WSSV from crustaceans 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 (Flegel 2001; Tang et al. 2012; Oakey & Smith 2018; Oakey et al. 2019). DNA sequence variation was 0.68% among completely sequenced WSSV strains originating from Thailand, China and Taiwan (Marks et al. 2004). However, over time and with infection of a wider range of hosts, accumulation of deletions in the WSSV genome have resulted in significant genome shrinkage due to loss of selected (possibly redundant) genes

(Zwart et al. 2010; Tang et al. 2012; Jiang et al. 2017; Oakey & Smith 2018; Kawato et al. 2019). Sequencing of variable regions of the genome such as repeated DNA sequences within open reading frames (ORFs) and short tandem repeats (STRs) may provide an opportunity to discriminate between different WSSV strains (Wongteerasupaya et al. 2003), thus providing a means of epidemiological tracing of infection origins and transmission (Pradeep et al. 2008; Tang et al. 2012; Jiang et al. 2017; Oakey & Smith 2018; Oakey et al. 2019). Sequencing found the WSSV strain isolated from cultured Australian *P. monodon* on the Logan River had a relatively small but unique genome (286 kbp) with 91-97% homology compared to previously reported WSSV sequences, with the Australian genome clustering with two WSSV isolates from China (Oakey & Smith 2018). The source of the WSSV incursion on the Logan River and in Moreton Bay remains cryptic (Oakey et al. 2019), but is widely considered most likely to be linked to widespread use of frozen imported uncooked WSSV positive prawns as bait by recreational anglers (Diggles 2017; Scott-Orr et al. 2017).

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 a general rule of thumb, WSSV variants with smaller genomes have increased virulence (Marks et al. 2004), possibly because they may replicate more efficiently than those with larger genomes, however environmental and host factors will also influence the outcomes of WSSV disease incursions (Jiang et al. 2017; Kawato et al. 2019; Oakey et al. 2019). 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 and potentially other decapod hosts.

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*) (see Flegel 2007), 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 2019; Pradeep et al. 2012; Stentiford et al. 2009). 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 (Table 1), including *Penaeus monodon* (giant tiger prawn), *P. merguiensis* (banana prawn) and *P. japonicus* (kuruma prawn)—as well as *Metapenaeus* spp., mud crabs (*Scylla* spp.), sand crabs (*Portunus* spp.), and several freshwater crustacean species including *Macrobrachium rosenbergii* (giant freshwater prawn), *Cherax quadricarinatus* (red claw crayfish), *C. destructor albidus* (yabby) and *C. tenuimanus (*marron). Other non-decapod species can also be potential carriers of the virus (Table 2).

WSD has not been officially reported in wild (as opposed to farmed) prawns, however there is evidence of WSSV incursions into new geographic areas causing disease outbreaks in wild crayfish (Baumgartner et al. 2009). It is notable that some wild prawns in Moreton Bay had WSSV qPCR Ct values similar to those of *P. monodon* which were dying in prawn ponds on the Logan River

(Biosecurity Queensland 2018). It is also well known that many species of wild crustaceans can develop clinical WSD soon after they are bought into captivity (Lo et al. 1996; Brummett et al. 2014), hence it appears possible that wild crustaceans can experience mortality due to WSD if the virus is introduced into naïve host populations and/or wild hosts are exposed to adverse environmental conditions. WSSV infection can be transmitted to prawns, crabs, crayfish and lobsters 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; Bateman et al. 2012; Raja et al. 2015). Susceptibility to clinical disease and mortality through ingestion (*per-os* exposure) has been demonstrated in freshwater crayfish species indigenous to North America such as *Procambarus clarkii* (red swamp crayfish; see Wang et al. 1998; Jiang et al. 2017) and *Orconectes punctimanus* (spothanded crayfish; see Richman et al. 1997), 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), and several European marine and freshwater crustacean species including European lobsters (*Homarus gammarus*)(see Corbel et al. 2001; Bateman et al. 2012). The *per-os* route is more effective for establishing WSSV infections than horizontal exposure through the water (Soto & Lotz 2001, Raja et al. 2015), but less effective than injection (Thuong et al. 2016). Ingestion of infected tissue can 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 exposure can occur in *C. quadricarinatus* and *C. destructor albidus* (see Shi et al. 2000; Soowannayan & Phanthura 2011). Mortalities reached 100% in *C. quadricarinatus* in Thailand within 16 days of being fed WSSV infected prawn meat (Soowannayan & Phanthura 2011). In contrast, ingestion of WSSV-infected tissue by *C. destructor albidus* established a subclinical infection that required stress to promote disease and mortality, suggesting this species might have higher resilience to WSD than penaeid prawns infected via the *per-os* route (Edgerton 2004). However, Bateman et al. (2012) found that the limiting factor in the rapid appearance of WSD in European lobsters fed WSSV infected tissue was the initial dose; a low-level infectious dose established latent infection, while a high-level dose progressed more rapidly to disease. The susceptibility of many other Australian freshwater crayfish species to WSD remains unknown, however they are all assumed to be susceptible to infection and disease.

WSSV infection can occur at high prevalence in wild prawns and other crustaceans in regions where WSD is endemic in farmed crustaceans (Baumgartner et al. 2009; Cavalli et al. 2010; Chapman et al. 2004; de la Peña et al. 2007; Lo & Kou 1998; Withyachumnarnkul et al. 2003), often with evidence of significant seasonal variation (such as prevalences as high as 75-93% in broodstock *P. monodon* during the summer monsoon season, see Aftabuddin et al. 2014, Debnath et al. 2014). 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). In contrast, adverse impacts at the population level have not been reported in wild crustaceans in areas where WSSV has been introduced (de la Peña et al. 2007; Flegel 2009; Maeda et al. 1998b). Nevertheless, sub-clinical WSSV infections can revert to the disease state after periods of stress (Lo et al. 1996). This suggests that populations of wild crustaceans adversely affected by environmental stressors (e.g. adverse environmental conditions, rapid drops in water temperature or exposure to pollutants such as pesticides and

Table 1 Decapod crustaceans endemic or introduced into Australia which are known to be susceptible to infection with WSSV (Department of Agriculture 2019)

Species	Subclinical infection	Clinical disease	Comments
Black tiger prawn[a] (*Penaeus monodon*)	Yes	Yes	Highly susceptible
Brown tiger prawn[a] (*Penaeus esculentus*)	Yes	Yes	
European shore crab (*Carcinus maenas*)	Yes	No	Potential carrier
Fiddler crab (*Uca* spp.)	Yes	Yes	Disease at high doses only
Freshwater crayfish[a] (*Cherax* spp.)	Yes	Yes	
Giant crayfish (*Astacopsis* spp.)	Yes	Yes	Assumed susceptible
Giant freshwater prawn[a] (*Macrobrachium rosenbergii*)	Yes	Yes	
Giant Tasmanian crayfish (*Astacopsis gouldi*)	Yes	Yes	Assumed susceptible
Gippsland spiny crayfish (*Euastacus kershawi*)	Yes	Yes	Assumed susceptible
Green tiger prawn[a] (*Penaeus semisulcatus*)	Yes	Yes	
Greentail prawn[a] (*Metapenaeus bennettae*)	Yes	Yes	
Gulf banana prawn[a] (*Penaeus merguiensis*)	Yes	Yes	
Jelly prawns[a] (*Acetes* spp.)	Yes	Yes	
Kuruma prawn[a] (*Penaeus japonicus*)	Yes	Yes	
Mangrove swimming crab (*Thalamita crenata*)	Yes	No	Potential carrier
Mud crab[a] (*Scylla serrata*)	Yes	Yes	Disease at high viral loads
Red claw crayfish[a] (*Cherax quadricarinatus*)	Yes	Yes	
Red endeavour (greasyback) prawn[a] (*Metapenaeus ensis*)	Yes	Yes	
Sand crab[a] (*Portunus pelagicus*)	Yes	Yes	Disease at high viral loads
Smooth crayfish (*Geocherax* spp.)	Yes	Yes	Assumed susceptible
Spiny crayfish (*Euastacus* spp.)	Yes	Yes	Assumed susceptible
Three spot swimming crab[a] (*Portunus sanguinolentus*)	Yes	Yes	Disease at high viral loads
Tropical spiny lobster (*Panulirus* spp.)	Yes	Yes	Disease at high viral loads
Yabbies (freshwater) (*Cherax destructor albidus*)	Yes	Yes	

[a] Naturally susceptible. Note: Other species are likely to be susceptible or shown to be experimentally susceptible

Table 2 Non-decapod carriers of WSSV (Department of Agriculture 2019)

Common name	Subclinical infection	Clinical disease	Comments
Annelids/polychaetes	Yes	No	Potential carriers
Aquatic insects	Yes	No	Potential carriers
Bivalve molluscs	No	No	Mechanical vectors
Brine shrimp (*Artemia* spp.)	Yes	No	Potential carriers
Copepods- planktonic	Yes	No	Potential carriers
Copepods- parasitic	Yes	No	Potential carriers
Piscivorous birds	No	No	Mechanical vectors
Rotifers	Yes	No	Potential carriers

herbicides) may experience disease and/or "silent mortalities" due to WSSV infection (Stentiford et al. 2012, Shields 2012, OIE 2019), as was reported for prawns infected with *Baculovirus penaei* (BP) by Couch and Courtney (1977). Viral disease is therefore likely a contributing factor to fluctuations in populations of wild crustaceans; however, it is often overlooked in fisheries research and stock assessment (Harvell et al. 2002, 2004) because of the difficulty in gathering convincing evidence. The difficulty in gathering this evidence may be due to 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. The main reason for the limited evidence of WSD in wild populations is likely 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).

There is no evidence that WSSV can infect or cause disease in higher organisms, including humans.

1.3 World distribution

WSD 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). WSSV was also detected in Spain and Australia in 2000–01, however in both cases, successful containment and eradication were reported (East et al. 2004; Stentiford and Lightner 2011). The spread of WSSV between countries and regions has been linked primarily to translocations of live prawns for aquaculture or to imported uncooked frozen WSSV infected prawns used either as feed for broodstock crustaceans or finding their way into aquatic environments as processing waste or bait (Durand et al. 2000; Nunan et al. 1998; Diggles 2017; Scott-Orr et al. 2017).

The presence of WSSV has been reported officially by many countries in the Asia–Pacific region (Australia, Bangladesh, Brunei, China, India, Indonesia, Japan, Malaysia, Pakistan, Philippines, Singapore, South Korea, Sri Lanka, Taiwan, Thailand, Vietnam), Africa and the Middle East (Egypt, Madagascar, Mozambique, Saudi Arabia), and the Americas (Argentina, Brazil, Colombia, Ecuador, Guatemala, Honduras, Mexico, Nicaragua, Panama, Peru and the United States) (Martorelli et al 2010; Muller et al. 2010; OIE 2019, Tang et al. 2012). WSD has also 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 (Stentiford & Lightner 2011).

New Zealand, South Pacific island countries and most of Australia, are currently free of WSSV (East et al. 2004; OIE 2019). In Australia, 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; Scott-Orr et al. 2017). Australia was subsequently considered free of WSSV until late November 2016, when an outbreak of WSD was recorded in black tiger prawns (*Penaeus monodon*) cultured along the Logan River, in South East Queensland (DAF QLD 2017, Diggles 2017; Scott-Orr et al. 2017). Surveillance of wild populations of crustaceans in early 2017 confirmed the presence of WSSV infections in wild prawns and crabs in the Logan River and also in Deception Bay, in northern Moreton Bay, around 70km north from the affected aquaculture farms. The WSSV infections in wild crustaceans in Deception Bay persisted into March 2018, but were not detected in March 2019. Furthermore, WSSV was never detected from wild crustaceans in Moreton Bay during sampling undertaken during the winter months (Biosecurity QLD 2018a, 2018b).

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 may 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, molecular testing at a national reference laboratory using OIE approved methods is recommended (OIE 2019). 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 Case definition

1. **A suspect case**:
   - Gross signs of WSD (clinical signs and behavioural changes).

   OR

   - Hypertrophied nuclei in squash preparations of gill or cuticular epithelium; unusual aggregates in haemolymph by dark-field microscopy; inclusion bodies in histological sections in target tissues.

   OR

   - A positive or inconclusive result by any conventional PCR, nested PCR or qPCR assay for WSSV.

2. **A confirmed case:**

- Meets the criteria above for a Suspect Case

AND

- The sample is tested at AAHL in both the original assay that was employed and an alternative validated assay – e.g. either the CSIRO WSSV qPCR test (Sritunyalucksana et al. 2006) or the OIE qPCR (Durand and Lightner 2002), and shows a positive result in either test based on a typical amplification curve at any stage before PCR cycling is completed (not more than 45 cycles).

AND

- For an index case from an area previously considered uninfected, additional diagnostic evidence on repeat sampling is required including:
  a. Detection of virus in multiple animals or multiple species AND
  b. Conventional PCR amplicons are sequenced and found to be consistent with WSSV.

  Results need to be carefully assessed to ensure that they are unlikely to be the result of contamination.

1.4.2 Field methods: clinical signs and gross pathology

Clinical signs and gross lesions associated with WSD (Table 3) can vary between outbreaks and, alone, are insufficient for diagnosis. 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).

Table 3 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

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 (Department of Agriculture 2019). 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 2019) 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 enlarged in the cuticular epithelium.

It is also important to note that prawns dying from acute WSSV infection may not exhibit any clinical signs.

1.4.3 Laboratory methods

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.

Sample submission

In the first instance, where WSSV is suspected, clinical specimens must be sent to 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 national reference laboratory, the CSIRO Australian Animal Health Laboratory Fish Diseases Laboratory (AFDL), Geelong, for diagnostic confirmation.

The AFDL should be contacted directly to confirm information on what clinical material is required, how it should be collected and transported and provide notification of the number and type of samples being submitted, to satisfy AFDL requirements for rapid confirmation of a preliminary diagnosis of WSSV infection.

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 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 tissues placed in, fixative.

Samples for PCR testing
For larvae and postlarvae, immerse live animals directly in a minimum of 10 volumes of 70% v.v ethanol (analytical grade ethanol, not technical grade). For live juvenile and adult prawns, dissect either gill tissue (whole filaments) or pleopods (the paired swimming legs on the underside of the abdomen) and immediately place into a minimum of 10 volumes of 70% v.v. ethanol.

Samples for histopathology
For larvae and small postlarvae, live animals can be immersed directly into Davidson's fixative solution and left for 24–72 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 Davidson's AFA fixative (330 ml 95% ethanol, 220 ml of 100% formalin (37% formaldehyde), 115 ml glacial acetic acid and 335 ml tap water) ensuring that the hepatopancreas is injected liberally first, and that the whole specimen is thoroughly injected thereafter with 5-10% of its body weight of fixative. 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

Ideally, all laboratory procedures should comply with the OIE *Manual of diagnostic tests for aquatic animals 2018* (Aquatic Manual; OIE 2019). 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. The laboratory the samples are being sent to should be consulted if there is any doubt regarding the number of specimens to be submitted.

Table 4 compares the suitability of the different methods for screening and diagnosis.

Microscopy

There are two rapid microscopy 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 2019).

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).

Table 4 Comparison of advantages and disadvantages of white spot syndrome virus screening and diagnostic methods

Method	Surveillance in apparently healthy animals			Presumptive diagnosis of clinically affected animals			Confirmatory diagnosis of a suspect result from surveillance or presumptive diagnosis			Advantages of diagnostic method	Disadvantages of diagnostic method
	Early life stages	Juveniles	Adults	Early life stages	Juveniles	Adults	Early life stages	Juveniles	Adults		
Gross signs				+	+	+				Simple field technique	Poor specificity, presumptive only
Wet mounts				+	+	+				Simple field technique	Poor specificity, presumptive only
Histology		+	+	++	++	++				Low probability of misdiagnosis in high-level infections	Might not detect low-level infections; not field friendly; several days for result
TEM				+	+	+	++	++	++	Visualisation of virus	Sophisticated equipment required; laborious and technically complex; expensive; may not detect low-level infections; low throughput
Real time PCR	+++	+++	+++	+++	+++	+++				More sensitive than conventional PCR, quantitative, high throughput	As for conventional PCR
Conventional PCR	-	-	-	++	++	++				Sensitive and specific, capable of detecting low pathogen levels; rapid results	Hypersensitive; prone to contamination; relatively costly; does not discriminate between presence of infectious virus and non-infectious nucleic acid fragments
Sequencing							+++	+++	+++	Highly sensitive	Can be time delays getting results
In situ hybridisation				+	+	+	+++	+++	+++	Very sensitive; specific; reliable; can visualise infected tissues	Histological preparation required; laborious, low throughput
Bioassay				++	++	++				Demonstrates presence of viable pathogen	Several days/weeks for result; not specific; expensive; requires confirmation by other methods
LAMP	+	+	+	++	++	++				Rapid diagnostic results; cheap	May not detect low-level infection

PCR = polymerase chain reaction; TEM = transmission electron microscopy; LAMP = Loop-mediated isothermal amplification

Shaded area = the method is not recommended,

- the method is unavailable or unsuitable

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

++ the method has good diagnostic sensitivity and specificity,

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

Source: Modified from OIE (2019)- some of the methods listed in Table 4 may need CVO approval depending on Australian jurisdiction

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 WSSV infected 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 (Table 4; OIE 2019).

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 2019). 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.

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 flagella-like protrusion from the envelope (OIE 2019).

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 (OIE 2019). Details on how to perform these tests can be found in the original publications and in the OIE Aquatic Manual (Lo et al. 1996; Lo & Kou 1998; Sritunyalucksana et al. 2006; OIE 2019). Several PCR and real-time PCR kits for detection of WSSV DNA are also available from commercial 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.

Insitu 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 2019). 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.

Loop mediated isothermal amplification (LAMP)

The LAMP method amplifies the target nucleic acids under isothermal conditions, therefore needing no sophisticated machine for thermal cycling (OIE 2019). It is a relatively sensitive and rapid diagnostic method, and therefore may be useful for pond side testing, however this method may not detect low level infections (Table 4). Use of LAMP-based assays in Australia needs CVO approval, depending on jurisdiction.

Other methods

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 2019). 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 4.

In a suspected outbreak of WSD that is affecting any species of crustacean, PCR should be used initially as it provides the most rapid turnaround required to make a presumptive diagnosis. The standard approach is to screen with real time PCR (qPCR) with confirmation of positives by conventional PCR and sequence analysis of amplicons. Depending on samples collected, PCR-positive results may also be confirmed by histology and ISH. A definitive association can then be made between the presence of WSSV and observed histopathological tissue changes characteristic of WSD.

1.4.4 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.

OIE Definition of suspect case

For juvenile and adult shrimp: gross signs of WSD.

For shrimp at any life stage (larva to adult): mortality.

For shrimp and crab at any life stage (larva to adult): hypertrophied nuclei in squash preparations of gill and/or cuticular epithelium; unusual aggregates in haemolymph by dark-field microscopy; inclusion bodies in histological sections in target tissues.

OIE Definition of confirmed case

Suspect cases should first be checked by PCR or LAMP. If in a previously WSSV-free country/zone/compartment, where PCR results are positive, they should be confirmed by sequencing. Histopathology, probes and electron microscopy also can be used to confirm the case.

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

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
- quantitative polymerase chain reaction (qPCR) 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.

1.4.5 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 5 helps differentiate WSD from other significant exotic viral diseases most capable of causing mass mortalities in the penaeid species farmed in Australia— yellow head disease (YHD) caused by yellow head virus genotype 1 (YHV1), and Taura Syndrome (TS). 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, or yellowhead genotype 2 (YHV2)) associated with mid-crop mortality syndrome (Callinan & Jiang 2003; Callinan et al. 2003).

Three other viruses are also of interest—Taura syndrome virus (TSV), infectious myonecrosis virus (IMNV) and IHHNV. All three of these viruses 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 from TSV is considered low, as for WSSV, TSV can infect a wide range of crustaceans including not only cultured prawns, but also mud crabs (*Scylla serrata*) (Department of Agriculture 2019). Infectious myonecrosis virus (IMNV) can also infect a range of penaeids native to Australia (Gudkovs et al. 2015), while IHHNV has been reported from Australian-farmed prawns, where its presence causes deformities and significant decreases in pond productivity (Sellars et al. 2019).

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 2019). 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.

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.

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. Generally, when investigating high mortality rates in farmed prawns, initial screening of submissions for WSSV, YHV1, TSV, IMNV and GAV (YHV2) should be undertaken.

Treatment of infected crustaceans

Many and varied therapeutic treatments have been trialled experimentally to combat WSSV infection in prawns, with variable levels of efficacy. While various compounds have shown some levels of efficacy against WSSV *in-vitro* or in small scale laboratory experiments (e.g. Huang et al. 2019), there are currently no commercial reagents with proven abilities to completely clear WSSV infections in prawn ponds, or for prawn prophylaxis in the event of outbreaks of WSD (OIE 2019).

Table 5 Differential diagnosis of virus-induced mortalities that might occur in Australian-farmed prawns[a]

Disease name	White spot disease (WSD)	Yellow head disease (YHD)	Mid-crop mortality syndrome	Taura syndrome (TS)	Infectious myonecrosis (IMN)
Disease agent	WSSV	YHV1	YHV2 (GAV)	TSV	IMNV
Susceptible Australian-farmed species	*P. monodon*, *P. japonicus*, *P. merguiensis* *P. esculentus*	*P. monodon* *P. esculentus*	*P. monodon* *P. merguiensis* *P. esculentus*	*P. monodon,* *P. japonicus* *P. merguiensis*	*P. monodon*, *P. esculentus* *P. merguiensis*
Stage of grow-out	All	Usually 7–10 weeks poststocking	Usually >13 weeks poststocking	Usually 2–6 weeks post-stocking	Juveniles and subadults
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	Moderate (up to 70%) after stressful events
External appearance	Often white spots 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	Opaque white necrotic areas in abdominal muscle, red 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	Skeletal muscle
Inclusion body type	Intranuclear; eosinophilic (Cowdry type A) to basophilic	Intracytoplasmic; basophilic	Uncommon; intracytoplasmic ; basophilic	Intracytoplasmic; initially eosinophilic, then basophilic	Perinuclear basophilic inclusion bodies

YHV1= yellowhead virus genotype 1; GAV = gill-associated virus (also known as yellowhead virus 2, or YHV2); TSV = Taura syndrome virus, IMNV = infectious myonecrosis virus

a *Penaeus monodon*, *P. japonicus*, *P. merguiensis, P. esculentus* Note: TS features are as described for *P. vannamei*.

1.5 Resistance and immunity

Prawns possess pathogen defence systems that, although quite complex, differ substantially from those present in vertebrates (Flegel 2001; Musthaq & Kwang 2015). 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 relatively 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).

Heritable genetic components responsible for innate or adaptive immunity against WSSV in various crustacean species may explain how selective breeding from survivors of WSD epizootics can result in significant increases in resistance of subsequent generations to WSSV infection (Trang et al. 2019).

1.5.1 Responses to bacterial or fungal infections

The range of defence systems that prawns can mount against invading bacteria or fungi includes rapid haemolymph clotting, agglutination, antimicrobial peptides, 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 phagocytosis, apoptosis, encapsulation and haemocyte granuloma formation and melanisation via the prophenoloxidase cascade (Musthaq & Kwang 2015).

1.5.2 Responses to viral infections

The responses used by prawns to defend themselves against viral pathogens differ from those used in protection against bacterial or fungal pathogens (Wang et al. 2018). In prawns and other crustaceans, and perhaps arthropods in general, there is often no direct inflammatory response to viral infection. In the case of WSSV infection this is probably due to downregulation of genes responsible for inflammatory processes (Wang et al. 2018). As a result, persistent infection by one or more viruses can be common, which in the case of IHHNV + WSSV infections may result in viral interference that prolongs the lifespan of the host prior to eventual death from WSD (Bonnichon et al. 2006; Yan et al. 2016).

There is a general phenomenon that, when viruses like WSSV first emerge, epizootics ensue that are characterised by initial catastrophic and widespread crop losses (Lightner 2003). 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 naïve 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 appears capable of generating a 'quasi-immune' defence response after WSSV exposure, which can protect against mortality following subsequent challenge with WSSV (Venegas et al. 2000). 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

There are currently no specific vaccines commercially available to protect farmed prawns against WSSV infection (Feng et al. 2017). Typically, vaccination trials which administer prawns with inactivated WSSV prior to exposure to the pathogen only delay mortality, but do not prevent it (Melena et al. 2015), although exposure to a DNA vaccine comprised of recombinant baculovirus envelope protein (VP-28) has increased short term (2 week) survival in some trials (Musthaq & Kwang 2015).

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 (Musthaq & Kwang 2015). This defence system also 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; Feng et al. 2017; Heidarieh et al. 2010; Huang & Song 1999). Musthaq & Kwang (2015) found that "specific immune priming" from administration of immunostimulating compounds can increase short term survival after challenge with WSSV, however there remains little published evidence that such an approach can significantly increase survival through a full pond production cycle. Similarly, various attempts to synthesise vaccines using RNAi (dsRNA) and recombinant DNA technologies have shown promise in the laboratory, but to date none have been developed into a successful commercial product (Feng et al., 2017).

1.6 Epidemiology

WSSV can infect a wide variety of decapod crustaceans, but causes significant disease and economic impact mainly in farmed prawns. In the 1990s, the disease exhibited pandemic behaviour in Asia and caused substantial economic impacts following its emergence in the Americas (Lightner 2003). When first apparent in any particular region, WSD characteristically caused epizootics in farmed prawns. The epizootics 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 epizootics. 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 (Gao et al. 2011; Jiravanichpaisal et al. 2004; Rahman et al. 2007; Tendencia et al. 2010; Vidal et al. 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), but can also occur later in the production cycle. For example, during the WSD outbreak on the Logan River, prawns in the index pond of the first affected farm started dying 69 days post stocking, 6 days after intake of unfiltered water from the river, and 4 days after a transient drop in water temperature from 24.6°C to 21.9°C (Diggles 2017). 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). A reduction in water temperature is an important risk factor for initiation of disease in populations of cultured prawns that are exposed to WSSV (Corsin et al. 2005).

1.6.2 Persistence of the pathogen

The OIE Aquatic Manual (OIE 2019) provides information, summarised in Table 6, on protocols for chemical and physical treatment that effectively destroy WSSV infectivity. Over the years a large number of research groups have examined how to inactivate WSSV under various laboratory conditions, resulting in publication of a range of effective dose rates (Table 6). The data presented in Table 6 are conservative. In some cases, lower doses have been found to inactivate WSSV (OIE 2019), however these are not reported in Table 6, because other studies found the same dose rates were not 100% effective for inactivation of WSSV. These differences between studies may be due to differences in methodology, pathogenicity of viral strains, initial viral dose studied or the susceptibility of hosts used in bioassays to determine virus viability post-treatment.

In water

There are reports that WSSV particles kept in sterile sea water in the dark can remain infectious at temperatures of 30°C for up to 30 days, for over 40 days at 25°C and 120 days at 15°C (Maeda et al. 1998a; Momoyama et al. 1998; Oidtmann et al. 2018). However, other studies have reported WSSV infectivity for only 48 hours in sea water (Wang et al. 2002). Free WSSV particles released into pond water during WSD outbreaks have been shown to remain infectious for at least 3–4 days (Flegel et al. 1997), and up to 12 days at 29-33°C (Kumar et al. 2013), however the presence of planktonic non-penaeid hosts which can act as vectors and reservoirs of infection increases the

risk of longer term persistence of WSSV within the pond environment (Esparza-Leal et al. 2009; Mendoza Cano et al. 2014).

Table 6 Agents and conditions that inactivate free WSSV virions in the environment

Physical agents	**Inactivation conditions**			**Reference**
Heat	>55°C for 90 min or >70°C for 5 min			1
	>60°C for 20 min or >70°C for 10 min			3
Dessication	>3 hrs			2
UV light [a]	**Intensity**	**Time (sec)**	**UV dose (mJ/cm²)**	
	2.56 x 10^2 µW/sec	3600	921 mJ/cm²	1
	1 x 10^2 µW/sec	100	10 mJ/cm²	2
	2.56 x 10^2 µW/sec	1200	307 mJ/cm²	3
	1 x 10^2 µW/sec	300	30 mJ/cm²	4
pH	Inactivated by pH 1 for 10 min, pH 3 for 60 min or pH 12 for 25 min at 25°C			1, 3
Chemical agents	**Inactivation conditions**			**Reference**
Chlorine in tanks	Inactivated by sodium hypochlorite at 200 ppm for 10 min			3
	Inactivated by sodium hypochlorite at 0.5-1 ppm for 10 min			2, 4
Chlorine in ponds	Achieve 30 ppm sodium hypochlorite in ponds, ensure residual of > 5 ppm after 24 hrs. Apply chlorine in evening to reduce photodegradation. Leave treated pond for 40 days to dispose of prawn carcasses, then re-treat pond water (10 ppm chlorine>30 min) and discharge once chlorine <3 ppm			5
Ethanol	Inactivated by 30% ethanol for 1 minute			2
	Inactivated by 30% ethanol for 10 minutes			4
Formalin	Inactivated by formalin at 5 g/L (5000 ppm) for 10 minutes			2
	Inactivated by formalin at 0.25 % v/v (2500 ppm) for 10 minutes			4
Iodine	Inactivated by povidone iodine at 200 ppm for 10 min			3
	Inactivated by povidone iodine at 2.5 ppm for 10 min			2, 4
Quaternary ammonia	Inactivated by benzalkonium chloride at 75 ppm for 10 min			1
	Inactivated by benzalkonium chloride at 200 ppm for 10 min			3

a UV = ultraviolet, Total UV dose (in mJ/cm2) = $\frac{\text{intensity } (\mu W/cm^2) \times \text{duration of exposure (sec)}}{1000}$

ppm = parts per million = mg/L

References: 1 = Chang et al. (1998), 2 = Nakano et al. (1998), 3 = Balasubramanian et al. (2006), 4 = Oseko et al. (2006), 5 = Diggles (2017) describing Biosecurity QLD "destroy and let lie" response to WSD outbreak on Logan River.

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 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 (Raja et al. 2015; 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 tissue

Prior and Browdy (2000) found that WSSV could remain infectious in decaying prawn tail tissue at ambient temperatures 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 one potential reason for the inconsistent data. Importantly, both studies clearly indicate that dead and decaying prawn carcasses are a significant risk factor in transmitting WSSV. Furthermore, Reddy et al. (2011) found that some WSSV in prawn carcasses cooked at 100°C for up to 30 minutes remained viable, suggesting that the virus remains well protected while within infected tissues inside prawn carcasses.

In sediment

It is known that WSSV can remain viable for at least 19 days in dried pond sediments, and between 35 and 40 days in moist pond sediments under non-drainable conditions (Kumar et al. 2013). The presence of viable WSSV in benthic sediment dwelling vectors likely to act as reservoirs of infection such as polychaetes (Desrina et al. 2013) and rotifers or their eggs (Yan et al. 2004; Zhang et al. 2006) means that the virus is likely to be very persistent in untreated pond environments which cannot be completely dried out.

On farm equipment

It is highly likely that contaminated farm equipment such as nets or other equipment moved between ponds can pose a substantial risk of transmitting WSSV infection, if not disinfected appropriately between uses.

1.6.3 Modes of transmission

WSSV infection has been documented in all life stages of penaeid prawns and other decapod hosts, and can be transmitted both vertically and horizontally.

Vertical transmission

Prawn larvae can become infected during spawning, however true vertical transmission (intra-ovum) of WSSV to the progeny has not been demonstrated (OIE 2019). 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), while others suggest transmission is due to larvae contacting viral particles shed by infected broodstock (Pradeep et al. 2012; Verbruggen et al. 2016). For these reasons, 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) which can have very high viral loads (Oidtmann and Stentiford 2011), 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). Other decapods including crabs and lobsters are also highly susceptible to infection with WSSV via ingestion of infected tissues (Oidtmann and Stentiford 2011). For example, Bateman et al. (2012) found that 94% of European lobsters (*Homarus gammarus*) fed 50 mg of infected commodity prawn tissues became infected and experienced slightly increased mortality rates compared to uninfected lobsters. They concluded that the limiting factor in the rapid appearance of WSD in European lobsters was the initial dose; a low-level infectious dose establishes latent infection, while a high-level dose progresses more rapidly to disease (Bateman et al. 2012).

Reservoirs of virus

Wild broodstock

The prevalence of WSSV in wild crustaceans from various parts of the world is shown in Table 7. 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 crustacean populations in prawn-farming regions in which WSSV remains endemic (Lo & Kou 1999). There is also a large body of evidence showing seasonal differences in WSSV infection prevalence (de la Peña et al. 2007; Lo et al. 1997a; Mushiake et al. 1998; Withyachumnarkul et al. 2003), with highest prevalences often occurring during the summer monsoon season (Aftabuddin et al. 2014, Debnath et al. 2014). It is notable that during the incursion in Moreton Bay, WSSV infections were only detected in samples of wild crustaceans taken during late summer, and no infections were detected during the winter months (Biosecurity QLD 2018a, 2018b). Variable rates of infection of wild crustaceans, particularly when prevalence may be very low due to seasonal conditions, may reduce the success of detection of infection in surveillance programs using sample sizes based on a higher assumed infection rate.

In regions where WSSV has been endemic in wild crustaceans for some time, ISH and qPCR 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). However, it is notable that some wild prawns in Moreton Bay had WSSV qPCR Ct values similar to those of *P. monodon* which were dying in prawn ponds on the Logan River (Biosecurity Queensland 2018), suggesting that WSD probably does occur in at least some individuals within naïve populations of wild crustaceans during WSD incursions.

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

Table 7 Published prevalence estimates for WSSV in wild crustaceans

Species	Prevalence (%)	Location	Reference
Prawns			
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]	Thailand	Withyachumnarnkul et al. (2003)
Penaeus monodon	Wet season 10 (*n* = 713) [a] Dry season 0.3 (*n* = 714) [a]	Philippines	de la Peña et al. (2007)
Penaeus monodon	Shallow water 63.3 (*n* = 90) [b] Deep water 23.3 (*n* = 90) [b]	Bangladesh	Debnath et al. (2014)
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)
Penaeus esculentus [d]	Summer 25.7 (*n* = 4432) [d] Winter 0 (*n* = 347) [d]	Australia	Biosecurity Queensland (2018)
Metapenaeus bennettae [d]	Summer 25.7 (*n* = 4432) [d] Winter 0 (*n* = 347) [d]	Australia	Biosecurity Queensland (2018)
Crabs			
Portunus sanguinolentus	87.5 (*n* = 48)	Taiwan	Lo & Kou (1998)
Portunus pelagicus	60 (*n* = 5)	Taiwan	Lo & Kou (1998)
Scylla serrata	60 (*n* = 10)	Taiwan	Lo & Kou (1998)
Charybdis feriatus	80 (*n* = 5)	Taiwan	Lo & Kou (1998)
Thalamita crenata [d]	Summer 25.7 (*n* = 4432) [d] Winter 0 (*n* = 347) [d]	Australia	Biosecurity Queensland (2018)
Portunus pelagicus [d]	Summer 25.7 (*n* = 4432) [d] Winter 0 (*n* = 347) [d]	Australia	Biosecurity Queensland (2018)

n = number of prawns in study

a One-step PCR

b Two-step PCR

c PCR protocol not specified

d qPCR done on pooled samples – results may significantly overestimate actual prevalence by assuming all crustaceans in positive pools were infected – for more information on pooling samples see Laurin et al. (2019).

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

(69%) of ponds stocked with one-step PCR-negative postlarvae. A subsequent study in Taiwan had similar outcomes (Peng et al. 2001). 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 (Diggles 2017).

Other decapod crustaceans

All decapod crustaceans including not only penaeids (*Penaeus* spp., *Metapenaeus* spp.), but also freshwater shrimp (*Macrobrachium* spp.), glass shrimp or jelly prawns (*Acetes* spp.), crabs (mud crab *Scylla serrata*, blue swimmer crabs *Portunus* spp., mangrove swimming crab *Thalamita crenata*), lobsters (*Panulirus* spp., *Homarus* spp.) and crayfish (*Cherax* spp., *Procambarus* spp.) can carry subclinical WSSV infections (Bateman et al. 2012; Pradeep et al. 2012; OIE 2019). Many of these species can enter prawn ponds either via unfiltered 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; Soowannayan & Phanthura 2011; 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 suggest that WSD 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 relatively low, but depend on the virulence of the strain, the prevalence of infection and the infection load in the carriers (Bateman et al. 2012).

Other carriers

Planktonic copepods, parasitic copepods, barnacles, rotifers, insect larvae or other zooplankton can act as reservoir hosts or mechanical vectors and are potential sources of WSSV infection in farmed prawns (Corre et al. 2012; Esparza-Leal et al. 2009; Liu et al. 2000; Lo et al. 1996; Mendoza Cano et al. 2014; Overstreet et al. 2009; Pradeep et al. 2012; Stentiford et al. 2009; Yan et al. 2004; Zhang et al. 2006) (Table 2). WSSV can also replicate in polychaete worms (*Marphysa* spp., *Dendronereis* spp.) which are another potential source of WSSV infection (Desrina et al. 2013; Haryadi et al. 2015; 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. (Chang et al. 2002; Hameed et al. 2000), the evidence that WSSV positive *Artemia* can transmit infection to prawns and cause disease is equivocal (Zhang et al 2010).

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). Vazquez-Boucard et al (2012) found that Pacific oysters (*Crassostrea gigas*) can accumulate WSSV in their gills and digestive glands, and could be used to detect the presence of WSSV in water intakes up to 16 days prior to WSD outbreaks in cultured prawns. It is possible, therefore, that a range of filter feeding bivalve species could also act as mechanical vectors for WSSV, as well as sentinel species for surveillance monitoring.

1.6.4 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). It appears that freshwater prawns, crabs, lobsters and crayfishes are more tolerant of WSSV than penaeid prawns (Bateman et al. 2012; Rajendran et al. 1999; Stentiford et al. 2009). Moreover, within species and life stages, the outcome of challenge by WSSV depends on their general health, the infective dose they are exposed to, and history of exposure to non-lethal infection with WSSV or other viruses (Section 1.5) (Bateman et al. 2012; 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 2019). 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 (Gao et al. 2011; Lin et al. 2011; Rahman et al. 2007; Vidal et al. 2001). Gao et al. (2011) found the optimum pH for WSSV replication was 8, and that viral replication was reduced at pH >8.5, however in contrast changes in salinity within the range of 15-35 ‰ had no significant effect on viral replication.

1.7 Impact

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 (Lightner 2003). 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 (Stentiford et al. 2012). 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.

In Australia, the WSD incursion and its associated biosecurity response in 2016-17 resulted in complete shutdown of the prawn farming industry along the Logan River, as well as complete shutdown of bait prawn and bait worm fisheries within Moreton Bay. Production losses for the Logan River prawn farming industry in 2016–17 were estimated to be around $43 million (Scott-Orr et al. 2017). Furthermore, the economic impact of the biosecurity control zone on the gross value of production of commercial wild fisheries for polychaetes, yabbies, mud and blue swimmer crabs, and prawns between December 2016 and April 2017 alone was estimated as $20.5 million (Scott-Orr et al. 2017). Recreational fishing activities were also affected throughout south-east Queensland, due to restrictions on movements of crustaceans and loss of bait prawn, yabby and worm supplies, and the cost of the biosecurity response by the Queensland Government was estimated to be in excess of $26 million (Scott-Orr et al. 2017). The Seafood Importers Association of Australasia Inc. estimated that the 6 month suspension of imported uncooked prawns that was enacted at the time to allow biosecurity measures at the border to be repaired and upgraded resulted in a failure to sell several thousand tonnes of seafood product estimated to cost $383 million to Australian businesses.

WSD outbreaks have been managed overseas by the widespread use of specific pathogen-free (SPF) white shrimp (*Penaeus vannamei*). White shrimp are less susceptible to WSD than *P. monodon*, grow faster and thus can usually be grown to a marketable size and emergency harvested before significant production losses occur due to WSD. These sort of management strategies which farm "around the disease" but do not prevent the introduction of WSSV into production ponds result in introduction of large quantities of WSSV infected prawns into commodity markets (Bateman et al. 2012; Scott-Orr et al. 2017), but such strategies have alleviated the economic impact of WSD in most countries in which farmed prawn production has been seriously affected. However, *P. vannamei* is not native to Australia, therefore this option is not available to the prawn farming industry here. The WSD incursion in Moreton Bay caused significant disruption to Australian *P. monodon* selective breeding programmes and has renewed interest in accessing or developing supplies of SPF *P. monodon* broodstock in Australia (ProAqua 2018). Even today with the emergence of bacterial diseases such as early mortality syndrome (EMS) and other viral diseases such as shrimp haemocyte iridescent virus (SHIV, also known as Decapod iridescent virus 1 (DIV1)) in Asia, WSSV remains 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

2.1 Introduction

History has shown that 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* (see Diggles 2017). However, it cannot be discounted that disease may emerge in other crustaceans being farmed or reared by hobbyists in aquaria. 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) may persist and be detected in surveillance surveys (Biosecurity QLD 2018a, 2018b). 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 would also be applied to other crustacean aquaculture enterprises or to wild crustacean populations.

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.

Control options

The feasibility of containing a detection of WSSV or 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 may have 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.

2.2 Methods to prevent spread and eliminate pathogens

2.2.1 Quarantine and movement controls

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; and vector control. These methods are discussed in the following sections.

Farm types

All commercial 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 subclassified 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.

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.

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.

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 unlikely, unless heavy rainfall causes overflows from ponds or reservoirs into natural watercourses.

Hatcheries

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

Wild populations

The waterways adjacent to aquaculture farms from which water is taken in and which receive effluent water from those farms are most likely to be fully open systems, in which the wild crustaceans within them are able to move freely throughout their natural ranges.

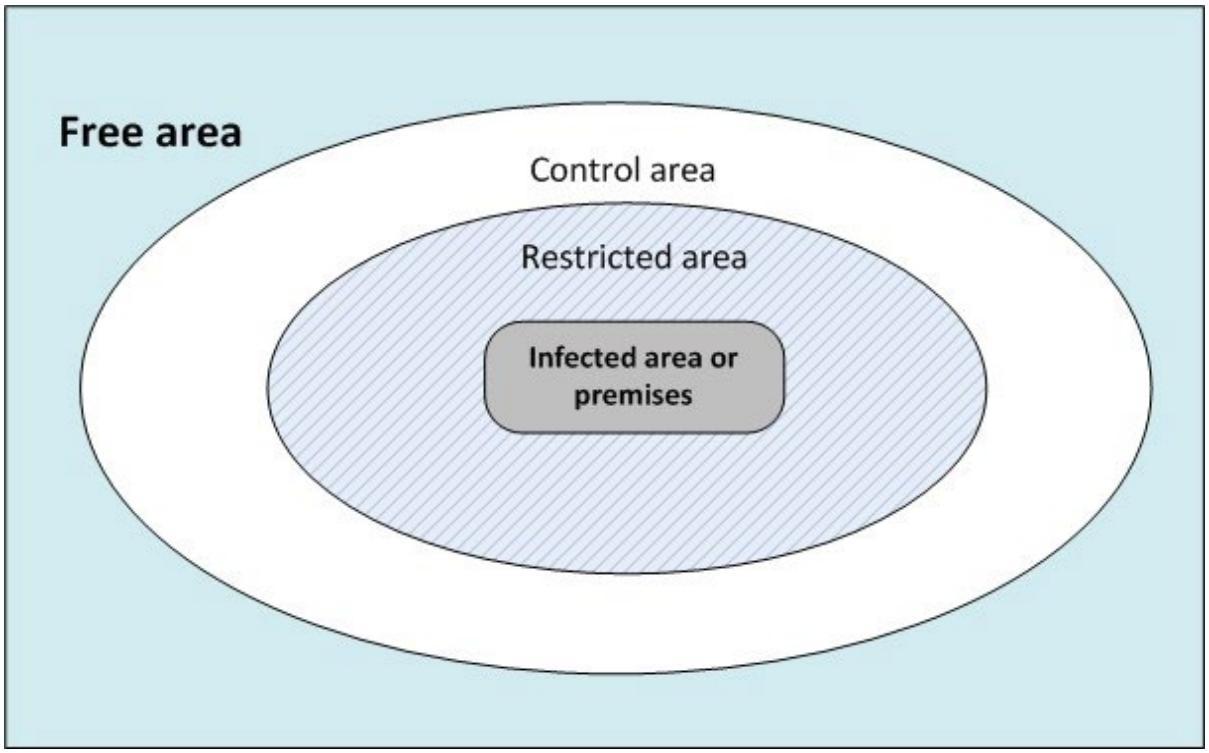
Establishment of quarantine areas

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

Establishment of specified areas (see AQUAVETPLAN Enterprise Manual Section A for more details), including:

- declared area—infected, restricted and control areas
  - infected area or premises—the premises (for example, farm) or area (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 WSSV remains unassessed).

Figure 1 Establishment of specified areas to control white spot disease



In the declaration of quarantine areas, the factors listed in this section need to be taken into account.

Epidemiological and surveillance information

While epidemiological and surveillance data on infection distribution and susceptible populations is the best means of establishing zones, this is unlikely to be comprehensively available, especially early in an epizootic. Consideration of the factors in this section may allow prediction of likely transmission/dispersal and early establishment of zones in the absence of good surveillance data.

Natural factors that could facilitate or hinder transmission:

- the contiguous distribution of wild crustacean populations
- movements of crustacean reservoirs
- water movements that can disperse virus (use sea distances and current directions instead of Euclidean distance between farms to set declared areas)
- natural catchment divisions that may contain infection because water or crustaceans do not move between catchments.

Anthropogenic factors (industry)

- connectedness of aquaculture facilities and transmission of infection between facilities—for example, company structure and movement of stock or equipment/fomites and product to processing, shared jetty
- presence of other aquaculture facilities with susceptible species
- movement of consumer products
- facilitation of business continuity where possible—for example, where possible, declared areas should facilitate movement of essential equipment, personnel and product between farms or to processing plants.

Anthropogenic factors (recreational fishing)

- establish areas based on likely historical movement of fishers that may have transmitted infection
- structure areas to minimise disruption to recreational fishing (where possible)
- structure areas so that movement bans can be legally and practically enforced.

Practices that must be considered when implementing response options include:

- possibility of the existence and presence of a previously unknown susceptible species of asymptomatic carriers
- other water user movements
- discharge of effluent from processors and farms
- disposal of dead crustaceans and products
- decontamination.

Movement controls

2.2.2 Zoning and compartmentalisation

It is sometimes possible to maintain a sub-population of crustaceans with distinct aquatic animal health status (for example, infected or free of WSSV). This can be done on a geographical basis (referred to as zoning) or on a common biosecurity basis such as management practices (referred to as compartmentalisation).

Zoning

If WSSV were to become endemic in regions of Australia, a zoning policy specific for WSSV would 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 WSSV 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*[1] and in the OIE *Aquatic Animal Health Code* (OIE 2018a).

Zoning for WSSV might prove problematic, 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, and access to SPF broodstock would be highly recommended.

The varied transmission possibilities, the many susceptible hosts and the difficulty of disinfecting aquatic environments make it difficult to protect WSSV-free zones in the long term. Zoning programs for WSSV have not been implemented successfully in any other 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 eventually introduced, purportedly by illegal imports of infected postlarvae (Magbanua et al. 2000). However, the ultimate success of any zoning strategy will depend significantly on geographic and hydrological factors, as well as population characteristics of endemic crustaceans, all of which are likely to be very different in Australia compared to other parts of the world.

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1 http://www.agriculture.gov.au/animal/aquatic/aquaplan

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 (compartments) rather than impose zones accompanied by restrictions in crustacean product 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 SPF or WSSV-free *P. monodon* broodstock reared in biosecure facilities would provide similar benefits. Domesticated, selectively bred *P. monodon* became available in 2010 and, despite the setback of the 2016-17 WSD incursion, these may eventually replace the use of wild-caught broodstock.

Compartmentalisation

A compartment means one or more aquaculture establishments under a common biosecurity management system containing an 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.

A compartment does not have to be contiguous facilities—it can apply to a series of farms over a large area, including over several jurisdictions. It must have in place a biosecurity management system that meets guidelines provided in Chapters 4.1 and 4.2 of the OIE Aquatic Animal Health Code (OIE 2018c) and this system must have been documented by the competent authority (that is, the veterinary authority of the jurisdiction).

Disease management in aquatic environments

The establishment of Disease Management Area (DMA) boundaries during an Emergency Aquatic Animal Disease event presents particular difficulties requiring detailed consideration beyond that normally required for terrestrial animal disease control. Water movement through and around farms, within streams or rivers, and in the marine environment represents a substantial risk for spread of disease through transfer of infectious pathogens in the water column, movement of infected material (particularly on suspended organic and inorganic matter), and any infected wild organisms.

For example, although an infected area may be established around an individual land-based hatchery or farm, natural water bodies adjacent to the infected area as well as in the same catchment should be considered for monitoring and control measures. The establishment of DMA boundaries around marine farms or wild fisheries may need comparatively large areas that must take into consideration local currents, natural barriers and the normal range of susceptible wild species.

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

Thus, rather than property boundaries, the geography, water flow, distance between farming areas and the range of susceptible species will define where DMA boundaries are placed, and the maintenance of those boundaries should be informed by surveillance. An example of how this can

be done comes from Moreton Bay where the original disease management area around the Logan River prawn farms established in December 2016 was extended to include all of Moreton Bay and its catchments in March 2017 once crustaceans infected with WSSV were found in Deception Bay, 70 km to the north (Biosecurity QLD 2018; Diggles 2017).

Establishment of DMA boundaries and their classification must also take into account potential mechanisms by which disease may move beyond these boundaries. In most circumstances it is advisable to overestimate the size of DMAs and reduce their area as the response takes effect. In most cases, in the initial response, the DMA boundaries will need to include the whole of a catchment area in freshwater systems and complete bays or regions in marine environments.

2.2.3 Tracing

Thorough investigation of any WSSV 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 a disease outbreak is the process of retrospectively determining the method, route and pattern of disease spread. Tracing investigations are crucial in determining all confirmed and potential locations of the disease and its causative agent, as well as defining restricted and control areas. The information gathered from these investigations will assist in determining the most appropriate response action. The immediate steps required are to trace-back all contacts with infected crustaceans, premises and sites (to establish the origin of the outbreak) and to trace-forward all contacts with infected fish, premises and sites (to establish the current location and potential spread of infection).

These items 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 or burley (if properly 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.
- natural movements of wild or feral crustaceans and water should be modelled and surveyed.

Wild and farmed crustaceans in close proximity to a farm or locality in which WSD or WSSV infection has been diagnosed are 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).

2.2.4 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 2019). The manual also provides specific information on surveillance for WSSV. For information on pooling samples during surveillance, see Laurin et al. 2019.

2.2.5 Treatment of infected host species

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

2.2.6 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. Emergency permit use may be required when unapproved chemicals are best suited for the purposes of disinfection or control in an emergency response situation.

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 – small prawns may be suitable for the *in situ* kill and let lie method of destruction in aquaculture ponds (see Table 6)
- Emergency harvest if product large enough and processed onto cooking at the at risk premise.
- desirability of removing some or all dead animals from the pond bottom before disinfecting the water, this may be required if very large prawns or large prawn biomasses are present during very late stages of the production cycle
- the need to prevent scavengers, particularly birds, from spreading infection mechanically during the destruction process – permits will be required for bird mitigation
- deadline for slaughter—depends on the risk of further spread posed by the particular infected population
- slaughter facilities—site, equipment and methods available – onsite cooking preferred
- 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 if they can achieve complete destruction within appropriate time frames, 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), emergency harvesting into cooking can be considered.

If prawns are sufficiently large, emergency harvesting followed by cooking of clinically normal prawns infected with WSSV on site prior to retail sale may 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). Whole prawns and prawn products should be thoroughly cooked (100°C for >5 minutes) to attempt to destroy virus infectivity before they leave an infected farm/location.

Data from studies on the heat stability of semi-purified WSSV suspensions are summarised in Table 6. 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, within 5 minutes at 70°C (Chang et al. 1998). However, these studies examined free virus particles in suspension. When WSSV is present in tissue, host protein mass will help protect viral infectivity against thermal destruction. For example, Reddy et al. (2011) found that some WSSV in prawn carcasses cooked at 100°C for up to 30 minutes remained viable and could be used to infect other crustaceans via injection. While the infectivity of the cooked product via the ingestion (*per-os*) pathway was not established by Reddy et al. (2011), these data suggest that WSSV remains well protected while within infected tissues inside prawn carcasses. Because of this unknown, biological waste derived from crustaceans potentially infected with WSSV should be heated to 100°C for at least 5 minutes to increase the probability that the virus is destroyed.

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 4.4 of the OIE Aquatic Manual (OIE 2019).

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.

2.2.7 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. In the outbreak of WSD on prawn farms in the Logan River in Moreton Bay, a form of "destroy and let lie" was employed, involving destruction of carcasses within rearing ponds by chlorination followed by *insitu* decomposition for 4-6 weeks to reduce the volume of waste requiring off-site disposal (Table 6).

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

2.2.8 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, and possibly over 12 days (Flegel et al. 1997; Kumar et al. 2013). 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 at least 4 days. If the "destroy and let lie" method of carcass disposal is being used, this time period increases to 40 days before retreating with chlorine prior to discharge of the pond water (Table 6). The requirements allowing discharge of residual chlorine into the environment are likely to differ across jurisdictions in relation to State environmental legislation (e.g. drinking water standards).

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. It is known that WSSV can remain viable for at least 19 days in dried pond sediments, and between 35 and 40 days in moist pond sediments under non-drainable conditions (Kumar et al. 2013). If pond sludge is removed, it should be disposed of appropriately due to the possibility of the presence of viable WSSV inside the tissues of benthic sediment dwelling vectors such as polychaetes (Desrina et al. 2013) and rotifers or their eggs (Yan et al. 2004; Zhang et al. 2006). 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 (Table 6).

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

2.2.9 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. Results clearly demonstrate 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. Even if this was achieved, regulatory approval would then be required before commercial use.

2.2.10 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, as can broadcasting of recorded bird distress calls, but effectiveness of these methods usually diminishes quickly.

Firearms can be used as an alternative to noisemakers and, if approved, killing a limited number of birds is very effective for reinforcing fear instincts within flocks (Littauer 1990). In most regions of Australia, however, the use of firearms 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**). Use of professional bird control contractors may also be expensive. If live ammunition is used, extreme care must be exercised and all staff briefed beforehand in safety procedures to ensure high visibility clothing are used and all other relevant OH&S requirements are followed.

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. Smooth plastic fences 30–40 cm high are difficult for crabs to climb, while shade-cloth–type netting (2 mm mesh size, see Fegan & Clifford 2001) is climbable and thus less effective for this purpose.

2.3 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**.

2.4 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. Experience following the WSD incursion on the Logan River suggests that fallowing sites for at least 1 year to skip an entire production cycle may be highly effective, however financial assistance for farmers may be required to enforce this. The duration of fallowing 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). Studies in India have found that over 25 days sun drying

is required to eliminate WSSV infectivity in dry pond sediments, extending to 40 days when the sediments remain moist (Kumar et al. 2013).

For any attempts to eradicate WSD, it is important that prawns restocked into ponds are free of WSSV 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.

2.4.1 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 need to be emphasised on an ongoing basis. Also, public awareness information should emphasise that WSSV is harmless to humans.

2.5 Control or eradication of WSD in Australia

The circumstances surrounding an outbreak of WSD will greatly influence selection of the most suitable response option. If a diagnosis of WSSV infection has been obtained, there are generally 3 options to consider, namely eradication with a view to returning to disease freedom, containment and control via zoning and compartmentalisation, or control and mitigation of the disease.

2.5.1 Response option 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.
- Failure to detect WSSV infected wild crustaceans in the Logan River in the two years following eradication on the prawn farms affected by the WSD incursion into Moreton Bay in 2016-17.

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 rigorous biosecurity standards are adopted, including:

- the farm perimeter is fenced to exclude crabs and other potential vectors
- intake water is filtered through 250–500 µm screens or less (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 12 days) to eliminate free WSSV and to allow disinfectant chemicals to degrade to an acceptable level before pond stocking. See Appendix 2 for information on approval for using drugs and chemicals in Australia.

Eradication is less likely 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
- 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 (ProAqua 2018). 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 also 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.

Unexposed prawns

Ponds at an infected farm containing pre-market size juvenile prawns in which there is no evidence of exposure to WSSV may still be eradicated during a response situation.

At sites where WSSV infections have occurred, but unexposed prawns are of marketable size, emergency harvest is recommended provided effective farm, transportation and processing hygiene protocols are available, with on-farm processing and cooking being preferable at the infected premise 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
- emergency 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.

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

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 and adopt rigorous biosecurity standards (Sub-Committee on Aquatic Animal Health 2016)
- 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 biosecurity 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 (most likely) 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.

2.5.3 Response option 3: Control and mitigation

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 biosecurity 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 prescribed free and infected zones
- the compulsory requirement for minimum biosecurity standards for prawn farms (Sub-Committee on Aquatic Animal Health 2016)
- the disinfection of all water to destroy WSSV and WSSV carriers before discharge (at farms using partial recirculation systems).

2.5.4 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.

Export markets

WSSV is listed by the World Organisation for Animal Health (OIE) as a notifiable disease (OIE 2018a, 2018b, 2019). Potentially infectious WSSV commonly occurs in uncooked commodity prawns sold through retail outlets (Nunan et al. 1998; Scott-Orr et al. 2017). 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. Failures of these import conditions was the most likely source of the WSD incursion that occurred in the Logan River and Moreton Bay in 2016-17 (Scott-Orr et al. 2017). 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 would, however, affect pricing because of increased costs of production for Australian exporters, and reduced market prices as they would no longer being able to differentiate their products from those of competitors on the basis of guaranteed freedom from WSSV. For these reasons, establishment of WSSV in Australia would be extremely detrimental to the profitability and hence viability of the Australian prawn farming industry. The presence of WSSV in Australia would be unlikely to impact adversely on market access for exported cooked prawns, however again the increased costs of production for Australian exporters would be extremely detrimental to an industry that already has to contend with some of the tightest environmental requirements and highest costs of prawn production in the world.

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 (http://www.agriculture.gov.au/export).

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 (Bateman et al. 2012; Pradeep et al. 2012; Raja et al. 2015), any crustacean species, particularly intensively farmed species, but also those decapods maintained at high densities in captivity (e.g. live lobsters and crabs being held prior to export), are at risk in Australia. Introduction of any diseases that impact captive crustaceans held at high densities would be potentially devastating to Australia's live crustacean export industries.

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.

3 Preferred Australian response options

3.1 Overall policy for white spot syndrome virus

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 in prawn farms and in other circumstances where decapods are held in captivity at high densities. WSSV is considered exotic to Australia, and is listed as a notifiable disease in Australia's National List of Reportable Diseases of Aquatic Animals and by the OIE (World Organisation for Animal Health). Outbreaks of WSSV in farmed prawns both overseas and in Australia have been associated with high mortality rates of up to 100%. The recent incursion of WSD into the Logan River and Moreton Bay demonstrated that WSD can devastate the Australian prawn farming industry, while biosecurity responses would pose severe restrictions to the activities of Australian wild catch prawn fisheries in regions where WSD incursions have occurred. Moreover, experience on the Logan River has shown that clean-up and control of any major outbreak of WSD will result in substantial human and financial costs to both industry and government.

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 under some circumstances, 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 disease response plan (EAD Response Plan). This plan will be submitted to the Aquatic Consultative Committee on Emergency Animal Diseases (AqCCEAD), who will provide advice on the technical soundness of the plan and its consistency with AQUAVETPLAN.

Directors of fisheries and/or 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 AqCCEAD. The detailed response measures adopted will be determined using the principles of disease eradication, containment, control and mitigation (see Section 2), depending on the strain of virus detected, epidemiological information about the outbreak and the financial and logistical feasibility of the selected option.

For information on the responsibilities of the other state or territory disease control headquarters and local disease control centres, see the AQUAVETPLAN **Control Centres Management Manual** (DAFF 2001).

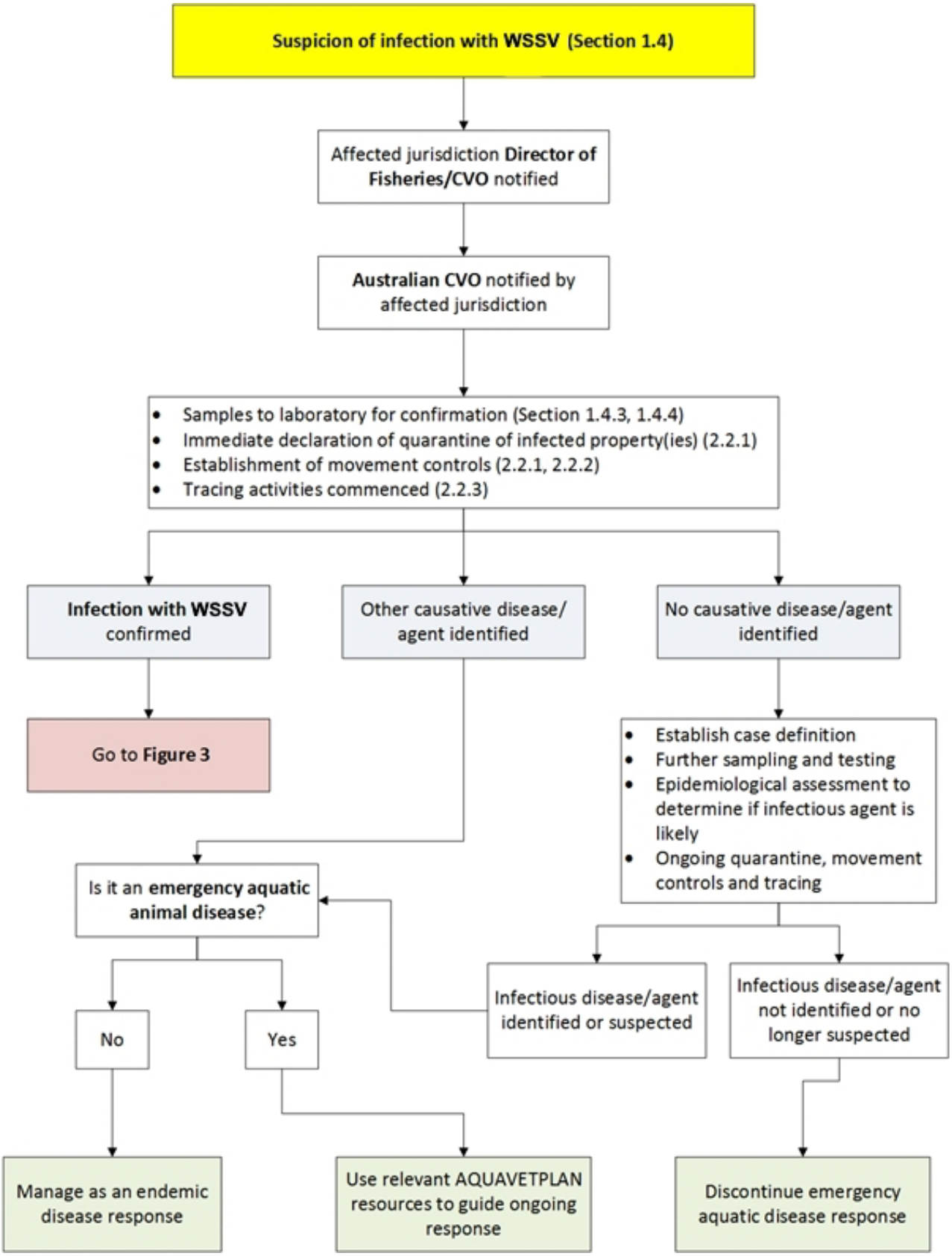
3.2 Response options

The circumstances surrounding an outbreak of WSD will greatly influence selection of the most suitable response option. Figure 2 details the actions that should occur on initial suspicion of WSD in Australia.

As soon as adequate information becomes available and the presence of WSSV is confirmed, a decision will be made on the appropriate response, based on the reasoning shown in Figure 3.

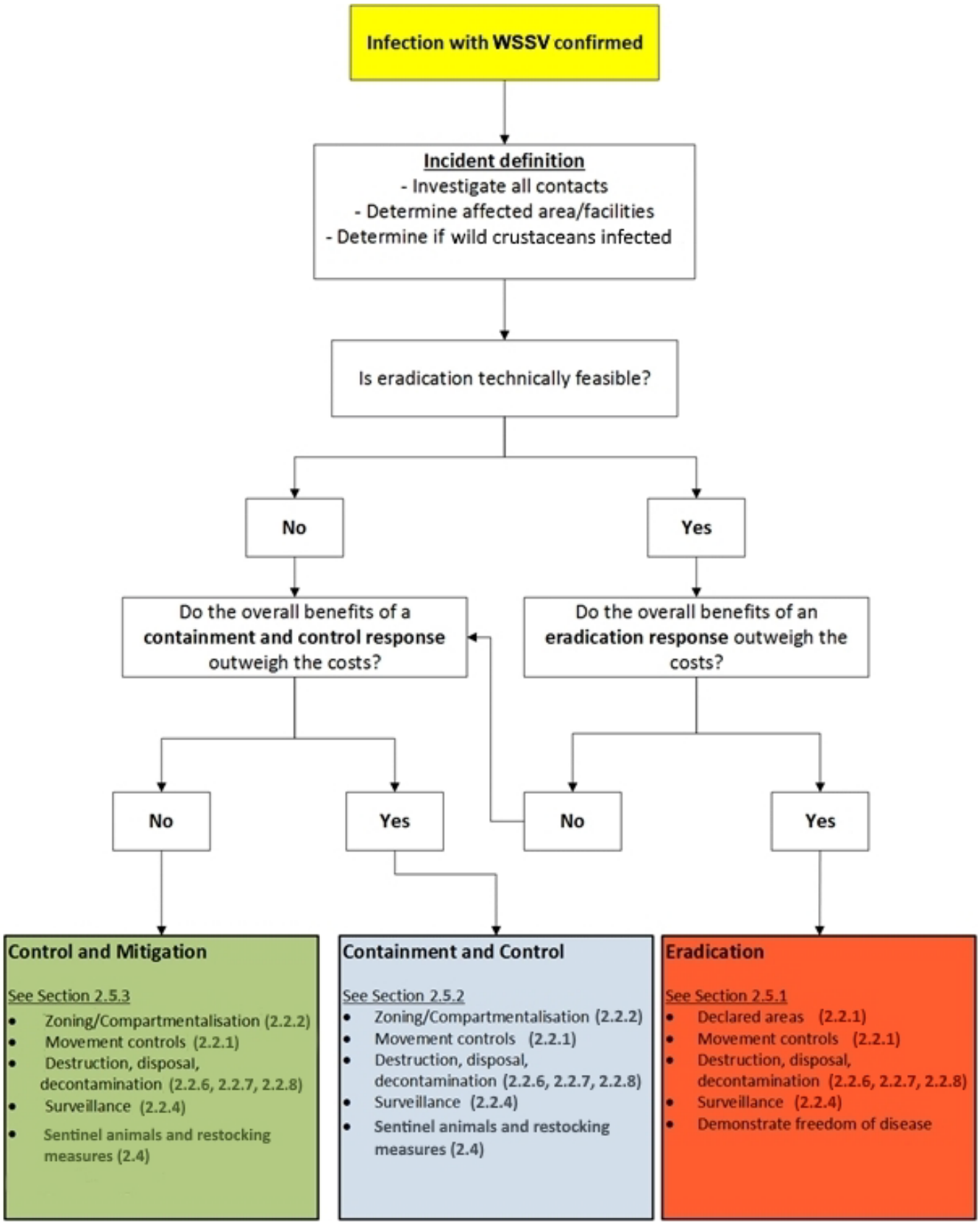
Eradication will generally be attempted if the infection appears to be limited to prawns farmed at one or a small number of localised facilities, and/or if eradication is deemed to be achievable. If infection is detected across a larger number of widely distributed farms or extensively in wild prawns,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

Figure 2 Decision flow chart for suspected white spot syndrome virus infection



CVO Chief veterinary officer. **WSSV** white spot syndrome virus.

Figure 3 Determining response to outbreak or confirmed white spot syndrome virus infection



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 crustaceans. However, the potential constraint on eradication posed by the presence of infection in wild crustaceans 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, adjust import risk analyses and import health standards as applicable, and to provide proof of freedom from the disease
- a public awareness campaign to encourage cooperation from industry and the community.

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 2.2.8 and Table 6. At all stages of decontamination, steps must be taken to prevent any spread of infection via water, wastes or materials, especially into natural waterways. All infected prawns and other crustaceans, 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.

3.2.2 Option 2: containment and control

If infection is widespread in wild crustacean 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 Australian crustacean industries to maintain premium pricing in export markets. Restrictions on the movement of prawns and crustacean products, and a surveillance program, will be necessary to support zoning.

Farms, wild fisheries and premises handling crustaceans 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 and other crustaceans, water and any other potential vectors (including reservoir hosts as well as 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 crustaceans in the infected zone, followed by clean-up and disinfection
- use of closed-production systems and adoption of rigorous on-farm biosecurity standards (Sub-Committee on Aquatic Animal Health 2016)
- 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

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 rigorous on-farm biosecurity standards and any other 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, and/or adoption of rigorous on-farm biosecurity standards (Sub-Committee on Aquatic Animal Health 2016) 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.

The characteristics of the various options for eradication, containment or mitigation of WSD are summarised in Table 3.1

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

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	Yes	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 crustaceans	Yes	Yes	Yes
Destruction of unexposed crustaceans on WSSV infected premises	Yes	No	No
Destruction or harvest with on-farm cooking of exposed or potentially exposed but clinically normal crustaceans, depending on size	Yes	In free zones only	No
On-farm processing (e.g. by cooking)	Yes	Optional	Optional
Disposal of infected crustaceans 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
Biosecure farming systems	n.a.	Yes	Yes
Specific farm-level biosecurity measures	Yes	Yes	Yes

n.a. = not applicable; WSSV = white spot syndrome virus

3.3 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 2018a). 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.

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.

3.4 Funding and compensation

There are currently no agreed national cost-sharing agreements in place for emergency responses to white spot disease. However, in late 2014 the Federal government began development of a formal industry-government aquatic emergency animal disease response agreement (the Aquatic Deed). Until the Aquatic Deed arrangements have been agreed upon and finalised between government and the relevant Australian crustacean farming and crustacean fisheries industries, 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.

3.5 Export markets

WSSV is listed by the World Organisation for Animal Health (OIE) as a notifiable disease (OIE 2018a, 2018b, 2019). Potentially infectious WSSV commonly occurs in uncooked commodity prawns sold through retail outlets (Bateman et al. 2012; Durand et al. 2000; McColl et al. 2004; Nunan et al. 1998; Scott-Orr et al. 2017). 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 would, however, affect costs of production for industry and export 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. These increased costs of production and reduced market prices would, in combination, be extremely detrimental to the profitability and hence viability of the Australian prawn farming industry, as well as potentially other live crustacean export industries (live lobsters, crabs). However, the presence of WSSV in Australia would be unlikely to impact adversely on market access for exported cooked prawns, or other crustacean products.

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 (http://www.agriculture.gov.au/export).

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

OIE Aquatic Code

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

Chapter 9.8 of the 2018 OIE Aquatic Animal Health Code (21st edition) (Infection with White Spot Syndrome Virus) is relevant to this manual.

OIE Aquatic Manual

The purpose of the OIE Manual of Diagnostic Tests for Aquatic Animals is to contribute to the international harmonisation of methods for the surveillance and control of the most important aquatic animal diseases (OIE 2019). Standards are described for laboratory diagnostic tests and the production and control of biological products (principally vaccines) for veterinary use across the globe. Chapter 2.2.8 of the manual is most relevant to this document.

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 B: Approval of chemicals for use in Australia

The Australian Pesticides and Veterinary Medicines Authority (APVMA) evaluates, registers and regulates agricultural and veterinary chemicals. Before an antibiotic or chemical 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. Since the assessment process is so detailed, the evaluation may take some time to complete.

Registration

Registration is the default method for APVMA to allow the use of a veterinary chemical in Australia. Registration is time consuming and expensive and it may be necessary to apply for a minor or emergency use permit during an emergency.

Minor use permit system

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

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

Emergency use permits

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

For further details or permit application forms, visit the APVMA website at https://apvma.gov.au/.

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