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REPORT OF THE MEETING OF THE OIE *AD HOC* GROUP ON SUSCEPTIBILITY OF CRUSTACEAN SPECIES TO INFECTION WITH OIE LISTED DISEASES¹

Paris, 1–3 June 2016

The OIE *ad hoc* Group on Susceptibility of Crustacean Species to Infection with OIE Listed Diseases (the *ad hoc* Group) met at OIE Headquarters on 1–3 June 2016.

The members of the *ad hoc* Group, the adopted agenda and the Terms of Reference are presented at Annex 1, Annex 2 and Annex 3 respectively.

Dr Gillian Mylrea, Deputy Head of the OIE International Trade Department, welcomed members and thanked them for their willingness to work on this important topic. Dr Mylrea informed members that recommendations from their second meeting in October 2015 regarding the list of susceptible species for seven of the OIE listed crustacean diseases (acute hepatopancreatic necrosis disease; crayfish plague; infectious hypodermal and haematopoietic necrosis; infectious myonecrosis; necrotising hepatopancreatitis; Taura syndrome; and white tail disease) had been considered by the OIE Aquatic Animal Health Standards Commission (Aquatic Animals Commission) at their February 2016 meeting. The Commission had amended the disease-specific chapters for these diseases in the OIE *Aquatic Animal Health Code (Aquatic Code)* and the OIE *Manual of Diagnostic Tests for Aquatic Animals (Aquatic Manual)* in line with *ad hoc* group recommendations and had circulated these for Member Countries' comments in their February 2016 report.

The chair of the *ad hoc* Group, Dr Grant Stentiford, thanked the members for all their continued support and for participation in the third meeting of the *ad hoc* Group. Dr Stentiford clarified that the purpose of this meeting was to review the literature and develop a list of susceptible species for white spot syndrome virus for inclusion in the relevant chapters of the OIE *Aquatic Code* and *Aquatic Manual*.

The *ad hoc* Group applied the three-stage approach, outlined in Article 1.5.3. in Chapter 1.5. of the *Aquatic Code*, to assess susceptibility of a species to infection with white spot syndrome virus (WSSV).

The “Criteria for listing species as susceptible to infection with a specific pathogen” as described in Chapter 1.5. of the *Aquatic Code* are as follows:

- 1) criteria to determine whether the route of transmission is consistent with natural pathways for the infection (as described in Article 1.5.4.);
- 2) criteria to determine whether the pathogenic agent has been adequately identified (as described in Article 1.5.5.);

¹ Note: This *ad hoc* Group report reflects the views of its members and may not necessarily reflect the views of the OIE. This report should be read in conjunction with the September 2016 report of the Aquatic Animal Health Standards Commission because this report provides its considerations and comments. It is available at <http://www.oie.int/en/international-standard-setting/specialists-commissions-groups/aquatic-animal-commission-reports/meeting-reports/>

Hosts that were classified as species for which there is incomplete evidence for susceptibility (as described in Article 1.5.8.) were proposed for inclusion in a new Section 2.2.2. *Species with incomplete evidence for susceptibility* of Chapter 2.2.7. of the *Aquatic Manual* entitled White spot disease.

In addition, organisms producing pathogen-specific positive PCR results (without confirmation of an active infection) were identified and listed in a new sub point 2.2.2.2. of the *Aquatic Manual* chapter.

The assessment for infection with WSSV conducted by the *ad hoc* Group is provided in [Annex 4](#).

The *ad hoc* Group wished to note the following:

- 1) In many of the older publications accurate pathogen identification was not carried out because molecular typing techniques were not available at the time. This is particularly so for many of the original studies in Penaeidae. Therefore, for many of these cases, a weight of evidence approach using combined data from relevant studies was used to assess susceptibility.
- 2) Species categorised as ‘2’ (i.e. species for which there is incomplete evidence for susceptibility (those species for which criteria A-D were not fully met) includes a wide range of species, from those for which susceptibility to disease is low (e.g. reservoir species) through to those that do not meet category 1 because there is insufficient data available.
- 3) The *ad hoc* Group worked on the assumption that authors had correctly identified the host species on which they were reporting.

The *ad hoc* Group made the following recommendations:

- 1) That species categorised as ‘3’ (i.e. species for which only PCR based survey results are available) be listed in a new section in the relevant chapter of the *Aquatic Manual* to more clearly differentiate ‘2s’ and ‘3s’ because studies that only detected the nucleic acid of the pathogen (e.g. by PCR) cannot be used as evidence of infection. However, they are important to include because they provide some indication of the presence of the target pathogen in the host or the environment.

The *ad hoc* Group suggested this approach be included in the relevant *Aquatic Manual* chapter as a new point 2.2.2.2. as shown below:

“2.2.2.2. Pathogen-specific positive PCR results (without confirmation of an active infection) have been reported in the following organisms: species X, Y and Z.”

- 2) The following amendment be made to Chapter 1.5. *Criteria for listing species as susceptible to infection with a specific pathogen* to improve the applicability of this criterion:

In point A of Article 1.5.6. add the words “(and for viruses in host cells)” to clarify that the pathogen of interest is replicating in host cells and not potentially in symbionts:

i.e. “A. the *pathogenic agent* is multiplying in the host (and for viruses in host cells), or developing stages of the *pathogenic agent* are present in or on the host;”

- 3) The words ‘in host cells’ be added to criteria A for Table 1. *Criteria for susceptibility to infection with pathogen X* in all Tables developed by this *ad hoc* Group at their October 2015 meeting for: TSV, YHV, IMNV, IHHNV, MrNV and NHP to read as: “A: Replication in host cells”.
- 4) Section 7 of crustacean chapters of the *Aquatic Manual* be amended to take account of the requirement for accurate systematics of the pathogen. At present it confuses confirmation of a case with identification of the pathogen of concern.

.../Annexes

**MEETING OF THE OIE AD HOC GROUP ON SUSCEPTIBILITY OF CRUSTACEAN SPECIES
TO INFECTION WITH OIE LISTED DISEASES**

Paris, 1–3 June 2016

List of participants

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**MEETING OF THE OIE AD HOC GROUP ON SUSCEPTIBILITY OF CRUSTACEAN SPECIES
TO INFECTION WITH OIE LISTED DISEASES**

Paris, 1–3 June 2016

Adopted agenda

1. Conduct an assessment for species susceptibility as described in Chapter 1.5. of the *Aquatic Code* entitled White spot disease (Chapter 9.7.).
2. Draft a report to be considered by the Aquatic Animals Commission at their September 2016 meeting.

**MEETING OF THE OIE AD HOC GROUP ON SUSCEPTIBILITY OF CRUSTACEAN SPECIES
TO INFECTION WITH OIE LISTED DISEASES**

Paris, 1–3 June 2016

Terms of Reference

Background

A new Chapter 1.5. *Criteria for listing species as susceptible to infection with a specific pathogen* was introduced into the 2014 edition of the *Aquatic Code*. The purpose of this chapter is to provide criteria for determining which host species are listed as susceptible in Article X.X.2. of each disease-specific chapter in the *Aquatic Code*. The criteria are to be applied progressively to each disease-specific chapter in the *Aquatic Code*.

This *ad hoc* Group on Susceptibility of Crustacean Species to Infection with OIE Listed Diseases has undertaken assessments on susceptibility of crustacean species for eight of the OIE listed crustacean diseases: acute hepatopancreatic necrosis disease; crayfish plague (*Aphanomyces astaci*); infection with yellow head virus genotype 1; infectious hypodermal and haematopoietic necrosis; infectious myonecrosis; necrotising hepatopancreatitis; Taura syndrome; and white tail disease.

The assessments have been reviewed by the Aquatic Animals Commission to amend in the list of susceptible species in Article X.X.2. of the disease-specific chapters in the *Aquatic Code*.

In addition, for species where there is some evidence of susceptibility but insufficient evidence to demonstrate susceptibility through the approach described in Article 1.5.3., information has been proposed to be included in the relevant disease-specific chapter in the *Aquatic Manual*.

Terms of Reference

- 1) Consider standards of evidence required to satisfy the criteria in Chapter 1.5.
- 2) Review relevant literature documenting susceptibility of species.
- 3) Propose susceptible species for infection with white spot syndrome virus (WSSV) based on Article 1.5.7.
- 4) Propose susceptible species for infection with WSSV based on Article 1.5.8.

Expected outputs

- 1) Develop a list of susceptible species for inclusion in the relevant articles of the chapter in the *Aquatic Code* and *Manual* for white spot disease.
- 2) Draft a report for consideration by the Aquatic Animals Commission at their September 2016 meeting.

ASSESSMENT OF HOST SUSCEPTIBILITY TO INFECTION WITH WHITE SPOT SYNDROME VIRUS (WSSV)

The objectives of this assessment were to: (1) determine susceptibility of given host taxa to infection with white spot syndrome virus (WSSV) by applying the three-stage approach for as described in Article 1.5.3. of the *Aquatic Code* and (2) to provide the OIE with recommendations regarding revision of the relevant sections of the *Aquatic Code* and the *Aquatic Manual* with respect to host species susceptibility.

The *ad hoc* Group based pathogen identification on Section 7 of Chapter 2.2.7. of the *Aquatic Manual* with the exception of histology because it may not be specific for the identification of WSSV in non-penaeid species.

Criteria for susceptibility to infection with WSSV are detailed in Table 1 (as per Article 1.5.6. of the *Aquatic Code*). This table includes Replication in host cells (A), Viability/Infectivity (B), Pathology/Clinical Signs (C) and Location (D).

Hosts were considered to be infected with WSSV if they fulfilled either criterion A, or at least two of criteria B, C and D (as per point 3 of Article 1.5.7. of the *Aquatic Code*).

Table 1. Criteria for susceptibility to infection with WSSV

| A: Replication in host cells | B: Viability/Infectivity | C: Pathology/Clinical signs | D: Location |
|--|---|---|---|
| Presence of characteristic inclusion bodies and ideally positive labelling of inclusion bodies by ISH or IFAT; OR Presence of virions in inclusion bodies by TEM; OR Demonstration of increasing copy number over time with qPCR with confirmatory PCR/sequencing specific for infectious virus; OR Serial passage from individual to SPF individual of the same species*. | Single passage bioassay to a SPF (target pathogen) of any susceptible host species and confirmation of pathogen identification**. | Inclusions (eosinophilic to basophilic) within nuclei of cells in target organs and tissues. Host nuclei hypertrophic with marginated chromatin with/without the presence of clinical signs (e.g. white spots on cuticle, moribund, lethargic)***. | Cells of tissues and organs of ectodermic and mesodermic origin. Target sites include cuticular epithelium (gills, pleopods, appendages), connective tissues, the haematopoietic tissues, the antennal gland and lymphoid organ****. |

Key:

- * To demonstrate replication by this approach requires evidence for multiple passages in confirmed target pathogen-free hosts of the same species as being assessed.
- ** To demonstrate viability or infectivity of the target pathogen within the host being assessed, single passage in any known susceptible SPF host is required.
- *** Clinical signs accorder 2.2.7. of the *Aquatic Manual* may not present equally in all host taxa and are not specific for infection with WSSV.
- **** Lymphoid organ not present in most non-penaeid host taxa. For non-crustacean host taxa other organs and tissues may show evidence of infection with WSSV.

Annex 4 (contd)

The assessment for host susceptibility to infection with WSSV is provided in Table 2.

Table 2. Outcome of assessment for host susceptibility to infection with WSSV

| Family | Genus | Species | Stage 1: Route of infection* | Stage 2: Pathogen identification | Stage 3: Evidence for infection | | | | Outcome** | References |
|----------------|-------------------------|---|------------------------------------|--|------------------------------------|-----------|-----------|-----|-----------|-----------------------------|
| | | | | | A | B | C | D | | |
| Alpheidae | <i>Alpheus</i> | <i>brevicristatus</i> | nd | nest PCR | No | No | No | No | 2 | 63 |
| Alpheidae | <i>Alpheus</i> | <i>brevicristatus</i> | I | nest PCR/dot blot/ ISH | Yes | Yes | Yes | Yes | 2 | 63, 76 |
| Alpheidae | <i>Alpheus</i> | <i>lobidens</i> | nd | nest PCR | No | No | No | No | 3 | 63 |
| Ameiridae | <i>Nitocra</i> | sp. | E (per os) | PCR | No | No | No | No | 3 | 74 |
| Artemiidae | <i>Artemia</i> | <i>salina</i> | nd | nest PCR | No | No | No | No | 3 | 49 |
| Artemiidae | <i>Artemia</i> | sp. | N/E (bath) | dot blot/ISH | No | No | No | No | 3 | 76 |
| Astacidae | <i>Astacus</i> | <i>astacus</i> | E (per os)/I | nest PCR | No | No | No | No | 3 | 33 |
| Astacidae | <i>Astacus</i> | <i>leptodactylus</i> | E (per os) | ISH/TEM/dot blot | Yes | No | Yes | Yes | 1 | 12 |
| Astacidae | <i>Austropotamobius</i> | <i>pallipes</i> | E (per os)/I | PCR/sequencing | Yes | Yes | Yes | Yes | 1 | 2 |
| Astacidae | <i>Pacifastacus</i> | <i>leniusculus</i> | E (per os) | PCR/sequencing | Yes | Yes | Yes | Yes | 1 | 2 |
| Balanidae | <i>Balanus</i> | sp. | N/E (bath)/I | PCR/sequencing/dot blot/ISH | No Yes | No Yes | No Yes | No | 3 | 55, 76 |
| Calanidae | <i>Calanus</i> | <i>pacificus</i> <i>californicus</i> | E (per os) | RT-qPCR of VP28 transcripts | Yes | No | No | No | 1 | 46 |
| Calappidae | <i>Calappa</i> | <i>lophos</i> | N/E (per os/bath) | PCR | No | No | No | No | 3 | 66 |
| Calappidae | <i>Calappa</i> | <i>philarigus</i> | E (per os)/I | PCR | Yes | No | Yes | Yes | 2 | 58 |
| Callianassidae | <i>Callianassa</i> | <i>harmandi</i> | I | dot blot/ISH | Yes | Yes | Yes | Yes | 2 | 76 |
| Cambaridae | <i>Orconectes</i> | <i>limosus</i> | E (per os)/I | TEM/dot blot | Yes | No | Yes | Yes | 1 | 12 |
| Cambaridae | <i>Orconectes</i> | <i>punctimanus</i> | N | PCR/probe | No | No | No | No | 3 | 42 |
| Cambaridae | <i>Procambarus</i> | <i>clarkii</i> | N/E (per os)/I | PCR/ISH/dot blot | Yes | No Yes | Yes | Yes | 1 | 3, 6, 18, 31, 66, 69, 76 |

| Family | Genus | Species | Stage 1: Route of infection* | Stage 2: Pathogen identification | Stage 3: Evidence for infection | | | | Outcome** | References |
|-----------------------------|--|-------------------------------|------------------------------------|--|------------------------------------|-----|-----|-----|-----------|------------|
| | | | | | A | B | C | D | | |
| Cambaridae | <i>Procambarus</i> | <i>zonangulus</i> | N | PCR/sequencing | Yes | No | Yes | Yes | 1 | 3 |
| Carcinidae | <i>Carcinus</i> | <i>maenas</i> | E (per os)/I | PCR | Yes | Yes | Yes | Yes | 2 | 2, 12 |
| Cancridae | <i>Cancer</i> | <i>pagurus</i> | E (per os)/I | ISH/TEM/dot blot | Yes | Yes | Yes | Yes | 1 | 2, 12 |
| Coleoptera (Ephydriidae) | | | N | PCR | No | No | No | No | 3 | 41 |
| Crangonidae | <i>Crangon</i> | <i>affinis</i> | E (bath) | PCR/monoclonal antibody | No | No | Yes | No | 3 | 26 |
| Cyclopidae | <i>Apocyclops</i> | <i>royi</i> | E (bath) | PCR/sequencing | Yes | No | No | No | 3 | 8 |
| Decapoda (order) | <i>Paratelphusa</i> | <i>hydrodomous</i> | E (per os)/I | PCR | Yes | Yes | Yes | Yes | 2 | 52, 57 |
| Decapoda (order) | <i>Paratelphusa</i> (<i>Barytelphusa</i>) | <i>pulvinata</i> | E (per os)/I | PCR | Yes | No | Yes | Yes | 2 | 57 |
| Diogenidae | <i>Diogenes</i> | <i>nitidimanus</i> | I | PCR | No | No | No | No | 3 | 9 |
| Dorippidae | <i>Paradorippe</i> | <i>granulata</i> | E (per os)/I | PCR | Yes | No | Yes | Yes | 2 | 58 |
| Epialtidae | <i>Doclea</i> | <i>muricata</i> (=hybrida) | E (per os)/I | PCR | Yes | No | Yes | Yes | 2 | 58 |
| Ergasilidae | <i>Ergasilus</i> | <i>manicatus</i> | E (bath) | qPCR—no sequence | Yes | No | No | No | 2 | 50 |
| Galenidae | <i>Halimede</i> | <i>ochtodes</i> | E (per os)/I | PCR | Yes | No | Yes | Yes | 2 | 58 |
| Grapsidae | <i>Grapsus</i> | <i>albolineatus</i> | E (per os)/I | PCR | Yes | No | Yes | Yes | 2 | 58 |
| Grapsidae | <i>Metopograpsus</i> | sp. | E (per os) | EM in <i>P. vannamei</i> . No PCR or sequence | Yes | Yes | Yes | Yes | 2 | 54 |
| Grapsidae | <i>Metopograpsus</i> | <i>messor</i> | N | PCR | No | No | No | No | 3 | 29 |
| Grapsidae | <i>Hemigrapsus</i> | <i>sanguineus</i> | I | dot blot/ISH | Yes | Yes | Yes | Yes | 2 | 76 |
| Leucosiidae | <i>Philyra</i> | <i>syndactyla</i> | E (per os) | PCR | Yes | No | Yes | Yes | 2 | 58 |
| Lithodidae | <i>Lithodes</i> | <i>maja</i> | E (per os) | PCR | Yes | No | Yes | Yes | 2 | 58 |
| Macrophthalmidae | <i>Macrophthalmus</i> | <i>sulcatus</i> | N | PCR | No | No | No | No | 3 | 29 |

Annex 4 (contd)

| Family | Genus | Species | Stage 1: Route of infection* | Stage 2: Pathogen identification | Stage 3: Evidence for infection | | | | Outcome** | References |
|--------------|-------------------------|---|------------------------------------|---|------------------------------------|-----|-----------|-----|-----------|---------------------------|
| | | | | | A | B | C | D | | |
| Matutidae | <i>Ashtoret</i> | <i>miersii</i> | E (per os) | PCR | Yes | No | Yes | Yes | 2 | 58 |
| Matutidae | <i>Matuta</i> | <i>planipes</i> | N | PCR | No | No | No | No | 3 | 49 |
| Menippidae | <i>Menippe</i> | <i>rumphii</i> | E (per os) | PCR | No | No | No | No | 3 | 58 |
| Nephropidae | <i>Homarus</i> | <i>gammarus</i> | E (per os)/I | PCR/sequencing | Yes | Yes | Yes | Yes | 1 | 1, 2 |
| Nephropidae | <i>Nephrops</i> | <i>norvegicus</i> | E (per os)/I | PCR/sequencing | Yes | Yes | Yes | Yes | 1 | 2 |
| Nereididae | <i>Dendronereis</i> | sp. | N | PCR/sequencing | Yes | No | Yes | No | 1 | 15, 16, 28 |
| Ocypodidae | <i>Macrophthalmus</i> | <i>japonicus</i> | N | dot blot/ISH | Yes | No | Yes | Yes | 2 | 76 |
| Ocypodidae | <i>Uca (=Gelasimus)</i> | <i>vocans</i> (= <i>marionis nitidus</i>) | N | PCR | No | No | No | No | 3 | 29 |
| Ocypodidae | <i>Uca (=Leptuca)</i> | <i>pugillator</i> | E/I | PCR/ISH | Yes | Yes | Yes | Yes | 2 | 35 |
| Paguridae | <i>Pagurus</i> | <i>angustus</i> | I | PCR | No | No | No | No | 3 | 9 |
| Paguridae | <i>Pagurus</i> | <i>minutus</i> | N/I | PCR/TEM | Yes | No | No | No | 1 | 9 |
| Palaemonidae | <i>Exopalaemon</i> | <i>carinicauda</i> | N/E (per os) | RT-qPCR/dot blot/ ISH | Yes | Yes | No Yes | Yes | 1 | 19, 76 |
| Palaemonidae | <i>Exopalaemon</i> | <i>orientis</i> | E (per os) | PCR/ISH | Yes | No | Yes | Yes | 1 | 7, 66 |
| Palaemonidae | <i>Macrobrachium</i> | <i>idella</i> | E (per os) | Typical histopathology and Western blot. No PCR | Yes | Yes | Yes | Yes | 2 | 54, 56 |
| Palaemonidae | <i>Macrobrachium</i> | <i>lamerrae</i> | E (per os) | Typical histopathology and Western blot. No PCR | Yes | Yes | Yes | Yes | 2 | 56 |
| Palaemonidae | <i>Macrobrachium</i> | <i>nipponense</i> | E (per os) | PCR | Yes | No | Yes | Yes | 2 | 72 |
| Palaemonidae | <i>Macrobrachium</i> | <i>rosenbergii</i> | E (per os)/I | Various methods used | Yes | Yes | Yes | Yes | 2 | 13, 27, 29, 40, 54, 56 |
| Palaemonidae | <i>Palaemon</i> | sp. | N | PCR | No | No | No | No | 3 | 40 |
| Palaemonidae | <i>Palaemon</i> | <i>adpersus</i> | E/I | PCR/TEM/ISH/dot blot | Yes | Yes | Yes | Yes | 2 | 12 |
| Palaemonidae | <i>Palaemon</i> | <i>macroductylus</i> | N | PCR/qPCR | No | No | No | No | 3 | 45 |

| Family | Genus | Species | Stage 1: Route of infection* | Stage 2: Pathogen identification | Stage 3: Evidence for infection | | | | Outcome** | References |
|---------------|-----------------------|------------------------|------------------------------------|--|------------------------------------|-----|-----|-----|-----------|------------------|
| | | | | | A | B | C | D | | |
| Palaemonidae | <i>Palaemon</i> | <i>ritteri</i> | E (per os) | PCR/sequencing | Yes | No | Yes | No | 1 | 59 |
| Palaemonidae | <i>Palaemonetes</i> | <i>pugio</i> | N/I | qPCR | No | No | Yes | No | 3 | 48 |
| Palinuridae | <i>Panulirus</i> | <i>homarus</i> | I | EM in <i>P. vannamei</i> . No PCR or sequence | Yes | Yes | Yes | Yes | 2 | 54 |
| Palinuridae | <i>Panulirus</i> | <i>longipes</i> | E (per os) | EM in <i>P. vannamei</i> . No PCR or sequence | Yes | Yes | Yes | Yes | 3 | 54, 66 |
| Palinuridae | <i>Panulirus</i> | <i>ornatus</i> | E (per os) | EM in <i>P. vannamei</i> . No PCR or sequence | Yes | Yes | Yes | Yes | 3 | 54, 66 |
| Palinuridae | <i>Panulirus</i> | <i>penicillatus</i> | E (per os) | PCR/ISH | Yes | No | Yes | Yes | 1 | 6, 7, 66 |
| Palinuridae | <i>Panulirus</i> | <i>polyphagus</i> | E (per os) | EM in <i>P. vannamei</i> . No PCR or sequence | Yes | Yes | Yes | Yes | 2 | 54 |
| Palinuridae | <i>Panulirus</i> | <i>versicolor</i> | E (per os) | PCR/ISH | Yes | No | Yes | Yes | 1 | 6, 7, 66 |
| Parastacidae | <i>Cherax</i> | <i>destructor</i> | I | dot blot | Yes | No | Yes | Yes | 2 | 20 |
| Parastacidae | <i>Cherax</i> | <i>quadricarinatus</i> | E (per os)/I | PCR/qPCR/IHC | Yes | Yes | Yes | Yes | 1 | 24, 61 |
| Parthenopidae | <i>Parthenope</i> | <i>prensor</i> | E (per os)/I | PCR | Yes | No | Yes | Yes | 2 | 58 |
| Penaeidae | <i>Artemesia</i> | <i>longinaris</i> | N | PCR/qPCR | No | No | No | No | 3 | 45 |
| Penaeidae | <i>Metapenaeus</i> | <i>affinis</i> | N | PCR | No | No | No | No | 3 | 25 |
| Penaeidae | <i>Metapenaeus</i> | <i>brevicornis</i> | N | PCR | No | No | No | No | 3 | 30 |
| Penaeidae | <i>Metapenaeus</i> | <i>dobsoni</i> | N/E (per os) | PCR | Yes | Yes | Yes | Yes | 2 | 29, 54 |
| Penaeidae | <i>Metapenaeus</i> | <i>ensis</i> | N/E (per os) | PCR/ISH/dot blot/ISH | Yes | No | Yes | Yes | 1 | 6, 7, 66, 67, 76 |
| Penaeidae | <i>Metapenaeus</i> | <i>monoceros</i> | N/E (per os) | PCR | Yes | Yes | Yes | Yes | 2 | 34, 54, 70 |
| Penaeidae | <i>Parapenaeopsis</i> | <i>stylifera</i> | N | PCR/gene probes | No | No | No | No | 3 | 25, 29 |
| Penaeidae | <i>Penaeus</i> | <i>californiensis</i> | N | PCR/sequencing | No | No | No | No | 3 | 43 |
| Penaeidae | <i>Penaeus</i> | <i>paulensis</i> | N | PCR/sequencing | Yes | No | Yes | Yes | 1 | 4 |

Annex 4 (contd)

| Family | Genus | Species | Stage 1: Route of infection* | Stage 2: Pathogen identification | Stage 3: Evidence for infection | | | | Outcome** | References |
|-----------|------------------------|---------------------|------------------------------------|---|------------------------------------|-----|-----|-----|-----------|--------------------------------|
| | | | | | A | B | C | D | | |
| Penaeidae | <i>Penaeus</i> | <i>aztecus</i> | E (per os) | Inoculum not characterised; typical histopathology only | Yes | No | Yes | Yes | 2 | 37 |
| Penaeidae | <i>Penaeus</i> | <i>chinensis</i> | N/I | qPCR/TEM/dot blot/ISH | Yes | Yes | Yes | Yes | 1 | 23, 31, 32, 73, 76 |
| Penaeidae | <i>Penaeus</i> | <i>duorarum</i> | E (per os) | Inoculum not characterised; typical histopathology only | Yes | No | Yes | Yes | 2 | 37 |
| Penaeidae | <i>Penaeus</i> | <i>indicus</i> | N | PCR/sequencing | Yes | No | Yes | Yes | 1 | 34, 53, 54, 56, 64 |
| Penaeidae | <i>Penaeus</i> | <i>japonicus</i> | N/E (per os) | PCR | Yes | Yes | Yes | Yes | 1 | 11, 21, 40, 67, 71, 73, 74 |
| Penaeidae | <i>Penaeus</i> | <i>merguiensis</i> | N/E | PCR/TEM/IFA | Yes | Yes | Yes | Yes | 2 | 22, 68 |
| Penaeidae | <i>Penaeus</i> | <i>monodon</i> | N | PCR/ISH/TEM/dot blot/ISH | Yes | Yes | Yes | Yes | 1 | 34, 40, 54, 56, 66, 67, 73, 76 |
| Penaeidae | <i>Penaeus</i> | <i>penicillatus</i> | N/E (per os) | PCR | No | No | No | No | 3 | 11, 40, 66 |
| Penaeidae | <i>Penaeus</i> | <i>semisulcatus</i> | N/E (per os) | PCR | No | No | No | No | 3 | 40, 54, 66 |
| Penaeidae | <i>Penaeus</i> | <i>setiferus</i> | E (per os) | Inoculum not characterised; typical histopathology only | Yes | Yes | Yes | Yes | 2 | 37 |
| Penaeidae | <i>Penaeus</i> | <i>stylirostris</i> | E (per os) | Inoculum not characterised; typical histopathology only | Yes | Yes | Yes | Yes | 2 | 37 |
| Penaeidae | <i>Penaeus</i> | <i>vannamei</i> | N/E (per os) | PCR/ISH/Histology/dot blot | Yes | Yes | Yes | Yes | 1 | 14, 37, 42, 67, 76 |
| Penaeidae | <i>Trachysalambria</i> | <i>curvirostris</i> | E (per os) | PCR/ISH | Yes | No | Yes | Yes | 1 | 7, 66 |

| Family | Genus | Species | Stage 1: Route of infection* | Stage 2: Pathogen identification | Stage 3: Evidence for infection | | | | Outcome** | References |
|------------|--|------------------------|------------------------------------|-------------------------------------|------------------------------------|-----|-----|-----|-----------|------------|
| | | | | | A | B | C | D | | |
| Polybiidae | <i>Liocarcinus</i> | <i>depurator</i> | E (per os) | TEM/ISH/dot blot | Yes | No | Yes | Yes | 1 | 12 |
| Polybiidae | <i>Necora</i> (= <i>Liocarcinus</i>) | <i>puber</i> | E (per os) | PCR/TEM/ISH/dot blot | Yes | No | Yes | Yes | 1 | 12 |
| Polychaeta | <i>Marphysa</i> | <i>gravelyi</i> | N/E (per os) | PCR | No | Yes | No | No | 3 | 65 |
| Portunidae | <i>Callinectes</i> | <i>arcuatus</i> | N | PCR/sequencing | No | No | No | No | 3 | 43 |
| Portunidae | <i>Callinectes</i> | <i>sapidus</i> | N | PCR/sequencing | No | Yes | No | No | 3 | 51 |
| Portunidae | <i>Charybdis</i> | <i>annulata</i> | E (per os)/I | PCR | Yes | No | Yes | Yes | 2 | 58 |
| Portunidae | <i>Charybdis</i> | <i>cruciata</i> | N | PCR | No | No | No | No | 3 | 29 |
| Portunidae | <i>Charybdis</i> | <i>granulata</i> | E (per os) | PCR/ISH | Yes | No | Yes | Yes | 1 | 7, 66 |
| Portunidae | <i>Charybdis</i> | <i>feriata</i> | E (per os) | PCR/ISH | Yes | No | Yes | Yes | 2 | 36, 40, 66 |
| Portunidae | <i>Charybdis</i> | <i>japonica</i> | N | PCR | No | No | No | No | 3 | 63 |
| Portunidae | <i>Charybdis</i> | <i>lucifera</i> | E (per os)/I | PCR | Yes | No | Yes | Yes | 2 | 58 |
| Portunidae | <i>Charybdis</i> | <i>natator</i> | N/E (per os) | PCR | No | No | No | No | 3 | 36, 58 |
| Portunidae | <i>Podophthalmus</i> | <i>vigil</i> | E (per os)/I | PCR | Yes | No | Yes | Yes | 2 | 58 |
| Portunidae | <i>Portunus</i> | <i>trituberculatus</i> | N | qPCR | No | No | No | No | 2 | 47 |
| Portunidae | <i>Portunus</i> | <i>trituberculatus</i> | N/E (per os)/I | qPCR/TEM/histopathology | Yes | No | Yes | Yes | 3 | 48, 75 |

Annex 4 (contd)

| Family | Genus | Species | Stage 1: Route of infection* | Stage 2: Pathogen identification | Stage 3: Evidence for infection | | | | Outcome** | References |
|-------------------|---------------------|-----------------------|------------------------------------|-------------------------------------|------------------------------------|-----|-----|-----|-----------|--|
| | | | | | A | B | C | D | | |
| Portunidae | <i>Portunus</i> | <i>pelagicus</i> | N/E (per os)/I | PCR | Yes | No | Yes | Yes | 2 | 36, 62 |
| Portunidae | <i>Portunus</i> | <i>sanguinolentus</i> | N/E (per os)/I | PCR/ISH | Yes | No | Yes | Yes | 1 | 6, 7, 36, 40, 41, 58, 67 |
| Portunidae | <i>Scylla</i> | <i>olivacea</i> | I | qPCR | Yes | No | Yes | Yes | 2 | 60 |
| Portunidae | <i>Scylla</i> | <i>serrata</i> | N/E (per os) | PCR/ISH | Yes | Yes | Yes | Yes | 1 | 10, 34, 35, 38, 39, 40, 41, 54, 50, 62 |
| Portunidae | <i>Scylla</i> | <i>tranquebarica</i> | N/E (per os)/I | PCR (natural only) | Yes | Yes | Yes | Yes | 2 | 34, 54 |
| Portunidae | <i>Thalamita</i> | <i>danae</i> | E (per os)/I | PCR | Yes | No | Yes | Yes | 2 | 58 |
| Rotifera (phylum) | <i>Brachionus</i> | <i>urceus</i> | N | PCR | No | No | No | No | 3 | 70 |
| Scyllaridae | <i>Scyllarus</i> | <i>arctus</i> | E (per os)/I | TEM/dot blot | Yes | No | Yes | No | 2 | 12 |
| Sergestidae | <i>Acetes</i> | sp. | E (per os)/I | PCR | Yes | No | Yes | Yes | 2 | 62 |
| Sesarmidae | <i>Labuanium</i> | <i>rotundatum</i> | N | PCR | No | No | No | No | 3 | 49 |
| Sesarmidae | <i>Sesarma</i> | sp. | E (per os)/I | PCR | Yes | Yes | Yes | Yes | 2 | 35, 54 |
| Solenoceridae | <i>Solenocera</i> | <i>crassicornis</i> | N | PCR | No | No | No | No | 3 | 29 |
| Squillidae | <i>Squilla</i> | <i>mantis</i> | N | PCR | No | No | No | No | 3 | 29 |
| Varunidae | <i>Cyrtograpsus</i> | <i>angulatus</i> | N | PCR/qPCR | No | No | No | No | 3 | 45 |
| Varunidae | <i>Eriocheir</i> | <i>sinensis</i> | N/E (per os)/I | PCR/sequencing | Yes | Yes | Yes | Yes | 1 | 2, 17 |

| Family | Genus | Species | Stage 1: Route of infection* | Stage 2: Pathogen identification | Stage 3: Evidence for infection | | | | Outcome** | References |
|-----------|---|----------------------|------------------------------------|-------------------------------------|------------------------------------|----|-----|-----|-----------|------------|
| | | | | | A | B | C | D | | |
| Varunidae | <i>Helice</i> | <i>tridens</i> | N | PCR | No | No | No | No | 3 | 36 |
| Grapsidae | <i>Helice</i> | <i>tientsinensis</i> | N | dot blot/ISH | Yes | No | Yes | Yes | 2 | 76 |
| Varunidae | <i>Neohelice</i> (= <i>Chasmagnathus</i>) | <i>granulata</i> | N | PCR/sequencing | No | No | No | No | 3 | 5, 44 |
| Varunidae | <i>Pseudograpsus</i> | <i>intermedius</i> | N | PCR | No | No | No | No | 3 | 29, 30 |
| Xanthidae | <i>Atergatis</i> | <i>integerrimus</i> | E (per os)/I | PCR | No | No | No | No | 3 | 58 |
| Xanthidae | <i>Demania</i> | <i>splendida</i> | E (per os)/I | PCR | No | No | No | No | 3 | 58 |
| Xanthidae | <i>Liagore</i> | <i>rubronaculata</i> | E (per os)/I | PCR | Yes | No | Yes | Yes | 2 | 58 |

Route of infection Key*

N: Natural infection

E (per os/bath): Experimental infection per os/bath

I: Injection

nd: not determined

Outcome Key**

Outcome 1: Host species proposed to be listed in Article 9.7.2. of the Aquatic Code.

Outcome 2: Host species proposed to be listed in Chapter 2.2.7. of the Aquatic Manual under the revised Section 2.2.2. 'Species with incomplete evidence for susceptibility'.

Outcome 3: Host species proposed to be listed in Chapter 2.2.7. of the Aquatic Manual under the revised Section 2.2.2. 'Species with incomplete evidence for susceptibility' where pathogen-specific positive PCR results (but an active infection has not been demonstrated) have been reported.

Additional information relevant to WSSV

Host species to be included in Article 9.7.2. of the Aquatic Code

The *ad hoc* Group proposed amendments to the list of susceptible host species to be included in Article 9.7.2. of the *Aquatic Code*. Refer to Annex 5.

Host species to be included in Chapter 2.2.7. of the Aquatic Manual

The *ad hoc* Group proposed amendments to the list of species included in the revised Section 2.2.2. of Chapter 2.2.7. of the *Aquatic Manual*. Refer to Annex 6.

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CHAPTER 9.7.

INFECTION WITH WHITE SPOT SYNDROME VIRUS DISEASE

Article 9.7.1.

For the purposes of the *Aquatic Code*, white spot disease (WSD) means *infection* with white spot syndrome virus (WSSV). White spot syndrome virus 1 is classified as a species in the genus *Whispovirus* of the family *Nimaviridae*. Common synonyms are listed in the corresponding chapter of the *Aquatic Manual*.

Information on methods for *diagnosis* are provided in the *Aquatic Manual*.

Article 9.7.2.

Scope

The recommendations in this chapter apply to the following susceptible species which meet the criteria for listing species as susceptible in Chapter 1.5: all decapod (order Decapoda) crustaceans from marine, brackish and freshwater sources. These recommendations also apply to any other susceptible species referred to in the Aquatic Manual when traded internationally. blue-leg swimcrab (*Liocarcinus depurator*), Chinese mitten crab (*Eriocheir sinensis*), Danube crayfish (*Astacus leptodactylus*), edible crab (*Cancer pagurus*), European lobster (*Homarus gammarus*), fleshy prawn (*Penaeus chinensis*), giant tiger prawn (*Penaeus monodon*), Indian white prawn (*Penaeus indicus*), Kuruma prawn (*Penaeus japonicus*), Indo-Pacific swamp crab (*Scylla serrata*), Norway lobster (*Nephrops norvegicus*), painted spiny lobster (*Panulirus versicolor*), pronghorn spiny lobster (*Panulirus penicillatus*), red claw crayfish (*Cherax quadricarinatus*), threespot swimming crab (*Portunus sanguinolentus*), red swamp crayfish (*Procambarus clarkii*), greasyback shrimp (*Metapenaeus ensis*), signal crayfish (*Pacifastacus leniusculus*), southern rough shrimp (*Trachysalambria curvirostris*), spinycheek crayfish (*Orconectes limosus*), white-clawed crayfish (*Austropotamobius pallipes*), whiteleg shrimp (*Penaeus vannamei*), velvet swimcrab (*Necora—puber*), *Calanus pacificus californicus*, *Charybdis granulata*, *Dendronereis sp.*, ridgetail prawn (*Exopalaemon carinicauda*), Oriental prawn (*Exopalaemon orientis*), *Pagurus minutus*, barred grass shrimp (*Palaemon ritteri*), Sao Paulo shrimp (*Penaeus paulensis*), *Procambarus zonangulus*

For the purposes of this chapter, the terms shrimp and prawn are used interchangeably.

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CHAPTER 2.2.6.

WHITE SPOT DISEASE

1. Scope

For the purpose of this chapter, white spot disease (WSD) is considered to be infection with white spot syndrome virus (WSSV).

2.2. Host factors

WSSV has an extremely wide host range. The virus can infect a wide range of aquatic crustaceans especially decapod, including marine, brackish and freshwater prawns, crabs, crayfish and lobsters (Maeda *et al.*, 2000).

2.2.1. Susceptible host species

Species that fulfil the criteria for listing a species as susceptible to infection with WSSV according to Chapter 1.5. of the *Aquatic Animal Health Code (Aquatic Code)* include: blue-leg swimcrab (*Liocarcinus depurator*), Chinese mitten crab (*Eriocheir sinensis*), Danube crayfish (*Astacus leptodactylus*), edible crab (*Cancer pagurus*), European lobster (*Homarus gammarus*), fleshy prawn (*Penaeus chinensis*), giant tiger prawn (*Penaeus monodon*), Indian white prawn (*Penaeus indicus*), Kuruma prawn (*Penaeus japonicus*), Indo-Pacific swamp crab (*Scylla serrata*), Norway lobster (*Nephrops norvegicus*), Painted spiny lobster (*Panulirus versicolor*), pronghorn spiny lobster (*Panulirus penicillatus*), red claw crayfish (*Cherax quadricarinatus*), threespot swimming crab (*Portunus sanguinolentus*), red swamp crayfish (*Procambarus clarkii*), greasyback shrimp (*Metapenaeus ensis*), signal crayfish (*Pacifastacus leniusculus*), southern rough shrimp (*Trachysalambria curvirostris*), spinycheek crayfish (*Orconectes limosus*), white-clawed crayfish (*Austropotamobius pallipes*), whiteleg shrimp (*Penaeus vannamei*), velvet swimcrab (*Necora puber*), Calanus pacificus californicus, Charybdis granulata, Dendronereis sp., ridgetail prawn (*Exopalaemon carinicauda*), Oriental prawn (*Exopalaemon orientis*), Pagurus minutus, barred grass shrimp (*Palaemon ritteri*), Sao Paulo shrimp (*Penaeus paulensis*), Procambarus zonangulus.

To date, no decapod (order Decapoda) crustacean from marine and brackish or freshwater sources has been reported to be resistant (Flegel, 1997; Lightner, 1996; Lo & Kou, 1998; Maeda *et al.*, 2000; Stentiford *et al.*, 2009).

2.2.2. Species with incomplete evidence for susceptibility

Evidence is lacking for the following species to either confirm that the identity of the pathogenic agent is WSSV, transmission mimics natural pathways of infection, or presence of the pathogenic agent constitutes an infection:

2.2.2.1. Species for which there is incomplete evidence to fulfil the criteria for listing a species as susceptible to infection with WSSV according to Chapter 1.5. of the *Aquatic Code* include: Asian shore crab (*Hemigrapsus sanguineus*), banana prawn (*Penaeus merguensis*), blue shrimp (*Penaeus stylirostris*), blue swimming crab (*Portunus pelagicus*), banded-legged swimming crab (*Charybdis annulata*), calico fiddler crab (*Uca (=Leptuca) pugilator*), green crab (*Garcinus maenas*), crucifix crab (*Charybdis feriataus*), giant river prawn (*Macrobrachium rosenbergii*), freshwater crab (*Paratelphusa (Barytelphusa) pulvinata*), freshwater field crab (*Paratelphusa hydrodomous*), Japanese ghost shrimp (*Callinassa japonica*), Kadal shrimp (*Metapenaeus dobsoni*), Krill (*Acetes* sp.), lesser slipper lobster (*Scyllarus arctus*), mangrove crab (*Sesarma* sp.), Baltic prawn (*Palaemon adspersus*), mud spiny lobster (*Panulirus polyphagus*), northern brown shrimp (*Penaeus aztecus*), northern pink shrimp (*Penaeus duorarum*), stone king crab (*Lithodes maja*), northern white shrimp (*Penaeus setiferus*), scalloped spiny lobster (*Panulirus homarus*), periscope crab (*Podophthalmus vigil*), teppo snapping shrimp (*Alpheus brevicristatus*), speckled shrimp (*Metapenaeus monoceros*), swimming brachyuran crab (*Charybdis lucifera*), yabby crayfish (*Cherax destructor*), Ashtoret miersii, spectacled box crab (*Calappa philargius*), Doclea muricata (=hybrida), Ergasilus manicatus, mottled crab (*Grapsus albolineatus*), Halimede ochtodes, Helice tientsinensis, Liagore rubronaculata, slender river prawn (*Macrobrachium idella*), Kuncho river prawn (*Macrobrachium lamerraei*), Oriental river prawn (*Macrobrachium nipponense*), Macrophthalmus japonicus, Metopograpsus sp., Paradorippe granulata, Parthenope prensor, Philyra syndactyla, swimming crab (*Portunus trituberculatus*), orange mud crab (*Scylla olivacea*), purple mud crab (*Scylla tranquebarica*), Thalamita danae.

Annex 6 (contd)

2.2.2.2. Pathogen-specific positive PCR results (without confirmation of an active infection) have been reported in the following organisms: blue crab (*Callinectes sapidus*), common Bbox crab (*Calappa lophos*), Indian fiddler crab (*Uca (=Gelasimus) vocans (=marionis nitidus)*), swimming crab (*Portunus trituberculatus*), green tiger prawn (*Penaeus semisulcatus*), kiddi shrimp (*Parapenaeopsis stylifera*), longlegged spiny lobster (*Panulirus longipes*), mangrove rock crab (*Metopograpsus messon*), flower moon crab (*Matuta planipes*), noble crayfish (*Astacus astacus*), Ornate spiny lobster (*Panulirus ornatus*), redbtail prawn (*Penaeus penicillatus*), yellow shrimp (*Metapenaeus brevicornis*), yellowleg shrimp (*Penaeus californiensis*), *Alpheus lobidens*, *Apocyclops royi*, Argentine stiletto shrimp (*Artemesia longinaris*), brine shrimp (*Artemia salina*), brine shrimps (*Artemia sp.*), *Atergatis integerrimus*, *Balanus sp.*, *Brachionus urceus*, *Cuata swimcrab (Callinectes arcuatus)*, *Charybdis cruciata*, Japanese swimming crab (*Charybdis japonica*), ridged swimming crab (*Charybdis natator*), *Coleoptera*, *Ephydridae*, Japanese sand shrimp (*Crangon affinis*), *Demania splendida*, *Diogenes nitidimanus*, *Helice tridens*, *Labuanium rotundatum*, *Macrophthalmus sulcatus*, *Marphysa gravelyi*, maroon stone crab (*Menippe rumphii*), Jinga shrimp (*Metapenaeus affinis*), *Nechelice (=Chasmagnathus) granulata*, *Nitocra sp.*, *Orconectes punctimanus*, Palaemon shrimps (*Palaemon sp.*), migrant prawn (*Palaemon macrodactylus*), *Palaemonetes pugio*, *Pagurus angustus*, *Pseudograpsus intermedius*, coastal mud shrimp (*Solenocera crassicornis*), spottail mantis squillid (*Squilla mantis*).

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