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# REPORT OF THE MEETING OF THE OIE *AD HOC* GROUP ON SUSCEPTIBILITY OF CRUSTACEAN SPECIES TO INFECTION WITH OIE LISTED DISEASES[1]

**Paris, 13–15 October 2015**

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 13–15 October 2015.

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 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 first meeting in February 2015 regarding the list of susceptible species for infection with yellow head virus had been considered by the Aquatic Animal Health Standards Commission at their March 2015 meeting.

The chair of the *ad hoc* Group, Dr Grant Stentiford, thanked the members for all their hard work prior to the physical meeting in undertaking literature reviews and preparing assessments for seven of the OIE listed crustaceans diseases (Acute hepatopancreatic necrosis disease; Crayfish plague; Infectious hypodermal and haematopoietic necrosis; Infectious myonecrosis; Necrotising hepatopancreatitis; Taura syndrome; and White tail disease). Dr Stentiford clarified that the purpose of this meeting was to review these assessments in order to finalise the lists of susceptible species for the pathogens associated with these diseases for inclusion in the OIE *Aquatic Animal Health Code (Aquatic Code)* and *Manual of Diagnostic Tests for Aquatic Animals (Aquatic Manual)*.

The *ad hoc* Group applied the 3-stage approach, outlined in Article 1.5.3. of Chapter 1.5 of the *Aquatic Code*, to assess susceptibility of a species to infection with a specified pathogenic agent. The "Criteria for listing species as susceptible to infection with a specific pathogen" in 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.);

3) criteria to determine whether the evidence indicates that presence of the pathogenic agent constitutes an infection (as described in Article 1.5.6.).

Hosts that were classified as susceptible species (as described in Article 1.5.7.) were proposed for inclusion in Article X.X.2. of the *Aquatic Code.*

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 February 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-animalcommission-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 Article 2.2.2. 'Species with incomplete evidence for susceptibility' of the *Aquatic Manual*.

In addition, the *ad hoc* Group identified hosts where there was only evidence for criteria in Article 1.5.4. ('natural pathways for infection') and 1.5.5. ('pathogenic agent has been adequately identified'), but not 1.5.6. ('presence of the pathogenic agent constitutes an infection'). The *ad hoc* Group proposed that these hosts be included in the relevant *Aquatic Manual* chapter under the proposed new sub-heading '2.2.2. Species with incomplete evidence for susceptibility' in section '2.2. Host factors' in the following manner:

'In addition, pathogen-specific positive PCR results have been reported in the following organisms but an active infection has not been demonstrated: …'

The detailed assessments for each specific pathogenic agent assessed by the *ad hoc* Group are provided in Annexes 4 to 10.

| **Disease** | **Annex Number** |
|---|---|
| Crayfish plague (*Aphanomyces astaci*) | Annex 4 |
| Infectious hypodermal and haematopoietic necrosis | Annex 5 |
| Infectious myonecrosis | Annex 6 |
| Necrotising hepatopancreatitis | Annex 7 |
| Taura syndrome | Annex 8 |
| White tail disease | Annex 9 |
| Acute hepatopancreatic necrosis disease | Annex 10 |

The *ad hoc* Group noted that some text in Section '2.2. Host Factors' of the *Aquatic Manual* includes text and references to susceptible species. Given the proposed revised lists of susceptible species, the *ad hoc* Group made the following recommendations:

1) Section '2.2.5. Persistent infection with lifelong carriers'. The *ad hoc* Group recommended this title be amended to 'Persistent infection ~~carriers~~' because it is unknown whether persistence is lifelong. In addition they recommended that the relevant Reference Laboratory expert, includes in this section, a simple statement about persistent infection status supported by references, and that any text referring to susceptibility be deleted.

2) Section '2.2.7. Known or suspected wild aquatic animal carriers'. The *ad hoc* Group proposed that this section be deleted as it is covered under sections on susceptible hosts and persistent carriers and as currently written creates confusion.

The *ad hoc* Group noted that the only crustacean OIE listed disease yet to be assessed was white spot disease (WSD) caused by white spot syndrome virus (WSSV). The *ad hoc* Group agreed to commence this work electronically and requested that a physical meeting be held in early 2016 to finalise this work.

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.../Annexes

Annex 1

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

**Paris (France), 13–15 October 2015**

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## List of participants

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Annex 2

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

**Paris (France), 13–15 October 2015**

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## Agenda

1. Welcome

2. Review assessments for species susceptibility as described in Chapter 1.5. of the *Aquatic Code* for:

    2.1. Crayfish plague (Chapter 9.1.)

    2.2. Infectious hypodermal and haematopoietic necrosis (Chapter 9.3.)

    2.3. Infectious myonecrosis (Chapter 9.4.)

    2.4. Necrotising hepatopancreatitis (Chapter 9.5.)

    2.5. Taura syndrome (Chapter 9.6.)

    2.6. White tail disease (Chapter 9.8.)

    2.7. Acute hepatopancreatic necrosis disease (Chapter 9.X.)

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

**Paris (France), 13–15 October 2015**

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## 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*.

Assessments will be undertaken by *ad hoc* Groups and the assessments will be provided to Member Countries for comment prior to any change in the list of susceptible species in Article X.X.2. of the disease-specific chapters in the *Aquatic Code*.

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 will be included in the relevant disease-specific chapter in the *Aquatic Manual*.

### Purpose

The *ad hoc* Group on Susceptibility of crustacean species to infection with OIE listed diseases will undertake this task for OIE listed crustacean diseases.

### 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 OIE listed diseases based on Article 1.5.7.

4) Propose susceptible species for OIE listed diseases based on Article 1.5.8.

### Expected outputs of the October 2015 meeting of the *ad hoc* Group

1) Develop a list of susceptible species for inclusion in the relevant articles of crustacean disease-specific chapters in the *Aquatic Code* and *Manual* for Crayfish plague (Chapter 9.1.); Infectious hypodermal and haematopoietic necrosis (Chapter 9.3.); Infectious myonecrosis (Chapter 9.4.); Necrotising hepatopancreatitis (Chapter 9.5.); Taura syndrome (Chapter 9.6.); White tail disease (Chapter 9.8.); and Acute hepatopancreatic necrosis disease (Chapter 9.X.).

2) Draft a report for consideration by the Aquatic Animals Commission at their February 2016 meeting.

# ASSESSMENT OF HOST SUSCEPTIBILITY TO INFECTION WITH *APHANOMYCES ASTACI*

The objectives of this assessment were (1) to determine susceptibility of given host taxa to infection with *Aphanomyces astaci* , the causative agent of crayfish plague, by applying the 3-stage approach 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 *Aquatic Manual* with respect to host species susceptibility.

In this assessment the confirmation for susceptibility to infection with *A. astaci* (Stage 2) is based on Chapter 2.2.1. in the *Aquatic Manual* which states that a presumptive diagnosis of *A. astaci* can be made based on the presence of hyphae penetrating the cuticle resulting in a host tissue response (i.e. haemocytes and melanisation) and the presence of sporangia that morphologically correspond to *A. astaci* However, 'confirmation' of *A. astaci* should be based on PCR and sequence authentication.

Criteria for susceptibility to infection with *A. astaci* (stage 3) are detailed in Table 1 (as per Article 1.5.6. of the *Aquatic Code*). This table includes Replication (A), Viability/Infectivity (B), Pathology/Clinical Signs (C) and Location (D). Hosts were considered to be infected with *A. astaci* 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 A. astaci (Stage 3)*

| **A: Replication♯** | **B: Viability/Infectivity** | **C: Pathology/Clinical Signs** | **D: Location** |
|---|---|---|---|
| Presence of *A. astaci* developing hyphae with/without sporulation in the cuticle and/or underlying tissues; | *Aphanomyces* can be cultured in artificial media (Alderman and Polglase, 1986); | Presence of fungal hyphae 7 to 9 um wide in the cuticle and/or underlying tissues associated with haemocytic infiltration with/without melanisation. | Soft cuticle is usually the first tissue to be affected; however, *A. astaci* will eventually spread throughout connective tissue and haemal sinuses. |
| OR | 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**. | Clinical signs include localised whitening of the muscle under the infected cuticle. | |
| AND | | | |
| 1. Positive labelling of hyphae with ISH or IFAT; | | | |
| OR | | | |
| 2. Demonstration of increased copy number over time with qPCR with confirmatory PCR/sequencing specific for *A. astaci*. | | | |

Key:

♯ For this pathogen, the *ad hoc* Group agreed to forgo requirement for confirmation of replication using molecular or antibody labelling because these techniques were not utilized historically for this pathogen.

* To demonstrate replication by this approach requires evidence for passage in confirmed 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.

# ASSESSMENT FOR HOST SUSCEPTIBILITY

The assessment for host susceptibility to infection with *A. astaci* is provided in Table 2 (nd - not determined).

***Table 2.*** *Outcome of assessment for host susceptibility to infection with A. astaci*

| **Family** | **Genus** | **Species** | **Stage 1: Route of infection** | **Stage 2: Pathogen identification** | **Stage 3: Evidence for infection** | | | | **Outcome*** | **References** |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | **A** | **B** | **C** | **D** | | |
| Astacidae | *Austropotamobius* | *pallipes* | Natural | PCR | Yes | Yes | Yes | Yes | 1 | 1,3 |
| Astacidae | *Austropotamobius* | *torrentium* | Natural | PCR and sequencing | nd | nd | nd | Yes | 2 | |
| Astacidae | *Astacus* | *leptodactylus* | Experiment non-invasive | PCR and sequencing | nd | Yes | Yes | Yes | 1 | 1 6 |
| Astacidae | *Astacus* | *astacus* | Natural; experimental non-invasive | No | Yes | Yes | Yes | Yes | 1 | 4, 9, 15 |
| Astacidae | *Pacifastacus* | *leniusculus* | Natural | PCR | Yes | Yes | Yes | Yes | 1 | 7, 9, 14,16 |
| Cambaridae | *Procambarus* | *clarkii* | Natural | No | Yes | Yes | Yes | Yes | 2 | 4, 9, 14 |
| Cambaridae | *Procambarus* | *alleni* | Natural | PCR and sequencing | nd | Yes | Yes | Yes | 1 | 8 |
| Cambaridae | *Procambarus* | *fallax virginalis* | Natural | PCR | nd | nd | nd | Yes | 3 | 5, 8 |
| Cambaridae | *Orconectes* | *limosus* | Natural | PCR | Yes | Yes | Yes | Yes | 1 | 9, 14 |
| Cambaridae | *Orconectes* | *cf. virilis* | Natural | PCR | nd | nd | nd | nd | 3 | 14 |
| Cambaridae | *Orconectes* | *immunis* | Natural | PCR | nd | nd | nd | Yes | 2 | 10 |

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 | | |
| Parastacidae | *Cherax* | *quadricarinatus* | Natural; experimental non-invasive | No** | Yes | nd | Yes | Yes | 2 | 8, 15 |
| Parastacidae | *Cherax* | *destructor* | Experimental non-invasive | No | Yes | nd | Yes | Yes | 2 | 15 |
| Parastacidae | *Cherax* | *papuanus* | Experimental non-invasive | No | Yes | nd | Yes | Yes | 2 | 15 |
| Parastacidae | *Euastacus* | *kershawi* | Experimental non-invasive | No | Yes | nd | Yes | Yes | 2 | 15 |
| Parastacidae | *Euastacus* | *claydensis* | Experimental non-invasive | No | Yes | nd | Yes | Yes | 2 | 15 |
| Parastacidae | *Euastacus* | *crassus* | Experimental non-invasive | No | Yes | nd | Yes | Yes | 2 | 15 |
| Parastacidae | *Geocherax* | *gracilis* | Experimental non-invasive | No | Yes | nd | Yes | Yes | 2 | 15 |
| Parastacidae | *Astacopsis* | *gouldi* | Experimental non-invasive | No | Yes | nd | Yes | Yes | 2 | 15 |
| Parastacidae | *Astacopsis* | *fluviatilis* | Experimental non-invasive | No | Yes | nd | Yes | Yes | 2 | 15 |
| Palaemonidae | *Macrobrachium* | *dayanum* | Experimental non-invasive | PCR | nd | nd | Nd | Nd | 3 | 13 |
| Varunidae | *Eriocheir* | *sinensis* | Natural; experimental | PCR and sequencing | nd | Yes | No | Yes | 2 | 2, 14, 11, 13 |
| Potamidae | *Potamon* | *potamios* | Natural | PCR and sequencing | Yes | nd | Yes | Yes | 1 | 12 |

** PCR positive in one study but could have been contamination and no stage 3.

Outcome Key*:
Outcome 1: Host species proposed to be listed in Article 9.1.2. of the *Aquatic Code.*
Outcome 2: Host species proposed to be listed in Chapter 2.2.1. 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 2.2.1. 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.
Note: No differentiation has been made between different groups (A-D) of *A. astaci* in this report because strain types are not generally reported in the literature.

**Additional information relevant to *A. astaci***

Many of the early studies in the literature did not confirm the pathogen using molecular techniques that would differentiate it from other oomycetes or fungi. In most of these cases, with the exception of the crayfish species in Australia (i.e. *Cherax* spp.), the *ad hoc* Group were also able to confirm the susceptibility of the host taxa with more recent studies that utilised PCR and sequencing. Further, this pathogen infects the cuticle of crayfish so it is particularly difficult to establish whether an animal is infected with the pathogen versus surface contamination when no other diagnostic evaluation other than a molecular test was undertaken on the exoskeleton of the animal. The *ad hoc* Group relied on evidence of replication and invasion of the tissue to differentiate between these two scenarios but in many instances reference to pathology pertaining to crayfish plague was absent from the reports.

**Host species to be included in Article 9.1.2. of the *Aquatic Code***

The *ad hoc* Group proposed that the following host species be included in Article 9.1.2. of the *Aquatic Code*: Noble crayfish (*Astacus astacus*), Danube crayfish (*Astacus leptodactylus*), Signal crayfish (*Pacifastacus leniusculus*), Red swamp crawfish (*Procambarus clarkii*), *Austropotamobius torrentium, Austropotamobius pallipes, Orconectes limosus, Orconectes immunis, Procambarus alleni* and *Potamon potamios.*

Note: The *ad hoc* Group found research indicating that several species of crayfish in the families Cambaridae, and Astacidae met our criteria for susceptibility (listed as '1' in Table 2). However, they also found research indicating several species in both of these families had evidence of susceptibility to infection with *A. astaci*, but the extent of the information provided was insufficient on its own to meet the criteria for listing these hosts in the *Aquatic Code.* Given the numerous species in both these families that either fully or partially met the criteria for susceptibility (listed as "1" or "2" in Table 2) the *ad hoc* Group recommends that all species belonging to these two families (Cambaridae and Astacidae) be included in the list of susceptible species in Article 9.1.2. of the *Aquatic Code*.

**Host species to be included in Chapter 2.2.1. of the *Aquatic Manual***

The *ad hoc* Group proposed that the following host species be included in the revised Section 2.2.2. of Chapter 2.2.1. of the *Aquatic Manual* as species with only partially evidence for susceptibility to *A. astaci*: *Astacopsis fluviatilis*, *Astacopsis gouldi*, Red claw crayfish (*Cherax quadricarinatus*), Yabby crayfish (*Cherax destructor*), *Cherax papuanus*, *Euastacus crassus*, *Euastacus claydensis*, *Euastacus kershawi*, *Geocherax gracilis*, and Chinese mitten crab (*Eriocheir sinensis*).

Note: Crayfish belonging to the family Parastacidae did not meet the criteria for listing as susceptible species; however, there are very few studies that have evaluated these species. The only reports available did not confirm the presence of *A. astaci* using molecular tools and/or they did not clearly meet the criteria for infection. The *ad hoc* Group therefore recommends that species belonging to the family Parastacidae and the crab species *Eriocheir sinensis* be included in the *Aquatic Manual* under species with incomplete or partial evidence for susceptibility until new evidence is available. Given that at least one non-crayfish species (i.e. *Potamon potamios*) met the criteria for a susceptible host for *A. astaci* and a second crab species *(Eriocheir sinensis)* in the infraorder Brachyura met several of the criteria for susceptibility, the *ad hoc* Group recommends that text be added to the *Aquatic Manual* noting that any crustaceans from *A. astaci* positive watersheds could pose a risk of transmitting *A. astaci* either as vectors or potential susceptible hosts.

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# ASSESSMENT OF HOST SUSCEPTIBILITY TO INFECTION WITH INFECTIOUS HYPODERMAL AND HAEMATOPOIETIC NECROSIS VIRUS (IHHNV)

The objectives of this assessment were (1) to determine susceptibility of given host taxa to infection with infectious hypodermal and haematopoietic necrosis virus (IHHNV) by applying the 3-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 *Aquatic Manual* with respect to host species susceptibility.

In this assessment the confirmation for susceptibility to infection with IHHNV infection is based on Chapter 2.2.2. in the *Aquatic Manual* which states that a confirmed diagnosis is:

"Infectious hypodermal and haematopoietic necrosis (IHHN) is considered to be confirmed if two of the following criteria are met:

i) positive result by *in-situ* hybridization;

ii) positive result by PCR (always genotype specific);

iii) sequence analysis to confirm IHHNV nucleic acid sequence.

The two methods must target different areas of the genome."

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

| **A: Replication** | **B: Viability/Infectivity** | **C: Pathology/Clinical Signs** | **D: Location** |
|---|---|---|---|
| Presence of characteristic inclusion bodies and positive labelling of inclusion bodies by ISH or IFAT.<br>Presence of virions in inclusion bodies by TEM.<br>Demonstration of increasing copy number over time with qPCR with confirmatory PCR/ sequencing specific for infectious virus.<br>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**. | Numerous necrotic cells with pyknotic nuclei or characteristic eosinophilic inclusion bodies, with within chromatin-marginated, hypertrophied nuclei of cells in target tissues and/or clinical signs (e.g. runt deformity syndrome)***. | Gills, cuticular epithelium (or hypodermis), all connective tissues, the haematopoietic tissues, the lymphoid organ, antennal gland, and the ventral nerve cord, its branches and its ganglia****. |

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 typical of IHHNV may provide evidence for fulfillment of this category when evidence from histopathology is not available. However, clinical signs according to the *Manual* chapter may not present equally in all host taxa and may not be specific for infection with IHHNV.

**** Lymphoid organ not present in most non-penaeid host taxa.

# ASSESSMENT FOR HOST SUSCEPTIBILITY

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

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

| **Genus** | **Species** | **Stage 1: Transmission*** | **Stage 2: Pathogen identification** | **Stage 3: Evidence for infection** | | | | **Outcome**** | **Refs** |
|---|---|---|---|---|---|---|---|---|---|
| | | | | **A** | **B** | **C** | **D** | | |
| *Penaeus* | *vannamei* | N, E (*per os*) | PCR | ISH; TEM | Yes | Yes | Yes | 1 | 3, 8, 10, 13 |
| | *aztecus* | N | PCR | No | No | No | Yes | 2 | 1 |
| | *stylirostris* | N, E (*per os*) | PCR | ISH; TEM | Yes | Yes | Yes | 1 | 3, 7, 8 |
| | *californiensis* | N | PCR | ISH | No | No | Yes | 1 | 4, 5, 9 |
| | *setiferus* | N | PCR | No | No | Yes | Yes | 1 | 1 |
| | *duorarum* | E | No | No | No | No | No | 3 | 4 |
| | *monodon* | N, E (*per os*) | PCR | ISH | No | Yes | Yes | 1 | 8, 13 |
| | *occidentalis* | N | No | No | No | No | No | 3 | 4 |
| | *semisulcatus* | N | No | No | No | No | No | 3 | 4 |
| | *japonicus* | N | No | No | No | No | No | 3 | 4 |
| *Macrobrachium* | *rosenbergii* | N | PCR | ISH | No | Yes | Yes | 1 | 2 |
| *Hemigrapsus* | *penicillatus* | N | PCR | No | No | No | No | 3 | 14 |
| *Artemesia* | *longinaris* | N | PCR | No | No | No | No | 3 | 6 |
| *Callinectes* | *arcuatus* | N | PCR | No | No | No | No | 3 | 5 |
| *Achirus* | *mazatlanus* | N | PCR | No | No | No | No | 3 | 5 |
| *Gerres* | *cinerus* | N | PCR | No | No | No | No | 3 | 5 |
| *Oreochromis* | sp. | N | PCR | No | No | No | No | 3 | 5 |
| *Lile* | *stolifera* | N | PCR | No | No | No | No | 3 | 5 |
| *Centropomus* | *medius* | N | PCR | No | No | No | No | 3 | 5 |

Transmission Key*:
N: Natural infection
E: Experimental infection

Outcome Key**
Outcome 1: Host species proposed to be listed in Article 9.3.2. of the *Aquatic Code.*
Outcome 2: Host species proposed to be listed in Chapter 2.2.2. 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 2.2.2. 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 IHHNV**

Presence of IHHNV nucleic acid sequences integrated into the host genome were not considered to be infection with IHHNV and were not part of this assessment (Tang & Lightner, 2006; Tang *et al.*, 2007).

**Host species to be included in Article 9.3.2. of the *Aquatic Code***

The *ad hoc* Group proposed that the following host species be included in Article 9.3.2. of the *Aquatic Code*:

*Penaeus vannamei*, *P. stylirostris*, *P. californiensis*, *P. setiferus*, *P. monodon* and *Macrobrachium rosenbergii*.

**Host species to be included in Chapter 2.2.2. of *Aquatic Manual***

The *ad hoc* Group proposed that the following host species be included in the revised Section 2.2.2. of the *Aquatic Manual*:

*P. aztecus*, *P. duorarum*, *P. occidentalis*, *P. japonicus*, *P. semisulcatus*, *Hemigrapsus penicillatus*, *Artemesia longinarus*, *Callinectes arcuatus*, *Archirus mazatlanus*, *Gerres cinerus*, *Oreochromis* sp., *Lile stolifera* and *Centropomus medius*.

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# ASSESSMENT OF HOST SUSCEPTIBILITY TO INFECTION WITH INFECTIOUS MYONECROSIS VIRUS (IMNV)

The objectives of this assessment were (1) to determine susceptibility of given host taxa to infection with infectious myonecrosis virus (IMNV) by applying the 3-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 *Aquatic Manual* with respect to host species susceptibility.

In this assessment the confirmation for susceptibility to infection with IMNV infection is based on Chapter 2.2.3. in the *Aquatic Manual* which states that a confirmed diagnosis is: "Any combination of a molecular (PCR or ISH) test and a morphological (histology) test using at least two of the following three methods (with positive results):

- histological demonstration of diagnostic acute, transition or chronic-phase IMNV lesions in the striated muscles and/or the LO;
- ISH positive (with an IMNV-specific cDNA probe) signal to IMNV-type lesions in striated necrotic muscle fibres or to distinctive LOS in the lymphoid organs of shrimp with transition or chronic-phase IMNV infections in histological sections;
- one step or nested RT-PCR, or real time RT-qPCR with positive results for IMNV."

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

| **A: Replication** | **B: Viability/Infectivity** | **C: Pathology/Clinical signs** | **D: Location** |
|---|---|---|---|
| Presence of characteristic inclusion bodies and positive labelling of inclusion bodies by ISH or IFAT.<br>Presence of virions in inclusion bodies by TEM.<br>Demonstration of increasing copy number over time with RT-qPCR with confirmatory RT-PCR/ sequencing specific for infectious virus.<br>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**. | Multifocal to diffuse with characteristic coagulative necrosis of skeletal muscle fibres, often with marked edema. The main sign is whitish opaque lesions in skeletal tail muscle; infected shrimp may present lethargy. Shrimp may present a mix of acute and older lesions. In these shrimp, the affected muscle fibres appear to progress from presenting coagulative necrosis to presenting liquefactive necrosis, which is accompanied by moderate infiltration and accumulation of haemocytes, fibrosis and presence of basophilic inclusion bodies within cytoplasm of haemocytes, muscle and connective tissue cells. Lymphoid organ spheroids and ectopic spheroids are also a frequent finding. In the most advanced lesions, haemocytes and inflamed muscle fibres are replaced by a loose matrix of fibrocytes and connective tissue fibres that are interspersed with haemocytes and foci of (presumed) regenerating muscle fibres***. | Striated muscle (skeletal and less often cardiac),<br>connective tissue, haemocytes, and the lymphoid organ parenchymal cells****. |

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 typical of IMNV may provide evidence for fulfillment of this category when evidence from histopathology is not available. However, clinical signs according to the *Manual* chapter may not present equally in all host taxa and may not be specific for infection with IMNV.

**** Lymphoid organ not present in most non-penaeid host taxa.

# ASSESSMENT FOR HOST SUSCEPTIBILITY

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

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

| **Genus** | **Species** | **Stage 1: Transmission*** | **Stage 2: Pathogen identification** | **Stage 3: Evidence for infection** | | | | **Outcome**** | **Refs** |
|---|---|---|---|---|---|---|---|---|---|
| | | | | **A** | **B** | **C** | **D** | | |
| *Penaeus* | *vannamei* | N, E (*per os*) | RT-PCR | ISH | Yes | Yes | Yes | 1 | 3-5 |
| | *stylirostris* | E (injection) | RT-PCR | ISH | No | Yes | Yes | 2 | 4 |
| | *monodon* | E (injection) | RT-PCR | ISH | No | No | Yes | 2 | 4 |
| | *subtilis* | E (*per os*) | RT-PCR | No | No | No | No | 3 | 1 |
| | *esculentus* | E (injection, immersion; *per os*) | RT-PCR | ISH | No | Yes | Yes | 1 | 2 |
| | *merguiensis* | E (injection; immersion; *per os*) | RT-PCR | ISH | No | Yes | Yes | 1 | 2 |

Transmission Key*:
N: natural infection
E: experimental infection

Outcome Key**:
Outcome 1: Host species proposed to be listed in Article 9.4.2. of the *Aquatic Code*.
Outcome 2: Host species proposed to be listed in 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 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 IMNV**

Not applicable.

**Host species to be included in Article 9.4.2. of the *Aquatic Code***

The *ad hoc* Group proposed that the following host species be included in Article 9.4.2. of the *Aquatic Code*:

*Penaeus vannamei*, *P. esculentus* and *P. merguiensis*.

**Host species to be included in Chapter 2.2.3. of the *Aquatic Manual***

The *ad hoc* Group proposed that the following host species be included in the revised Section 2.2.2. of Chapter 2.2.3. of the *Aquatic Manual*:

*P. monodon*, *P. stylirostris* and *P. subtilis*.

**References**

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# ASSESSMENT OF HOST SUSCEPTIBILITY TO INFECTION WITH CANDIDATUS *HEPATOBACTER PENAEI*

The objectives of this assessment were (1) to determine susceptibility of given host taxa to infection Candidatus *Hepatobacter penaei* (hereafter 'susceptibility to NHP') by applying the 3-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 *Aquatic Manual* with respect to host species susceptibility.

In this assessment the confirmation for susceptibility to infection with NHP is based on Chapter 2.2.4. in the *Aquatic Manual* which states that a confirmed diagnosis is:

Histological demonstration of diagnostic acute-phase NHPB lesions in (especially) the atrophied hepatopancreas with moderate atrophy of the tubule mucosa, presence of bacteria and infiltrating haemocytes involving one or more of the tubules (multifocal encapsulations) AND, ISH positive histological signal to NHPB-type lesions, OR PCR positive result for the causative agent Candidatus *Hepatobacter penaei*.

Criteria for susceptibility to NHP are detailed in Table 1 (as per Article 1.5.6. of the *Aquatic Code*). This Table includes Replication (A), Viability/Infectivity (B), Pathology/Clinical Signs (C) and Location (D). Hosts were considered to susceptible to NHP 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 NHP*

| **A: Replication** | **B: Viability/Infectivity** | **C: Pathology/Clinical Signs** | **D: Location** |
|---|---|---|---|
| Presence of Candidatus *Hepatobacter penaei* colonies observed in HP epithelial cell cytoplasm observed by histology. Colonies confirmed to be C.*H.p.* via positive labelling by ISH or IFAT.<br><br>Demonstration of increasing copy number of bacterial target genes (16s rRNA) over time with qPCR.<br><br>Serial passage from individual to SPF individual of the same species*. | Single passage bioassay to a SPF (target pathogen) population of any susceptible host species (e.g. *P. vannamei*) and, confirmation of pathogen synonymy in donor and recipient population using PCR and sequencing of the 16s rRNA gene**. | Distinct phases of pathogenesis: **initial** (low presence of HP tubule epithelial desquamation), **acute** (atrophied hepatopancreas, increase in desquamation of epithelial cells, bacterial colonies and haemocytic infiltration), **transition** (widespread necrosis/sloughing of epithelial cells, edema, widespread haemocyte infiltration and encapsulation of HP tubules) and, **chronic** (HP lesions less abundant but organ is infiltrated by haemocytes and fibrosis apparent). Clinical signs (e.g slow growth, a soft cuticle and a flaccid body) and an acute, usually catastrophic disease with mortalities approaching 100% may indicate NHP but are not pathognomonic***. | Hepatopancreatic tubules. Intracellular infection of hepatopancreatic tubule epithelial cytoplasm by colonies of C.*H.p.* Pronounced encapsulation of infected hepatopancreatic tubules during acute and transition phases of the disease. |

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 to a known susceptible SPF host is required.

*** Clinical signs typical of NHP may provide some evidence for fulfillment of this category when evidence from histopathology is not available. However, clinical signs according to the *Manual* chapter are not pathognomonic for NHP and further, may not present equally in all host taxa.

# ASSESSMENT FOR HOST SUSCEPTIBILITY

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

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

| **Genus** | **Species** | **Stage 1: Transmission** | **Stage 2: Pathogen Identification** | **Stage 3: Evidence for infection** | | | | **Outcome*** | **References** |
|---|---|---|---|---|---|---|---|---|---|
| | | | | A | B | C | D | | |
| *Penaeus* | *vannamei* | Natural | Yes | Yes | Yes | Yes | Yes | 1 | 1,2,3,4,5 |
| | *setiferus* | Natural | Partial | No | No | No | No | 2 | 6 |
| | *aztecus* | Natural | Partial | No | No | No | No | 2 | 6 |
| | *duorarum* | Natural | Partial | No | No | No | No | 2 | 6 |
| | *merguiensis* | Natural | No | No | No | Yes | Yes | 2 | 7 |
| | *marginatus* | Natural | No | No | No | Yes | Yes | 2 | 7 |
| | *stylirostris* | Natural | No | n | n | y | y | 2 | 7,8 |
| | *monodon* | Natural | No | n | n | Y | y | 2 | 7 |
| *Homarus* | *americanus* | Experimental/ non-invasive | Yes | n | n | n | n | 3 | 9 |

*Outcome key:

1: Host species proposed to be listed in Article 9.5.2. of the *Aquatic Code.*

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

3: Host species proposed to be listed in 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 NHP**

Chapter 2.2.4. of the OIE *Aquatic Manual* states that the following may be used to confirm NHPB identity:

*'any combination of a molecular (PCR or ISH) test and a morphological (histology) test using at least two of the following three methods (with positive results): Histological demonstration of diagnostic acute-phase NHPB lesions in (especially) the atrophied hepatopancreas with moderate atrophy of the tubule mucosa, presence of bacteria and infiltrating haemocytes involving one or more of the tubules (multifocal encapsulations); ISH positive histological signal to NHPB-type lesions; PCR positive results for NHPB.'*A recent paper by Nunan *et al.* (2013) (10) has classified the causative agent of NHPB as Candidatus *Hepatobacter penaei*. Systematics based upon the 16S rRNA and gyrase B genes have placed the agent within the class *Alphaproteobacteria*, order *Rickettsiales*.

The authors state that 'classifying and provisionally naming the bacteria responsible for NHP will help eliminate future confusion with other pathogenic bacteria that can cause similar pathology of the HP in *P. vannamei*'. Lightner (1996) (7) previously stated that 'Similar, if not identical NHP bacteria, have been found to be associated with serious epizootic disease in shrimp farms in Peru, Ecuador, Venezuela, Brazil, Panama, and Costa Rica'.

Based upon this evidence, for the purposes of this exercise, 'Confirmation' of pathogen identification within a potential susceptible host is based upon characterisation of *Hepatobacter penaei* via the method of Nunan *et al.* (2013) (10), or by a previously published PCR (and sequencing) approach developed by the same group (Nunan *et al.*, 2008) (11).

**Host species to be included in Article 9.5.2. of the *Aquatic Code***

The *ad hoc* Group proposed that the following host species be included in Article 9.5.2. of the *Aquatic Code*: *Penaeus vannamei*.

**Host species to be included in Chapter 2.2.4. of the *Aquatic Manual***

The *ad hoc* Group proposed that the following host species be included in the revised Section 2.2.2. of Chapter 2.2.4. of the *Aquatic Manual*:

*Penaeus aztecus*, *Penaeus setiferus*, *Penaeus stylirostris*, *Penaeus duorarum*, *Penaeus merguiensis*, *Penaeus marginatus* and *Penaeus monodon.*

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# ASSESSMENT OF HOST SUSCEPTIBILITY TO INFECTION WITH TAURA SYNDROME VIRUS (TSV)

The objectives of this assessment were (1) to determine susceptibility of given host taxa to infection with Taura syndrome virus (TSV) by applying the 3-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 *Aquatic Manual* with respect to host species susceptibility.

In this assessment the confirmation for susceptibility to infection with TSV is based on Chapter 2.2.5. in the *Aquatic Manual* which states that a confirmed diagnosis is:

"Any combination of a molecular (PCR or ISH) test and a morphological (histology) test using at least two of the following three methods (with positive results):

- histological demonstration of diagnostic acute-phase TSV lesions in (especially) the cuticular epithelium of the foregut (oesophagus, anterior, or posterior chambers of the stomach) and/or in the gills, appendages, or general cuticle. Such TSV lesions are pathognomonic for TSV only when they occur without accompanying severe acute necrosis (with nuclear pyknosis and karyorrhexis) of the parenchymal cells of the lymphoid organ tubules (which may occur in acute-phase yellow head virus infections);

- ISH-positive (with a TSV-specific cDNA probe) signal to TSV-type lesions in histological sections (i.e. cuticular acute-phase TS lesions) or to distinctive lymphoid organ spheroids (LOS) in the lymphoid organs of shrimp with chronic phase TS lesions;

- RT-PCR positive results for TSV;

- sequencing of PCR product encompassing CP2 may be accomplished, as needed, to determine the TSV genotype."

Criteria for susceptibility to infection with TSV are detailed in Table 1 (as per Article 1.5.6. of the *Aquatic Code*). This table includes Replication (A), Viability/Infectivity (B), Pathology/Clinical signs (C) and Location (D). Hosts were considered to be infected by TSV 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 TSV*

| **A: Replication** | **B: Viability/Infectivity** | **C: Pathology/Clinical Signs** | **D: Location** |
|---|---|---|---|
| Presence of characteristic inclusion bodies and positive labelling of inclusion bodies by ISH or IFAT.<br><br>Presence of virions in inclusion bodies by TEM.<br><br>Demonstration of increasing copy number over time with qPCR with confirmatory RT-PCR/sequencing specific for infectious virus.<br><br>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**. | Characteristic inclusion bodies, with pyknosis and karyorrhectic nuclei (“peppered” or “buckshot”) in target tissues. No haemocytic infiltration. Clinical signs include lethargy, low feeding rate, static activity on pond edge when moribund, red pale body and appendage discoloration, intensely red tail fan and pleopods, soft shell, empty gut, multifocal irregular melanized cuticle lesions. TS in *P. vannamei* has three phases clinically and histologically differentiated: peracute-to-acute phase, displaying lethargy, anorexia, empty midgut, atactic swimming, flaccid bodies, soft cuticles, muscle opacity, and chromatophore expansion resulting in red or dark discoloration of uropods, antennae and general body. A transition/recovery phase with generalized multifocal irregular black melanized cuticular lesions; lethargy and anorexia may occur; mortality continues. Chronic, sub-clinical carrier phase starting post-molt and loss of melanized cuticle; this phase can persist for the remainder of the shrimp’s life. Typical histological lesions can be observed only during acute phase.<br><br>In severely infected shrimp, lymphoid organ spheroids are sometimes observed in association with tegmental glands and within connective tissues of the cephalothorax and appendages (ectopic spheroids)***. | Cells of tissues of ectodermic and endodermic origin that include cuticular epithelium (or hypodermis) of most exoskeleton, foregut, hindgut, gills, appendages, connective tissue, haematopoietic tissues, lymphoid organ and antennal gland****. |

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 typical of TSV may provide evidence for fulfillment of this category when evidence from histopathology is not available. However, clinical signs according to the *Manual* chapter may not present equally in all host taxa and may not be specific for infection with TSV.

**** Lymphoid organ not present in most non-penaeid host taxa.

# ASSESSMENT FOR HOST SUSCEPTIBILITY

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

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

| **Genus** | **Species** | **Stage 1 Transmission*** | **Stage 2 Pathogen identification** | **Stage 3 Evidence for infection** | | | | **Outcome**** | **Refs** |
|---|---|---|---|---|---|---|---|---|---|
| | | | | **A** | **B** | **C** | **D** | | |
| *Penaeus* | *vannamei* | N | RT-PCR | IHC | No | Yes | Yes | 1 | 7, 8 |
| | *aztecus* | E (*per os*) | RT-PCR | ISH | No | Yes | Yes | 1 | 6 |
| | *stylirostris* | N | RT-PCR | IHC | No | Yes | Yes | 1 | 8 |
| | *setiferus* | E (*per os*) | RT-PCR | ISH | No | Yes | Yes | 1 | 6 |
| | *duorarum* | E (*per os*) | RT-PCR | No | No | No | No | 3 | 6 |
| | *monodon* | N, E (*per os*) | RT-PCR | ISH | No | Yes | Yes | 1 | 2, 7 |
| | *japonicus* | E (injection) | RT-PCR | No | No | No | No | 3 | 1 |
| | *ensis* | N | RT-PCR | No | No | Yes | Yes | 1 | 1 |
| | *chinensis* | E (injection) | RT-PCR | No | No | Yes | Yes | 2 | 6 |
| | *schmitti* | N | RT-PCR | No | No | No | No | 3 | 3 |
| *Macrobrachium* | *rosenbergii* | E (injection) | RT-PCR | ISH | No | Yes | Yes | 2 | 2 |
| *Fundulus* | *grandis* | E (*per os*) | RT-PCR | No | No | No | No | 3 | 5 |
| *Ergasilus* | *manicatus* | E (*per os*) | RT-PCR | RT-qPCR | No | No | No | 2 | 5 |
| *Chelonibia* | *patula* | E (*per os*) | RT-PCR | ISH | No | No | No | 2 | 5 |
| *Callinectes* | *sapidus* | E (per os) | RT-PCR | No | No | No | No | 3 | 5 |
| *Octolasmis* | *muelleri* | E (*per os*) | RT-PCR | ISH | No | No | No | 2 | 5 |
| *Uca* | *vocans* | E (injection and *per os*) | RT-PCR | No | No | No | No | 3 | 4 |
| *Sesarma* | *mederi* | E (injection and *per os*) | RT-PCR | No | No | No | No | 3 | 4 |
| *Scylla* | *serrata* | E (injection and *per os*) | RT-PCR | No | No | No | No | 3 | 4 |

Transmission Key*:
N: Natural infection
E: Experimental infection

Outcome Key**:
Outcome 1: Host species proposed to be listed in Article 9.6.2. of the *Aquatic Code.*
Outcome 2: Host species proposed to be listed in 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 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 TSV**

Not applicable.

**Host species to be included in Article 9.6.2. of the *Aquatic Code***

The *ad hoc* Group proposed that the following host species be included in Article 9.6.2. of the *Aquatic Code*:

*Penaeus vannamei*, *P. aztecus*, *P. stylirostris*, *P. setiferus*, *P. monodon* and *P. ensis*.

**Host species to be included in Chapter 2.2.5. of the *Aquatic Manual***

The *ad hoc* Group proposed that the following host species be included in the revised Section 2.2.2. of Chapter 2.2.5. of the *Aquatic Manual*:

*P. duorarum*, *P. japonicus*, *P. chinensis*, *P. schmitti*, *Macrobrachium rosenbergii*, *Fundulus grandis*, *Ergasilus manicatus*, *Chelonibia patula*, *Callinectes* sapidus, *Octolasmis* muelleri, *Uca vocans*, *Sesarma mederi* and *Scylla serrata*.

**References**

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# ASSESSMENT OF HOST SUSCEPTIBILITY TO INFECTION WITH MACROBRACHIUM NODAVIRUS (MRNV)

The objectives of this assessment were (1) to determine susceptibility of given host taxa to infection with *Macrobrachium rosenbergii* nodavirus (hereafter 'susceptibility to WTD') by applying the 3-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 *Aquatic Manual* with respect to host species susceptibility.

In this assessment the confirmation for susceptibility to infection with WTD is based on Chapter 2.2.7. in the *Aquatic Manual* which states that a confirmed diagnosis is: Suspect cases should first be checked by RT-PCR and confirmed by nRT-PCR, sequencing, TEM and DNA probes.

Criteria for susceptibility to WTD are detailed in Table 1 (as per Article 1.5.6. of the *Aquatic Code*). This Table includes Replication (A), Viability/Infectivity (B), Pathology/Clinical Signs (C) and Location (D). Hosts were considered to susceptible to NHP 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 WTD*

| **A: Replication** | **B: Viability/Infectivity** | **C: Pathology/Clinical signs** | **D: Location** |
|---|---|---|---|
| Presence of characteristic lesions and positive labelling by ISH or IFAT;<br><br>OR<br><br>Presence of virions by TEM;<br><br>OR<br><br>Demonstration of increased copy number with RT-qPCR;<br><br>OR<br><br>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**;<br><br>OR<br><br>Replication in C6/36 sub clones of *Aedes albopictus* mosquito cell line. | Characteristic pathology including progressive segmental myofibre degeneration of striated muscle and necrotic myopathy. Basophilic cytoplasmic inclusions in striated muscles of abdomen, cephalothorax and intratubular connective tissue of the hepatopancreas;<br><br>AND/OR<br><br>Clinical signs including lethargy, anorexia, opaqueness of abdominal muscle and degeneration of telson and uropods. | Striated muscle. |

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.

# ASSESSMENT FOR HOST SUSCEPTIBILITY

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

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

| **Genus** | **Species** | **Stage 1: Transmission** | **Stage 2: Pathogen identification** | **Stage 3: Evidence for infection** | | | | **Outcome*** | **References** |
|---|---|---|---|---|---|---|---|---|---|
| | | | | A | B | C | D | | |
| *Macrobrachium* | *rosenbergii* | Experimental (immersion„ oral, injection) | Northern blotting, RT-PCR, real time RT-PCR, nested RT-PCR, ISH) ; Natural | Yes | Yes | Yes | Yes | 1 | 3,4,5,7,10,13 ,17,18,19 |
| *Penaeus* | *japonicus* | Experimental (oral & intramuscular injection) | RT-PCR | No | No | No | No | 3 | 16 |
| *Penaeus* | *indicus* | Natural & experimental infection | RT-PCR | No | Yes | No | No | 3 | 6, 16 |
| *Penaeus* | *monodon* | Natural & experimental infection | RT-PCR | No | Yes | Yes | No | 3 | 6, 16 |
| *Penaeus* | *vannamei* | Natural and Experimental (oral) | Nested RT-PCR | No | Yes | Yes | Yes | 2 | 11, 12 |
| *Belostoma* | sp. | Experimental challenges with C6/36 cells | RT-PCR, Nested RT-PCR, TEM | No | Yes | No | No | 3 | 15 |
| *Aesohna* | sp. | Experimental challenges with C6/36 cells | RT-PCR, Nested RT-PCR, TEM | No | Yes | No | No | 3 | 15 |
| *Cybister* | sp. | Experimental challenges with C6/36 cells | RT-PCR, Nested RT-PCR, TEM | No | Yes | No | No | 3 | 15 |
| *Notonecta* | sp. | Experimental challenges with C6/36 cells | RT-PCR, nested RT-PCR, TEM | No | yes | No | No | 3 | 15 |
| *Macrobrachium* | *malcolmsonii* | Experimental (oral and intramuscularly injection) | RT-PCR | No | No | No | No | 3 | 8 |
| *Macrobrachium* | *rude* | Experimental (oral and intramuscularly injection) | RT-PCR | No | No | No | No | 3 | 8 |

| Genus | Species | Stage 1: Transmission | Stage 2: Pathogen identification | Stage 3: Evidence for infection | | | | Outcome* | References |
|---|---|---|---|---|---|---|---|---|---|
| | | | | A | B | C | D | | |
| *Artemia* | sp. | Experimental (oral) | RT-PCR, nested RT-PCR | No | No | No | No | 3 | 16 |
| *Cherax* | *quadricarinatus* | Experimental (feed & intramuscular injection) | qRT-PCR | No | No | Yes | No | 3 | 2 |

*Outcome key:

1: Host species proposed to be listed in Article 9.8.2. of the *Aquatic Code.*

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

3: Host species proposed to be listed in 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 WTD**

Outbreaks of white tail disease have never been reported in any species of crustacean other than *Macrobrachium rosenbergii*. *Mr*NV has been observed in several species of crustacean but they did not show mortality or any clinical sign. These species could be reservoirs for *Mr*NV.

Only post larvae of *Macrobrachium rosenbergii* exhibited specific clinical signs. Muscle turned opaque from tail to head and mortality reached 100% within 2–5 days. No outbreaks have been reported in adult stages.

The *Aquatic Manual* for WTD states that suspect cases should first be checked by RT-PCR (9, 10, 21) and confirmed by nRT-PCR (14), sequencing, TEM and DNA probes (20, 21).

White tail disease is associated with two aetiological agents: *Macrobrachium rosenbergii* nodavirus (*Mr*NV) and extra small virus (XSV). The role of XSV in pathogenicity remains unclear. *Mr*NV genome is composed of two linear ss-RNAs present in equimolar ratio and exhibiting a length of ca 3200 (RNA-1) and ca 1250 nucleotides (RNA-2), respectively (1).

**Host species to be included in Article 9.8.2. of the *Aquatic Code***

The *ad hoc* Group proposed that the following host species be included in Article 9.8.2. of the *Aquatic Code*: giant freshwater prawn *(Macrobrachium rosenbergii)*.

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

The *ad hoc* Group proposed that the following host species be included in the revised Section 2.2.2. of Chapter 2.2.7. of the *Aquatic Manual*:

*Penaeus vannamei*, *Penaeus japonicus*, *Penaeus indicus*, *Penaeus monodon*, *Belostoma* sp., *Aesohna* sp., *Cybister* sp., *Notonecta* sp., *Macrobrachium rude*, *M. malcolmsonii*, *Artemia* sp. and *Cherax quadricarinatus*.

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Annex 9 (contd)


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# ASSESSMENT OF HOST SUSCEPTIBILITY TO ACUTE HEPATO-PANCREATIC NECROSIS DISEASE (AHPND)

The objectives of this assessment were (1) to determine susceptibility of given host taxa for Acute Hepatopancreatic Necrosis Disease (AHPND) by applying the 3-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 *Aquatic Manual* with respect to host species susceptibility.

In this assessment the confirmation for susceptibility to AHPND is based on draft OIE *Aquatic Manual* chapter (in preparation) which states that a confirmed diagnosis is:

"in addition to the criteria in Section 7.1, two or more of the following criteria are met:

- histopathology indicative of AHPND;
- detection of PiR toxin gene by PCR and sequence analysis;
- positive results (clinical signs/mortality/histopathology/PCR and sequence) by bioassay."

Criteria for susceptibility to infection with AHPND causing bacteria are detailed in Table 1 (as per Article 1.5.6. of the *Aquatic Code*). This table includes Replication (A), Viability/Infectivity (B), Pathology/Clinical Signs (C) and Location (D). Hosts were considered to be infected with AHPND causing bacteria 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 AHPND causing bacteria*

| **A: Replication** | **B: Viability/Infectivity** | **C: Pathology/Clinical signs** | **D: Location** |
|---|---|---|---|
| Presence of characteristic histopathology.<br>Demonstration of increasing copy number over time with qPCR with confirmatory PCR/ sequencing specific for Pir toxin gene.<br>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**. | Clinical signs and mortality can start as early as 10 days post-stocking. Clinical signs include a pale-to-white hepatopancreas (HP), significant HP atrophy, soft shells, guts with discontinuous, or no, contents, black spots or streaks visible within the HP (due to melanised tubules).<br>Acute phase: Characterised by a massive and progressive degeneration of the HP tubules from proximal to distal, with significant rounding and sloughing of HP tubule epithelial cells into the HP tubules, HP collecting ducts and posterior stomach in the absence of bacterial cells.<br>Terminal phase: Characterised by marked intra-tubular haemocytic inflammation and development of massive secondary bacterial infections that occur in association with the necrotic and sloughed HP tubule cells***. | Gut-associated tissues and organs, such as hepatopancreas (HP), stomach, the midgut and the hindgut. |

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.

*** Demonstration of terminal phase is insufficient evidence for fulfillment of this category when evidence from acute phase histopathology is not available.

# ASSESSMENT FOR HOST SUSCEPTIBILITY

The assessment for host susceptibility to infection with AHPND causing bacteria is provided in Table 2.

***Table 2.*** *Outcome of assessment for host susceptibility to AHPND*

| **Genus** | **Species** | **Stage 1: Transmission*** | **Stage 2: Toxin gene identification** | **Stage 3: Evidence for infection** | | | | **Outcome**** | **References** |
|---|---|---|---|---|---|---|---|---|---|
| | | | | **A** | **B** | **C** | **D** | | |
| *Penaeus* | *vannamei* | N, E (immersion and *per os*) | PCR | Yes | Yes | Yes | Yes | 1 | 3, 5, 6 |
| | *monodon* | N, E (immersion) | PCR | Yes | Yes | Yes | Yes | 1 | 1, 2 |
| | *chinensis* | N | nd | histo | No | Yes | Yes | 2 | 4 |

Transmission Key*:
N: Natural infection
E: Experimental infection

Outcome Key**:
Outcome 1: Host species proposed to be listed in Article 9.3.2. of the *Aquatic Code.*
Outcome 2: Host species proposed to be listed in Chapter X.X.X. 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 X.X.X. 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 AHPND**

AHPND is caused by infection with unique strains of *Vibrio* species, including *V. parahaemolyticus* ($VP_{AHPND}$), *V. harveyi*, and possibly others, that contain a ~70-kbp plasmid with genes that encode homologues of the *Photorhabdus* insect-related (Pir) toxins, PirA and PirB.

**Host species to be included in Article 9.3.2. of the *Aquatic Code***

The *ad hoc* Group proposed that the following host species be included in Article 9.3.2. of the *Aquatic Code*:

*Penaeus vannamei* and *P. monodon*.

**Host species to be included in the new draft Section 2.2.2. of the *Aquatic Manual* Chapter X.X.X.**

The *ad hoc* Group proposed that the following host species be included in the revised Section 2.2.2. of the *Aquatic Manual*:

*P. chinensis*.

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