

Report of the WOAAH *ad hoc* Group on susceptibility of crustacean species to infection with WOAAH listed diseases

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1. Introduction

This report covers the work of the WOAH *ad hoc* Group on Susceptibility of crustacean species to infection with WOAH listed diseases (the *ad hoc* Group) who met virtually on 14, 21 and 23 March 2023.

The list of participants and the Terms of Reference are presented in Annex 1 and Annex 2, respectively.

2. Methodology

The *ad hoc* Group applied criteria, as outlined in Chapter 1.5. Criteria for listing species as susceptible to infection with a specific pathogen, of the WOAH *Aquatic Animal Health Code* (the *Aquatic Code*), to potential host species in order to determine susceptibility to infection with decapod iridescent virus 1 (DIV1).

A three-stage approach, as described in Article 1.5.3, was used to assess the susceptibility of a species to infection with DIV1 and was based on:

Stage 1. criteria to determine whether the route of transmission is consistent with natural pathways for the infection (as described in Article 1.5.4.);

Stage 2. criteria to determine whether the pathogenic agent has been adequately identified (as described in Article 1.5.5.);

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

- A. The pathogenic agent is multiplying in the host, or developing stages of the pathogenic agent are present in or on the host;
- B. Viable pathogenic agent is isolated from the proposed susceptible species, or infectivity is demonstrated by way of transmission to naïve individuals;
- C. Clinical or pathological changes are associated with the infection;
- D. The specific location of the pathogen corresponds with the expected target tissues.

Details of the three-stage approach applied by the *ad hoc* Group for infection with DIV1, including any additional considerations are described below:

2.1. Stage 1: Criteria to determine whether the route of transmission is consistent with natural pathways for the infection:

Table 1 describes the route of transmission for infection with DIV1 used by the *ad hoc* Group when applying Stage 1 to assess susceptibility to infection with DIV1, as well as some considerations.

Table 1: Route of transmission for infection with DIV1

Route of transmission	Considerations
Natural exposure included situations where infection had occurred without experimental intervention (e.g. infection in wild or farmed populations) OR Non-invasive experimental procedures such as cohabitation with infected hosts or infection by immersion or <i>per os</i> .	Experimental infection via invasive routes (i.e. injection) was not considered a natural route of transmission and therefore such studies were not evaluated.

2.2. Stage 2: Criteria to determine whether the pathogenic agent has been adequately identified:

Table 2 describes the pathogen identification methods for infection with DIV1 used by the *ad hoc* Group when applying Stage 2 to assess susceptibility to infection with DIV1, as well as some considerations. These criteria was developed in consultation with the Reference Laboratory expert for DIV1 to ensure alignment between these criteria and the pathogen identification methods included in the draft chapter of the *Manual of Diagnostic Tests for Aquatic Animals (the Aquatic Manual)*.

Table 2: Pathogen identification for infection with DIV1

Pathogen Identification (DIV1)	Considerations
Specific TaqMan real-time PCR (e.g. Qiu <i>et al.</i> , 2020) OR PCR or nested PCR followed by sequence analysis (e.g. Qiu <i>et al.</i> , 2017) OR <i>In-situ</i> hybridisation using a DIV1-specific probe (e.g. Qiu <i>et al.</i> , 2017) OR <i>In-situ</i> DIG-labelling-loop-mediated DNA amplification (ISDL) (e.g. Chen <i>et al.</i> , 2019)	Because of the specificity of the TaqMan real-time PCR, sequence analysis was not considered necessary for pathogen confirmation. Recombinase polymerase amplification (RPA) targeting the ATPase gene was used in some studies for pathogen identification, which was deemed acceptable when there was supporting evidence of infection with DIV1.

2.3. Stage 3: Criteria to determine whether the evidence indicates that presence of the pathogenic agent constitutes an infection:

Table 3 describes the evidence of infection used by the *ad hoc* Group when applying Stage 3 to assess susceptibility to infection with DIV1.

Table 3: Evidence of infection with DIV1

Evidence of infection			
A: Replication	B: Viability / Infectivity	C: Pathology / Clinical signs*	D: Location
<p>Presence of characteristic inclusion bodies and positive labelling of inclusion bodies by ISH or IFAT</p> <p>OR</p> <p>Presence of virions in inclusion bodies by TEM</p> <p>OR</p> <p>Demonstration of high copy number by a specific TaqMan PCR (e.g. Qiu <i>et al.</i>, 2020)</p> <p>OR</p> <p>Demonstration of increasing copy number over time with qPCR with confirmatory PCR/sequencing specific for infectious virus</p> <p>OR</p> <p>Serial passage from individual to SPF individual of the same species.</p>	<p>Single passage bioassay to a SPF (target pathogen) of any susceptible host species and confirmation of pathogen identification.</p>	<p>Reddish body; hepatopancreatic atrophy with faded colour; empty digestive system; In <i>M. rosenbergii</i>: white triangular area under the carapace at the base of the rostrum.</p>	<p>Haematopoietic tissues, cuticular epithelium, lymphoid organ, haemocytes in gills, pereopods, hepatopancreatic sinus.</p>

* Pathology/Clinical signs may be non-specific, variable and include some or all of the characteristics listed.

3. Scoring and assessments

Table 4 describes the different scores and outcomes of the assessments undertaken by the *ad hoc* Group.

Table 4: Scores

Score	Outcome
1	Species assessed as susceptible (as described in Article 1.5.7.). These species were proposed for inclusion in Article 9.3.2. of Chapter 9.3., Infection with DIV1, of the <i>Aquatic Code</i> and Section 2.2.1. of Chapter 2.2.X., Infection with DIV1, of the <i>Aquatic Manual</i> .
2	Species assessed as having incomplete evidence for susceptibility (as described in Article 1.5.8.) were proposed for inclusion in Section 2.2.2., Species with incomplete evidence for susceptibility of Chapter 2.2.X., Infection with DIV1, of the <i>Aquatic Manual</i> .
3	Species assessed as not meeting the criteria or for which there was unresolved or conflicting information. These species were not proposed for inclusion in either the <i>Aquatic Code</i> or the <i>Aquatic Manual</i> . The exceptions were species where pathogen-specific positive PCR results have been reported but an active infection has not been demonstrated. These species were proposed for inclusion in the second paragraph in Section 2.2.2. Species with incomplete evidence for susceptibility of Chapter 2.2.X. Infection with DIV1, of the <i>Aquatic Manual</i> .
4	Species assessed as non-susceptible.
NS	Species not scored due to insufficient or irrelevant information.

Table 5 summarises all of the assessments for host susceptibility to infection with DIV1 undertaken by the *ad hoc* Group together with the outcomes and relevant references. For Stage 3, as described in Chapter 1.5. of the *Aquatic Code*, evidence to support criterion A alone was sufficient to determine infection. In the absence of evidence to meet criterion A, satisfying at least two of criteria B, C or D were required to determine evidence of infection.

Table 5: Assessments for infection with DIV1

Family	Scientific name	Common name	Stage 1: Route of infection	Stage 2: Pathogen Identification	Stage 3: Evidence of Infection				Outcome	References
					A	B	C	D		
Score 1										
Cambaridae	<i>Procambarus clarkii</i>	red swamp crawfish	N	TaqMan real-time PCR	YES	ND	ND	YES	1	Qui <i>et al.</i> , 2019
Palaemonidae	<i>Macrobrachium nipponense</i>	Oriental river prawn	N	TaqMan real-time PCR	YES	ND	ND	ND	1	Qui <i>et al.</i> , 2019
	<i>Macrobrachium rosenbergii</i>	giant river prawn	N	nested-PCR and RPA (ATPase gene)	YES	ND	YES	YES	1	Guixiang <i>et al.</i> , 2022

Family	Scientific name	Common name	Stage 1: Route of infection	Stage 2: Pathogen Identification	Stage 3: Evidence of Infection				Outcome	References
					A	B	C	D		
			N	TaqMan real-time PCR	YES	ND	YES	YES	1	Qui <i>et al.</i> , 2019
	<i>Palaemon carinicauda</i>	ridgetail prawn	E	TaqMan real-time PCR	YES	ND	YES	YES	1	Chen <i>et al.</i> , 2019
Parastacidae	<i>Cherax quadricarinatus</i> ¹	red claw crayfish	N	PCR (MCP gene) and sequencing	YES	ND	ND	YES	1	Xu <i>et al.</i> , 2016
			N	NO – nested-PCR	ND	ND	ND	ND	NS	Yang <i>et al.</i> , 2020
Penaeidae	<i>Penaeus chinensis</i>	fleshy prawn	N	nested-PCR and RPA (ATPase gene)	YES	ND	YES	YES	1	Guixiang <i>et al.</i> , 2022
			N	TaqMan real-time PCR	ND	ND	ND	YES	2	Qiu <i>et al.</i> , 2018a
	<i>Penaeus japonicus</i>	kuruma prawn	E and EI	TaqMan real-time PCR	YES	YES	YES	YES	1	Qiu <i>et al.</i> , 2023
			EI	TaqMan real-time PCR	N/A	N/A	N/A	N/A	NS	He <i>et al.</i> , 2021b
	<i>Penaeus vannamei</i> ¹	whiteleg shrimp	N and EI	semi-nested-PCR (MCP gene) and sequencing	YES	YES	YES	YES	1	Sanguanrut <i>et al.</i> , 2022
			N	TaqMan real-time PCR, sequencing	YES	ND	YES	YES	1	Qui <i>et al.</i> , 2021
			N and E	TaqMan real-time PCR	YES	YES	ND	YES	1	Qiu <i>et al.</i> , 2018a
			N and E and EI	nested-PCR and sequencing	YES	YES	YES	YES	1	Qiu <i>et al.</i> , 2017
Portunidae	<i>Portunus trituberculatus</i>	gazami crab	E and EI	TaqMan real-time PCR, nested-PCR	YES	YES	I ²	YES	1	Qiu <i>et al.</i> , 2022
Score 2										
Penaeidae	<i>Penaeus monodon</i>	giant tiger prawn	E and EI	TaqMan real-time PCR, nested-PCR	YES	YES	ND	ND	1 ³	He <i>et al.</i> , 2021a

Family	Scientific name	Common name	Stage 1: Route of infection	Stage 2: Pathogen Identification	Stage 3: Evidence of Infection				Outcome	References
					A	B	C	D		
			N	nested-PCR (ATPase and MCP genes) and sequencing	I ⁴	ND	NO	YES	2	Srisala et al., 2021
			N	nested-PCR and sequencing	ND	ND	ND	ND	3	Srisala et al., 2020
Score 3										
Ampullariidae	<i>Pomacea canaliculata</i>	channeled applesnail	N	TaqMan real-time PCR	ND	ND	ND	NO	3	Qiu et al., 2021
Palaemonidae	<i>Macrobrachium superbum</i> ⁵		N	TaqMan real-time PCR	ND	ND	ND	ND	3	Qui et al., 2019
Salticidae	<i>Plexippus paykulli</i> ⁵		N	TaqMan real-time PCR	ND	ND	ND	NO	3	Qiu et al., 2021
Varunidae	<i>Helice tientsinensis</i> ⁵		N	TaqMan real-time PCR	ND	ND	ND	ND	3	Qiu et al., 2022
	<i>Hemigrapsus penicillatus</i>	Japanese shore crab	N	TaqMan real-time PCR	ND	ND	ND	ND	3	Qiu et al., 2022
Not scored (NS)										
Penaeidae	<i>Penaeus merguensis</i>	banana prawn	EI	nested-PCR and sequencing	N/A	N/A	N/A	N/A	NS	Liao et al., 2020

¹ An outbreak was reported in this host species (WAHIS report IDs IN_17915, FUR_18004, FUR_152709) and DIV1 infection was confirmed by the laboratory, which became the WOAHA Reference Laboratory in 2022.

² Non-specific clinical signs; i.e. decreased motility, retarded reaction and anorexia.

³ The *ad hoc* Group determined that the evidence in the paper scored '1' is not sufficient for a final assessment of '1' as the paper had no details of any disease in the animals fed with the infected tissues although infectious virus was re-isolated by injecting a large inoculum into experimental animals. There are no other reports to corroborate susceptibility therefore the *ad hoc* Group assessed this species as an overall score of '2'.

⁴ In situ hybridisation showed positive results in nuclei only.

⁵ No common name was available on FAOTerm or www.sealifebase.se.

Assessment Table Key

N: Natural infection

E: Experimental (non-invasive)

EI: Experimental invasive

YES: Demonstrates criterion is met

NO: Criterion is not met

I: Inconclusive

ND: Not determined

NS: Not scored

N/A: Not applicable

4. Results

The *ad hoc* Group agreed that nine species, fleshy prawn (*Penaeus chinensis*), gazami crab (*Portunus trituberculatus*), giant river prawn (*Macrobrachium rosenbergii*), kuruma prawn (*Penaeus japonicus*), Oriental river prawn (*Macrobrachium nipponense*), red claw crayfish (*Cherax quadricarinatus*), red swamp crawfish (*Procambarus clarkii*), ridgetail prawn (*Palaemon carinicauda*), and whiteleg shrimp (*Penaeus vannamei*), meet the criteria for listing as susceptible to infection with DIV1 in accordance with Chapter 1.5. and therefore should be proposed to be included in Article 9.3.2. of the *Aquatic Code*. All of these species, except fleshy prawn (*Penaeus chinensis*), gazami crab (*Portunus trituberculatus*) and kuruma prawn (*Penaeus japonicus*) are currently listed in Article 9.3.2. 'under study'.

Giant tiger prawn (*Penaeus monodon*) which is currently listed in Article 9.3.2. 'under study' was assessed as having incomplete evidence of susceptibility and was therefore proposed to be removed from Article 9.3.2. and included in Section 2.2.2. of Chapter 2.2.X., Infection with DIV1 of the *Aquatic Manual*.

Pathogen-specific positive PCR results have been reported in the following five species, channeled applesnail (*Pomacea canaliculata*), *Helice tientsinensis*, Japanese shore crab (*Hemigrapsus penicillatus*), *Macrobrachium superbum* and *Plexippus paykulli*, but an active infection has not been demonstrated. Therefore, these species were proposed to be included in the second paragraph of Section 2.2.2. of Chapter 2.2.X., Infection with DIV1 of the *Aquatic Manual*.

5. Naming convention for susceptible species

The scientific names of the host species are in accordance with the World Register of Marine Species (WoRMS) <https://www.marinespecies.org/index.php>.

The common names of host species are in accordance with FAOTERM (<http://www.fao.org/faoterm/collection/faoterm/en/>). Where a common name was not found in FAOTERM, the naming was done in accordance with <https://www.sealifebase.ca>.

6. Comments on the *ad hoc* Group's rationale and decision-making

'Inconclusive' was used to distinguish situations where more information was provided than would have been assessed as 'Not-determined' but the *ad hoc* Group could not conclude that the criterion was met. Each time inconclusive was used within the assessment table, the *ad hoc* Group provided additional information in a footnote. The *ad hoc* Group treated 'Inconclusive' as 'Not-determined' when making their final assessment.

The *ad hoc* Group agreed that while the ideal situation was two papers with a score of '1', a single robust study scoring '1' was also enough to conclude susceptibility of a species in the absence of conflicting evidence. Additional studies were still reviewed to check for any supporting or conflicting evidence. When additional papers were identified but the *ad hoc* Group did not feel that they were necessary to assess comprehensively because the species had already been determined as susceptible by other studies, these references were only noted in the list of references (Section 7).

7. Article 1.5.9 Listing of Susceptible species at a taxonomic ranking of Genus or higher

The *ad hoc* Group considered Article 1.5.9., Listing of susceptible species at a taxonomic ranking of Genus or higher, and determined that it was not applicable for the susceptible species for DIV1 identified at this time.

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.../Annexes

Annex 1. List of Participants

MEETING OF THE WOAAH *AD HOC* GROUP ON SUSCEPTIBILITY OF CRUSTACEAN SPECIES TO WOAAH LISTED DISEASES

14, 21 and 23 March 2023 (virtual)

List of Participants

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Annex 2. Terms of Reference

MEETING OF THE WOAAH *AD HOC* GROUP ON SUSCEPTIBILITY OF CRUSTACEAN SPECIES TO WOAAH LISTED DISEASES

14, 21 and 23 March 2023 (virtual)

Terms of reference

Background

Chapter 1.5. Criteria for listing species as susceptible to infection with a specific pathogenic agent of the *Aquatic Code*, provides criteria for determining which host species are listed as susceptible in Article X.X.2. of each disease-specific chapter in the *Aquatic Code*.

Assessments for all of the WOAAH listed diseases are being undertaken progressively by *ad hoc* Groups. Once completed, the revised list of susceptible species in the relevant Article X.X.2. of the *Aquatic Code* is circulated for Member comment and then presented for adoption.

Species, where there is some evidence of susceptibility but insufficient evidence to demonstrate susceptibility are included in the relevant disease-specific chapter in the *Aquatic Manual*.

The *ad hoc* Group on Susceptibility of crustacean species to infection with WOAAH listed diseases completed assessments for most WOAAH-listed diseases in 2015-2016, but has not met since.

Purpose

The *ad hoc* Group on Susceptibility of crustacean species to infection with WOAAH listed diseases will undertake assessments for infection with decapod iridescent virus 1 (DIV1), a new WOAAH listed disease, adopted in 2021.

Terms of Reference

- 1) Review relevant literature documenting susceptibility of species for infection with DIV1 and apply the criteria, as outlined in Chapter 1.5. Criteria for listing species as susceptible to infection with a specific pathogen, to potential host species.
- 2) Determine susceptible species for infection with DIV1 based on Article 1.5.7.
- 3) Determine species with incomplete evidence for susceptibility for infection with DIV1 based on Article 1.5.8.

Expected outputs of the *ad hoc* Group

- 1) Propose a list of susceptible species for inclusion in the Article 9.3.2. of Chapter 9.3., Infection with DIV1, of the *Aquatic Code*.
 - 2) Propose a list of species with incomplete evidence for susceptibility for inclusion in Section 2.2.2 of Chapter 2.2.X. Infection with DIV1 of the *Aquatic Manual*.
 - 3) A report for consideration by the Aquatic Animals Commission at its September 2023 meeting.
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