



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

November–December 2020

This report covers the work of the OIE *ad hoc* Group on Susceptibility of mollusc species to infection with OIE listed diseases (the *ad hoc* Group) who met electronically between November and December 2020.

The list of participants and the Terms of Reference are presented in Annex I and Annex II, respectively.

Methodology

The *ad hoc* Group applied criteria, as outlined in Article 1.5.3 of the OIE *Aquatic Animal Health Code* (the *Aquatic Code*), to potential host species in order to determine susceptibility and non-susceptibility to infection with *Bonamia exitiosa*. This was done by a three-stage approach, as described below:

1) 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):

Consideration was given to whether experimental procedures mimic natural pathways for disease transmission. Consideration was also given to environmental factors given that these may affect host response, virulence and transmission of infection with *B. exitiosa*.

The table below describes additional considerations made by the *ad hoc* Group when applying Stage 1 to support susceptibility to infection with *B. exitiosa*.

Source of infection	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 ² : cohabitation with infected hosts; infection by immersion or feeding.	<i>In vitro</i> experimental assays (contact between haemocytes and parasites) were not considered appropriate to answer the question of susceptibility or non-susceptibility.

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 2021 report of the Aquatic Animal Health Standards Commission because this report provides its considerations and comments. It is available at <https://www.oie.int/en/standard-setting/specialists-commissions-working-ad-hoc-groups/aquatic-animals-commission-reports/meeting-reports/>

² Invasive experimental procedures including injection were only used to demonstrate non-susceptibility.

2) **Stage 2: Criteria to determine whether the pathogenic agent has been adequately identified (as described in Article 1.5.5):**

The *ad hoc* Group noted that unambiguous pathogenic agent identification might not have been carried out in older publications because molecular techniques were not available at the time. In these circumstances a weight of evidence approach, whereby the combined information from subsequent studies and additional information provided by the authors, was considered and used to conclude sufficiency of pathogen identification.

The table below describes the pathogen identification methods used by the *ad hoc* Group as well as some considerations.

Pathogen Identification	Considerations
Molecular sequence information (species-specific regions of 18S sequence) OR PCR-RFLP (as described in Cochenec <i>et al.</i> , 2000) OR Species-specific Real-time or conventional PCR (for example Ramilo <i>et al.</i> , 2013) OR Observed parasite and morphology from histology was later characterised by linked molecular information from other studies	Molecular data should be associated with microscopical examination wherever possible to confirm the presence of the pathogen. ISH is currently not sufficiently specific to resolve species level identifications. For early studies without molecular information, corroborating evidence from later studies was also considered. ITS rDNA sequence has a higher resolution than 18s rDNA and therefore can provide information about the intra-species diversity between populations. Primers and probes from Carnegie <i>et al.</i> , 2008, are expected to be specific to <i>Bonamia exitiosa</i> but were not considered sufficient singular evidence of pathogen identification as they have not been formally validated to date.

3) **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):**

Criteria A to D, as described in Article 1.5.6 and presented below, were used to determine if there was sufficient evidence for infection with *B. exitiosa* in the suspected host species:

- A. The pathogenic agent is multiplying in the host, or developing stages of the pathogenic agent are present in or on the host³;
- 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.

Evidence to support criterion A alone was sufficient to determine infection. In the absence of evidence to meet criterion

³ For the purposes of the assessments for susceptibility to *B. exitiosa*, replication 'on the host' was not considered to apply.

A, satisfying at least two of criteria B, C or D were required to determine infection.

The table below describes the criteria for assessment of Stage 3 to support susceptibility to infection with *B. exitiosa*.

Evidence for infection			
A: Replication	B: Viability / Infectivity	C: Pathology / Clinical signs*	D: Location
1) Presence of multiple intracellular parasites or presence of multinucleated parasites (including plasmodial stage) demonstrated by: <ul style="list-style-type: none"> a) Histopathology OR <ul style="list-style-type: none"> b) Cytology (usually gill or heart imprint or haemolymph smears) OR <ul style="list-style-type: none"> c) <i>In-situ</i> hybridization (ISH) OR <ul style="list-style-type: none"> d) TEM OR 2) Demonstration of increasing copy number over time with qPCR (targeting DNA) or reverse transcription qPCR (targeting RNA) in tissues	1) Transmission via co-habitation with uninfected individuals of a known-susceptible (e.g. <i>Ostrea chilensis</i>) species OR 2) Demonstration of viability of cells isolated from tissues by: <ul style="list-style-type: none"> a) Flow cytometry OR <ul style="list-style-type: none"> a) Vital stains OR <ul style="list-style-type: none"> b) Successful infection of uninfected animals by inoculation 	1) Mortality OR 2) <u>Macroscopic lesions</u> such as: <ul style="list-style-type: none"> a. Discolouration of tissue b. Gill ulceration OR 3) Rapid loss of condition OR 4) <u>Microscopic lesions</u> such as generalized haemocyte infiltration in connective tissues of several organs including gills and mantle	Within haemocytes circulating in the connective tissue in different organs, in particular gills** or heart (rarely extracellular)

* Non-specific signs and inconsistent presentation.

** Inside gills, as opposed to potential external contaminant.

Results

The table below describes the different scores and outcomes of the assessments undertaken by the *ad hoc* Group.

Score	Outcome
1.	Species assessed as susceptible (as described in Article 1.5.7) and were proposed for inclusion in Article 11.2.2 of Chapter 11.2, Infection with <i>B. exitiosa</i> , of the <i>Aquatic Code</i> and Section 2.2.1 of Chapter 2.4.2, Infection with <i>B. exitiosa</i> , of the <i>Manual of Diagnostic Tests for Aquatic Animals</i> (the <i>Aquatic Manual</i>).

2.	Species assessed as having incomplete evidence for susceptibility (as described in Article 1.5.8).
3.	Species that were assessed as not meeting the criteria or for which there was unresolved or conflicting information were not proposed for inclusion in either the <i>Aquatic Code</i> or the <i>Aquatic Manual</i> . The exceptions were species where there had been reported pathogen-specific positive PCR results, but an active infection had not been demonstrated. These species were proposed for inclusion in a separate paragraph in Section 2.2.2, Species with incomplete evidence for susceptibility, of Chapter 2.4.2 of the <i>Aquatic Manual</i> .
4.	Species assessed as non-susceptible.
NS	Not scored due to insufficient or irrelevant information.

Evidence of infection Key Stage 3

Y: Demonstrates criterion is met.

N: Criterion is not met.

ND: Not determined.

Assessments of host susceptibility to infection with *B. exitiosa*

Summary

The *ad hoc* Group agreed that the two species currently included in Article 11.2.2 as susceptible to infection with *B. exitiosa*, the Australian mud oyster (*Ostrea angasi*) and Chilean flat oyster (*Ostrea chilensis*), meet the criteria for listing as susceptible to infection with *B. exitiosa* in accordance with Chapter 1.5 of the *Aquatic Code*. and were proposed to remain in Article 11.2.2.

Six additional species, the Argentinean flat oyster (*Ostrea puelchana*), Dwarf oyster (*Ostrea stentina*), Eastern oyster (*Crassostrea virginica*), European flat oyster (*Ostrea edulis*), Olympia oyster (*Ostrea lurida*) and the Suminoe oyster (*Crassostrea ariakensis*) were assessed to meet the criteria for listing as susceptible to infection with *B. exitiosa*, in accordance with Chapter 1.5, and were proposed to be included in Article 11.2.2.

Two species, Pacific cupped oyster (*Crassostrea gigas*) and Sydney rock oyster (*Saccostrea glomerata*), were assessed as having incomplete evidence of susceptibility and were proposed to be included in Section 2.2.2, of Chapter 2.4.2 of the *Aquatic Manual*.

The assessments for host susceptibility to infection with *B. exitiosa* conducted by the *ad hoc* Group together with the outcomes and relevant references are shown in the table below.

Family	Scientific name	Common name	Stages 1: Route of infection	Stage 2: Pathogen identification	Stage 3: Evidence for infection				Outcome	References
					A	B	C	D		
Score 1										
Ostreidae	<i>Ostrea edulis</i>	European flat oyster	YES	YES	YES	ND	YES	YES	1	Abollo <i>et al.</i> , 2008
			YES	YES	YES	ND	YES	YES	1	Carrasco <i>et al.</i> , 2012
Ostreidae	<i>Ostrea chilensis</i>	Chilean flat oyster	YES	YES	YES	ND	YES	YES	1	Hill <i>et al.</i> , 2014
			YES	YES	YES	ND	YES	YES	1	Lane <i>et al.</i> , 2016
Ostreidae	<i>Ostrea stentina</i>	Dwarf oyster	YES	YES	YES	ND	YES	YES	1	Hill <i>et al.</i> , 2014
			YES	YES	YES	ND	ND	YES	1	Hill <i>et al.</i> , 2010
Ostreidae	<i>Ostrea puelchana</i>	Argentinean flat oyster	YES	YES	YES	ND	YES	YES	1	Hill <i>et al.</i> , 2014
			YES	YES ⁴	YES	ND	YES	YES	1	Kroeck, 2010
Ostreidae	<i>Ostrea angasi</i>	Australian mud oyster	YES	YES	YES	ND	YES	YES	1	Hill <i>et al.</i> , 2014
			YES	YES ⁵	YES	ND	YES	YES	1	Heasman <i>et al.</i> , 2004
Ostreidae	<i>Crassostrea virginica</i>	Eastern Oyster	YES	YES	YES	ND	YES ⁶	YES	1	OIE, 2012 and personal communication (R. Carnegie)
			YES	YES	YES	ND	ND ⁷	YES	1	OIE, 2013 and personal communication (R. Carnegie)
			YES	YES	YES	ND	ND	YES	1	Hill <i>et al.</i> , 2014
			YES	YES	NO	ND	NO	NO	4	Dungan <i>et al.</i> , 2012

⁴ Pathogen identified on histology and was later characterized as *B. exitiosa* through molecular techniques in Hill *et al.*, 2014.

⁵ Pathogen identified on histology and was later characterized as *B. exitiosa* through molecular techniques in Hill *et al.*, 2014.

⁶ No morbidity, mortalities or lesions reported but infiltration of parasites in hemocytes was noted.

⁷ No mortality or lesions on histology was documented.

Family	Scientific name	Common name	Stages 1: Route of infection	Stage 2: Pathogen identification	Stage 3: Evidence for infection				Outcome	References
					A	B	C	D		
Ostreidae	<i>Crassostrea ariakensis</i>	Suminoe oyster	YES	YES	YES	ND	YES	YES	1	Burreson <i>et al.</i> , 2004
			YES	YES	YES	ND	YES	YES	1	Dungan <i>et al.</i> , 2012
Ostreidae	<i>Ostrea lurida</i>	Olympia oyster	YES	YES	YES	ND	YES	YES	1	Hill <i>et al.</i> , 2014
Score 3										
Ostreidae	<i>Crassostrea gigas</i>	Pacific cupped oyster	YES	YES	NO	ND	NO	NO	3	Lynch <i>et al.</i> , 2010
Ostreidae	<i>Saccostrea glomerata</i>	Sydney rock oyster	YES	YES	ND	ND	YES	YES ⁸	3	Hill <i>et al.</i> , 2014
			YES	YES	NO	ND	NO	NO	3	Carnegie <i>et al.</i> , 2014
			YES	YES	NO	ND	NO	NO	3	Spiers <i>et al.</i> , 2014
Not scored (NS) because pathogen ID was inconclusive										
Mytilidae	<i>Geukensia demissa</i>	Ribbed mussel	YES	NO ⁹	NO	ND	NO	NO	NS	Laramore <i>et al.</i> , 2017
Mytilidae	<i>Brachidontes exustus</i>	Scorched mussel	YES	NO	NO	ND	NO	NO	NS	Laramore <i>et al.</i> , 2017
Mytilidae	<i>Ischadium recurvum</i>	Hooked mussel	YES	NO	ND	ND	ND	ND	NS	Laramore <i>et al.</i> , 2017
Isognomonid	<i>Isognomon bicolor</i>	Bicolor purse-oyster	YES	NO	NO	ND	NO	NO	NS	Laramore <i>et al.</i> , 2017
Isognomonid	<i>Isognomon alatus</i>	Flat tree-oyster	YES	NO	NO	ND	NO	NO	NS	Laramore <i>et al.</i> , 2017

⁸ Microcells were identified but were not necessarily *B. exitiosa* as ISH was not completed. Pictures of histology were not provided and no specific description of microcells from *Saccostrea glomerata*.

⁹ The specificity for the PCR and ISH used in Laramore *et al.*, 2017, has not been formally validated for *B. exitiosa*.

Note:

The scientific names of the species are in line with World Register of Marine Species (WoRMS) <https://www.marinespecies.org/index.php> (for *Crassostrea gigas* and *Crassostrea ariakensis* see explanatory note below).

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

Comments on the *ad hoc* Group's rationale and decision-making:**General comments**

The *ad hoc* Group agreed to focus on studies published from 2000 onwards, when molecular testing was available. Papers published in earlier years were referred to when necessary to increase confidence of an assessment or when no recent paper was available for the assessment of a specific host species.

The *ad hoc* Group decided that either two papers with a score of '1', or a single study with corroborative evidence, were enough to conclude susceptibility of a species. Additional studies were still checked and considered for conflicting evidence. When a single publication provided evidence for a score of 1, some form of corroborating evidence was required in addition, specifically:

- Internal corroboration in the published study. Multiple lines of evidence within the same publication. This could result from i) a study that amasses positive molluscs from multiple dates and locations or ii) an experimental study testing several isolates or routes of exposure (e.g. immersion and cohab). In these instances, assuming the research is sound, the species was scored a 1 from a single peer-reviewed publication.
- External corroboration: evidence from other publications or sources. Examples might include data found in a government website, a separate publication that scores a 2 or better, or evidence of expert judgement (e.g. records from a reference lab).

When additional papers were identified but the *ad hoc* Group did not feel that they were necessary to assess as the species had already been determined as susceptible by other studies, these studies were included in the list of references.

Species-specific comments

- *Crassostrea virginica*: The *ad hoc* Group sought additional information from authors regarding infection of *Crassostrea virginica* with *Bonamia exitiosa* to enable an assessment for susceptibility. The *ad hoc* Group scored a '1' for this species but recognise that regression of infection without mortality appeared to occur. This suggests that *C. virginica* displays tolerance/resistance to infection as it supports replication without development of morbidity or mortality. *C. virginica* was proposed to be included in Article 11.2.2 of the *Aquatic Code*.
- *Ostrea lurida*: only one paper was available for assessment but was determined by the *ad hoc* Group as sufficiently having met the criteria for susceptibility to be scored as a '1' as there were multiple collections of oysters from different time periods. *O. lurida* was proposed to be included in Article 11.2.2 of the *Aquatic Code*.
- *Crassostrea gigas* is currently listed as a "possible carrier or reservoir" in the *Aquatic Manual*. The *ad hoc* Group felt that the Lynch *et al.*, 2010, paper reported pathogen specific positive PCR results, but an active infection had not been demonstrated. The *ad hoc* Group determined this met the criteria for susceptibility to be scored as a "3" and included in Section 2.2.2, Species with incomplete evidence for susceptibility, of the *Aquatic Manual*.
- According to WoRMS, the accepted Genus for *Crassostrea* should be *Magallana*. However, Bayne *et al.*, 2017, consider that the report by Salvi & Mariottini, 2017, is not sufficiently robust to support the proposed taxonomic change.

- According to WoRMS, *Ostrea stentina* and *Ostrea equestris* are considered distinct species, however there are some papers (Hill *et al.*, 2010; Shilts *et al.*, 2007) that consider them synonyms.

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, in the *Aquatic Code*, but felt that it was not applicable for the hosts of *B. exitiosa* identified at this time.

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**OIE AD HOC GROUP ON SUSCEPTIBILITY
OF MOLLUSCS SPECIES TO INFECTION WITH OIE LISTED DISEASES
November–December 2020**

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**OIE AD HOC GROUP ON SUSCEPTIBILITY OF
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Terms of reference

Background

Chapter 1.5, Criteria for listing species as susceptible to infection with a specific pathogen, was introduced in 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*.

These 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 mollusc species to infection with OIE listed diseases will undertake assessments for the seven OIE listed mollusc diseases.

Terms of Reference

- 1) Consider evidence required to satisfy the criteria in Chapter 1.5.
- 2) Review relevant literature documenting susceptibility of species for OIE listed mollusc diseases.
- 3) Propose susceptible species for OIE listed diseases for molluscs based on Article 1.5.7.
- 4) Propose susceptible species for OIE listed diseases for molluscs based on Article 1.5.8.

Expected outputs of the *ad hoc* Group

- 1) Develop a list of susceptible species for inclusion in the relevant Article X.X.2 of mollusc disease-specific chapters in the *Aquatic Code*.
 - 2) Develop a list of species with incomplete evidence for susceptibility for inclusion in Section 2.2.2 of the *Aquatic Manual*.
 - 3) Draft a report for consideration by the Aquatic Animals Commission at their September 2020 meeting.
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