Report of the WOAH *ad hoc* Group on susceptibility of mollusc species to infection with WOAH listed diseases

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World Organisation for Animal Health
Founded as OIE

Standards Department
[ACC.Secretariat@woah.org]

12, rue de Prony
75017 Paris, France

T. +33 (0)1 44 15 18 88
F. +33 (0)1 42 67 09 87
woah@woah.org
www.woah.org

1. Introduction

This report covers the work of the WOAH *ad hoc* Group on Susceptibility of mollusc species to infection with WOAH listed diseases (the *ad hoc* Group) who met physically in La Tremblade, France on 6-8 June 2023 and in Paris, France on 29, 30 November and 1 December 2023.

The list of participants for June and November/December 2023, and the Terms of Reference are presented in Annex I, II and Annex III, 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 *Perkinsus olseni*.

A three-stage approach, as described in Article 1.5.3., was used to assess the susceptibility of a species to infection with *P. olseni* 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 *P. olseni* 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 *P. olseni* used by the *ad hoc* Group when applying Stage 1 to assess susceptibility to infection with *P. olseni*, as well as some considerations.

Table 1: Route of transmission for infection with *P. olseni*

Route of transmission	Considerations
1. Natural exposure included situations where infection had occurred without experimental intervention (e.g., infection in wild or farmed populations) **OR** 2. Non-invasive experimental procedures: cohabitation with infected hosts or faeces of infected hosts; infection by immersion or feeding or mantle cavity inoculation, under conditions that mimic natural conditions for the host.	*In vitro* experimental assays (contact between haemocytes and parasites) are not considered appropriate to answer the question of susceptibility or non-susceptibility. Infection by injection into body tissue was considered to not mimic natural pathways so were not assessed by the *ad hoc* Group unless the studies provided evidence of non-susceptibility. For immersion, feeding or mantle cavity inoculation, the dose was considered to determine if it could mimic natural infections.

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

Table 2 describes the pathogen identification methods used by the *ad hoc* Group when applying Stage 2 to susceptibility to infection with *P. olseni*, as well as some considerations.

Table 2: Pathogen identification for infection with *P. olseni*

Pathogen Identification (*P. olseni*)	Considerations
1. PCR and sequencing of the ITS, NTS (a section within the IGS) (e.g. Casas *et al.*, 2002b). **OR** 2. PCR-RFLP (Abollo *et al.,* 2006) **OR** 3. Species-specific real-time (e.g. Itoiz *et al.* 2021; Rios *et al.,* 2020; Umeda *et al.*, 2012).	NTS though not highly sensitive has been demonstrated to have high species specificity (Villalba *et al.*, 2004). LSU, SSU and actin gene sequencing can be used in addition to ITS and NTS but on their own were not considered to have sufficient specificity to unambiguously define the species *P. olseni*. Histology and RFTM are not considered to be specific however historical records (if the same species, same locality, and information suggesting that no other *Perkinsus* spp. occur in the area) that were later confirmed through molecular work were considered. *Perkinsus atlanticus* has been synonymised with *P. olseni* based on molecular data. Based on this information previous studies on *P. atlanticus* were assessed (Murrell *et al.*, 2002). *In situ* hybridization using the *P. olseni* DNA probe of Moss *et al.*, 2006 was not used because of the limited information on its specificity.

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 with *P. olseni*, used by the *ad hoc* Group when applying Stage 3 to susceptibility to infection with *P. olseni*.

Table 3: Evidence of infection with *P. olseni*

Evidence of infection			
A: Replication	**B: Viability / Infectivity**	**C: Pathology / Clinical signs[1]**	**D: Location**
1. Presence of multinucleated cells or several aggregated uninucleate cells in the tissue as demonstrated by: a) Histopathology OR b) *In situ* hybridization (ISH) OR c) TEM **OR** 2. Demonstration of high-intensity natural infections by histology, RFTM, or ISH. **OR** 3. Demonstration of increasing copy number over time with qPCR or RFTM.	1. Transmission to uninfected individuals via co-habitation or through immersion or inoculation[2] with infective material from the host in question **OR** 2. Demonstration of viability through development of cells isolated or cultivated from tissues (e.g. RFTM). **OR** 3. Flow cytometry with markers. **OR** 4. Vital stains.	1. Mortality[3] **OR** 2. Macroscopic lesions such as pustules/cysts (i) in the foot and mantle of abalone (ii) on the gills, foot, gut, digestive gland, kidney, gonad and mantle of heavily infected clams **OR** 3. Microscopic lesions such as haemocyte infiltration, or haemocyte-encapsulated granulomatous cysts in haemocyte-infiltrated connective tissues.	1. With microscopic techniques, the parasite can be observed within connective tissues and occasionally, the *Perkinsus* cells occur within haemocytes in different organs including (i) gills, foot, gut, digestive gland, kidney, gonad and mantle for bivalves and (ii) mostly in the foot and mantle in abalone. **OR** 2. Without microscopic techniques, results from external tissue(s) (i.e. gills or mantle) need to be accompanied by a positive result from internal tissue(s), except in cases of high intensity infection (e.g. exceeding the dose or concentration of the initial challenge).

[1] Pathology and clinical signs may be non-specific, variable and include some, or all, of the characteristics listed.

[2] Inoculation in this instance is only used to demonstrate viability.

[3] It is sometimes difficult to correlate the presence of the pathogen with mortality. In this case mortality alone was not sufficient when other pathogens or environmental factors were documented.

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 11.6.2. of Chapter 11.6., Infection with *P. olseni*, of the *Aquatic Code* and Section 2.2.1. of Chapter 2.4.6., Infection with *P. olseni*, of the Manual of Diagnostic Tests for Aquatic Animals (the *Aquatic Manual*).
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 2.4.6., Infection with *P. olseni*, of the *Aquatic Manual*.
3	Species assessed as having unresolved or conflicting information. These species were not proposed for inclusion in the *Aquatic Manual*. Species in which the identity of the pathogen has been confirmed 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 2.4.6. Infection with *P. olseni*, of the *Aquatic Manual*.
4	Species assessed as non-susceptible.
5	Species not scored due to insufficient or irrelevant information.

Table 5 summarises the assessments for host susceptibility to infection with *P. olseni* 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 *P. olseni*

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

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										
Arcidae	*Anadara kagoshimensis*	half-crenated ark	N	ITS PCR and sequencing	YES	YES	YES	YES	1	Cho *et al.*., 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	ITS PCR and sequencing	ND	ND	ND	ND	3	Ye *et al.*, 2022
	Anadara trapezia	no common name	N and E	ITS PCR and sequencing[4]	YES	YES[5]	ND	YES	1	Goggin *et al.*, 1989
			N	ITS PCR and sequencing	I[6]	YES	I[6]	NO	2	Dang *et al.*, 2015
Cardiidae	*Tridacna crocea*	crocus giant clam	N	IGS & NTS PCR and sequencing[7]	YES	ND	YES	YES	1	Sheppard & Phillips, 2008
			N	ITS PCR and sequencing	NO	ND	YES	YES	1[8]	WOAH-WAHIS event ID#5517, 2024
			N	NO (RFTM)	ND	I[9]	ND	ND	NS	Goggin *et al.*, 1989
Haliotidae	*Haliotis laevigata*	greenlip abalone	N and E	ITS PCR and sequencing[4]	YES	YES[5]	ND	YES	1	Goggin *et al.*, 1989
			N	NTS PCR and sequencing	ND	ND	ND	ND	3	Murrell *et al.*, 2002
			N	ITS PCR and sequencing	ND	ND	ND	ND	3	Goggin *et al.*, 1994
	Haliotis rubra	blacklip abalone	N	NTS PCR and sequencing	ND	YES	YES	I[10]	1	Lester & Hayward, 2005
			N	ITS PCR and sequencing	I[11]	YES	YES	YES	1	Gudkovs *et al.*, 2016

[4] Pathogen identification completed in Goggin et al., 1994.

[5] The host species met criterion 3B via positive RFTM, but also potentially via apparent transmission to alternate hosts (though this study lacked controls).

[6] As *P. chesapeaki* was also detected in the studied animals, the histopathological changes are not clearly linked to infection with *P. olseni*.

[7] Sequences later submitted to GenBank (Accession numbers EU871715 and FJ477549).

[8] Only based on one animal.

[9] There was unsuccessful transmission from this host species to other potential hosts.

[10] Tissues included gills however the paper provided no information regarding rinsing or separation of tissues. The study did not specify the location of the abscesses.

[11] Histology completed was not described in Gudkovs *et al.*, 2016; however, pictures of the histology from this paper were provided in Handlinger, 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		
Margaritidae	*Pinctada fucata*	Japanese pearl oyster	N	ITS PCR and sequencing	NO	YES	NO	YES	1[12]	Sanil *et al*., 2010
			N	ITS PCR and sequencing	ND	ND	ND	ND	3	Yang *et al*., 2022
Mytilidae	*Mytilus galloprovincialis*	Mediterranean mussel	N	ITS PCR and sequencing	YES	YES	YES	YES	1	Carella *et al*., 2023
			N	ITS PCR and sequencing	I[13]	YES	I[13]	I[13]	2	Itoh *et al*., 2019
			N	qPCR, ITS PCR and sequencing	NO	ND	NO	NO	3	Ríos-Castro *et al*., 2022
	Perna canaliculus	New Zealand mussel	N	qPCR[14], PCR, RFTM, histology	NO	YES	NO	YES	1	Lane *et al*., 2023
			N	ITS PCR and sequencing	ND	ND	ND	YES	2	WOAH-WAHIS event ID#1600, 2014a
			N	NO (ISH - Moss *et al*., 2006)	YES	ND	YES	YES	NS	Muznebin *et al*., 2023b
Veneridae	*Austrovenus stutchburyi*	Stutchbury's venus	N	ITS PCR and sequencing	YES	YES	YES	YES	1	Dungan *et al*., 2007
			N	NO (RFTM, histology)	YES	YES	YES	YES	NS	Hine & Diggles, 2002
	Leukoma jedoensis	Jedo venus	N	ITS and NTS PCR and sequencing	YES	YES	YES	YES	1	Park *et al*., 2006
	Paratapes undulatus	undulate venus	N	sequencing[15]	YES	YES	YES	YES	1	Leethochavalit *et al*., 2004
	Protapes gallus	rooster venus	N	ITS PCR and sequencing	YES	YES	YES	YES	1	Shamal *et al*., 2018

[12] See section 6.2. of this report for further information on the assessment of this host species.

[13] The histopathological changes cannot be clearly linked to *P. olseni* as there is a co-infection with *P. beihaiensis*.

[14] The qPCR used in this study targets the 5.8S region (Gias & Johnston, 2011), and the *ad hoc* Group agreed that the combination of primers and probe used was shown to be specific to *P. olseni*.

[15] The parasite was only identified with RFTM and histology in the study; however, was later sequenced and deposited in GenBank (AF522321.2).

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		
	Proteopitar patagonicus	no common name	N	ITS PCR and sequencing	YES	NO	YES	YES	1	Cremonte *et al*., 2005
	Ruditapes decussatus[16]	grooved carpet shell	N	qPCR	YES	YES	ND	YES	1	Estevao *et al*., 2023
			N	IGS PCR and sequencing[17]	YES	ND	YES	YES	1	Costa *et al*., 2012
			N	ITS PCR and sequencing	YES	YES	YES	YES	1	Elandaloussi *et al*. 2009a
			N	ITS PCR and sequencing	YES	YES	YES	YES	1	Casas *et al*., 2002a
	Ruditapes philippinarum[16]	Japanese carpet clam	N	ITS PCR and sequencing	ND	YES	ND	YES	1	Itoiz *et al*., 2021
			N	ITS PCR and sequencing	YES	YES	YES	YES	1	Pretto *et al*., 2014
			N	ITS PCR and sequencing	ND	YES	ND	YES	1	Wu *et al*., 2011
			N	ITS PCR and sequencing	YES	YES	YES	YES	1	Hamagushi *et al*., 1998
Score 2										
Cardiidae	*Cerastoderma edule*	common edible cockle	N	ITS PCR and sequencing analysis	ND	ND	YES	YES	2	Ríos-Castro *et al*., 2022
Mytilidae	*Mytilus chilensis*	Chilean mussel	N	ITS PCR and sequencing analysis	YES	ND	YES	YES	1[12]	Vázquez *et al*., 2022
Ostreidae	*Crassostrea gasar*[16]	gasar cupped oyster	N	ITS PCR and sequencing analysis	YES	YES	I[18]	YES	1[12]	da Silva *et al*., 2014
			N	NO (PCR, RFTM)	YES	YES	NO	NO	NS	da Silva *et al*., 2016
	Ostrea angasi	Australian mud oyster	N	qPCR, PCR and sequencing	ND	ND	ND	YES	2	WOAH-WAHIS event ID#1743, 2015

[16] See section 6.3. of this report for further information on host identification.

[17] The NTS region is included within the IGS one.

[18] Histopathological changes were present; however, as a co-infection with *P. marinus* was reported, these changes cannot be clearly linked to infection with *P. olseni*.

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		
Pectinidae	*Pecten novaezelandiae*	New Zealand scallop	N	PCR and sequencing	ND	ND	ND	YES	2	WOAH-WAHIS event ID#1672, 2014b
Psammobiidae	*Hiatula acuta*	No common name	N	ITS PCR and sequencing analysis	ND	ND	ND	YES	2	Cui *et al*., 2018
Veneridae	*Venerupis corrugata*	corrugated venus	N	ITS PCR and sequencing analysis	ND	ND	ND	NO	3[12]	Ramilo *et al*., 2016
			N	NO (PCR, RFTM, histology)	ND	YES	ND	ND	NS	Balseiro *et al*., 2010
			EI	NO (infective material from *R. decussatus*)	YES	YES	ND	ND	NS	Rodriguez *et al*., 1994
			N	NO (RFTM, histology)	ND	YES	YES	YES	NS	Navas *et al*., 1992
Score 3										
Cardiidae	*Cerastoderma glaucum*	olive green cockle	N	ITS PCR and sequencing analysis	ND	ND	ND	ND	3	Ramilo *et al*., 2015
Chamidae	*Chama pacifica*	reflexed jewel box	N	ITS PCR and sequencing analysis	ND	ND	ND	ND	3	Goggin *et al*., 1994
			N	ITS PCR and sequencing[4]	ND[19]	I[20]	ND	ND	NS	Goggin *et al*., 1989
Haliotidae	*Haliotis diversicolor*	small abalone	N	ITS PCR and sequencing	ND	ND	ND	ND	3	Yang *et al*., 2022
			N	ITS PCR and sequencing	ND	ND	NO	ND	3	Ye *et al*., 2022
Isognomonidae	*Isognomon alatus*	flat tree oyster	N	ITS PCR and sequencing	ND	ND	ND	ND	3	Pagenkopp Lohan *et al*., 2016
	Isognomon sp. (origin: Panama)	N/A	N	ITS PCR and sequencing	ND	ND	ND	ND	3	Pagenkopp Lohan *et al*., 2016
Margaritidae	*Pinctada imbricata*	Atlantic pearl oyster	N	ITS PCR and sequencing	ND	ND	ND	ND	3	Pagenkopp Lohan *et al*., 2016

[19] Low infection intensity with a RFTM score of 0.1-1.9 was reported in the study.

[20] *Chama pacifica* acted as a donor transmitter in Goggin *et al*., 1989, but there were no controls in the study, so the infection status prior to the experiment was unknown.

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		
Ostreidae	*Crassostrea rhizophorae*	mangrove cupped oyster	N	ITS PCR and sequencing	ND	ND	ND	ND	3	Pagenkopp Lohan *et al*., 2016
	Dendostrea frons	Frons oyster	N	ITS PCR and sequencing	ND	ND	ND	ND	3	Pagenkopp Lohan *et al*., 2016
	Magallana [Syn. *Crassostrea*] *gigas*	Pacific oyster	N	ITS PCR and sequencing	ND	ND	ND	ND	3	Ye *et al*., 2022
	Magallana [Syn. *Crassostrea*] *hongkongensis*	no common name	N	ITS PCR and sequencing	NO	ND	NO	NO	3	Moss *et al*., 2007
	Saccostrea sp. (origin: Panama)	N/A	N	ITS PCR and sequencing	ND	ND	ND	ND	3	Pagenkopp Lohan *et al*., 2016
Pectinidae	*Mimachlamys crassicostata*	noble scallop	N	ITS PCR and sequencing	ND	ND	ND	ND	3	Yang *et al*., 2022
Pharidae	*Sinonovacula constricta*	constricted tagelus	N	ITS PCR and sequencing	ND	ND	ND	ND	3	Ye *et al*., 2022
Veneridae	*Meretrix lyrata*	lyrate hard clam	N	ITS PCR and sequencing	ND	ND	ND	ND	3	Ye *et al*., 2022
			N	NO (histology)	ND	ND	ND	ND	NS	WOAH-WAHIS event ID #1077, 2011
	Polititapes aureus	golden carpet shell	N	ITS PCR and sequencing analysis	ND	ND	ND	ND	3	Ramilo *et al*., 2015
			N	NO (RFTM, histology)	ND	YES	YES	YES	NS	Navas *et al*., 1992
	Venus verrucosa	warty venus	N	ITS PCR and sequencing analysis	I[21]	ND	I[21]	I[21]	3	Ramilo *et al*., 2015
Not scored (NS) because pathogen ID was inconclusive										
Arcidae	*Barbatia candida*	white-beard ark	N	NO (histology)	YES	ND	YES	YES	NS	Hine & Thorne, 2000
	Barbatia foliata	decussate ark	N	NO (RFTM)	ND[19]	I[22]	ND	ND	NS	Goggin *et al*., 1989

[21] As a co-infection with *Perkinsus mediterraneus* was reported, the histological changes cannot be clearly linked to infection with *P. olseni*.

[22] There was apparent transmission from *Barbatia foliata* to *Saccostrea cucullata*, but there were no controls in the study, so the infection status prior to the experiment was unknown.

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		
	Barbatia novaezealandiae	ark shell	N	NO (histology)	YES	ND	ND	ND	NS	Hine, 2002
Batillariidae	*Pyrazus ebeninus*	no common name	E	NO (RFTM)	I[23]	YES	ND	ND	NS	Goggin *et al*., 1989
Cardiidae	*Tridacna gigas*	giant clam	N	NO (RFTM)	ND	I[24]	ND	ND	NS	Goggin *et al*., 1989
	Tridacna maxima	elongate giant clam	N	NO (RFTM)	ND	I[9]	ND	ND	NS	Goggin *et al*., 1989
			N	NO (PCR, histology)	ND	ND	YES	ND	2	WOAH-WAHIS event ID#1316, 2012
Haliotidae	*Haliotis cyclobates*	no common name	N and E	NO (RFTM)	I[19]	YES[5]	ND	ND	NS	Goggin *et al*., 1989
	Haliotis iris	rainbow abalone	N	NO[25]	YES	ND	YES	YES	NS[12]	Muznebin *et al*., 2023a
	Haliotis roei	Roe's abalone	N	NTS PCR and sequencing	ND	YES	ND	I[26]	2[12]	Lester & Hayward, 2005
	Haliotis scalaris	no common name	E	NO (RFTM)	I[23]	YES	ND	ND	NS	Goggin *et al*., 1989
Isognomonidae	*Isognomon isognomum*	wader tree oyster	N	NO (histology)	YES	ND	NO	YES	NS	Hine & Thorne, 2000
	Isognomon sp. (origin: New South Wales, Australia)	No common name	E	NO (RFTM)	I[19]	YES	ND	ND	NS	Goggin *et al*., 1989
Malleidae	*Malleus meridianus*	no common name	N	NO (histology)	YES	ND	NO	YES	NS	Hine & Thorne, 2000
Margaritidae	*Pinctada albina*	Sharks Bay pearl oyster	N	NO (histology)	YES	ND	YES	YES	NS	Hine & Thorne, 2000
	Pinctada margaritifera	blacklip pearl oyster	N	NO (PCR, histology)	ND	ND	NO	ND	NS	WOAH-WAHIS event ID#1372, 2013
	Pinctada maxima	silverlip pearl oyster	N	NO (histology)	YES	ND	NO	YES	NS	Hine & Thorne, 2000
	Pinctada sugillata	fringed pearl oyster	E	NO (RFTM)	YES	YES	ND	YES	NS	Goggin *et al*., 1989

[23] Moderate infection intensity with a RFTM score of 2.0-3.9 was reported.

[24] There was apparent transmission from *Tridacna gigas* to *Pinctada sugillata*, but there were no controls in the study, so the infection status prior to the experiment was unknown.

[25] Pathogen identification relied on the *P. olseni* DNA probe of Moss *et al*., 2006, which was not accepted as confirmation due to the limited information on its specificity.

[26] Tissues included gills; however, there was no information provided regarding rinsing or separation of tissues.

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		
Mesodesmatidae	*Paphies australis*	Pipi wedge clam	N	NO (RFTM, histology)	ND	ND	ND	ND	NS	Hine & Diggles, 2002
Mytilidae	*Septifer bilocularis*	box mussel	N	NO (histology)	YES	ND	ND	YES	NS	Hine & Thorne, 2000
	Trichomya hirsuta	no common name	E	NO (RFTM)	I[19]	YES	ND	ND	NS	Goggin *et al.*, 1989
Ostreidae	*Magallana ariakensis*	ariake cupped oyster	EI	ITS PCR and sequencing	NO	ND	NO	NO	NS[12]	Moss *et al.*, 2006
	Saccostrea cucullata	hooded oyster	N	NO (histology)	YES	ND	YES	YES	NS	Hine & Thorne, 2000
			E	NO (RFTM)	I[19]	YES	ND	ND	NS	Goggin *et al.*, 1989
	Saccostrea glomerata	New Zealand rock oyster	N	NO (histology)	YES	ND	NO	YES	NS	Hine & Thorne, 2000
			E	NO (RFTM)	I[19]	YES	ND	ND	NS	Goggin *et al.*, 1989
Pinnidae	*Pinna deltodes*	No common name	N	No (histology)	ND	ND	ND	ND	NS	Hine & Thorne, 2000
Spondylidae	*Spondylus sp.* (origin: Northwest Western Australia)	N/A	N	NO (histology)	YES	ND	YES	YES	NS	Hine & Thorne, 2000
Tellinidae	*Macomona liliana*	large wedge shell	N	NO[27] (RFTM, histology)	N/A	N/A	N/A	N/A	NS[12]	Hine & Diggles, 2002
Veneridae	*Callista chione*	smooth callista	N	NO (RFTM, squash)	ND	YES	ND	ND	NS	Canestri-Trotti *et al.*, 2000
	Meretrix taiwanica	no common name	N	NO (ITS qPCR)	ND	ND	I[28]	YES	NS	WOAH-WAHIS event ID#5233, 2023
	Polititapes rhomboides	banded carpet shell	N	NO (ITS PCR)	ND	ND	ND	ND	NS	Balseiro *et al.*, 2010

[27] The 24 animals of this species tested in this study by RFTM and histology were all negative.

[28] High mortality was associated with the presence of *P. olseni*; however, there was a co-infection with *Vibrio spp*.

4. Results

The *ad hoc* Group agreed that six of the species currently included in Article 11.6.2. as susceptible to infection with *Perkinsus olseni*, and nine additional species, not previously listed, meet the criteria for listing as susceptible to infection with *P. olseni* in accordance with Chapter 1.5. of the *Aquatic Code.* These are proposed to be listed in Article 11.6.2. of Chapter 11.6. Infection with *P. olseni*. These species are shown in the table below:

Family	Scientific name	Common name
Arcidae	*Anadara kagoshimensis*	half-crenated ark
	Anadara trapezia	no common name
Cardiidae	*Tridacna crocea*	crocus giant clam
Haliotidae	*Haliotis laevigata*	greenlip abalone
	Haliotis rubra	blacklip abalone
Margaritidae	*Pinctada fucata*	Japanese pearl oyster
Mytilidae	*Mytilus galloprovincialis*	Mediterranean mussel
	Perna canaliculus	New Zealand mussel
Veneridae	*Austrovenus stutchburyi*	Stutchbury's venus
	Leukoma jedoensis	Jedo venus
	Paratapes undulatus	undulate venus
	Protapes gallus	rooster venus
	Proteopitar patagonicus	no common name
	Ruditapes decussatus	grooved carpet shell
	Ruditapes philippinarum	Japanese carpet clam

Eight species currently included in Article 11.6.2. as susceptible to infection with *Perkinsus olseni*, Ariake cupped oyster (*Magallana ariakensis*) *Barbatia novaezealandiae*, corrugated venus (*Venerupis corrugata*), golden carpet shell (*Polititapes aureus*), *Haliotis cyclobates*, *Haliotis scalaris*, *Macomona liliana*, and pipi wedge clam (*Paphies australis*) were assessed as not meeting the criteria and were proposed to be removed from Article 11.6.2. of Chapter 11.6. of the *Aquatic Code*.

Seven species were assessed as having incomplete evidence of susceptibility and were proposed to be included in Section 2.2.2. of Chapter 2.4.6. of the *Aquatic Manual*. These species are shown in the table below:

Family	Scientific name	Common name
Cardiidae	*Cerastoderma edule*	common edible cockle
Mytilidae	*Mytilus chilensis*	Chilean mussel
Ostreidae	*Crassostrea gasar*	gasar cupped oyster
	Ostrea angasi	Australian mud oyster
Pectinidae	*Pecten novaezelandiae*	New Zealand scallop
Psammobiidae	*Hiatula acuta*	no common name
Veneridae	*Venerupis corrugata*	corrugated venus

The *ad hoc* Group found that the identity of the pathogen has been confirmed but an active infection has not been demonstrated in 16 species. These species were therefore proposed to be included in the second

paragraph of Section 2.2.2. of Chapter 2.4.6. of the *Aquatic Manual*. These species are shown in the table below:

Family	Scientific name	Common name
Cardiidae	*Cerastoderma glaucum*	olive green cockle
Chamidae	*Chama pacifica*	reflexed jewel box
Haliotidae	*Haliotis diversicolor*	small abalone
Isognomonidae	*Isognomon alatus*	flat tree oyster
	Isognomon sp.	N/A
Margaritidae	*Pinctada imbricata*	Atlantic pearl oyster
Ostreidae	*Crassostrea rhizophorae*	mangrove cupped oyster
	Dendostrea frons	Frons oyster
	Magallana [Syn. *Crassostrea*] *gigas*	Pacific oyster
	Magallana [Syn. *Crassostrea*] *hongkongensis*	no common name
	Saccostrea sp.	N/A
Pectinidae	*Mimachlamys crassicostata*	noble scallop
Pharidae	*Sinonovacula constricta*	constricted tagelus
Veneridae	*Meretrix lyrata*	lyrate hard clam
	Polititapes aureus	golden carpet shell
	Venus verrucosa	warty venus

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 mollusc species are in accordance with FAOTERM (http://www.fao.org/faoterm/collection/faoterm/en/). Where the common mollusc 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 'Non-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 'Non-Determined' when making their final assessment.

6.1. General comments

The *ad hoc* Group reviewed all available papers (refer to Table 5) but only fully assessed papers that provided sufficient evidence for susceptibility for each species assessed. Additional papers beyond those needed to provide sufficient evidence were reviewed to ensure the absence of conflicting evidence and retained in the list of references.

The *ad hoc* Group agreed to focus on studies published from 1994 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.

When necessary to corroborate pathogen identification, the *ad hoc* Group contacted authors of the studies to further describe pathogen identification methods.

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. Where sampling strategy was distributed across seasons or locations, and/or where a single paper provided all evidence (molecular with corresponding evidence from histology within the same animals) the *ad hoc* Group considered that one strong paper was sufficient to conclude susceptibility of a species. Additional studies were still reviewed to check for any supporting or conflicting evidence.

Seven WAHIS reports of notifications for infection with *P. olseni* were assessed by the *ad hoc* Group and these were predominantly found to be for new host species. Unfortunately, these did not provide enough detail on pathogen identification and/or infection criteria to support a decision regarding susceptibility. Consequently, the *ad hoc* Group recommends that when a notification is reported to WAHIS, that an appropriate level of detail is included to allow for an assessment to occur.

6.2. Species specific comments

Crassostrea gasar – In the da Silva *et al*., 2014 paper, despite the reported co-infection with *P. marinus* in the population (confirmed by sequencing), the *ad hoc* Group scored the paper as a '1' by using the results from the ISH probe (Moss *et al*., 2006) to link the histopathology to *P. olseni*. However, the *ad hoc* Group scored this host species as a '2' because the da Silva *et al*., 2014 conclusion was based on one animal out of the six tested with species-specific tools, and the other available paper was not scored.

Haliotis iris – Did not meet the pathogen identification criteria because it relied only on the ISH probe published by Moss *et al*., 2006. If more information becomes available on the molecular identity of the pathogen in this host, the score of this host species will be reviewed.

Haliotis roei – Only one animal was found to be positive in one study, which was assessed as a score '2'; therefore, the *ad hoc* Group assessed *Haliotis roei* as an overall score of "NS".

Macomona liliana – In Hine & Diggles, 2002, the introduction mentions a previous detection of *Perkinsus* in *Macomona liliana* in Kaipara Harbour in 1999, but no reference or further information is provided.

Magallana ariakensis – In Moss *et al*., 2006, the experimental infection does not mimic a natural infection and as such was not used to assess susceptibility in this host species. Furthermore, the low number of positives 72 days after inoculation precludes the conclusion of non-susceptibility, and rather indicates viability of *P. olseni* in this host.

Mytilus chilensis – The *ad hoc* Group concluded to score *Mytilus chilensis* as '2' because only one study was assessed which had only 2 out of 60 cultured animals and 0 out of 60 wild animals found to be infected with *P. olseni*. Also, the host identification was not confirmed and other *Mytilus* species are known to be present in Beagle Channel, Argentina.

Pinctada fucata – An atypical presentation of the parasite is seen in the study with a score of '1' (Sanil *et al*., 2010); however, as the study covers multiple locations with a large number of individuals found to be infected with the parasite, the host species was determined by the *ad hoc* Group to have an overall score of '1'.

Venerupis corrugata – In Ramilo *et al*., 2016, *P. chesapeaki* was not detected through molecular testing in *Ruditapes decussatus* from Galicia, Spain, which is the species and region used for the source material in the experimental trial described in Rodríguez et al., 1994. Based on this, the *ad hoc* Group determined that the *Perkinsus* sp. detected in the Rodríguez *et al*., 1994 in *V. corrugata* was highly likely to be *P. olseni*. Collectively these 2 studies provide enough information for the *ad hoc* Group to give the host species a score of '2'.

6.3. Host Identification

Ruditapes philippinarum and *R. decussatus* – The *ad hoc* Group noted that both *Ruditapes philippinarum* and *R. decussatus* can occur in the same locations and are morphologically similar. While in most papers there was no information provided on how the identity of the clams was determined, the *ad hoc* Group accepted the identifications provided by the authors.

In tropical regions the identification of some mollusc species is a recurring issue for specialists and the *ad hoc* Group asked for confirmation from authors of host identification if not provided in the papers. For example, *Crassostrea gasar* and *C. rhizophorae* live in sympatry in the same mangrove zone (Ferreira *et al*., 2023 and Diyie *et al*., 2023) and the *ad hoc* Group contacted DaSilva *et al*., 2014 to confirm that the host species in their study was *C. gasar*.

In addition, the *ad hoc* Group noted the need to replace *Crassostrea tulipa* by *C. gasar* in the *ad hoc* Group's assessments of the susceptibility of mollusc species to infection with *Perkinsus marinus* as they are distinct species as described in Ferreira *et al*., 2023. The original report on the susceptibility of mollusc species to infection with *P. marinus* referenced literature on *C. gasar*, but at the time the *ad hoc* Group changed the species name to *C. tulipa* due to information on WoRMS (now debated information on WoRMS).

6.4. Non-susceptibility

Despite the fact that a number of host species tested negative for *Perkinsus* spp. in known infected regions, the *ad hoc* Group felt that the sampling/experimental designs were not sufficiently robust to demonstrate freedom of infection and therefore do not provide evidence of non-susceptibility (e.g. Hine & Thorne, 2000; Pagenkopp Lohan *et al*., 2016; Goggin *et al*., 1989).

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 could be applicable for the susceptible species identified for infection with *Perkinsus olseni*. However the families (e.g. Veneridae and Haliotidae) with multiple susceptible species, also have a number of species with incomplete information regarding susceptibility to infection with *P. olseni*, and the *ad hoc* Group concluded that it would be more appropriate to list the susceptible species at the species level.

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…/Annexes

Annex 1. List of Participants June 2023

MEETING OF THE WOAH *AD HOC* GROUP ON SUSCEPTIBILITY OF MOLLUSC SPECIES TO WOAH LISTED DISEASES

La Tremblade, France, 6 to 8 June

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MEMBERS OF THE *AD HOC* GROUP

Dr Isabelle Arzul
(Chair)
IFREMER
Adaptation et Santé des Invertébrés Marins
La Tremblade,
FRANCE

Dr Robert Adlard
Marine Biodiversity at Queensland Museum Network,
South Brisbane,
AUSTRALIA

Dr Chang-Ming Bai
Yellow Sea Fisheries Research Institute, CAFS
Division of Maricultural Organism Disease control and Molecular Pathology
Qingdao,
CHINA (PEOPLE'S REPUBLIC OF)

Dr Lori Gustafson
Surveillance Design and Analysis
USDA/APHIS/VS/CEAH,
Fort Collins,
UNITED STATES OF AMERICA

Dr Karin B. Lohrmann
Departamento de Biología Marina,
Facultad de Ciencias del Mar,
Universidad Católica del Norte,
Coquimbo,
CHILE

MEMBERS OF THE COMMISSION

Dr Kevin William Christison
Department of Environment,
Forestry and Fisheries,
Directorate: Aquaculture Innovation and Technology Development,
Vlaeberg,
SOUTH AFRICA

WOAH HEADQUARTERS

Dr Bernita Giffin
Scientific Coordinator for Aquatic Animal Health
Standards Department

Dr Kathleen Frisch
Scientific Coordinator for Aquatic Animal Health
Standards Department

Annex 2. List of Participants November/December 2023

MEETING OF THE WOAH *AD HOC* GROUP ON SUSCEPTIBILITY OF MOLLUSC SPECIES TO WOAH LISTED DISEASES

Paris, France, 29, 30 November and 1 December 2023

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MEMBERS OF THE *AD HOC* GROUP

Dr Isabelle Arzul
(Chair)
IFREMER
Adaptation et Santé des Invertébrés Marins
La Tremblade,
FRANCE

Dr Robert Adlard
Marine Biodiversity at Queensland Museum Network,
South Brisbane,
AUSTRALIA

Dr Chang-Ming Bai
Yellow Sea Fisheries Research Institute, CAFS
Division of Maricultural Organism Disease control and Molecular Pathology
Qingdao,
CHINA (PEOPLE'S REPUBLIC OF)

Dr Lori Gustafson
Surveillance Design and Analysis
USDA/APHIS/VS/CEAH,
Fort Collins,
UNITED STATES OF AMERICA

Dr Karin B. Lohrmann
Departamento de Biología Marina,
Facultad de Ciencias del Mar,
Universidad Católica del Norte,
Coquimbo,
CHILE

MEMBERS OF THE COMMISSION

Dr Kevin William Christison
Department of Environment,
Forestry and Fisheries,
Directorate: Aquaculture Innovation and Technology Development,
Vlaeberg,
SOUTH AFRICA

WOAH HEADQUARTERS

Dr Kathleen Frisch
Scientific Coordinator for Aquatic Animal Health
Standards Department

Dr Patricia Kelly
Scientific Coordinator for Aquatic Animal Health
Standards Department

Annex 3. Terms of Reference

WOAH *AD HOC* GROUP ON SUSCEPTIBILITY OF MOLLUSC SPECIES TO INFECTION WITH WOAH LISTED DISEASES

June 6–8 2023

Terms of reference

Background

Chapter 1.5. Criteria for listing species as susceptible to infection with a specific pathogenic agent, 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 Members 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 WOAH listed diseases will undertake assessments for infection with *Perkinsus olseni* in molluscs.

Terms of Reference

1) Review relevant literature documenting susceptibility of species for infection with *Perkinsus olseni* and apply criteria, as outlined in Chapter 1.5. Criteria for listing species as susceptible to infection with a specific pathogen, to potential host species in order to determine susceptibility to infection with *Perkinsus olseni*.

2) Determine susceptible species for infection with *Perkinsus olseni* based on Article 1.5.7.

3) Determine species with incomplete evidence for susceptibility for infection with *Perkinsus olseni* 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 11.6.2. of Chapter 11.6., Infection with *Perkinsus olseni*, in the *Aquatic Code.*

2) Propose a list of species with incomplete evidence for susceptibility for inclusion in Section 2.2.2. of Chapter 2.4.6. Infection with *Perkinsus olseni* of the *Aquatic Manual.*

3) A report for consideration by the Aquatic Animals Commission at its September 2023 meeting.
