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

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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 in person in Paris, France, from 11 to 13 June 2024.

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

For the purpose of this report, *Xenohaliotis californiensis* refers to the pathogenic agent *Candidatus* Xenohaliotis californiensis, which is the accepted name of the pathogenic agent.

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 *Xenohaliotis californiensis*.

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

Table 1: Route of transmission for infection with *X. californiensis*

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; infection by immersion or exposure to effluent water from infected individuals, under conditions that mimic natural conditions for the host.	Infection by injection into body tissue was considered to not mimic natural pathways. The dose was considered to determine if exposure by immersion or oral delivery mimicked levels expected in 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 *X. californiensis*, as well as some considerations.

Table 2: Pathogen identification for infection with *X. californiensis*

Pathogen Identification (*X. californiensis*)	Considerations
1. PCR and sequencing of the 16S rDNA (e.g. Andree *et al*., 2000; Cicala *et al*., 2017). **OR** 2. Species-specific real-time PCR using primers/probe designed based on 16S rDNA (e.g. Friedman *et al*., 2014). **OR** 3. *in situ* hybridization (ISH) using all four probes (RA5.1, RA3.6, RA3.8 and RA5.6) developed by Antonio *et al*., 2000.	Cicala *et al*., 2017 reported a high frequency of false negative when using the RA5.1/RA3.6 primer sets developed by Andree *et al*., 2000.; however, this is not reported by other studies. As a result Cicala *et al.*, 2017 designed new primers based on 16S rDNA (ss16S-F/R) which were accepted by the *ad hoc* Group as a method for pathogen identification when combined with sequencing. For studies without molecular information, corroborating evidence from other studies (same location and host species) was also considered for pathogen identification.

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

Table 3: Evidence of infection with *X. californiensis*

Evidence of infection			
A: Replication	**B: Viability / Infectivity**	**C: Pathology / Clinical signs[1]**	**D: Location**
1. Presence of WS-RLO[2] intracellular inclusions within vacuoles in the tissue as demonstrated by: a. Histopathology OR b. ISH OR c. Transmission electron microscopy (TEM). **OR** 2. Demonstration of high-intensity natural infections by histology, or ISH. **OR** 3. Demonstration using qPCR of increasing infection intensity over time following exposure.	Transmission to uninfected individuals via co-habitation or through exposure to infective material from the host being assessed.	1. Clinical signs, such as: a. Weak, or loss of, righting reflex b. Reduced, or loss of, pedal adhesion c. Mortality[3] d. Anorexia[3] **OR** 2. Macroscopic lesions, such as: a. Atrophied foot muscle b. Dark pigmentation of the foot c. Mottled digestive gland (dark brown with small foci of tan coloured tissue) **OR** 3. Microscopic lesions, such as: a. Digestive organs degeneration (e.g. atrophy of digestive tubules, inflammation) and/or metaplasia of the digestive gland. b. In the foot: reduction in number and organisation of muscle fibres and serous cell abundance may increase.	With microscopic techniques[4], the presence of intracellular WS-RLO inclusions within the gastrointestinal (i.e. oesophagus, stomach, digestive gland, intestine) epithelial cells, particularly in the posterior oesophagus.

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

[2] WS-RLO (Withering-Syndrome Rickettsia-Like Organisms) inclusions consist of multiple bacteria within vacuoles.

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

[4] Without microscopic or other corroborating information including gross pathological signs, the *ad hoc* Group determined that positive molecular results from the digestive organs could not be used alone to assess stage 3D.

3. Scoring and assessments

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

Table 4: Scores and outcomes of the assessments

Score	Outcome
1	Species assessed as susceptible (as described in Article 1.5.7.). These species were proposed for inclusion in Article 11.7.2. of Chapter 11.7. 'Infection with *X. californiensis*' of the *Aquatic Code* and Section 2.2.1. of Chapter 2.4.7. 'Infection with *X. californiensis'* 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 2.4.7., Infection with *X. californiensis*, 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 2.4.7. Infection with *X. californiensis*, of the *Aquatic Manual*.
4	Species assessed as non-susceptible.
NS	Species not scored due to insufficient or irrelevant information.

Table 5 summarises the assessments for host susceptibility to infection with *X. californiensis* 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 *X. californiensis*

Assessment Table Key:

N:	Natural infection	YES:	Demonstrates criterion is met	NS:	Not scored
E:	Experimental (non-invasive)	NO:	Criterion is not met	N/A:	Not applicable
EI:	Experimental invasive	ND:	Not determined		

Family	Scientific name	Common name	Subspecies (if applicable)	Stage 1: Route of infection	Stage 2: Pathogen Identification	Stage 3: Evidence of Infection				Outcome	References
						A	B	C	D		
Score 1											
Haliotidae	*Haliotis corrugata*	pink abalone	N/A	N	PCR and sequencing	ND	ND	YES	YES	1	Cicala *et al*., 2018b
				E	qPCR	YES	ND	YES	YES	1	Crosson & Friedman, 2018
				N	PCR and sequencing	YES	ND	ND	YES	1	Cruz-Flores *et al*., 2016b
	Haliotis cracherodii	black abalone	N/A	N and E	PCR and sequencing[5]	YES	YES	YES	YES	1	Friedman *et al*., 2002
				N	PCR and sequencing	YES	ND	ND	YES	1	Andree *et al*., 2000
				N	ISH	YES	ND	YES	YES	1	Antonio *et al*., 2000
				N	PCR and sequencing	YES	ND	ND	YES	1	Friedman *et al*., 2000
	Haliotis discus	Japanese abalone	*H. discus discus*	N	PCR and sequencing	ND	YES	ND	YES	1	Nishioka *et al*., 2016
				N	PCR and sequencing	YES	ND	YES	YES	1	Kiryu *et al*., 2013
	Haliotis diversicolor [6]	small abalone	*H. diversicolor aquatilis*	N	PCR and sequencing	ND	ND	ND	ND	3	Nishioka *et al*., 2016
			H. diversicolor diversicolor	N and E	PCR and sequencing	ND	YES	ND	ND	2	Nishioka *et al*., 2016
			H. diversicolor supertexta	N	PCR and sequencing	YES	ND	NO	YES	1	Wetchateng *et al*., 2010
	Haliotis fulgens	green abalone	N/A	N	PCR and sequencing	ND	ND	YES	YES	1	Cicala *et al*., 2018b
				N	PCR and sequencing	YES	ND	ND	YES	1	Cruz-Flores *et al*., 2016b
	Haliotis kamtschatkana	pinto abalone	N/A	E	qPCR	YES	ND	NO	YES	1	Frederick *et al*., 2022
				E	qPCR	YES	ND	YES	YES	1	Crosson & Friedman, 2018
	Haliotis rufescens	red abalone	N/A	N	ISH	YES	ND	YES	YES	1	Cáceres-Martínez *et al*., 2021

[5] Pathogen identification performed in Antonio *et al*., 2000 (see species specific note in Section 6.3 of this report).

[6] As a result of the taxonomic uncertainty, the *ad hoc* Group scored *Haliotis diversicolor* at the species level with an overall score of '1' (see species specific note in Section 6.3 of this report).

Family	Scientific name	Common name	Subspecies (if applicable)	Stage 1: Route of infection	Stage 2: Pathogen Identification	Stage 3: Evidence of Infection				Outcome	References
						A	B	C	D		
				N and E	qPCR	YES	YES	YES	YES	1	Crosson & Friedman, 2018
				E	PCR and sequencing	YES	YES	YES	YES	1	González *et al*., 2012
	Haliotis rufescens X *Haliotis discus hannai* hybrid	hybrid red and Japanese abalone	N/A	E	PCR and sequencing[7]	YES	ND	ND	YES	1	González *et al*., 2014
	Haliotis sorenseni	white abalone	N/A	E	qPCR	YES	ND	YES	YES	1	Vater *et al*., 2018
				N	NO (PCR)	YES	ND	ND	YES	NS	Friedman *et al*., 2007
	Haliotis tuberculata	tuberculate abalone	N/A	N	PCR and sequencing	YES	ND	YES	YES	1	Balseiro *et al*., 2006
				N	PCR and sequencing; qPCR	YES	ND	NO	YES	1	WOAH-WAHIS event ID#212, 2006
Score 2											
Haliotidae	*Haliotis gigantea*	giant abalone	N/A	N	PCR and sequencing	ND	ND	ND	YES	2	Kiryu *et al*., 2014
				N and E	PCR and sequencing	ND	ND	ND	ND	3	Nishioka *et al*., 2016
Score 3											
Haliotidae	*Haliotis discus*	Japanese abalone	*Haliotis discus hannai*	N	PCR and sequencing	ND	ND	ND	NO	3	Nishioka *et al*., 2016
				E	PCR and sequencing	NO	ND	NO	NO	3	González *et al*., 2012
				E	PCR and sequencing[7]	NO	ND	ND	NO	4	González *et al*., 2014

[7] Pathogen identification performed in González et al., 2012 (see species specific note in Section 6.3 of this report).

4. Results

The *ad hoc* Group agreed that six of the species currently included in Article 11.7.2. as susceptible to infection with *X. californiensis*, and four additional species, not previously listed, meet the criteria for listing as susceptible to infection with *X. californiensis* in accordance with Chapter 1.5. of the *Aquatic Code.* These are proposed to be listed in Article 11.7.2. of Chapter 11.7. 'Infection with *X. californiensis*'. These species are shown in Table 6 below.

Table 6: Species susceptible to infection with *X. californiensis*

Family	Scientific name	Common name
Haliotidae	*Haliotis corrugata*	pink abalone
	Haliotis cracherodii	black abalone
	Haliotis discus discus	Japanese abalone
	Haliotis diversicolor	small abalone
	Haliotis fulgens	green abalone
	Haliotis kamtschatkana	pinto abalone
	Haliotis rufescens	red abalone
	Haliotis rufescens X *Haliotis discus hannai* hybrid	hybrid red and Japanese abalone
	Haliotis sorenseni	white abalone
	Haliotis tuberculata	tuberculate abalone

Haliotis discus hannai currently included in Article 11.7.2. was assessed as not meeting the criteria and was proposed to be removed from Article 11.7.2. of Chapter 11.7. of the *Aquatic Code*.

The *ad hoc* Group could not find any publications to assess the susceptibility of *Haliotis walallensis* which is also currently included in Article 11.7.2. and therefore proposed to remove this species from Article 11.7.2. of Chapter 11.7. of the *Aquatic Code*.

Haliotis gigantea was assessed as having incomplete evidence of susceptibility and was proposed to be included in Section 2.2.2. of Chapter 2.4.7. of the *Aquatic Manual*.

The *ad hoc* Group found that the identity of the pathogenic agent, *X. californiensis* has been confirmed but an active infection has not been demonstrated in *Haliotis discus hannai*. This species was therefore proposed to be included in the second paragraph of Section 2.2.2. of Chapter 2.4.7. 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 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

6.1. General comments

The *ad hoc* Group reviewed all available papers but only summarized papers that provided sufficient evidence for susceptibility for each species assessed (Table 5). 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 focused on studies published 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 the sampling strategy was distributed across seasons or locations, 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.

The *ad hoc* Group considered two notifications for infection with *X. californiensis* reported in the World Animal Health Information System (WAHIS). For one of these reports the *ad hoc* Group requested additional supporting information from the Member to enable it to assess the host's susceptibility. The second report referred to an event for which a publication was available so no additional information was required to undertake an assessment. Consequently, the *ad hoc* Group notes that when a notification is reported to WAHIS, that an appropriate level of detail is included to support future species susceptibility assessments.

6.2. Host Identification (species and subspecies)

The *ad hoc* Group accepted the host identification as stated by the authors noting that the evaluated studies did not report methods of host identification.

6.3. Species specific comments

Haliotis cracherodii

No molecular methods were used for pathogen identification in the study by Friedman *et al*., 2002; however, the animals collected were from the same population as those reported in Antonio *et al*., 2000. The *ad hoc* Group therefore determined pathogen identification to be confirmed.

Haliotis diversicolor

The subspecies *Haliotis diversicolor aquatilis* and *Haliotis diversicolor supertexta* mentioned in some published studies could not be found or are unaccepted names in WoRMS. In the assessments (Table 5), the *ad hoc* Group retained the host names used by the authors in order to best reflect the information presented in the studies.

As a result of the taxonomic uncertainty and the absence of conflicting information, the *ad hoc* Group scored *Haliotis diversicolor* at the species level with an overall score of '1'. However, if further taxonomic information on this species becomes available, the *ad hoc* Group recommends re-evaluating at the subspecies level.

Haliotis discus

The two subspecies *Haliotis discus discus* and *Haliotis discus hannai* are accepted names in WoRMS; therefore, the *ad hoc* Group assessed and scored the subspecies separately. The *ad hoc* Group does not recommend assessing *Haliotis discus* at the species level as there is preliminary evidence of resistance to infection for one of the two subspecies.

Haliotis discus hannai

No molecular methods were used for pathogen identification in González *et al*., 2014; however, the histology samples assessed in this study were from the same individuals as those reported in González *et al*., 2012 (communication with author). The *ad hoc* Group therefore determined pathogen identification to be confirmed.

In the challenge trial described in González *et al*., 2014, while *H. discus hannai* did not show any histological evidence of infection, the two other exposed groups (*H. rufescens* and *H. rufescens* X *H. discus hannai* hybrid) showed WS-RLOs histologically. The *ad hoc* Group concluded that González *et al*., 2014 should have a score of '4' (non-susceptible) though the study was not designed to demonstrate non-susceptibility.

The *ad hoc* Group gave an overall score of '3' to *H. discus hannai* because González *et al*., 2012 and Nishioka *et al*., 2016 detected the pathogen using PCR.

***Haliotis rufescens* X *Haliotis discus hannai* hybrid**

No molecular methods were used for pathogen identification in González *et al*., 2014; however, the histology samples assessed in this study were from the same individuals as those reported in González *et al*., 2012 (communication with author). The *ad hoc* Group therefore determined pathogen identification to be confirmed.

Haliotis walallensis

In Crosson *et al*., 2014, the introduction mentions that *H. walallensis* is a potential host for WS-RLO, but no reference or further information could be found.

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' in the *Aquatic Code,* and determined that it was not applicable for the susceptible host species for *X. californiensis* identified at this time.

The rationale for not applying the criteria in Article 1.5.9. was that *Haliotis discus hannai* may not meet the criteria for susceptibility to infection with *X. californiensis*. In addition, there are over 200 *Haliotis* species but the level of information available only allowed the *ad hoc* Group to assess 11 species. Also, the *Haliotis* species assessed are in the same phylogenetic clade (Tshilate *et al*., 2023) and there are multiple *Haliotis* clades.

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…/Annexes

Annex 1. List of Participants

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

Paris, France, 11 to 13 June 2024

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Annex 2. Terms of Reference

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

Paris, France, 11 to 13 June 2024

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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 WOAH listed diseases are being undertaken progressively by dedicated *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 comment and then presented for adoption.

Species, where there is some evidence of susceptibility but insufficient evidence to demonstrate susceptibility are included in Section 2.2.2 of the relevant disease-specific chapter of the *Aquatic Manual*.

The *ad hoc* Group on Susceptibility of mollusc species to infection with WOAH listed diseases has undertaken assessments for all of the WOAH listed diseases of molluscs except for infection with *Xenohaliotis californiensis*.

Purpose

The *ad hoc* Group on Susceptibility of mollusc species to infection with WOAH listed diseases will undertake assessments for infection with *Xenohaliotis californiensis* in molluscs.

Terms of Reference

1) Review relevant literature documenting susceptibility of species for infection with *Xenohaliotis californiensis* 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.

2) Determine susceptible species for infection with *Xenohaliotis californiensis* based on Article 1.5.7.

3) Determine species with incomplete evidence for susceptibility for infection with *Xenohaliotis californiensis* based on Article 1.5.8.

Expected outputs of the *ad hoc* Group

1) Propose a list of susceptible species for inclusion in Article 11.7.2. of Chapter 11.7. 'Infection with *Xenohaliotis californiensis*' in the *Aquatic Code*.

2) Propose a list of species with incomplete evidence for susceptibility for inclusion in Section 2.2.2. of Chapter 2.4.7. 'Infection with *Xenohaliotis californiensis*' of the *Aquatic Manual.*

3) A report for consideration by the Aquatic Animals Commission at its September 2024 meeting.

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