WORLD ORGANISATION FOR ANIMAL HEALTH
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Annex 2

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REPORT OF THE MEETING OF THE OIE AQUATIC ANIMAL HEALTH STANDARDS COMMISSION

Paris, 17–24 February 2021

PART B – Texts for Member comments and information

EU comment

The EU would like to commend the OIE Aquatic Animal Health Standards Commission for its work and for having taken into consideration EU comments on the Aquatic Code and Manual submitted previously.

A number of general comments on this report of the September 2021 meeting of the Aquatic Animals Commission are inserted in the text below, while specific comments are inserted in the text of the respective annexes to the report.

The EU would like to stress again its continued commitment to participate in the work of the OIE and to offer all technical support needed by the Aquatic Animals Commission and its ad hoc groups for future work on the Aquatic Code and Manual.

The OIE Aquatic Animal Health Standards Commission (the Aquatic Animals Commission) held its meeting electronically from 17 to 24 February 2021. The list of participants is attached as Annex 1.

To facilitate the virtual 88th Annual General Session, the February 2021 meeting report of the Aquatic Animals Commission is being published in two parts: **Part A** (available on the OIE website) provides information about the new and revised texts for the *Aquatic Code* and the *Aquatic Manual* that will be proposed for adoption at the 88th General Session; and **Part B** (herewith) will provide information about other topics discussed at the Commission's February 2021 meeting including texts presented for comments and for information.

The Aquatic Animals Commission thanked the following Members for providing written comments on draft texts for the OIE *Aquatic Animal Health Code* (hereinafter referred to as the *Aquatic Code*) and OIE *Manual of Diagnostic Tests for Aquatic Animals* (hereinafter referred to as the *Aquatic Manual*) circulated in the Commission's September 2020 meeting report: Armenia, Australia, Canada, China (People's Rep of), Chinese Taipei, Cuba, Japan, Korea (Rep. of), New Caledonia, Switzerland, Thailand, the United Kingdom (the UK), the United States of America (the USA), Members of the OIE Americas region, the Member States of European Union (the EU) and the African Union Inter-African Bureau for Animal Resources (AU-IBAR) on behalf of African Members. The Commission also wished to acknowledge the valuable advice and contributions from numerous experts of the OIE scientific network.

The Commission reviewed all comments that were submitted on time and were supported by a rationale. The Commission made amendments to draft texts, where relevant, in the usual manner by '<u>double underline</u>' and '~~strikethrough~~'. In the Annexes, amendments proposed at this meeting are highlighted with a coloured background in order to distinguish them from those made previously. The Commission did not consider comments where a rationale

had not been provided or that were unclear. Due to the large volume of work, the Commission was not able to draft a detailed explanation for the reasons for accepting or not each of the comments received, and focused its explanations on the major comments. Where amendments were of an editorial nature, no explanatory text has been provided. The Commission wished to note that not all texts proposed by Members to improve clarity were accepted; in these cases, it considered the text clear as currently written.

The Commission encourages Members to consider relevant information in previous Commission and *ad hoc* Group reports when preparing comments, especially on longstanding issues. These reports are available on the OIE Website.

The table below lists the meeting agenda items presented in Part B (herewith) of the Commission's February report and includes links to relevant items within this report. Members should note that texts in **Annexes 2 to 6** and **Annexes 9 to 10** are presented for Member comments, and **Annexes 7 to 8** for information.

Comments on relevant texts in this report must reach OIE Headquarters by the **6 August 2021** to be considered at the September 2021 meeting of the Aquatic Animals Commission.

All comments should be sent to the OIE Standards Department at: AAC.Secretariat@oie.int.

Comments should be submitted as Word files rather than pdf files because pdf files are difficult to incorporate into the Commission's working documents.

Comments should be presented in the relevant Annex, and include new proposed text, supported by a structured rationale or by published scientific references. Proposed deletions should be indicated in '~~strikethrough~~' and proposed additions with 'double underline'. Members should not use the automatic 'track-changes' function provided by Word processing software, as such changes may be lost in the process of collating Members' submissions into the Aquatic Animals Commission's working documents. Members are also requested not to reproduce the full text of a chapter as this makes it easy to miss comments while preparing the working documents.

The Aquatic Animals Commission strongly encourages Members to participate in the development of the OIE's international standards by submitting comments on this report and participate in the process of adoption at the General Session.

Agenda

1. THE OIE *AQUATIC ANIMAL HEALTH CODE* - Texts for Member comments

The Aquatic Animals Commission thanked Members for highlighting some translation issues in some of the Annexes circulated for comment in the French and Spanish versions, and reported that these have been reviewed and corrected.

1.1. Glossary definitions 'Basic biosecurity conditions', 'Early detection system' and 'Passive surveillance'

The Aquatic Animals Commission informed Members that as a consequence of the revision of Chapter 1.4 (see Item 1.2.1), the Commission agreed to propose amendments to the Glossary terms 'Basic biosecurity conditions' and 'Early detection system' and propose a new Glossary term for 'Passive surveillance' to ensure alignment with proposed amendments to Chapter 1.4.

'Basic biosecurity conditions'

The Commission proposed to simplify the definition and delete the specific requirements for basic biosecurity conditions from the definition and reference Article 1.4.6 of the amended Chapter 1.4, where these requirements are described.

'Early detection system'

The Commission proposed to simplify the definition and delete text in the definition referring to the characteristics of an early detection system. The Commission noted that these characteristics are described in Article 1.4.7 of the amended Chapter 1.4.

'Passive surveillance'

The Commission proposed a new definition for 'Passive surveillance' to facilitate better understanding of different types of surveillance in the *Aquatic Code*.

The amended and new Glossary definitions for 'Basic biosecurity conditions', Early detection system' and Passive surveillance' are presented as **Annex 2** for Member comments.

EU comment

The EU in general supports the proposed changes to the Glossary.

One comment is inserted in the text of Annex 2.

1.2. Approaches to demonstrating disease freedom

Comments were received from Australia, Canada, China (People's Rep. of), Japan, Switzerland, the UK, the USA and the EU.

The Aquatic Animals Commission thanked Members for their constructive comments on the discussion paper and model articles as well as the experts from the OIE Collaborating Centres on Epidemiology and Risk Assessment of Aquatic Animal Diseases, for their review and comments on Chapter 1.4.

Background

A discussion paper on approaches for determining periods required to demonstrate disease freedom, developed by the Commission, was first circulated for comments in the Commission's September 2018 report. The Commission considered comments received and circulated a revised discussion paper in its September 2019 report, and presented model Articles X.X.4, X.X.5 and X.X.6 for the disease-specific chapters of the *Aquatic Code* for Member comments in its February 2020 report.

At its September 2020 meeting, the Commission considered all comments received and agreed that a review of Chapter 1.4, Aquatic animal health surveillance, was required to ensure that all comments were addressed appropriately. It agreed that the response to these comments, including the revised Chapter 1.4, Aquatic animal health surveillance, and the model Articles X.X.4, X.X.5 and X.X.6, would be provided to Members in its February 2021 report.

Previous Commission reports where this item was discussed:

September 2018 report (Item 2.10, page 11); September 2019 report (Item 6.6, page 9); February 2020 report (Item 7.2.2, page 15); September 2020 (Item 6.2, page 16).

1.2.1. Chapter 1.4. Aquatic Animal Health Surveillance

The Aquatic Animals Commission noted that it completed a substantial revision of Chapter 1.4, Aquatic Animal Health Surveillance. This work was required as the chapter has not been substantially revised since first adoption in 2008 and that amendments were needed to support the work being undertaken to revise the articles on demonstration of freedom in disease-specific chapters (see Item 1.2.2).

The Commission wished to advise Members that the revisions to Chapter 1.4 are intended to align with the approaches proposed in the discussion paper previously provided to Members for comments. The revised Chapter 1.4 is more directly focused on providing guidance for self-declaration of freedom from disease, rather than providing general guidance on aquatic animal health surveillance. For these reasons, the changes to Chapter 1.4 are substantial and a version with tracked changes is not provided.

The Commission noted that a new Article 1.4.4, Publication by the OIE of a self-declaration of freedom from disease by a Member Country, was included to align with text to be proposed for adoption in Article 1.6.3 of the *Terrestrial Animal Health Code*. This new Article 1.4.4 clarifies the self-declaration of disease freedom process, information that should be included in self-declarations and consequences of an outbreak in a self-declared free country, zone or compartment.

Information addressed in the current Article 1.4.6, Pathways to demonstrate freedom from disease, was expanded and included in several articles and includes criteria and approaches set out in the earlier discussion paper sent to Members (e.g. guidance on surveillance to achieve disease freedom). These new articles are cross referenced from Article 1.4.3 in the revised chapter.

The Commission noted that new and revised articles on surveillance system requirements are included in the revised chapter. These requirements are cross-referenced from Article 1.4.5. Additionally, new articles are included on requirements for passive surveillance, and on how the required periods for basic biosecurity conditions and for targeted surveillance specified in each of the disease-specific chapters are to be determined.

The generic information on surveillance and examples of surveillance systems in the current Chapter 1.4 were considerably shortened or removed.

The Commission informed Members that the references included at the end of the current Chapter 1.4 for further reading have been removed to align with the approach of other chapters in the *Aquatic Code*. However the Commission recognised that these references provide additional guidance to Members for the development of surveillance systems and requested that these references be made available on the aquatic portal of the renovated OIE website.

The Commission agreed that given the extensive amendments made to this chapter only a clean version of the revised chapter will be provided for Member comments.

The revised Chapter 1.4, Aquatic animal health surveillance, is presented as **Annex 3** for Member comments.

EU comment

The EU in general supports the proposed changes to this chapter.

Comments are inserted in the text of Annex 3.

1.2.2. Model Articles X.X.4 to X.X.8 for disease-specific chapters to address declaration of freedom from [Pathogen X]

Article X.X.4. Requirements for declaration of freedom from infection with [PATHOGEN X]

In response to requests that additional details on requirements to demonstrate freedom be included in the model articles, the Commission highlighted that in most cases the information requested has been included in the amended Chapter 1.4 (see Item 1.2.1). Chapter 1.4 has also been referenced in the model articles.

Article X.X.5. Country free from infection with [PATHOGEN X]

In response to a number of comments on the first paragraph, the Commission agreed to revise the wording related to shared waterbodies. The current text requires that waterbodies shared between countries cannot have a different disease status in each country. The Commission agreed that in practice, this means that countries need to collaborate and coordinate country self-declarations of disease freedom (and for zones that include the shared waterbody). The Commission amended the text to better reflect this reality.

Regarding point 1 of Articles X.X.5 and X.X.6 for declaration of disease freedom that are based on the absence of susceptible species, some Members requested that these should require that a Member provides evidence of both the absence of the susceptible species as well as the likelihood of introduction. The Commission explained that these requirements are now included in the amended Chapter 1.4 (see Item 1.2.1).

A Member presented an argument that the standard of evidence to return to freedom after an outbreak should be the same as for the original declaration of country freedom, and as such should require the same period of targeted surveillance. The Commission explained that a similar standard of evidence for a geographically defined outbreak under certain circumstances could be provided in a reduced time frame, e.g. if the outbreak is restricted to a few closed systems. The Commission noted that this approach was consistent with its aim to have a more outcome focused approach, which is reflected in the revised requirements for declaration of freedom.

The Commission concurred with a comment that a country that has lost its disease free status will have to respond to the outbreak by creating geographically defined infected and protection zones, and further noted that following detection of an introduced pathogenic agent, surveillance is required to define infected and protection zones. The level of surveillance will be determined through contact tracing and other circumstances surrounding the outbreak. Once all infected aquaculture establishments have been detected and infected and protection zones established, other parts of the country could be considered to have regained their pathogen free status. The Commission considered that this issue is addressed by the Glossary definition of 'zone', the requirements of Article X.X.6, and the guidance provided in the revised Chapter 1.4.

In point 4 b) of Articles X.X.5 and X.X.6 and point 2 a) in Article X.X.7, the Commission reminded Members that the Glossary definition of 'disinfection' includes cleaning, and so it was not necessary to add 'cleaning' where the defined term for 'disinfection' is used.

In point 4) of Articles X.X.5 and X.X.6, the Commission agreed with a comment to include 'fallowing' as a procedure to be undertaken on farms following culling and disinfection for 'freedom to be re-established'. However, the Commission did not agree that these articles should stipulate that countries or zones seeking to re-establish freedom can only source aquatic animals from an approved pathogen free establishment. Relevant guidance is provided in Article X.X.8 in disease-specific chapters that clearly sets out the conditions under which stock can be imported for aquaculture from a source not approved free of the relevant disease(s) if freedom is to be regained. However, the Commission considered that compartments seeking to regain a disease free status should source stock from an approved pathogen free facility.

In response to a comment that 'conditions conducive to clinical expression' should include both environmental and host factors as necessary for the expression of clinical disease. The Commission agreed to address this point but to do so in the amended Chapter 1.4 (see Item 1.2.1).

Article X.X.6. Zone free from infection with [PATHOGEN X]

In point 2 b), the Commission did not agree with a request that 'vaccination has not been practised' be included to claim freedom, noting that this requirement has been included in the revised Chapter 1.4.

A Member commented that the historical freedom pathway should only be applicable if there are species present that would be expected to develop clinical signs, in addition to conditions that are conducive to clinical expression of the disease. The Commission noted that "conditions conducive to clinical expression of disease" is intended to include all host and environmental factors that would lead to clinical expression of the disease. The Commission noted that this has been addressed in the revision to Chapter 1.4, and agreed to include a reference to Article 1.4.8 of the revised Chapter 1.4 to address this issue.

In response to comments regarding the surveillance required to re-establish freedom, the Commission agreed to include relevant text in the revised Chapter 1.4 (see Item 1.2.1).

Article X.X.7. Compartment free from infection with [PATHOGEN X]

In point 2 c), the Commission did not agree with a comment suggesting deletion of the requirement for targeted surveillance if the compartment is epidemiologically isolated. The Commission noted that epidemiological isolation is a pre-requisite for the establishment of a compartment. In addition, the Commission considered that, if a disease has occurred within a compartment, testing is required to demonstrate that elimination of the pathogenic agent has been successful, before the disease-free status can be restored.

The Commission noted that the new Article X.X.7 point 2 b) refers to Articles X.X.9 and X.X.10 and reminded Members that these are the current Articles X.X.7 and X.X.8.

Article X.X.8. Maintenance of free status

In the second paragraph, the Commission agreed with a comment that guidance on continued surveillance to maintain disease freedom in zones was unclear and amended text to clarify that targeted surveillance cannot be discontinued in zones.

The revised model articles X.X.4 to X.X.8 for disease-specific chapters to address declaration of freedom from [Pathogen X] are presented as **Annex 4** for Member comments.

EU comment

The EU thanks the OIE and in general supports the proposed changes to this chapter.

One comment is inserted in the text of Annex 4.

1.3. New draft chapters on Emergency disease preparedness and Disease outbreak management

Background

At its September 2020 meeting, the Aquatic Animals Commission continued its work to develop the article structure for two new chapters of Section 4, Chapter 4.X, Emergency disease preparedness, and Chapter 4.Y, Disease outbreak management.

Previous Commission reports where this item was discussed:

February 2020 (Item 7.3.2, page 16), September 2020 (Item 6.1, page 16).

February 2021 meeting

The Commission further developed the article structure of the new draft Chapter 4.X, Emergency disease preparedness, and Chapter 4.Y, Disease outbreak management. The Commission requested an *ad hoc* Group to be convened to develop the specific text for these two chapters.

Given the importance of this work to support Members in these critical areas, the Commission agreed to circulate the article structure of the new draft chapters to Members for comments on the proposed structure, before the work to draft the detailed text was started.

CHAPTER 4.X.

EMERGENCY DISEASE PREPAREDNESS

Article 4.X.1. - **Purpose**

Describe a comprehensive emergency management framework to guide emergency *disease* preparedness by the *Competent Authority*.

Article 4.X.2. - **Scope**

Article 4.X.3. - **Introduction**

Article 4.X.4. - **General principles**

Article 4.X.5. - **Risk analysis**

Article 4.X.6. - **Emergency preparedness: authority, capacity and infrastructure**

Article 4.X.7. - **Contingency plan**

Article 4.X.8. - **Information systems**

Article 4.X.9. - **Recovery plan**

CHAPTER 4.Y

DISEASE OUTBREAK MANAGEMENT

Article 4.X.1. - **Purpose**

To provide recommendations to the *Competent Authorities* for the management of the emergency response to the occurrence of *disease outbreak*.

Article 4.X.2. - **Scope**

Article 4.X.3. - **General Principles**

Article 4.X.4. - **Alert and investigation phase**

Article 4.X.5. - **Operation Phase**

Article 4.X.6. - **Stand down phase**

Article 4.X.7. - **Communication**

Article 4.X.8. - **Recovery plan**

1.4. Safe commodities (Article X.X.3 of disease-specific chapters)

Comments were received from Armenia, Australia, Canada, Chinese Taipei, Cuba, New Caledonia, Switzerland, Thailand and the EU.

Background

At its September 2020 meeting, the Aquatic Animals Commission reviewed Article X.X.3 of all disease-specific chapters to address comments that the recommended time and temperature treatments in these articles represented different levels of thermal treatment and that some were not commercially feasible as they would diminish product quality.

The Commission noted that it was difficult to propose a uniform model Article X.X.3 because of differences in time/temperature treatments as well as products in Article X.X.3 between disease-specific chapters. Therefore, the Commission developed an example Article X.X.3 to state more clearly the heat treatment required (i.e. core temperature and time period) to inactivate the pathogenic agent, and an example article to Members to demonstrate the suggested approach. The Commission agreed to present Article 9.8.3 of Chapter 9.8, Infection with white spot syndrome virus, as the example article for Member comments.

Previous Commission reports where this item was discussed:

September 2020 (Item 4.7, page 10).

February 2021 meeting

Example Article 9.8.3.

The Commission noted that several Members supported the proposed amendments.

In point 1a) a comment suggested to delete 'cooked, canned, pasteurised or retorted' because listing these products was confusing given that these terms have a specific meaning in food manufacturing. The Commission did not agree and reiterated its rationale for its approach as noted in its September 2020 report, i.e. 'hermetically sealed' was replaced by 'canned or retorted' to specify more clearly the type of product that has been hermetically sealed. However, the Commission did agree to delete the word 'canned' noting that it is a type of retorted product and was therefore not necessary.

In response to a comment recommending that the Commission review the minimum temperature and time treatment regimens for all pathogenic agents with the latest scientific information, the Commission acknowledged that the assessments conducted for the aquatic animal products currently listed in Article X.X.3 needed to be reviewed given that that additional scientific evidence had likely been published since these assessments were conducted (between 2009 and 2011). It reiterated that this work had been added to its work plan. The Commission emphasised that until that time, existing assessments will continue to be used as the basis for the time/temperature treatments provided in Article X.X.3 of all disease-specific chapters.

In response to a comment requesting evidence that a heat treatment of 121°C for 3.6 minutes for a hermetically sealed product or that 90°C for 10 minutes during pasteurisation, results in a core temperature above 60°C for 1 minute, the Commission reminded Members that the original approach to this article had been to list product types (e.g. hermetically sealed, pasteurised, cooked) and the standard commercial temperature treatments for those product types. This approach had resulted in the apparent lack of equivalence in time/temperature treatments (for example, between pasteurisation and hermetically sealed products) and had also reduced flexibility for different product types to be considered safe even though the treatment applied might exceed the heat treatment required to deactivate the relevant pathogenic agent. The Commission had therefore proposed to amend Article X.X.3 of all disease-specific chapters to state more clearly the heat treatment required (i.e. core temperature and time period) to inactive each specific pathogenic agent rather than include industry standard treatments.

The Commission considered a request regarding availability of equivalence tables or a formula to check the equivalence of different time/treatments to the 60°C for 1 minute included in the Model Article X.X.3, that could be applied to any heat treatment for certification by an exporting country. The Commission noted that

there is a body of scientific literature on the thermal inactivation of microbes that informs methods for calculation of equivalence (for example, see the review by Smelt and Brul, 2014, Critical Reviews in Food Science and Nutrition, 54:10, 1371-1385). Unfortunately, for many aquatic animal pathogens, data are lacking and calculation of equivalence may be problematic.

In response to a comment to include a minimum temperature and time regime for the heat treatment of crustacean meal given that it may be manufactured through a low temperature drying process which may not be sufficient to inactivate WSSV, the Commission agreed to include a specific time/temperature heat treatment for meal in Article X.X.3 in each of the relevant disease-specific chapters. As a result of this amendment, the Commission will review the use of the definition of 'meal' throughout the *Aquatic Code* to determine if the addition of a core time/temperature for meal in Article X.X.3 will require the Glossary definition be amended. This review will be discussed at the Commission's September 2021 meeting.

The Commission did not agree with comments requesting to reinstate point 3, noting that the issue of risk analysis applies to all aspects of the standards, not just to aquatic animal products, and that it is addressed in Articles 5.3.1 and 5.3.2 of Chapter 5.3, OIE procedures relevant to the Agreement on the Application of Sanitary and Phytosanitary Measures of the World Trade Organization.

Application of example article to crustacean disease-specific chapters

At its September 2020 meeting, the Commission agreed that it would circulate the amended Article X.X.3 for each disease-specific chapter, for Member comments, following its review of comments received on the example Article X.X.3. The time/temperature treatments provided in Article X.X.3 of all disease-specific chapters were adjusted in line with the information provided in the 2016 Safe commodity assessments for OIE listed aquatic animal diseases. However, given the complexity of applying these changes to all the disease-specific chapters, the Commission agreed to implement these amendments one section at a time, starting with crustacean disease-specific chapters.

The Commission wished to reiterate that thermal treatments recommended in the revised articles are based on the assessments adopted in 2011 and now available in a consolidated document that was published on the OIE website in 2016 (Safe commodity assessments for OIE listed aquatic animal diseases).

The amended Article X.X.3 for the crustacean disease-specific chapters are presented as **Annex 5** as clean and track changes versions for Member comments.

EU comment

The EU thanks the OIE and supports the proposed changes to Chapter 9.1. to 9.9.

1.5. Articles 11.2.1 and 11.2.2 of Chapter 11.2 Infection with *Bonamia exitiosa*

The Aquatic Animals Commission reviewed the report of the *ad hoc* Group on Susceptibility of mollusc species to infection with OIE listed diseases. The *ad hoc* Group had applied the criteria for listing species as susceptible to infection with a specific pathogenic agent in accordance with Chapter 1.5 of the *Aquatic Code* for infection with *Bonamia exitiosa*.

The Commission amended Article 11.2.1 to ensure consistency with the other amended mollusc disease-specific chapters.

The Commission agreed to amend the list of susceptible species in Article 11.2.2 in line with recommendations made by the *ad hoc* Group. It noted that, in addition to the Australian mud oyster (*Ostrea angasi*) and Chilean flat oyster (*Ostrea chilensis*) currently listed in Article 11.2.2, six new susceptible 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*, and are therefore proposed to be added to Article 11.2.2.

Relevant sections of Chapter 2.4.2, Infection with *Bonamia exitiosa*, in the *Aquatic Manual* were also amended in line with the recommendations of the *ad hoc* Group (see Item 3.2).

The report of the *ad hoc* Group on Susceptibility of mollusc species to infection with OIE listed diseases is presented as **Annex 6** for Members' information.

The revised Articles 11.2.1 and 11.2.2 of Chapter 11.2, Infection with *Bonamia exitiosa*, are presented as **Annex 10** for Member comments.

EU comment

The EU supports the proposed changes to this chapter.

2. THE OIE *AQUATIC ANIMAL HEALTH CODE* - Text for Members' Information

2.1. De-listing of infection with infectious hypodermal and haematopoietic necrosis virus

Comments were received from Armenia, Australia, China (People's Rep. of), Chinese Taipei, Cuba, Korea (Rep. of), Switzerland, the UK, the USA, the EU and Members of the OIE Americas region.

Background

At its February 2020 meeting, the Aquatic Animals Commission considered a request from a Member to remove infection with infectious hypodermal and haematopoietic necrosis virus (IHHNV) from the list of diseases in Article 1.3.3 of Chapter 1.3, Diseases listed by the OIE.

At its September 2020 meeting, the Commission undertook an assessment of infection with IHHNV against the criteria for listing aquatic animal diseases in Article 1.2.2 of Chapter 1.2, Criteria for listing aquatic animal diseases, taking into consideration information provided by Members, relevant publications as well as advice from the OIE Reference Laboratory expert for this disease. The Commission concluded that infection with IHHNV meets the listing criteria and should therefore remain listed in Article 1.3.3.

Previous Commission reports where this item was discussed:

February 2020 (Item 7.3.1, page 16); September 2020 report (Item 4.6, page 10).

February 2021 meeting

The Commission noted the general support by Members to maintain infection with IHHNV as an OIE listed disease in Article 1.3.3. and agreed that it should remain as listed in Article 1.3.3.

The Commission acknowledged comments received on the assessment document and amended these accordingly, noting that none of these amendments influenced the outcome of the assessment.

The Commission reminded Members that should new scientific evidence become available that could affect the outcome of this assessment for listing, the Commission would review its assessment, and encouraged Members to provide any such information for its consideration.

The revised assessment for infection with IHHNV is presented, as a clean version, in **Annex 7** for Members' information.

2.2. Consideration of emerging diseases - Infection with carp edema virus (CEV)

Comments were received from Armenia, Cuba, Japan, New Caledonia and Switzerland.

Background

At its February 2020 meeting, the Aquatic Animals Commission reviewed scientific information on infection with carp edema virus (CEV), given that the disease had been recently reported in several countries in the Asia-Pacific region and appears to be extending its geographic range. Based on available scientific information, the Commission agreed that infection with CEV meets the OIE definition of an 'emerging disease'.

The Commission agreed that it would continue to monitor the situation and encouraged Members to investigate mortality and morbidity events in carp, emphasising that a better understanding of the virus is essential for efforts to control its possible spread. Members were reminded that detections of infection with CEV should be reported to the OIE as an emerging disease in accordance with Article 1.1.4 of the *Aquatic Code*.

Previous Commission reports where this item was discussed:

February 2020 report (Item 7.3.3, page 17); September 2020 (Item 6.3, page 17).

February 2021 meeting

The Commission was asked to justify why it regarded infection with CEV as meeting the definition of an emerging disease despite reports of low mortalities and low virulence from some countries. The Commission informed Members that it had based its conclusion on scientific evidence and have provided a list of references used in **Annex 8**.

The Commission noted that it had also considered that infection with CEV has spread from the Asia-Pacific region to many European countries and has caused mortalities in common carp and koi carp. While the mortality caused by infection with CEV in New Caledonia has demonstrated the virulence of CEV to koi carp, the spread of infection with CEV and mortalities caused by infection with CEV in many common and koi carp farms in China (People's Rep. of) has supported that this virus can have significant impacts.

The Commission agreed that the decrease in mortality rates in some countries was likely to be the result of successful mitigation measures.

The Commission reviewed the latest scientific evidence and agreed that infection with CEV should be considered an emerging disease in accordance with Article 1.1.4 of the *Aquatic Code* and noted that it will continue to review new scientific evidence.

The references considered for notifying infection with CEV as an emerging disease are provided in **Annex 8** for Members' information.

3. THE OIE *MANUAL OF DIAGNOSTIC TESTS FOR AQUATIC ANIMALS* - Texts for Member comments

3.1. The use of environmental DNA methods for aquatic animal disease surveillance

Background

The monitoring of aquatic systems using environmental DNA (eDNA) is a rapidly advancing research field that will provide opportunities for rapid, cost-effective, non-destructive methods to screen for pathogens, especially in wild aquatic populations where sampling may be difficult or removal of animals undesirable. The Aquatic Animals Commission is aware that eDNA methods exist for detecting pathogenic agents of

several listed diseases, including *Xenohaliotis californiensis*, *Batrachochytrium dendrobatidis*, *Aphanomyces astaci* and *Gyrodactylus salaris*.

The Commission agreed that as these methods are available and currently in use, it would be advisable for guidance to be provided on appropriate application and potential limitations. The Commission noted that as accurate estimates of diagnostic performance are not available for designing surveillance programmes using eDNA assays, data obtained from eDNA methods may not be suitable to support declaration of freedom from listed diseases. The Commission also noted that confirmation of infection by listed diseases could not be made using eDNA methods; however, positive results could be appropriate criteria for a suspect case.

The Commission agreed to develop a guidance document to outline considerations for the appropriate purposes of use, benefits and limitations of eDNA methods. The use of an eDNA method for the detection of *G. salaris* is proposed for inclusion in the *Aquatic Manual* chapter for Infection with *G. salaris*.

The Commission prioritised other agenda items at the September 2020 meeting and decided to work on the discussion paper on guidance for the use of environmental DNA methods for aquatic animal disease surveillance at its February 2021 meeting

Previous Commission reports where this item was discussed: February 2020 (Item 8.4.2, page 22), September 2020 (Item 6.4, page 17)

February 2021 meeting

The Commission has developed a discussion document outlining the benefits and limitations of eDNA detection within a diagnostic or disease surveillance context. This document is intended to guide the appropriate purposes of use and assay performance reporting required for an eDNA assay to be considered for inclusion in the *Aquatic Manual*.

The guidance document for the use of environmental DNA methods for aquatic animal disease surveillance is presented as **Annex 9** for Member comments.

EU comment

We thank the OIE AAHSC for this very useful paper which explores the potential use of eDNA methods with respect to the standards of the Code and the Manual and which outlines their benefits and limitations. Each of the conclusions which are listed in Point 11, have been explored in the paper, are concise and well founded, and can therefore be fully supported by the EU.

3.2. Sections 2.2.1 and 2.2.2 of Chapter 2.4.2 Infection with *Bonamia exitiosa*

The Aquatic Animals Commission amended Sections 2.2.1 and 2.2.2 of Chapter 2.4.2, Infection with *Bonamia exitiosa*, in line with the recommendations of the *ad hoc* Group on Susceptibility of mollusc species to infection with OIE listed diseases, as described in Item 1.5.

The report of the *ad hoc* Group is presented as **Annex 6** for Member's information.

The amended Sections 2.2.1 and 2.2.2 of Chapter 2.4.3, Infection with *Bonamia exitiosa*, are presented as **Annex 10** for Member comments.

EU comment

The EU supports the proposed changes to this chapter.

4. *AD HOC* GROUP REPORT AND OTHER DOCUMENTS FOR INFORMATION

4.1. Status on the *ad hoc* Group on Susceptibility of mollusc species to infection with OIE listed diseases

The *ad hoc* Group on Susceptibility of mollusc species to infection with OIE listed diseases has met twice and finalized reports for susceptibility of mollusc species to infection with *B.ostreae* and *B. exitiosa*. The *ad hoc* Group is planning two meetings in 2021 to continue its work assessing species susceptible to listed OIE mollusc diseases.

The report of the *ad hoc* Group on Susceptibility of mollusc species to infection with OIE listed diseases is presented as **Annex 6** for Members' information.

4.2. *Ad hoc* Group on New draft chapters on emergency disease preparedness and disease outbreak management

The Aquatic Animals Commission agreed that a new *ad hoc* Group be convened to commence work on developing the two new chapters on emergency disease preparedness and disease outbreak management based on the article structure developed by the Commission. This new *ad hoc* Group is anticipated to commence work in 2021.

5. OTHER ISSUES

5.1. Endorsement of updated SOP for OIE Register of diagnostic kits

The Secretariat for Registration of Diagnostic Kits (OIE SRDK) had introduced changes to the *Standard Operating Procedure* (*SOPs*) *for OIE Registration of Diagnostic Kits* and the *Application Form for the Certification of Diagnostic Kits validated as fit for specific purposes* (*Application Form*) after consultation with OIE Collaborating Centres and the industry association for animal diagnostic kits.

These changes aimed to bring the guidance in these documents up to date with the application of the current procedure, recognising that a more thorough update of the SOP may need to be scheduled in the future. The proposed changes to the SOPs concerned principally the addition of information regarding provisional recognition, and the allowed timeframe for applicants to prepare responses to the Review Panel's questions. The proposed changes to the Application Form related principally to the addition of information to the instructions provided to applicants in Sections 2, 3 and 4 to assist applicants in preparing their response, the addition of more detailed references to the OIE *Terrestrial Manual* and *Aquatic Manual*, and changes to the question on the intended purpose of test (Section 2.2.3).

The Aquatic Animals Commission agreed with the proposal and, as the amendments had also been endorsed by the Biological Standards Commission, agreed that the revised SOP should be posted on the OIE website to replace the current version, so all applicants will be fully informed of the new procedure.

https://www.oie.int/en/scientific-expertise/registration-of-diagnostic-kits/procedure-for-submission/

https://www.oie.int/en/scientific-expertise/registration-of-diagnostic-kits/download-application-form/

The amended documents are also annexed to the report of the Biological Standards Commission's February 2021 meeting.

6. OIE REFERENCE CENTRES OR CHANGE OF EXPERTS

6.1. Evaluation of applications for OIE Reference Centres for Aquatic Animal Health issues or change of experts

The Aquatic Animals Commission reviewed an application for an OIE Collaborating Centre for Economics of Animal Health. The Commission was impressed with this strong application, which is linked to the OIE-

led project on the Global Burden of Animal Disease (GBADs). The Commission was pleased that aquatics was one of the central targeted areas of activity. The Commission fully endorsed the application and recommended its acceptance:

OIE *Collaborating Centre for Economics of Animal Health*

University of Liverpool, Centre of Excellence for Sustainable Food Systems, Global Burden of Animal Diseases Programme, Institute of Infection, Veterinary and Ecological Sciences, Liverpool, UNITED KINGDOM
Tel.: (+44-151) 794.61.13
E-mail: j.rushton@liverpool.ac.uk
Web site: www.liverpool.ac.uk
Designated Contact Point: Prof. Jonathan Rushton.

This multi-national OIE Collaborating Centre will include participation from the following institutions:

Norwegian Veterinary Institute, P.O. Box 750 Sentrum, 0106 Oslo, NORWAY
Tel: (+47-91) 61.85.87
E-mail: edgar.brun@vetinst.no
Web site: www.vetinst.no
Designated Contact Point: Dr Edgar Brun.

Utrecht University, Department of Population Health Services, Utrecht, NETHERLANDS
Tel.: (+31-30) 253.10.91
E-mail: j.a.stegeman@uu.nl
Web site: www.uu.nl
Designated Contact Point: Prof. Arjan Stegeman.

An OIE Reference Laboratory had informed the Commission that it had undergone a restructuring and reorganisation within its governing body and facilities. The laboratory had submitted information on its new organisation. The Commission was satisfied that the facilities continued to meet the standards expected of an OIE Reference Laboratory.

6.2. Evaluation of annual reports from the OIE Reference Centres

Annual reports had been received from all OIE Reference Laboratories for diseases of aquatic animals and all Collaborating Centres for aquatic animal issues.

In accordance with the adopted *Procedures for designation of OIE Reference Laboratories* (the SOPs) (http://www.oie.int/en/scientific-expertise/reference-laboratories/sops/) and the *Procedures for designation of OIE Collaborating Centres* http://www.oie.int/en/scientific-expertise/collaborating-centres/sops/, the Aquatic Animals Commission reviewed all the reports received, noting in particular the performance of each Reference Centre with regard to fulfilling the Terms of Reference (ToR) to the benefit of OIE Members.

The Commission noted the significant contributions that had been made by Reference Laboratories during 2020 despite the difficulty situation posed by the Covid-19 pandemic, and wished to thank designated experts for leading these valuable contributions to the OIE mission.

Two Reference Laboratories that reported very little activity would be requested to provide an explanation of their situation and possible reasons for the lack of activity. The Commission expressed its on-going appreciation for the enthusiastic support and expert advice given to the OIE by the Reference Centres.

6.3. Twinning projects

As of February 2021, 66 projects have been completed, 29 projects are underway and 11 are awaiting funding before beginning.

One Laboratory Twinning project proposal was presented for the Aquatic Animals Commission's review:

- ***United States of America – Colombia*** for shrimp and fish diseases with emphasises on pathology, isolation and diagnosis of: acute hepatopancreatic necrosis disease, infection with *Hepatobacter penaei* (necrotising hepatopancreatitis), infection with *Enterocytozoon hepatopenaei*, infection with Tilapia lake virus and infectious spleen and kidney necrosis virus. The Commission supported the technical contents of this project.

7. NEXT MEETING

To be confirmed.

MEETING OF THE OIE
AQUATIC ANIMAL HEALTH STANDARDS COMMISSION

Paris, 17–24 February 2021

List of participants

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GLOSSARY

EU comment

The EU in general supports the proposed changes to the Glossary.

One comment is inserted in the text below.

BASIC BIOSECURITY CONDITIONS

means a minimum set of conditions, as described in Article 1.4.6., required to ensure *biosecurity* for a particular *disease*, in a country, *zone* or *compartment*, ~~that should include:~~

~~a)~~ ~~compulsory notification of the disease or suspicion of the disease to the Competent Authority; and~~

~~b)~~ ~~an early detection system; and~~

~~c)~~ ~~requirements to prevent the introduction of the pathogenic agent into a free country, zone or compartment, or the spread within or from infected zones and protection zones, in accordance with the relevant disease-specific chapter.~~

EARLY DETECTION SYSTEM

means an efficient system, as described in Article 1.4.7., for ensuring the rapid recognition of signs that are suspicious of a *listed disease*, or an *emerging disease* ~~situation~~, or unexplained mortality, in *aquatic animals* in an *aquaculture establishment* or in the wild, and the rapid communication of the event to the *Competent Authority*, with the aim of activating diagnostic investigation by the *Aquatic Animal Health Services* with minimal delay. ~~Such a system will include the following characteristics:~~

~~a)~~ ~~broad awareness, e.g. among the personnel employed at *aquaculture establishments* or involved in processing, of the characteristic signs of the *listed diseases* and *emerging diseases*;~~

~~b)~~ ~~*veterinarians* or *aquatic animal health professionals* trained in recognising and reporting suspicions of *disease* occurrence;~~

~~c)~~ ~~ability of the *Aquatic Animal Health Services* to undertake rapid and effective *disease* investigation based on a national chain of command;~~

~~d)~~ ~~access by the *Aquatic Animal Health Services* to laboratories with the facilities for diagnosing and differentiating *listed diseases* and *emerging diseases*;~~

~~e)~~ ~~the legal obligation of private *veterinarians* or *aquatic animal health professionals* to report suspicions of *disease* occurrence to the *Competent Authority*.~~

EU comment

For reasons of clarity, the EU suggests a slight rewording of the first sentence of the definition above, as follows:

"means an efficient system, as described in Article 1.4.7., ~~for ensuring~~ which ensures the rapid recognition of signs that ~~are suspicious of~~ create suspicion of the presence of a listed disease, or an emerging disease [...]".

PASSIVE SURVEILLANCE

means the generation of observer-initiated *aquatic animal* health data by an *early detection system.*

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CHAPTER 1.4.

AQUATIC ANIMAL DISEASE SURVEILLANCE

EU comment

The EU in general supports the proposed changes to this chapter.

Comments are inserted in the text below.

Article 1.4.1.

Purpose

This chapter provides guidance on the *surveillance* approaches to be used by a *Competent Authority* to make a *self-declaration of freedom from disease* or to confirm the occurrence of a *listed disease* or an *emerging disease*.

Article 1.4.2.

Introduction and scope

This chapter supports the *Competent Authority* to meet the requirements for *self-declaration of freedom from disease* at the level of a country, *zone* or *compartment*, and for maintenance of freedom, that are presented in each *disease*-specific chapter. It also provides the *Competent Authority* with guidance to meet the requirements of *notification* of a *listed disease* or an *emerging disease* in accordance with Chapter 1.1.

This chapter is not intended to provide detailed technical guidance on *surveillance* design or analysis. The *Competent Authority* is encouraged to consult published literature and seek appropriate expertise to design and analyse *surveillance* programmes that meet the requirements of the *Aquatic Code*.

1) The general requirements of a *surveillance* system necessary to support a *self-declaration of freedom from disease* are specified in Article 1.4.5. to Article 1.4.8. .

2) The criteria that have been used to set the periods specified in each *disease*-specific chapter for *basic biosecurity conditions* to be in place, or for *targeted surveillance* that should be undertaken, prior to claiming freedom, are included in EU comment

The EU suggests adding the words "<u>in a timely fashion and in accordance with a protocol which has been designed for that purpose</u>" at the end of point (6) above, similar to the EU comment above (see Article 1.4.5.5.).

Article 1.4.9. and 1.4.10.

3) The requirements for each of the four pathways for claiming freedom, and for maintaining freedom, are described in EU comment

The EU suggests inserting the words "<u>using a suitable sample size, and under conditions including water temperature, which are conducive to the clinical expression of the disease</u>" after "round of testing" in the last sentence of the paragraph above, as these elements are relevant in this contaxt.

Article 1.4.11. to EU comment

There is an error in the first sentence of point (3) above. It should be re-worded as follows: "Once the infected populations have been depopulated and affected aquaculture

establishments disinfected, ~~and fallowed~~ as described in Chapter 4.3 and fallowed as described in Chapter 4.6, [...]".

Article 1.4.15.

4) Guidance on the design of surveys to demonstrate freedom from *disease*, and for combining multiple sources of *surveillance* information are provided in Article 1.4.16. and EU comment

In the first column of Table 1.2. above, please insert "(%)" after "design prevalence".

Article 1.4.17., respectively.

5) Article 1.4.18. provides guidance on diagnostic confirmation of *listed diseases* or an *emerging disease*.

The *Competent Authority* should refer to the relevant *disease*-specific chapter of the *Aquatic Manual* for recommendations on sample collection and appropriate diagnostic methods for *surveillance* and diagnosis of *listed diseases*. The relevant *disease*-specific chapter of the *Aquatic Manual* should also be consulted for the necessary information on epidemiology and diagnostic performance of assays required for *surveillance* programme design.

Article 1.4.3.

Pathways for demonstrating freedom from disease

The *Competent Authority* may use one of four pathways to make a *self-declaration of freedom from disease*. Each pathway outlines the *aquatic animal* health circumstances and requirements that should be met for a self-declaration to be made. Any one of these four pathways may be utilised; however, the *Competent Authority* should provide evidence that all relevant requirements to demonstrate *disease* freedom have been met as described in this chapter and the relevant *disease*-specific chapter of the *Aquatic Code*. The four pathways are:

1. Absence of susceptible species

   This pathway may be utilised if, as described in EU comment

The EU suggests inserting the words "using a suitable sample size, and under conditions including water temperature, which are conducive to the clinical expression of the disease" after "round of testing" in the last sentence of the paragraph above, as these elements are relevant in this contaxt.

   Article 1.4.11., it can be demonstrated that no *susceptible species* are present.

2. Historical freedom

   This pathway may be utilised if, as described in Article 1.4.12., there is evidence of historical absence of a *disease* that is supported primarily by *passive surveillance* data generated by a country's *early detection system*.

3. Surveillance

   This pathway may be utilised if the requirements of pathway 1 (absence of *susceptible species*) or pathway 2 (historical freedom) cannot be met. The pathway primarily uses targeted *surveillance* data, but other sources of evidence may be utilised as described in Article 1.4.13.

4. Returning to freedom

   This pathway may be utilised, as described in Article 1.4.14., in circumstances where a self-declaration had been made, but free status was subsequently lost due to detection of the *disease*.

Table 1.1. A summary of the four pathways for *self-declaration of freedom from disease*, including the types of primary and secondary *surveillance* information, and the applicable level of application for either a country, *zone* or *compartment*.

Pathway	**Primary surveillance evidence to claim disease freedom**	**Proposed secondary evidence to claim freedom (if required)**	**Applicable level of application**
1. Absence of susceptible species	*Active surveillance*	None	Country, *zone*
2. Historical freedom	*Passive surveillance*	*Targeted surveillance* (in populations where *passive surveillance* is not appropriate)	Country, *zone*
3. Surveillance	*Targeted surveillance*	*Passive surveillance* (in appropriate populations)	Country, *zone*, *compartment*
4. Returning to freedom	*Targeted surveillance*	*Passive surveillance* (in appropriate populations)	Country, *zone*, *compartment*

EU comment

For self-declaration of freedom based on the absence of susceptible species, the supporting documentary evidence which is referred to in Article 1.4.11., is required. It would therefore, make sense to refer to that evidence in Table 1.1. above, in reference to Pathway 1.

Article 1.4.4.

Publication by the OIE of a self-declaration of freedom from disease by a Member Country

A Member Country may make a *self-declaration of freedom from disease* in a country, *zone* or *compartment*. The Member Country may inform the OIE of the claimed status and the OIE may publish the self-declaration.

A Member Country requesting the publication of a self-declaration should follow the Standard Operating Procedure (under development) for submission and provide documented information on its compliance with the relevant chapters of the *Aquatic Code.* This information should include, but is not limited to the following:

EU comment

The EU does not support referring to Standard Operating Procedures in OIE standards. Indeed, it is not established practice in OIE standards to refer to external guidance documents. This is neither necessary nor appropriate, especially when these documents are not yet available and are not part of the standard setting process (i.e. they are not submitted for comments to members nor for adoption by the World Assembly).

1) the scope of the declaration, i.e. the specific *disease*, the level of freedom (country, *zone* or *compartment*) and the pathway utilised to claim freedom;

2) information to confirm that the general requirements of *biosecurity* and *surveillance* systems have been met;

3) details of the *surveillance* design and assumptions;

4) the *surveillance* analysis and results;

5) the measures implemented to maintain freedom.

The *self-declaration of freedom from disease* may be published only after all the information provided has been received and administrative and technical screening has been performed by the OIE. Publication does not imply endorsement of the claim of freedom by the OIE and does not reflect the official opinion of the OIE. Responsibility for the accuracy of the information contained in a self-declaration lies entirely with the OIE Delegate of the Member Country concerned.

EU comment

The documented information, which must be submitted by a Member Country which is making a self-declaration of freedom, should include information on the geographical scope of the zone or compartment concerned. In particular, the documentary evidence should demonstrate that the zone or compartment in question, complies with the relevant definition in the Glossary, and that it demonstrates adequate disease-specific separation from the surrounding waters. This should preferably be specified in the paragraph above, or in a separate new paragraph in this article.

Except when otherwise provided for in the *disease*-specific chapter, an *outbreak* in a Member Country, a *zone* or a *compartment* having a self-declared free status results in the loss of the self-declared free status. A Member Country wishing to reclaim a lost free status should submit a new self-declaration following the procedure described in this chapter.

Article 1.4.5.

Biosecurity and surveillance system requirements

The following *surveillance* system requirements should be met for any *self-declaration of freedom from disease.*

1) the quality of *Aquatic Animal Health Services* can be substantiated to meet the requirements of Chapter 3.1.:

2) *basic biosecurity conditions* as described in Article 1.4.6. are in place;

3) an *early detection system* as described in Article 1.4.7. is in place;

4) there has been no vaccination of *susceptible aquatic animals* for the specific *disease* for at least the period that *basic biosecurity conditions* have been applied prior to self-declaration;

5) the *Aquatic Animal Health Services* have sufficient capacity to investigate and report *disease* events to the *Competent Authority*;

EU comment

The EU suggests inserting the words "<u>and expertise</u>" after "capacity" in point (5) above, as this is also needed for disease investigation.

6) the *Competent Authority* has access to appropriate diagnostic capability to confirm or exclude cases of *listed diseases* and *emerging diseases* in accordance with Article 1.4.18.

Article 1.4.6.

Basic biosecurity conditions

Basic biosecurity conditions include requirements for preventing the introduction and spread of a specific *disease* and for detection of the *disease* should it occur. The requirements for *basic biosecurity conditions* include:

1) a compulsory requirement for *notification* of a specific *disease*, or suspicion of the *disease*, to the *Competent Authority*;

2) an *early detection system* (as described in Article 1.4.7.);

3) measures to prevent the introduction of the *pathogenic agent* into a country, *zone* or *compartment*, or the spread within or from *infected zones* and *protection zones*, in accordance with the relevant *disease*-specific chapter.

In making a *self-declaration of freedom from disease* for a country, *zone* or *compartment,* the C*ompetent Authority* should describe the *basic biosecurity conditions* relevant to its declaration, and ensure all requirements for *basic biosecurity conditions* described in this chapter are met.

Article 1.4.7.

Early detection system

The *early detection system* of the *Competent Authority* underpins any *passive surveillance* data utilised by a *Competent Authority* to make a *self-declaration of freedom from disease*.

A *self-declaration of freedom from disease* needs to document that the *early detection system* fulfils each of the five characteristics below:

1) broad awareness, e.g. among the personnel employed at *aquaculture establishments* or involved in processing, of the characteristic signs of *listed diseases* and *emerging diseases*;

EU comment

The EU suggests inserting the words "<u>, transportation or the provision of other services</u>" after "processing" in point (1) above, as these personnel should also be included here.

2) *veterinarians* and *aquatic animal health professionals* are trained in recognising and reporting suspicion of *disease* occurrence;

3) the *Aquatic Animal Health Services* have capacity to undertake rapid and effective *disease* investigation based on a national chain of command;

4) the *Aquatic Animal Health Services* have access to sufficient diagnostic capability to confirm or exclude cases of *listed diseases* and *emerging diseases* as described in Article 1.4.18.;

EU comment

The EU suggests inserting the words "<u>and expertise</u>" after "capability" in point (4) above, similar to the EU comment above (see Article 1.4.5.5.).

5) *veterinarians* and *aquatic animal health professionals* have a legal obligation to report suspicions of *disease* occurrence to the *Competent Authority*.

EU comment

We suggest including reference to farmers' legal obligation to report suspicion in point (5) above. This is referred to in the following paragraph, but Point (5) and the following paragraph should be reflective of each other.

The sensitivity of an *early detection system* is the likelihood that the *disease* will be detected if present. Of fundamental importance is *disease* reporting by farmers to initiate the necessary steps of *passive surveillance*. Specifically, the

Competent Authority should be able to demonstrate that efforts have been made to make farmers aware of signs of *listed diseases* and *emerging diseases*, and secondly the obligation of farmers, *aquatic animal health professionals* and others to report suspicion. The underpinning legal instruments should be cited.

The capacity of the *Aquatic Animal Health Services* to respond to suspicion of a *listed disease* can be evidenced by response plans, and a descriptive chain of command that will result in an official declaration that the *pathogenic agent* has been detected. Standard operating procedures for diagnostic assays for *listed diseases* and accreditation to internationally recognised laboratory standards can demonstrate the capacity of the *Aquatic Animal Health Services* to detect *listed diseases*. In addition, the effective function of the *early detection system* is best illustrated through examples of investigations in response to reported suspicion of *disease*. Ideally, the sensitivity of an *early detection system* (i.e. the likelihood of *pathogenic agent* detection following introduction) should be quantified, for example, by use of a scenario tree model.

Article 1.4.8.

Requirements for passive surveillance

1) In addition to the characteristics of an *early detection system* described in Article 1.4.7., the conditions described in this article should be met for *passive surveillance* data to be utilised for a *self-declaration of freedom from disease.* The conditions, which apply to each defined *study population* of *susceptible species* of a specific *disease*, are that:

   a) conditions (biotic and abiotic) are conducive to clinical expression of the *infection*, such that if the *pathogenic agent* were present within the population of *susceptible species*, it would produce clinical signs of the *disease*;

   b) there should be sufficient awareness by potential observers of the *study population*, such that observation of clinical signs of the *disease*, which may include increased mortality, would lead to reporting;

   c) populations of susceptible farmed *aquatic animals* should be under sufficient observation in all relevant production systems, such that, if clinical signs of the *disease* were to occur, they would be observed;

EU comment

The EU suggests inserting the words "<u>and during transportation</u>" after "production systems" in point (c) above, similar to the EU comment above (see Article 1.4.5.5.).

   d) for populations of susceptible wild *aquatic animals*, they should:

      i) be under sufficient observation, such that if clinical signs of the *disease* were to occur, they would be observed and reported, or

      ii) be epidemiologically linked to farmed populations, such that the *disease* would occur and be observed and reported in farmed populations if it were to occur in adjacent wild *aquatic animal* populations.

2) *Passive surveillance* depends primarily on observers (e.g. farmers, *aquatic animal health professionals*) reporting suspicion of *disease* and unexplained increased mortality to the *Competent Authority*. For wild populations, the requirements of point 4 a) above are unlikely to be met under most circumstances and, therefore, *passive surveillance* will be insufficiently sensitive. If a *Competent Authority* utilises *passive surveillance* data for defined populations of wild *aquatic animals*, it should demonstrate that the conditions of this article have been met, and that the *early detection system* provides appropriate *sensitivity* for detection of the *disease* should it occur.

EU comment

It seems that the reference to "point 4(a) above" in point (2) above is an error. The reference should be to "<u>point (d)(i) above</u>" instead.

3) Awareness of clinical signs of *disease* and the necessary level of observation is best demonstrated through examples of reporting by farmers, *aquatic animal health professionals* and others to the *Competent Authority*. In addition to reporting, information for *passive surveillance* may originate from inspections at processing plants,

routine visits by government officials and surveys (e.g. of wild populations), submissions to laboratories, *aquaculture establishment* records (e.g. mortality, medicine use, etc.).

4) *Passive surveillance* is only effective if conditions are conducive to clinical expressions of *disease*, which include:

   a) environmental conditions (e.g. water temperatures) being permissive for the development of clinical signs during at least a period of the year; and

   b) the presence of *susceptible species* in which *infection* results in clinical signs.

5) Evidence from published literature will generally be sufficient to demonstrate the environmental conditions over which clinical signs appear, and in which *infection* of *susceptible species* will result in clinical signs. This information should be supplemented with data on the environmental conditions for the *target populations*.

6) *Passive surveillance* only contributes to the *early detection system* if investigations by the *Competent Authority* follow reports of *disease*.

EU comment

The EU suggests adding the words "<u>in a timely fashion and in accordance with a protocol which has been designed for that purpose</u>" at the end of point (6) above, similar to the EU comment above (see Article 1.4.5.5.).

Article 1.4.9.

Required periods for basic biosecurity conditions

1) Prior to a Member Country making a *self-declaration of freedom from disease*, *basic biosecurity conditions* should be in place for a defined period. *Basic biosecurity conditions* should be applied for sufficient duration prior to a self-declaration*,* so that, by the end of the period, should the *disease* have been introduced before the *basic biosecurity conditions* began:

   a) no *pathogenic agent* would remain present in the environment (see pathway 1 – absence of *susceptible species*),

   b) the *disease* would manifest clinically and be detected by the country's *early detection system* (see pathway 2 – historical freedom), and

   c) by the time targeted *surveillance* commenced (see pathway 3 – *surveillance*), *infection* levels would have reached the minimum *prevalence* estimate (i.e. the design *prevalence*) used in the survey design to calculate the sample sizes (e.g. of *aquaculture establishments* and *aquatic animals* needed to demonstrate freedom).

2) Each *disease*-specific chapter of the *Aquatic Code* includes minimum periods that *basic biosecurity conditions* should be in place prior to a *self-declaration of freedom from disease*. These periods are determined based on the factors described below.

   a) For pathway 1, the default minimum period that *basic biosecurity conditions* should be in place prior to a *self-declaration of freedom from disease* is six months. It is expected that this period will be sufficient for most *diseases* to ensure that no viable *pathogenic agent* introduced via *aquatic animal* commodities has remained present in the environment, and the *early detection system* was well established and demonstrated to be functioning. The required period that *basic biosecurity conditions* should be in place prior to making a self-declaration*,* using this pathway, is determined for each *pathogenic agent* based on its epidemiology (e.g. agent stability in the environment, presence of resistant life stages, *vectors*), and is specified in the relevant *disease*-specific chapter of the *Aquatic Code*.

   b) For pathway 2, the default minimum period that *basic biosecurity conditions* should be in place prior to a self-declaration, for all *listed diseases,* is ten years. This period is the minimum required to achieve 95% likelihood of freedom, if the annual likelihood of detection is 30%. However, if the average annual likelihood of detection by a country's *early detection system* is considered to be less than 30% in the period preceding declaration

(following consideration of the factors below), the minimum period required for *basic biosecurity conditions* defined in the relevant *disease*-specific chapter of the *Aquatic Code* will be set to a period greater than ten years, as appropriate. An evaluation of the following factors will determine whether a period longer than ten years is required:

i) the maximum duration of the production cycle for the *susceptible species*;

ii) the life stages at which *aquatic animals* are susceptible;

iii) the variation in predilection to clinical *disease* among *susceptible species*;

iv) the expected severity and duration of clinical signs in the *susceptible species* (and therefore the likelihood of detection);

v) environmental conditions that influence levels of *infection* and clinical expression, including seasonality of the *disease* (period of the year when clinical *disease* occurs, e.g. when water temperatures are permissive);

vi) factors specific to the *pathogenic agent* (e.g. production of spores);

vii) production systems and management practices that would affect observation of clinical signs if they were to occur;

viii) any other relevant factors that may influence presentation of clinical signs and observation of the *disease* should it be present.

c) For pathway 3, the minimum period that *basic biosecurity conditions* should be in place prior to commencement of *targeted surveillance* will generally be one year. It is expected that this period will be sufficient under most circumstances for a *disease* to reach a *prevalence* sufficiently high to be detected by a survey designed in accordance with the recommendations of this chapter. However, different recommendations are provided in the *disease*-specific chapters of the *Aquatic Code* for some *diseases* where the epidemiology of a *disease* and nature of production systems would affect the expected transmission, and thus increase in *prevalence* and intensity of *infection* in the *susceptible species* following introduction of the *disease*. An evaluation of the following factors will determine whether a period longer than one year is required:

i) the maximum duration of the production cycle for the *susceptible species*;

ii) the life stages at which *aquatic animals* are susceptible;

iii) seasonality of the *disease* (periods of the year when *prevalence* and intensity of *infection* is highest and most conducive to detection);

iv) production systems and management practices that would affect occurrence of *infection*;

v) any other relevant factors that may influence the expected rate of increase in *prevalence* and intensity of *infection* in *susceptible species* following introduction of the *disease*.

d) Pathway 4 is only applicable following the loss of *disease* freedom due to a *disease outbreak*. This circumstance implies a failure of *basic biosecurity conditions* to prevent the introduction of the *disease*. The pathway of *disease* introduction should be investigated and *basic biosecurity conditions* should be reviewed and modified as necessary following eradication of the *disease*, and prior to commencement of any *targeted surveillance* that will be utilised as evidence for a subsequent self-declaration.

Article 1.4.10.

Required periods for targeted surveillance

Prior to a *Competent Authority* making a *self-declaration of freedom from disease* utilising pathway 3 or pathway 4, *targeted surveillance* should be conducted for a defined period, as described in the relevant *disease*-specific chapter of the *Aquatic Code*. The period of *targeted surveillance* is determined for each *disease*-specific chapter of the *Aquatic Code,* based on the factors described below:

1) the maximum duration of the production cycle for the *susceptible species*;

2) the life stages at which *aquatic animals* are susceptible;

3) seasonality of the *disease* (periods of the year when *prevalence* and intensity of *infection* is highest and most conducive to detection);

4) production systems and management practices that would affect the seasonal occurrence of *infection*.

For a country or *zone*, the minimum default period for which *targeted surveillance* should occur prior to a *self-declaration of freedom from disease* is two years. During the period of *targeted surveillance,* surveys should occur during defined time periods when conditions are optimal for detection of the *pathogenic agent* (e.g. seasons, temperatures, and life stages). All populations of *susceptible species* should be included in the scope of each survey. There should be a gap of at least three months between surveys and, if there are breaks in production, the surveys should also ideally span two production cycles.

For a country or *zone* to regain freedom in accordance with pathway 4, the required period of *targeted surveillance* specified in the *disease*-specific chapter of the *Aquatic Code* will be consistent with the original self-declaration of freedom.

For *compartments*, the minimum default period that *targeted surveillance* should occur prior to a *self-declaration of freedom from disease* is one year. This shorter period for a *compartment* reflects the more clearly defined populations, the *biosecurity* required to maintain its population's health status and a likely narrower variation in environmental variables. However, a different period (more or less than one year) may be stipulated in the *disease*-specific chapter of the *Aquatic Code* if warranted by the epidemiology of the *disease* and the criteria proposed above. For example, different requirements may be appropriate where *susceptible species* have a three-year production cycle, versus one that has a six-month production cycle; particularly if the *disease* is likely to occur at a very low *prevalence* until near the end of the production cycle.

EU comment

Shortening the period during which targeted surveillance should take place to one year for compartments, will depend very much on the nature of the compartment. This should only be considered when the nature of the compartment is such that the disease-specific separation from surrounding waters can be guaranteed. The EU suggests this be specified in the paragraph above.

For *compartments* to regain freedom in accordance with pathway 4, the required period of *targeted surveillance* specified in the *disease*-specific chapter of the *Aquatic Code* may be less than the original declaration of freedom (dependent on the nature of the specific *disease*). However, at least one round of testing is required to demonstrate that eradication has been successful and to test the reviewed *biosecurity* conditions.

EU comment

The EU suggests inserting the words "<u>using a suitable sample size, and under conditions including water temperature, which are conducive to the clinical expression of the disease</u>" after "round of testing" in the last sentence of the paragraph above, as these elements are relevant in this contaxt.

Article 1.4.11.

Pathway 1 – Absence of susceptible species

Unless otherwise specified in the relevant *disease*-specific chapter of the *Aquatic Code*, a self-declaration of freedom from a specific *disease* may be made for a country or *zone* without applying *targeted surveillance* if there are no *susceptible species* (as listed in Article X.X.2. of the relevant *disease*-specific chapter of the *Aquatic Code*) present in that country or *zone*.

Basic biosecurity conditions should be in place for a period of time prior to a *self-declaration of freedom from disease*.

This pathway relies on confidence that *susceptible species* are in fact absent from a country or *zone*. To be confident that *susceptible species* are absent there should be:

1) sound knowledge of the range of *susceptible species* of a *pathogenic agent;* and

2) sufficient knowledge, based on active *surveillance*, of the local *aquatic animal* fauna (including wild populations).

The forms of evidence that may be required to demonstrate absence of *susceptible species* include:

1) the absence of reports of the existence of the *susceptible species* in the country or *zone* from structured surveys (e.g. of fisheries and aquatic fauna surveys, historical fisheries data);

2) documentation from the relevant *Competent Authority* showing that those *susceptible species* have not been imported into the country or *zone*;

3) provision of documentation which sets out scientific evidence indicating that the likelihood of the presence of *susceptible species* in the country or *zone* is negligible (e.g. data on physiological requirements, oceanographic information, biodiversity databases).

EU comment

As indicated in the EU comment above in relation to Table 1.1., it would be important to refer to these forms of evidence in that table for Pathway 1.

This pathway cannot be used for *diseases* where there is uncertainty regarding the full range of *susceptible species* (e.g. *diseases* with a broad host range), or where the *pathogenic agent* may not be obligate (e.g. able to survive indefinitely outside the host). In these cases, the pathway will be absent from the relevant *disease*-specific chapter of the *Aquatic Code*, and alternative pathways to demonstrate freedom should be utilised.

The pathway is intended primarily to be used by the *Competent Authority* wishing to establish freedom ahead of farming a new species.

Article 1.4.12.

Pathway 2 – Historically free

Unless otherwise specified in the relevant *disease*-specific chapter of the *Aquatic Code*, a *self-declaration of freedom from disease* may be made for a country or *zone* on the basis of historical freedom. The primary evidence for historical freedom is *passive surveillance* data generated by a country's *early detection system*. For this pathway to be utilised, the following conditions should be met:

1) the country has *basic biosecurity conditions* in place, including an *early detection system*, that is sufficiently sensitive to detect the *disease* should it occur, and the conditions of Article 1.4.8. are met;

2) the *disease* has not been reported in the country or *zone* (including in wild *aquatic animal* populations) for the minimum period specified in the relevant *disease*-specific chapter of the *Aquatic Code*.

Requirements for passive surveillance

The level of confidence provided by *passive surveillance* data (generated by the *early detection system* of the *Competent Authority*) to demonstrate historical freedom should be set at 95%, equivalent to that of other pathways for which the evidence is provided by *targeted surveillance*. If a combination of *surveillance* data sources is to be used (e.g.

passive surveillance and *targeted surveillance*), the level of confidence should also be set at 95% that the *disease* is absent. The data sources for *passive surveillance* are described in Article 1.4.8. of this chapter.

A *Competent Authority* making a *self-declaration of freedom from disease* on the basis of historical freedom will need to provide an explanation of how the criteria (i.e. for *basic biosecurity conditions*) presented for this pathway have been met. Specifically, the *Competent Authority* needs to provide evidence that its *early detection system* meets the conditions as described in Article 1.4.7. (and ideally a quantitative assessment of *sensitivity* would be included). The *early detection system* needs to cover all the *susceptible species* populations in the country or *zone*. If the *Competent Authority* cannot demonstrate that the required characteristics are fulfilled, due to a country's circumstances (e.g. nature of the *early detection system*, environmental conditions, nature of the *aquaculture* industry), this pathway is not considered valid. Instead, an alternative pathway that utilises *targeted surveillance* data will be required, or the *passive surveillance* data will need to be supplemented with *targeted surveillance* data (see below).

Need for targeted surveillance

If the requirements for *passive surveillance* specified in points 1 and 2 above would not be met for some defined populations of *susceptible species* (e.g. for wild populations), *targeted surveillance* may be used to provide additional evidence of freedom for those populations. However, for this pathway to be utilised as the basis of a *self-declaration of freedom from disease*, it should be based primarily on *passive surveillance* data to demonstrate historical freedom; alternatively, pathway 3, as described in Article 1.4.13., should be used.

Article 1.4.13.

Pathway 3 – Surveillance

As specified in the relevant *disease*-specific chapter of the *Aquatic Code*, a *self-declaration of freedom from disease* may be made for a country, a *zone* or a *compartment* where the primary evidence for freedom is *targeted surveillance* data. For this pathway to be utilised, the following conditions should be met:

1) *basic biosecurity conditions* have been in place for a default minimum period as specified in the relevant *disease*-specific chapter of the *Aquatic Code*;

EU comment

Point (1) above appears to replicate the information set out in the subsequent paragraph entitled 'Requirements for basic biosecurity conditions'.

2) the *disease* has not been reported in the country, *zone* or *compartment,* despite *targeted surveillance* that has been conducted for a period as specified in the relevant *disease*-specific chapter of the *Aquatic Code,* and in accordance with the requirements below.

Requirements for basic biosecurity conditions

Targeted surveillance surveys should only commence following a period of time that *basic biosecurity conditions* have been in place, as specified in the relevant *disease*-specific chapter of the *Aquatic Code*.

Requirements for targeted surveillance

For many *diseases*, there will be significant temporal variability in the *prevalence* and intensity of *infection* (and therefore likelihood of detection by *targeted surveillance*). For example, the likelihood of detection may be greatest for a particular life stage, or during periods of the year when the rate *pathogenic agent* replication and transmission are at their highest.

Environmental variability from one year to another may also result in differences in *prevalence* and intensity between years that could affect likelihood of detection. Surveys should therefore be designed to account for such variability and sample populations in a manner to maximise the likelihood of detecting a *disease* should it occur. This may require targeting temporal windows such that sampling can only take place during limited periods within a single year. Based on an assessment of potential pathways of introduction of the *diseases*, high risk regions or *aquaculture establishments* should be identified and preferentially included in the *surveillance* programmes. For example, establishments near ports or processing facilities may have higher likelihood of exposure to introduced *pathogenic agents*.

To maximise the likelihood of *pathogenic agent* detection, surveys should select species and life stages most likely to be infected and take place at times of the year when temperature and season offer the best opportunity for detection. At least two surveys per year (for at least two consecutive years) need to be conducted three or more months apart to declare freedom unless *disease*-specific evidence supports an alternative strategy. The number of *aquaculture establishments* and *aquatic animals* sampled should be sufficient to generate an overall 95% confidence or greater that the *pathogenic agent* is at or below the design *prevalence*. Design *prevalence* at the animal and higher levels of aggregation (i.e. pond, *aquaculture establishment*, village, etc.) should be 2% or lower (a higher design *prevalence* can only be used if justified by epidemiological evidence). Surveys should be designed in accordance with the recommendations provided in Article 1.4.1.

For declared *free zones* or *free compartments* in infected countries, and in all cases where conditions are not conducive to clinical expression of the *pathogenic agent*, *targeted surveillance* needs to be continued at a level, determined by the *Competent Authority*, to generate an annual 95% confidence of detection.

Other sources of data

This pathway to *disease* freedom should be based primarily on the results of structured *surveillance*, however, the submission may also include an analysis of the *passive surveillance* data to provide supplemental evidence. This evidence may be used for defined populations of *susceptible species* where the *sensitivity* of *passive surveillance* is demonstrated to be sufficient (as described in Article 1.4.8. .).

Article 1.4.14.

Pathway 4 – Returning to freedom

As specified in the relevant *disease*-specific chapter of the *Aquatic Code*, a *self-declaration of freedom from disease* may be made for a country, a *zone* or a *compartment* for which a self-declaration had previously been made, but subsequently lost due to an *outbreak* of the *disease*.

For a *country or a zone,* the default minimum period of *surveillance* to regain freedom is consistent with the requirements for pathway 3. However, a self-declaration of freedom can be made sooner if the relevant *Competent Authority* can demonstrate that the approach would provide an appropriate standard of evidence for the circumstances of the *outbreak* and the *disease*.

Compartments are able to return to freedom relatively rapidly; however, a minimum period of time is required as specified in each *disease*-specific chapter of the *Aquatic Code* to test the reviewed *biosecurity* conditions, and to undertake sufficient testing to demonstrate that eradication has been successful.

For a country, *zone* or *compartment*, a self-declaration utilising this pathway should provide information on the process employed to review *basic biosecurity conditions*. This information should also address the outcomes of the review and any relevant *sanitary measures* implemented to strengthen *basic biosecurity conditions*.

1. Infected zone and protection zone

   Infected and *protection zones* should be established through exposure contact tracing from known infected *aquaculture establishments* (e.g. by following movements of *aquatic animals* or equipment to and from infected establishments) to identify all known infected establishments. Once contact tracing is complete and no new cases are being reported or detected through tracing, the boundaries of *infected zones* and *protection zones* can be finalised. The geographic extent of an *infected zone* should be based on the spatial distributions of infected and non-infected establishments within a region (e.g. river, estuary or bay). The *zone* should be defined to encompass geographically clustered infected populations.

   The geographic extent of a *protection zone* needs to provide a very high level of confidence that measures implemented within the *zone* will prevent spread from the *zone* and should be based on the epidemiology of the transmissible *pathogenic agent*, the potential for exposure of neighbouring *aquaculture establishments*, the influence of wild populations, and the local hydrology. In the marine environment, local hydrology (including tidal excursion), the distribution of suitable habitats for *susceptible species* and the movement of wild *susceptible species* should be considered. In the freshwater environment, the boundaries of the *protection zone* should be determined by the distance downstream that viable *pathogenic agent* is likely to spread on currents. If susceptible wild populations are present, their migratory patterns and ranges should be used.

Once *infected zones* and *protection zones* have been established, and no new cases have been detected for a period equal to or greater than the incubation period of the *pathogenic agent* (but no shorter than one month), the region outside of the *infected zones* and *protection zones* can be declared a *disease free zone*. Re-establishing *disease* freedom in the *infected* and *protection zones* requires *targeted surveillance*.

2. Requirements for targeted surveillance in a country or zone

Once all infected populations have been depopulated and affected *aquaculture establishments* have been disinfected, as described in Chapter 4.3., and synchronously fallowed as described in Chapter 4.6., for a period determined by the biophysical properties of the *pathogenic agent* (i.e. survival in the environment), a *surveillance* programme within the *protection* and *infected zones* should commence. The programme should include both farmed and wild populations of *susceptible species* in the *protection* and *infected zones*. A *risk*-based approach to the design of the survey is recommended (refer to Article 1.4.6.). The following *aquaculture establishments* or populations should be preferentially selected for sampling:

a) establishments which were depopulated (following restocking);

b) establishments and wild populations at greatest *risk* of exposure to *infection* during the *outbreak*, i.e. in close geographic proximity to infected establishments or with other epidemiological contacts such as sharing equipment or movements of *aquatic animals*;

c) wild populations of *susceptible species* downstream or in the immediate vicinity of previously infected establishments.

It is recommended that at least two negative surveys are conducted prior to reclaiming freedom. The second survey should start at least three months after completion of the first survey. Surveys should take place during optimum seasons, temperatures, and priority life stages to optimise *pathogenic agent* detection. If there are breaks in production, the surveys should also ideally span two production cycles. The number of *aquaculture establishments* and the samples taken per establishment in each survey should be sufficient to demonstrate with 95% confidence that the *pathogenic agent* is not present above a *prevalence* of 2% (a higher design *prevalence* can be used if justified by epidemiological evidence).

3. Requirements for targeted surveillance in a compartment

Once the infected populations have been depopulated and affected *aquaculture establishments* disinfected, and fallowed as described in Chapter 4.3. and fallowed as described in Chapter 4.6., for a period determined by the biophysical properties of the *pathogenic agent* (i.e. survival in the environment), the *compartment* can be restocked. A single survey is required following restocking to demonstrate that eradication has been successful. The survey should be undertaken at least 6 months after the *aquaculture establishment* has been restocked to ensure that the reviewed *basic biosecurity conditions* are effective; and should take place during optimum seasons, temperatures, and priority life stages to optimise *pathogenic agent* detection. The number of holding units (e.g. ponds, tanks) and the animals per holding unit sampled should be sufficient to demonstrate with 95% confidence that the *pathogenic agent* is not present above a *prevalence* of 2% (a higher design *prevalence* can be used if justified by epidemiological evidence).

EU comment

There is an error in the first sentence of point (3) above. It should be re-worded as follows: "Once the infected populations have been depopulated and affected aquaculture establishments disinfected, ~~and fallowed~~ as described in Chapter 4.3 and fallowed as described in Chapter 4.6, [...]".

Article 1.4.15.

Maintenance of disease free status

For maintenance of free status achieved via pathways 2, 3 and 4, the *Competent Authority* should provide evidence that *basic biosecurity conditions* are continuously maintained.

If *targeted surveillance*, that was required for initial demonstration of freedom, is to be discontinued for any identified population, evidence should be provided to demonstrate that conditions remain conducive to clinical expression of *disease*, and that *passive surveillance*, as provided by the country's *early detection system*, would rapidly detect the *disease* in those populations should it occur.

Any ongoing *targeted surveillance* to maintain freedom should be undertaken at a level necessary to maintain confidence of freedom, and should take into account the likelihood of *infection*.

Article 1.4.16.

Design of surveys to demonstrate freedom from disease

Surveys to demonstrate freedom from a specified *disease* (i.e. *targeted surveillance*) are required for pathway 3 as described in Article 1.4.13. to achieve a *disease* free status, and to regain a *disease* free status following detection of the *pathogenic agent* as described in Article 1.4.14.). Surveys may be required to supplement *passive surveillance* data generated by the *early detection system* required for pathway 2 as described in Article 1.4.12. In addition, where conditions are not conducive to clinical expression of *disease*, and, therefore, the *early detection system* cannot provide evidence for the maintenance of freedom, ongoing *targeted surveillance* is required.

It is not possible to provide absolute certainty of the absence of *disease*. Surveys can demonstrate freedom from *disease* by generating evidence that a *disease* is not present in a population at or above a predetermined *prevalence* (the design *prevalence*) and to an acceptable level of confidence. Apparent *disease* at any level in the *target population* automatically invalidates any freedom from *disease* claim, unless, on the basis of further testing, positive test results are accepted as false positives. A survey to demonstrate freedom from *disease* should meet the following requirements set out in this article:

1. Population

   The population of *epidemiological units* should be clearly defined. *Aquaculture establishments* and holding *units* (e.g. ponds, tanks) within establishments are the most commonly used *epidemiological unit* in surveys to demonstrate *disease* freedom. It is, therefore, important that *Competent Authorities* should keep registries of *aquaculture establishments*, which include geographic location and species held.

   The *target population* consists of all individuals of all *susceptible species* to the *disease* in a country, *zone* or *compartment,* to which the *surveillance* results apply. Exotic *disease* introduction may be more likely to occur in some components of the *target population* than others. In these cases, it is advisable to focus *surveillance* efforts on this part of the population.

   The design of the survey will depend on the size and structure of the population being studied. If the population is relatively small, and can be considered to be homogenous with regards to *risk* of *infection*, a single-stage survey can be used.

   Farmed *aquatic animals* are not individually identified and usually kept in holding *units* (e.g. ponds, tanks) which can lead to clusters of *infection* within *aquaculture establishments*. For these reasons, multi-stage sampling is recommended. In two-stage sampling, at the first stage of sampling, groups of animals (e.g. ponds, *aquaculture establishments* or villages) are selected. At the second stage, animals are selected for testing from each of the selected groups.

   In the case of a complex (e.g. multi-level) population structure, multi-stage sampling may be used, and the data analysed accordingly.

2. Dossier of evidence

   The sources of evidence should be fully described. A survey should include a description of the sampling strategy used for the selection of units for testing. For complex *surveillance* systems, a full description of the system is required, including consideration of any *biases* that may be inherent in the system. Evidence to support claims of freedom from *disease* can use non-random sources of information, provided that, overall, any *biases* introduced subsequently favour the detection.

3. Statistical methodology

The analysis and interpretation of test results from a survey shall be in accordance with the provisions of this chapter and consider the following factors:

a) the survey design;

b) the diagnostic *sensitivity* and *specificity* of the test or test system;

c) the design *prevalence* (or *prevalences* where a multi-stage design is used).

Analysis of data for evidence of freedom from *disease* involves estimating the probability (alpha) that the evidence observed (i.e. negative results for *disease* detection from *surveillance*) could have been produced assuming that *infection* is present in the population at or below the minimum specified *prevalence* (the design *prevalence*). The confidence in (or, equivalently, the *sensitivity* of) the survey that produced the evidence is equal to 1–alpha. If the confidence level exceeds a pre-set threshold, the evidence is deemed adequate to demonstrate freedom from *infection*. The required level of confidence (that the survey would detect *infection* if *infection* were present at or above the specified level) should be greater than or equal to 95%.

The power (probability that the survey would report that no *infection* is present if *infection* is truly not present) is by convention set to 80%, but may be adjusted in accordance with the country's or *zone*'s requirements.

Statistical analysis of *surveillance* data often requires assumptions about population parameters or test characteristics. These are usually based on expert opinion, previous studies on the same or similar populations, and epidemiology of the *disease*.

The values for design *prevalence* used in calculations should be those specified in the relevant *disease* chapter (if present) of the *Aquatic Manual*. If not specified for the particular *disease*, justification for the selection of design *prevalence* values should be provided, and should be based on the following recommendations:

a) At the individual animal level (e.g. *prevalence* of infected animals in a pond, tank or net pen, or cages), the design *prevalence* is based on the epidemiology of the *infection* in the population. It is equal to the minimum expected *prevalence* of *infection* in the *study population*, if the *infection* had become established in that population. A suitable design *prevalence* value at the animal level may be:

   i) between 1% and 5% for *infections* that are present in a small part of the population, e.g. are transmitted slowly or have been recently introduced, etc.;

   ii) over 5% for highly transmissible and persistent *infections*;

   iii) if reliable information, including expert opinion, on the expected *prevalence* in an infected population is not available, a value of 2% should be used for the design *prevalence*.

b) At higher levels (e.g. net pen or cage, pond, *aquaculture establishments*, village, etc.) the design *prevalence* should be based empirical evidence and reflect the expected behaviour of the *infection*. A higher establishment-level design *prevalence* can be used for diseases which spread rapidly between pens or cages, and establishments. *Diseases* which are transient require lower design *prevalences*:

   i) a suitable design *prevalence* value for the first level of clustering (e.g. proportion of infected establishments in a *zone*) is normally not greater than 2%. If a higher design *prevalence* is selected, it should be justified.

4. Risk based sampling

Risk-based sampling is an approach to identify and sample populations that have the greatest likelihood of *infection*. It can be applied to the design of surveys to demonstrate freedom from *disease* for a country, *zone* or *compartment*. A key advantage of *risk*-based sampling is that it can improve the efficiency of *surveillance* to demonstrate freedom from *disease* compared to random sampling approaches.

Risk-based sampling requires the identification of *risk*-factors that are applied to *bias* sample collection to populations of *aquatic animals* considered most likely to be infected if the specific *disease* had been introduced and had established. Where *risk*-based sampling is used for demonstration of freedom, the *risk* factors that

underpin survey design, and the evidence or assumptions for their selection, should be documented. Where existing *risk assessments* are available, these may be utilised to identify *risk* factors associated with introduction, exposure and establishment. The identification of appropriate *risk* factors may include consideration of:

a) the possible pathways of *disease* introduction (e.g. through imported *aquatic animals*, imported *aquatic animal products*, ship ballast water or biofouling);

b) proximity of susceptible populations to sources of exposure (e.g. to *quarantine* facilities, *aquatic animal* processing facilities, or ports);

c) environmental or husbandry conditions that are permissive for establishment (e.g. temperature, salinity, production system type, habitat type);

d) conditions that are conducive for development of clinical *disease*; including the species or life stages that are most susceptible to clinical *disease*.

5. Test characteristics

All *surveillance* involves performing one or more tests for evidence of the presence of current or past *infection*, ranging from laboratory assays to farmer observations. The performance level of a test is described in terms of its diagnostic *sensitivity* and *specificity*. Imperfect *sensitivity* or *specificity* impact on the interpretation of *surveillance* results, and should be taken into account in the analysis of *surveillance* data. For example, in the case of a test with imperfect diagnostic *specificity*, if the population is free of *disease* or has a very low *prevalence* of *infection*, all or a large proportion of positive tests will be false. Samples that test positive should be confirmed or refuted using a second highly specific test. Where more than one test is used (sometimes called using tests in series or parallel), the *sensitivity* and *specificity* of the test combination should be calculated.

All calculations should take the performance level (*sensitivity* and *specificity*) of any tests used into account. Information on test characteristics provided in the relevant disease-specific chapter of the *Aquatic Manual* should be used unless more appropriate information is available. The estimate of test *sensitivity* when the test was used in apparently healthy *aquatic animals* should be used. Samples should not be pooled before testing, unless approved in the relevant disease-specific chapter of the *Aquatic Manual*. If pooled testing is used, the results of testing should be interpreted using *sensitivity* and *specificity* values that have been determined or estimated for that particular pooled testing procedure, and for the applicable pool sizes being used.

6. Sample size

The number of units to be sampled from a population should be calculated, using a statistically valid technique that takes at least the following factors into account:

a) the *sensitivity* and *specificity* of the diagnostic test,

b) the design *prevalence* (or *prevalences* where a multi-stage design is used),

c) the level of confidence that is desired of the survey results.

Additionally, other factors may be considered in sample size calculations, including (but not limited to):

a) the size of the population (but it is acceptable to assume that the population is infinitely large),

b) the desired power of the survey.

Software for the calculation of sample sizes at varying parameter values are available. Table 1.1 provides examples of sample sizes generated by the software for a type I and type II error of 5% (i.e. 95% confidence and 95% statistical power). However, this does not mean that a type 1 and type 2 error of 0.05 should always be used. For example, using a test with *sensitivity* and *specificity* of 99%, 528 units should be sampled. If nine or less of those units test positive, the population can still be considered free of the *infection* at a design *prevalence* of 2%, provided that all efforts are made to ensure that all presumed false positives are indeed false (i.e. by use of a second highly specific assay). This means that there is a 95% confidence that the *prevalence* is 2% or lower,

which reflects the fact that false negative results can occur. Incorrectly concluding that a population is free can be reduce by increasing the sample size and using more than one assay but cannot be completely eliminated.

In the case in which the values of *sensitivity* and *specificity* are not known (e.g. no information is available in the relevant *disease*-specific chapter of the *Aquatic Manual*), they should not automatically be assumed to be 100%. All positive results should be included and discussed in any report regarding that particular survey, and all efforts should be made to ensure that all presumed false positives are indeed false.

7. Multi-stage structured survey design

In general, a survey to demonstrate freedom at *zone* or *country* level should use a multi-stage design. The first sampling level is often *aquaculture establishments* (or villages), and the second stage may be ponds or individual animals within the establishment (or village). At each level, design levels need to be set and sample sizes calculated.

8. Discounting

Where conditions are not conducive to clinical expression, ongoing *surveillance* is required. Regions and *aquaculture establishments* at high risk of introduction of *pathogenic agent* should be regularly sampled. *Targeted surveillance* required to maintain confidence in *disease* freedom at 95% can be determined based on estimates of the likelihood of introduction of *pathogenic agent* (low due to basic *biosecurity* measures) and the discounting of historic *surveillance*. Methods for using historical *surveillance* data have been developed.

9. Quality assurance

Surveys should include a documented quality assurance system, to ensure that field and other procedures conform to the specified survey design. Acceptable systems may be quite simple, as long as they provide verifiable documentation of procedures and basic checks to detect significant deviations of procedures from those documented in the survey design.

Table 1.2. Sample sizes for different design *prevalences* and test characteristics.

Design prevalence	**Sensitivity (%)**	**Specificity (%)**	**Sample size**	**Maximum number of false positive if the population is free**
2	100	100	149	0
2	100	99	524	9
2	100	95	1,671	98
2	99	100	150	0
2	99	99	528	9
2	99	95	1,707	100
2	95	100	157	0
2	95	99	542	9
2	95	95	1,854	108
2	90	100	165	0
2	90	99	607	10
2	90	95	2,059	119
2	80	100	186	0
2	80	99	750	12

2	80	95	2,599	148
5	100	100	59	0
5	100	99	128	3
5	100	95	330	23
5	99	100	59	0
5	99	99	129	3
5	99	95	331	23
5	95	100	62	0
5	95	99	134	3
5	95	95	351	24
5	90	100	66	0
5	90	99	166	4
5	90	95	398	27
5	80	100	74	0
5	80	99	183	4
5	80	95	486	32

EU comment

In the first column of Table 1.2. above, please insert "(%)" after "design prevalence".

Article 1.4.17.

Combining multiple sources of information

Pathway 1 to achieving *disease* freedom (absence of *susceptible species*) relies on a range of data sources. Pathway 2 to achieving *disease* freedom (historical freedom) will primarily use evidence from *passive surveillance,* which may come from multiple sources (as described in Article 1.4.8.). *Passive surveillance* data can also be used to provide additional support to case for *disease* freedom, primarily based on *targeted surveillance* (i.e. pathway 3). Estimates of the confidence in each data source may be combined to provide an overall level of confidence of freedom from *disease* for the combined data sources. The methodology used to combine the estimates from multiple data sources:

1) should be scientifically valid and fully documented, including references to published material; and

2) should, where possible, take into account any lack of statistical independence between different data sources.

A scenario tree modelling approach can be used to combine evidence from different sources including *passive* and *targeted surveillance*.

Article 1.4.18.

Diagnostic confirmation of a listed disease or an emerging disease

A *Competent Authority* is required to provide *disease notifications* as described in Chapter 1.1.

The relevant *disease*-specific chapter of the *Aquatic Manual* provide recommendations for the appropriate diagnostic methods for presumptive and confirmatory diagnostic purposes. The assays recommended for these purposes are presented in Table 4.1 of the relevant *disease*-specific chapter of the *Aquatic Manual*.

The recommended standards of diagnostic evidence to confirm *infection* in either apparently healthy or clinically diseased animals are provided in Section 6 of the relevant *disease*-specific chapter of the *Aquatic Manual*. These case definitions for suspect and confirmed cases have been developed to support decision making in relation to trade and for confirmation of *disease* status at the level of a country, *zone* or *compartment*. A *Competent Authority* may choose to apply a lower standard of evidence for *disease* confirmation within its *territory* for known endemic *diseases*.

If standards of evidence are not met to confirm a suspect case of *disease* in accordance with the case definitions in Section 6 of the relevant *disease*-specific chapter of the *Aquatic Manual*, ongoing investigation is required until sufficient evidence is obtained to either:

1) exclude the presence of a *listed disease* or an *emerging disease*, or;

2) to confirm the presence of a *listed disease* or an *emerging disease*.

If a laboratory does not have the capability to undertake the necessary diagnostic tests, it should seek advice from the relevant OIE Reference Laboratory.

In all circumstances, Member Countries should comply with the requirements described in Chapter 1.1. to provide transparent and timely *notification* to allow Member Countries to take appropriate action to prevent the transboundary spread of important *diseases* of *aquatic animals*.

EU comment

The EU suggests adding the words "and to ensure that appropriate disease control measures are put in place when the presence of a listed or emerging disease is suspected or has been confirmed" at the end of the paragraph above.

Back to Agenda

Model Articles X.X.4 to X.X.8 for disease-specific chapters to address declaration of freedom from [Pathogen X]

Note: time periods in these model articles will be determined by the Aquatic Animals Commission for each disease-specific chapter based on criteria that will be included in the revised Chapter 1.4. For this reason, periods are shown as [X] to indicate that the period is yet to be determined for each specific disease. Where a period is shown (e.g. 'the last [X] years') this indicates an intended default period that may vary depending on the circumstances of each disease.

EU comment

The EU thanks the OIE and in general supports the proposed changes to this chapter.

One comment is inserted in the text below.

Article X.X.4.

[**Note**: this is a new article that will outline general requirements for making a self-declaration of freedom for a country, zone or compartment.]

Requirements for self-declaration of freedom from infection with [PATHOGEN X]

A Member Country may make a self-declaration of freedom from infection with [PATHOGEN X] for the entire country, a *zone* or a *compartment* in accordance with the provisions of Articles X.X.5. to X.X.8., as relevant. The self-declaration of freedom must be made in accordance with other relevant requirements of the *Aquatic Code*, including that the Member Country meet the following conditions:

1) complies with the provisions of Chapter 3.1.; and

2) uses appropriate methods of diagnosis, as recommended in the *Aquatic Manual*; and

3) meets all requirements of Chapter 1.4. that are relevant to the self-declaration of freedom.

Article X.X.5.

[**Note**: this article will replace the existing Article X.X.4.]

Country free from infection with [PATHOGEN X]

If a country shares water bodies ~~a *zone*~~ with ~~one or more~~ other countries, it can only make a self-declaration of freedom from infection with [PATHOGEN X] if ~~the~~ all shared water bodies are within countries or *zones* declared free from infection with [PATHOGEN X] (see Article X.X.6.).

As described in Article 1.4.X., a Member Country may make a self-declaration of freedom from infection with [PATHOGEN X] for its entire *territory* if:

1) none of the *susceptible species* referred to in Article X.X.2. are present and *basic biosecurity conditions* have been continuously met for at least the last [two] years;

OR

2) there has been no occurrence of infection with [PATHOGEN X] for at least the last [ten] years, and:

a) the Member Country can demonstrate that conditions are conducive to the clinical expression of infection with [PATHOGEN X], as described in the corresponding chapter of the *Aquatic Manual*; and

b) *basic biosecurity conditions* as described in Chapter 1.4. have been continuously met for at least the last [ten] years;

OR

3) *targeted surveillance*, as described in Chapter 1.4., has been in place for at least the last [two] years without detection of [PATHOGEN X], and:

a) *basic biosecurity conditions* have been continuously met ~~from~~ for at least [one] year prior to commencement of *targeted surveillance*;

OR

4) it previously made a self-declaration of freedom from infection with [PATHOGEN X] and subsequently lost its free status due to the detection of [PATHOGEN X] but the following conditions have been met:

a) on detection of [PATHOGEN X], the affected area was declared an *infected zone* and a *protection zone* was established; and

b) infected populations within the *infected zone* have been killed and disposed of by means that minimise the likelihood of further transmission of [PATHOGEN X], and the appropriate *disinfection* procedures (as described in Chapter 4.3.) have been completed followed by fallowing as described in Chapter 4.6.; and

c) previously existing *basic biosecurity conditions* have been reviewed and modified as necessary and have continuously been in place since eradication of infection with [PATHOGEN X]; and

d) *targeted surveillance*, as described in Chapter 1.4., has been in place for i) at least the last [two] years without detection of [PATHOGEN X] or ii) at least the last [one] year without detection of [PATHOGEN X] if affected ~~farms~~ *aquaculture establishments* were not epidemiologically connected to wild populations of *susceptible species*.

In the meantime, part or all of the country, apart from the *infected* and *protection zones,* may be declared a free *zone* provided that such a part meets the conditions in point 2 of Article X.X.6.

Article X.X.6.

[**Note**: this new article for zone freedom is based on the existing Article X.X.5.]

Zone free from infection with [PATHOGEN X]

If a *zone* extends over the *territory* of more than one country, it can only be declared a *zone* free from infection with [PATHOGEN X] if all of the relevant *Competent Authorities* confirm that all relevant conditions have been met.

As described in Article 1.4.X., a Member Country may make a self-declaration of freedom from infection with [PATHOGEN X] for a *zone* within its *territory* if:

1) none of the *susceptible species* referred to in Article X.X.2. ~~10.6.2.~~ are present and *basic biosecurity conditions* have been continuously met for at least the last [two] years;

OR

2) there has been no occurrence of infection with [PATHOGEN X] for at least the last [ten] years, and;

a) the Member Country can demonstrate that conditions are conducive to the clinical expression of infection with [PATHOGEN X], as described in Article 1.4.8. of Chapter 1.4. ~~the corresponding chapter of the *Aquatic Manual*~~; and

b) *basic biosecurity conditions* as described in Chapter 1.4. have been continuously met for the *zone* for at least the last [ten] years;

OR

3) *targeted surveillance*, as described in Chapter 1.4., has been in place in the *zone* for at least the last [two] years without detection of [PATHOGEN X], and:

a) *basic biosecurity conditions* have been continuously met for at least [one] year prior to commencement of *targeted surveillance*;

OR

4) it previously made a self-declaration of freedom for a *zone* from infection with [PATHOGEN X] and subsequently lost its free status due to the detection of [PATHOGEN X] in the *zone* but the following conditions have been met:

a) on detection of [PATHOGEN X], the affected area was declared an *infected zone* and a *protection zone* was established; and

b) infected populations within the *infected zone* have been killed and disposed of by means that minimise the likelihood of further transmission of [PATHOGEN X], and the appropriate *disinfection* procedures (as described in Chapter 4.3.) have been completed followed by fallowing as described in Chapter 4.6.; and

c) previously existing *basic biosecurity conditions* have been reviewed and modified as necessary and have continuously been in place since eradication of infection with [PATHOGEN X]; and

d) *targeted surveillance*, as described in Chapter 1.4., has been in place for at least the last [two] years without detection of [PATHOGEN X].

Article X.X.7.

[**Note**: this is a new article to address free compartments]

Compartment free from infection with [PATHOGEN X]

As described in Article 1.4.X., a Member Country may make a self-declaration of freedom from infection with [PATHOGEN X] for a *compartment* within its *territory* if:

1) *targeted surveillance*, as described in Chapter 1.4., has been in place in the *compartment* for at least the last [two] years without detection of [PATHOGEN X], and:

a) *basic biosecurity conditions* have been continuously met for at least [one] year prior to commencement of *targeted surveillance*;

OR

2) it previously made a self-declaration of freedom for a *compartment* from infection with [PATHOGEN X] and subsequently lost its free status due to the detection of [PATHOGEN X] in the *compartment* ~~*zone*~~ but the following conditions have been met:

a) all *aquatic animals* within the *compartment* have been killed and disposed of by means that minimise the likelihood of further transmission of [PATHOGEN X], the appropriate *disinfection* procedures (as described in Chapter 4.3.) have been completed, and the *compartment* has been fallowed as described in Chapter 4.6. ~~for at least [X] weeks~~; and

b) previously existing *basic biosecurity conditions*, including the *biosecurity plan*, have been reviewed and modified as necessary and have continuously been in place from the time of restocking with animals from an approved pathogen free source in accordance with the requirements of Articles X.X.9. and X.X.10 as appropriate; and

c) *targeted surveillance*, as described in Chapter 1.4., has been in place for at least the last [one] year without detection of [PATHOGEN X].

EU comment

Reference is made to the final sentence of Article 1.4.10., of Chapter 1.4. and our comments on that article in Annex 3. This possibility should also be mentioned here in Article X.X.7.

Article X.X.8.

[**Note**: this article is based on the current Article X.X.6.]

Maintenance of free status

A country or *zone* that is declared free from infection with [PATHOGEN X] following the provisions of point 1 ~~of~~ in Articles X.X.5. or X.X.6. (as relevant) may maintain its status as free from infection with [PATHOGEN X] provided that *basic biosecurity conditions* are continuously maintained.

A country ~~or *zone*~~ that is declared free from infection with [PATHOGEN X] following the provisions of point 2 ~~of~~ in Article X.X.5. may discontinue *targeted surveillance* and maintain its free status provided that conditions are conducive to clinical expression of infection with [PATHOGEN X], as described in the corresponding chapter of the *Aquatic Manual*, and *basic biosecurity conditions* are continuously maintained.

For declared free *zones* or *compartments* within the *territory* of a country not declared free, *targeted surveillance* should be continued at a level determined by the *Aquatic Animal Health Service* on the basis of the likelihood of *infection.*

In all cases where conditions are not conducive to clinical expression of infection with [PATHOGEN X], ongoing *targeted surveillance,* as described in Chapter 1.4., is required at a level that maintains the level of confidence in freedom from infection with [PATHOGEN X] that was required for the initial declaration of freedom.

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EXAMPLE ARTICLE X.X.3 FOR DISEASE- SPECIFIC CHAPTERS
(TRACK CHANGES VERSION)

EU comment

The EU thanks the OIE and supports the proposed changes to Chapters 9.1. to 9.9.

CHAPTER 9.8.

INFECTION WITH WHITE SPOT SYNDROME VIRUS

[…]

Article 9.8.3.

Measures for the ~~I~~importation or transit of aquatic animal products for any purpose regardless of the infection with WSSV status of the exporting country, zone or compartment

1) The following *aquatic animal products* have been assessed as meeting the criteria for safety of *aquatic animal products* in accordance with Article 5.4.1. When authorising the importation or transit of the *aquatic animal products* listed below, *Competent Authorities* should not require any *sanitary measures* ~~conditions~~ related to WSSV, regardless of the infection with WSSV status of the *exporting country*, *zone* or *compartment*: ~~when authorising the importation or transit of the following *aquatic animal products* derived from a species referred to in Article 9.8.2. that are intended for any purpose and comply with Article 5.4.1.:~~

   a) cooked, ~~canned,~~ pasteurised or retorted *aquatic animal products* that have been subjected to a heat treatment sufficient to attain a core temperature of at least 60°C for at least one minute (or a time/temperature equivalent that has been demonstrated to inactivate WSSV);

   ~~a) heat sterilised hermetically sealed crustacean products (i.e. a heat treatment at 121°C for at least 3.6 minutes or any time/temperature equivalent that has been demonstrated to inactivate WSSV);~~

   ~~b) cooked crustacean products that have been subjected to heat treatment at 60°C for at least one minute (or any time/temperature equivalent that has been demonstrated to inactivate WSSV);~~

   ~~c) pasteurised crustacean products that have been subjected to heat treatment at 90°C for at least ten minutes (or any time/temperature equivalent that has been demonstrated to inactivate WSSV);~~

   ~~d)~~b) crustacean oil;

   ~~e)~~c) crustacean *meal* that has been heat treated to a core temperature of at least 60°C for at least one minute (or a time/temperature equivalent that has been demonstrated to inactivate WSSV);

   ~~f)~~d) chemically extracted chitin.

~~2) When authorising the importation or transit of *aquatic animal products* derived from a species referred to in Article 9.8.2., other than those referred to in point 1 of Article 9.8.3., *Competent Authorities* should require the conditions prescribed in Articles 9.8.7. to 9.8.12. relevant to the infection with WSSV status of the *exporting country*, *zone* or *compartment*.~~

~~3) When considering the importation or transit of *aquatic animal products* derived from a species not referred to in Article 9.8.2. but which could reasonably be expected to pose a risk of transmission of WSSV, the *Competent Authority* should conduct a *risk analysis* in accordance with the recommendations in Chapter 2.1. The *Competent Authority* of the *exporting country* should be informed of the outcome of this analysis.~~

[…]

EXAMPLE ARTICLE X.X.3 FOR DISEASE-SPECIFIC CHAPTERS (CLEAN VERSION)

CHAPTER 9.8.

INFECTION WITH WHITE SPOT SYNDROME VIRUS

[…]

Article 9.8.3.

Measures for the importation or transit of aquatic animal products for any purpose regardless of the infection with WSSV status of the exporting country, zone or compartment

1) The following *aquatic animal products* have been assessed as meeting the criteria for safety of *aquatic animal products* in accordance with Article 5.4.1. When authorising the importation or transit of the *aquatic animal products* listed below, *Competent Authorities* should not require any *sanitary measures* related to WSSV, regardless of the infection with WSSV status of the *exporting country*, *zone* or *compartment*:

   a) cooked, pasteurised or retorted *aquatic animal products* that have been subjected to a heat treatment sufficient to attain a core temperature of at least 60°C for at least one minute (or a time/temperature equivalent that has been demonstrated to inactivate WSSV);

   b) crustacean oil;

   c) crustacean *meal* that has been heat treated at a core temperature of at least 60°C for at least one minute (or a time/temperature equivalent that has been demonstrated to inactivate WSSV);

   d) chemically extracted chitin.

[…]

APPLICATION OF EXAMPLE ARTICLE TO CRUSTACEAN DISEASE-SPECIFIC CHAPTERS

(TRACK CHANGES VERSION)

CHAPTER 9.1.

ACUTE HEPATOPANCREATIC NECROSIS DISEASE

[…]

Article 9.1.3.

Measures for the i~~I~~mportation or transit of aquatic animal products for any purpose regardless of the AHPND status of the exporting country, zone or compartment

1) The following *aquatic animal products* have been assessed as meeting the criteria for safety of *aquatic animal products* in accordance with Article 5.4.1. When authorising the importation or transit of the *aquatic animal products* listed below, *Competent Authorities* should not require any *sanitary measures* ~~conditions~~ related to AHPND, regardless of the AHPND status of the *exporting country*, *zone* or *compartment*:~~, when authorising the importation or transit of the following *aquatic animal products* derived from a species referred to in Article 9.1.2. that are intended for any purpose and comply with Article 5.4.1.:~~

   a) cooked or retorted *aquatic animal products* that have been subjected to a heat treatment sufficient to attain a core temperature of at least 100°C for at least one minute (or a time/temperature equivalent that has been demonstrated to inactivate *Vp*$_{AHPND}$);

   ~~b) heat sterilised hermetically sealed crustacean products (i.e. a heat treatment at 121°C for at least 3.6 minutes or any time/temperature equivalent that has been demonstrated to inactivate *Vp*$_{AHPND}$);~~

   ~~c) cooked crustacean products that have been subjected to heat treatment at 100°C for at least one minute (or any time/temperature equivalent that has been demonstrated to inactivate *Vp*$_{AHPND}$);~~

   ~~d)~~b) crustacean oil;

   ~~e)~~c) crustacean *meal* that has been heat treated at a core temperature of at least 100°C for at least one minute (or a time/temperature equivalent that has been demonstrated to inactivate *Vp*$_{AHPND}$);

   ~~f)~~d) chemically extracted chitin.

~~2) When authorising the importation or transit of *aquatic animal products* derived from a species referred to in Article 9.1.2., other than those referred to in point 1 of Article 9.1.3., *Competent Authorities* should require the conditions prescribed in Articles 9.1.7. to 9.1.12. relevant to the AHPND status of the *exporting country*, *zone* or *compartment*.~~

~~3) When considering the importation or transit of *aquatic animal products* derived from a species not referred to in Article 9.1.2. but which could reasonably be expected to pose a *risk* of transmission of *Vp*$_{AHPND}$, the *Competent Authority* should conduct a *risk analysis* in accordance with the recommendations in Chapter 2.1. The *Competent Authority* of the *exporting country* should be informed of the outcome of this analysis.~~

[…]

(CLEAN VERSION)

CHAPTER 9.1.

ACUTE HEPATOPANCREATIC NECROSIS DISEASE

[…]

Article 9.1.3.

Measures for the importation or transit of aquatic animal products for any purpose regardless of the AHPND status of the exporting country, zone or compartment

1) The following *aquatic animal products* have been assessed as meeting the criteria for safety of *aquatic animal products* in accordance with Article 5.4.1. When authorising the importation or transit of the *aquatic animal products* listed below, *Competent Authorities* should not require any *sanitary measures* related to AHPND, regardless of the AHPND status of the *exporting country*, *zone* or *compartment*:

   a) cooked or retorted *aquatic animal products* that have been subjected to a heat treatment sufficient to attain a core temperature of at least 100°C for at least one minute (or a time/temperature equivalent that has been demonstrated to inactivate Vp_{AHPND});

   b) crustacean oil;

   c) crustacean *meal* that has been heat treated at a core temperature of at least 100°C for at least one minute (or a time/temperature equivalent that has been demonstrated to inactivate Vp_{AHPND});

   d) chemically extracted chitin.

[…]

(TRACK CHANGES VERSION)

CHAPTER 9.2.

INFECTION WITH *APHANOMYCES ASTACI* (CRAYFISH PLAGUE)

[…]

Article 9.2.3.

Measures for the i~~I~~mportation or transit of aquatic animal products for any purpose regardless of the infection with *A. astaci* status of the exporting country, zone or compartment

1) The following *aquatic animal products* have been assessed as meeting the criteria for safety of *aquatic animal products* in accordance with Article 5.4.1. When authorising the importation or transit of the *aquatic animal products* listed below, *Competent Authorities* should not require any *sanitary measures*~~conditions~~ related to *A. astaci*, regardless of the infection with *A. astaci* status of the *exporting country*, *zone* or *compartment*:~~, when authorising the importation or transit of the following *aquatic animal products* derived from a species referred to in Article 9.2.2. that are intended for any purpose and comply with Article 5.4.1.:~~

   a) cooked, pasteurised or retorted *aquatic animal products* that have been subjected to a heat treatment sufficient to attain a core temperature of at least 100°C for at least one minute (or a time/temperature equivalent that has been demonstrated to inactivate *A. astaci*;

   ~~a) heat sterilised hermetically sealed crayfish products (i.e. a heat treatment at 121°C for at least 3.6 minutes or any time/temperature equivalent that has been demonstrated to inactivate *A. astaci*);~~

   ~~b) cooked crayfish products that have been subjected to heat treatment at 100°C for at least one minute (or any time/temperature equivalent that has been demonstrated to inactivate *A. astaci*);~~

   ~~c) pasteurised crayfish products that have been subjected to heat treatment at 90°C for at least ten minutes (or any time/temperature equivalent that has been demonstrated to inactivate *A. astaci*);~~

   ~~d)~~b) frozen crayfish products that have been subjected to minus 20°C or lower temperatures for at least 72 hours;

   ~~e)~~c) crayfish oil;

   ~~f)~~d) crayfish *meal* that has been heat treated at a core temperature of at least 100°C for at least one minute (or a time/temperature equivalent that has been demonstrated to inactivate *A.astaci*);

   ~~g)~~e) chemically extracted chitin.

~~2) When authorising the importation or transit of *aquatic animal products* derived from a species referred to in Article 9.2.2., other than those referred to in point 1 of Article 9.2.3., *Competent Authorities* should require the conditions prescribed in Articles 9.2.7. to 9.2.12. relevant to the infection with *A. astaci* status of the *exporting country*, *zone* or *compartment*.~~

~~3) When considering the importation or transit of *aquatic animal products* derived from a species not referred to in Article 9.2.2. but which could reasonably be expected to pose a *risk* of transmission of *A. astaci*, the *Competent Authority* should conduct a *risk analysis* in accordance with the recommendations in Chapter 2.1. The *Competent Authority* of the *exporting country* should be informed of the outcome of this analysis.~~

[…]

(CLEAN VERSION)

CHAPTER 9.2.

INFECTION WITH *APHANOMYCES ASTACI* (CRAYFISH PLAGUE)

[…]

Article 9.2.3.

Measures for the importation or transit of aquatic animal products for any purpose regardless of the infection with *A. astaci* status of the exporting country, zone or compartment

1) The following *aquatic animal products* have been assessed as meeting the criteria for safety of *aquatic animal products* in accordance with Article 5.4.1. When authorising the importation or transit of the *aquatic animal products* listed below, *Competent Authorities* should not require any *sanitary measures* related to *A. astaci*, regardless of the infection with *A. astaci* status of the *exporting country*, *zone* or *compartment:*

   a) cooked, pasteurised or retorted *aquatic animal products* that have been subjected to a heat treatment sufficient to attain a core temperature of at least 100°C for at least one minute (or a time/temperature equivalent that has been demonstrated to inactivate *A. astaci*);

   b) frozen crayfish products that have been subjected to minus 20°C or lower temperatures for at least 72 hours;

   c) crayfish oil;

   d) crayfish *meal* that has been heat treated at a core temperature of at least 100°C for at least one minute (or a time/temperature equivalent that has been demonstrated to inactivate *A. astaci*);

   e) chemically extracted chitin.

[…]

(TRACK CHANGES VERSION)

CHAPTER 9.3.

INFECTION WITH *HEPATOBACTER PENAEI* (NECROTISING HEPATOPANCREATITIS)

[…]

Article 9.3.3

Measures for the i~~I~~mportation or transit of aquatic animal products for any purpose regardless of the infection with *H. penaei* status of the exporting country, zone or compartment

1) The following *aquatic animal products* have been assessed as meeting the criteria for safety of *aquatic animal products* in accordance with Article 5.4.1. When authorising the importation or transit of the *aquatic animal products* listed below, *Competent Authorities* should not require any *sanitary measures*~~conditions~~ related to *H. penaei*, regardless of the infection with *H. penaei* status of the *exporting country*, *zone* or *compartment*:~~, when authorising the importation or transit of the following *aquatic animal products* derived from a species referred to in Article 9.3.2. that are intended for any purpose and comply with Article 5.4.1.:~~

   a) cooked, pasteurised or retorted *aquatic animal products* that have been subjected to a heat treatment sufficient to attain a core temperature of at least 63°C for at least 30 minutes (or a time/temperature equivalent that has been demonstrated to inactivate *H. penaei*);

   ~~a) heat sterilised hermetically sealed crustacean products (i.e. a heat treatment at 121°C for at least 3.6 minutes or any time/temperature equivalent that has been demonstrated to inactivate *H. penaei*);~~

   ~~b) cooked crustacean products that have been subjected to heat treatment at 100°C for at least three minutes (or any time/temperature equivalent that has been demonstrated to inactivate *H. penaei*);~~

   ~~c) pasteurised crustacean products that have been subjected to heat treatment at 63°C for at least 30 minutes (or any time/temperature equivalent that has been demonstrated to inactivate *H. penaei*);~~

   ~~d)~~b) crustacean oil;

   ~~e)~~c) crustacean *meal* that has been heat treated at a core temperature of at least 63°C for at least 30 minutes (or a time/temperature equivalent that has been demonstrated to inactivate *H. penaei*);

   ~~f)~~d) chemically extracted chitin.

~~2) When authorising the importation or transit of *aquatic animal products* derived from a species referred to in Article 9.3.2., other than those referred to in point 1 of Article 9.3.3., *Competent Authorities* should require the conditions prescribed in Articles 9.3.7. to 9.3.12. relevant to the infection with *H. penaei* status of the *exporting country*, *zone* or *compartment*.~~

~~3) When considering the importation or transit of *aquatic animal products* derived from a species not referred to in Article 9.3.2. but which could reasonably be expected to pose a *risk* of transmission of *H. penaei*, the *Competent Authority* should conduct a *risk analysis* in accordance with the recommendations in Chapter 2.1. The *Competent Authority* of the *exporting country* should be informed of the outcome of this analysis.~~

[…]

(CLEAN VERSION)

CHAPTER 9.3.

INFECTION WITH *HEPATOBACTER PENAEI* (NECROTISING HEPATOPANCREATITIS)

[…]

Article 9.3.3

Measures for the importation or transit of aquatic animal products for any purpose regardless of the infection with *H. penaei* status of the exporting country, zone or compartment

1) The following *aquatic animal products* have been assessed as meeting the criteria for safety of *aquatic animal products* in accordance with Article 5.4.1. When authorising the importation or transit of the *aquatic animal products* listed below, *Competent Authorities* should not require any *sanitary measures* related to *H. penaei*, regardless of the infection with *H. penaei* status of the *exporting country*, *zone* or *compartment*:

   a) cooked, pasteurised or retorted *aquatic animal products* that have been subjected to a heat treatment sufficient to attain a core temperature of at least 63°C for at least 30 minutes (or a time/temperature equivalent that has been demonstrated to inactivate *H. penaei*);

   b) crustacean oil;

   c) crustacean *meal* that has been heat treated at a core temperature of at least 63°C for at least 30 minutes (or a time/temperature equivalent that has been demonstrated to inactivate *H. penaei*);

   d) chemically extracted chitin.

[…]

(TRACK CHANGES VERSION)

CHAPTER 9.4.

INFECTION WITH INFECTIOUS HYPODERMAL AND HAEMATOPOIETIC NECROSIS VIRUS

[…]

Article 9.4.3.

Measures for the i~~I~~mportation or transit of aquatic animal products for any purpose regardless of the infection with IHHNV status of the exporting country, zone or compartment

1) The following *aquatic animal products* have been assessed as meeting the criteria for safety of *aquatic animal products* in accordance with Article 5.4.1. When authorising the importation or transit of the *aquatic animal products* listed below, *Competent Authorities* should not require any *sanitary measures*~~conditions~~ related to IHHNV, regardless of the infection with IHHNV status of the *exporting country*, *zone* or *compartment*:~~, when authorising the importation or transit of the following *aquatic animal products* derived from a species referred to in Article 9.4.2. that are intended for any purpose and comply with Article 5.4.1.:~~

   a) cooked or retorted *aquatic animal products* that have been subjected to a heat treatment sufficient to attain a core temperature of at least 100°C for at least two minutes (or a time/temperature equivalent that has been demonstrated to inactivate IHHNV);

   ~~a) heat-sterilised hermetically sealed crustacean products (i.e. a heat treatment at 121°C for at least 3.6 minutes or any time/temperature equivalent that has been demonstrated to inactivate IHHNV);~~

   ~~b) cooked crustacean products that have been subjected to heat treatment at 90°C for at least 20 minutes (or any time/temperature equivalent that has been demonstrated to inactivate IHHNV);~~

   ~~c)~~b) crustacean oil;

   ~~d)~~c) crustacean *meal* that has been heat treated at a core temperature of at least 100°C for at least two minutes (or a time/temperature equivalent that has been demonstrated to inactivate IHHNV).

~~2) When authorising the importation or transit of *aquatic animal products* derived from a species referred to in Article 9.4.2., other than those referred to in point 1 of Article 9.4.3., *Competent Authorities* should require the conditions prescribed in Articles 9.4.7. to 9.4.12. relevant to the infection with IHHNV status of the *exporting country*, *zone* or *compartment*.~~

~~3) When considering the importation or transit of *aquatic animal products* derived from a species not referred to in Article 9.4.2. but which could reasonably be expected to pose a *risk* of transmission of IHHNV, the *Competent Authority* should conduct a *risk analysis* in accordance with the recommendations in Chapter 2.1. The *Competent Authority* of the *exporting country* should be informed of the outcome of this analysis.~~

[…]

(CLEAN VERSION)

CHAPTER 9.4.

INFECTION WITH INFECTIOUS HYPODERMAL AND HAEMATOPOIETIC NECROSIS VIRUS

[…]

Article 9.4.3.

Measures for the importation or transit of aquatic animal products for any purpose regardless of the infection with IHHNV status of the exporting country, zone or compartment

1) The following *aquatic animal products* have been assessed as meeting the criteria for safety of *aquatic animal products* in accordance with Article 5.4.1. When authorising the importation or transit of the *aquatic animal products* listed below, *Competent Authorities* should not require any *sanitary measures* related to IHHNV, regardless of the infection with IHHNV status of the *exporting country*, *zone* or *compartment:*

   a) cooked or retorted *aquatic animal products* that have been subjected to a heat treatment sufficient to attain a core temperature of at least 100°C for at least two minutes (or a time/temperature equivalent that has been demonstrated to inactivate IHHNV);

   b) crustacean oil;

   c) crustacean *meal* that has been heat treated at a core temperature of at least 100°C for at least two minutes (or a time/temperature equivalent that has been demonstrated to inactivate IHHNV).

[…]

(TRACK CHANGES VERSION)

CHAPTER 9.5.

INFECTION WITH INFECTIOUS MYONECROSIS VIRUS

[…]

Article 9.5.3.

Measures for the i~~I~~mportation or transit of aquatic animal products for any purpose regardless of the infection with IMNV status of the exporting country, zone or compartment

1) The following *aquatic animal products* have been assessed as meeting the criteria for safety of *aquatic animal products* in accordance with Article 5.4.1. When authorising the importation or transit of the *aquatic animal products* listed below, *Competent Authorities* should not require any *sanitary measures*~~conditions~~ related to IMNV, regardless of the infection with IMNV status of the *exporting country*, *zone* or *compartment*:~~, when authorising the importation or transit of the following *aquatic animal products* derived from a species referred to in Article 9.5.2. that are intended for any purpose and comply with Article 5.4.1.:~~

   a) cooked or retorted *aquatic animal products* that have been subjected to a heat treatment sufficient to attain a core temperature of at least 60°C for at least 60 minutes (or a time/temperature equivalent that has been demonstrated to inactivate IMNV);

   ~~a) heat sterilised hermetically sealed crustacean products (i.e. a heat treatment at 121°C for at least 3.6 minutes or any time/temperature equivalent that has been demonstrated to inactivate IMNV);~~

   ~~c) cooked crustacean products that have been subjected to heat treatment at 60°C for at least three minutes (or any time/temperature equivalent that has been demonstrated to inactivate IMNV);~~

   ~~d)~~b) crustacean oil;

   ~~e)~~c) crustacean *meal* that has been heat treated at a core temperature of at least 60°C for at least 60 minutes (or a time/temperature equivalent that has been demonstrated to inactivate IMNV);

   f)d) chemically extracted chitin.

~~2) When authorising the importation or transit of *aquatic animal products* derived from a species referred to in Article 9.5.2., other than those referred to in point 1 of Article 9.5.3., *Competent Authorities* should require the conditions prescribed in Articles 9.5.7. to 9.5.12. relevant to the infection with IMNV status of the *exporting country*, *zone* or *compartment*.~~

~~3) When considering the importation or transit of *aquatic animal products* derived from a species not referred to in Article 9.5.2. but which could reasonably be expected to pose a *risk* of transmission of IMNV, the *Competent Authority* should conduct a *risk analysis* in accordance with the recommendations in Chapter 2.1. The *Competent Authority* of the *exporting country* should be informed of the outcome of this analysis.~~

[…]

(CLEAN VERSION)

CHAPTER 9.5.

INFECTION WITH INFECTIOUS MYONECROSIS VIRUS

[…]

Article 9.5.3.

Measures for the importation or transit of aquatic animal products for any purpose regardless of the infection with IMNV status of the exporting country, zone or compartment

1) The following *aquatic animal products* have been assessed as meeting the criteria for safety of *aquatic animal products* in accordance with Article 5.4.1. When authorising the importation or transit of the *aquatic animal products* listed below, *Competent Authorities* should not require any *sanitary measures* related to IMNV, regardless of the infection with IMNV status of the *exporting country*, *zone* or *compartment*:

   a) cooked or retorted *aquatic animal products* that have been subjected to a heat treatment sufficient to attain a core temperature of at least 60°C for at least 60 minutes (or a time/temperature equivalent that has been demonstrated to inactivate IMNV);

   b) crustacean oil;

   c) crustacean *meal* that has been heat treated at a core temperature of at least 60°C for at least 60 minutes (or a time/temperature equivalent that has been demonstrated to inactivate IMNV);

   d) chemically extracted chitin.

[…]

(TRACK CHANGES VERSION)

CHAPTER 9.6.

INFECTION WITH *MACROBRACHIUM ROSENBERGII* NODAVIRUS (WHITE TAIL DISEASE)

[…]

Article 9.6.3.

Measures for the i~~I~~mportation or transit of aquatic animal products for any purpose regardless of the infection with MrNV status of the exporting country, zone or compartment

1) The following *aquatic animal products* have been assessed as meeting the criteria for safety of *aquatic animal products* in accordance with Article 5.4.1. When authorising the importation or transit of the *aquatic animal products* listed below, *Competent Authorities* should not require any *sanitary measures*~~conditions~~ related to MrNV, regardless of the infection with MrNV status of the *exporting country*, *zone* or *compartment*~~, when authorising the importation or transit of the following *aquatic animal products* derived from a species referred to in Article 9.6.2. that are intended for any purpose and comply with Article 5.4.1.~~:

   a) cooked, pasteurised or retorted *aquatic animal products* that have been subjected to a heat treatment sufficient to attain a core temperature of at least 60°C for at least 60 minutes (or a time/temperature equivalent that has been demonstrated to inactivate MrNV);

   ~~a) heat sterilised hermetically sealed crustacean products (i.e. a heat treatment at 121°C for at least 3.6 minutes or any time/temperature equivalent that has been demonstrated to inactivate MrNV);~~

   ~~b) cooked crustacean products that have been subjected to heat treatment at 60°C for at least 60 minutes (or any time/temperature equivalent that has been demonstrated to inactivate MrNV);~~

   ~~c) pasteurised crustacean products that have been subjected to heat treatment at 90°C for at least ten minutes (or any time/temperature equivalent that has been shown to inactivate MrNV);~~

   ~~d)~~b) crustacean oil;

   ~~e)~~c) crustacean *meal* that has been heat treated at a core temperature of at least 60°C for at least 60 minutes (or a time/temperature equivalent that has been demonstrated to inactivate MrNV);

   ~~f)~~d) chemically extracted chitin.

~~2) When authorising the importation or transit of *aquatic animal products* derived from a species referred to in Article 9.6.2., other than those referred to in point 1 of Article 9.6.3., *Competent Authorities* should require the conditions prescribed in Articles 9.6.7. to 9.6.12. relevant to the infection with MrNV status of the *exporting country*, *zone* or *compartment*.~~

~~3) When considering the importation or transit of *aquatic animal products* derived from a species not referred to in Article 9.6.2. but which could reasonably be expected to pose a *risk* of transmission of MrNV, the *Competent Authority* should conduct a *risk analysis* in accordance with the recommendations in Chapter 2.1. The *Competent Authority* of the *exporting country* should be informed of the outcome of this analysis.~~

[…]

(CLEAN VERSION)

CHAPTER 9.6.

INFECTION WITH *MACROBRACHIUM ROSENBERGII* NODAVIRUS (WHITE TAIL DISEASE)

[…]

Article 9.6.3.

Measures for the importation or transit of aquatic animal products for any purpose regardless of the infection with MrNV status of the exporting country, zone or compartment

1) The following *aquatic animal products* have been assessed as meeting the criteria for safety of *aquatic animal products* in accordance with Article 5.4.1. When authorising the importation or transit of the *aquatic animal products* listed below, *Competent Authorities* should not require any *sanitary measures* related to MrNV, regardless of the infection with MrNV status of the *exporting country*, *zone* or *compartment*:

   a) cooked, pasteurised or retorted *aquatic animal products* that have been subjected to a heat treatment sufficient to attain a core temperature of at least 60°C for at least 60 minutes (or a time/temperature equivalent that has been demonstrated to inactivate MrNV);

   b) crustacean oil;

   c) crustacean *meal* that has been heat treated at a core temperature of at least 60°C for at least 60 minutes (or a time/temperature equivalent that has been demonstrated to inactivate MrNV);

   d) chemically extracted chitin.

[…]

(TRACK CHANGES VERSION)

CHAPTER 9.7.

INFECTION WITH TAURA SYNDROME VIRUS

[…]

Article 9.7.3.

Measures for the i~~I~~mportation or transit of aquatic animal products for any purpose regardless of the infection with TSV status of the exporting country, zone or compartment

1) The following *aquatic animal products* have been assessed as meeting the criteria for safety of *aquatic animal products* in accordance with Article 5.4.1. When authorising the importation or transit of the *aquatic animal products* listed below, *Competent Authorities* should not require any *sanitary measures* ~~conditions~~ related to TSV, regardless of the infection with TSV status of the *exporting country*, *zone* or *compartment*:~~, when authorising the importation or transit of the following *aquatic animal products* derived from a species referred to in Article 9.7.2. that are intended for any purpose and comply with Article 5.4.1.:~~

   a) cooked, pasteurised or retorted *aquatic animal products* that have been subjected to a heat treatment sufficient to attain a core temperature of at least 70°C for at least 30 minutes (or a time/temperature equivalent that has been demonstrated to inactivate TSV);

   ~~a) heat sterilised hermetically sealed crustacean products (i.e. a heat treatment at 121°C for at least 3.6 minutes or any time/temperature equivalent that has been demonstrated to inactivate TSV);~~

   ~~b) cooked crustacean products that have been subjected to heat treatment at 70°C for at least 30 minutes (or any time/temperature equivalent that has been demonstrated to inactivate TSV);~~

   ~~c) pasteurised crustacean products that have been subjected to heat treatment at 90°C for at least ten minutes (or any time/temperature equivalent that has been demonstrated to inactivate TSV);~~

   ~~d)~~b) crustacean oil;

   e)c) crustacean *meal* that has been heat treated at a core temperature of at least 70°C for at least 30 minutes (or a time/temperature equivalent that has been demonstrated to inactivate TSV);

   f)d) chemically extracted chitin.

~~2) When authorising the importation or transit of *aquatic animal products* derived from a species referred to in Article 9.7.2., other than those referred to in point 1 of Article 9.7.3., *Competent Authorities* should require the conditions prescribed in Articles 9.7.7. to 9.7.12. relevant to the infection with TSV status of the *exporting country*, *zone* or *compartment*.~~

~~3) When considering the importation or transit of *aquatic animal products* derived from a species not referred to in Article 9.7.2. but which could reasonably be expected to pose a *risk* of transmission of TSV, the *Competent Authority* should conduct a *risk analysis* in accordance with the recommendations in Chapter 2.1. The *Competent Authority* of the *exporting country* should be informed of the outcome of this analysis.~~

[…]

(CLEAN VERSION)

CHAPTER 9.7.

INFECTION WITH TAURA SYNDROME VIRUS

[…]

Article 9.7.3.

Measures for the importation or transit of aquatic animal products for any purpose regardless of the infection with TSV status of the exporting country, zone or compartment

1) The following *aquatic animal products* have been assessed as meeting the criteria for safety of *aquatic animal products* in accordance with Article 5.4.1. When authorising the importation or transit of the *aquatic animal products* listed below, *Competent Authorities* should not require any *sanitary measures* related to TSV, regardless of the infection with TSV status of the *exporting country*, *zone* or *compartment*:

   a) cooked, pasteurised or retorted *aquatic animal products* that have been subjected to a heat treatment sufficient to attain a core temperature of at least 70°C for at least 30 minutes (or a time/temperature equivalent that has been demonstrated to inactivate TSV);

   b) crustacean oil;

   c) crustacean *meal* that has been heat treated at a core temperature of at least 70°C for at least 30 minutes (or a time/temperature equivalent that has been demonstrated to inactivate TSV);

   d) chemically extracted chitin.

[…]

(TRACK CHANGES VERSION)

CHAPTER 9.9.

INFECTION WITH YELLOW HEAD VIRUS GENOTYPE 1

[…]

Article 9.9.3.

Measures for the i~~I~~mportation or transit of aquatic animal products for any purpose regardless of the infection with YHV1 status of the exporting country, zone or compartment

1) The following *aquatic animal products* have been assessed as meeting the criteria for safety of *aquatic animal products* in accordance with Article 5.4.1. When authorising the importation or transit of the *aquatic animal products* listed below, *Competent Authorities* should not require any *sanitary measures*~~conditions~~ related to YHV1, regardless of the infection with YHV1 status of the *exporting country*, *zone* or *compartment*:~~, when authorising the importation or transit of the following *aquatic animal products* derived from a species referred to in Article 9.9.2. that are intended for any purpose and comply with Article 5.4.1.:~~

   a) cooked, pasteurised or retorted *aquatic animal products* that have been subjected to a heat treatment sufficient to attain a core temperature of at least 60°C for at least 15 minutes (or a time/temperature equivalent that has been demonstrated to inactivate YHV1);

   ~~a) heat sterilised hermetically sealed crustacean products (i.e. a heat treatment at 121°C for at least 3.6 minutes or any time/temperature equivalent that has been demonstrated to inactivate YHV1);~~

   ~~b) cooked crustacean products that have been subjected to heat treatment at 60°C for at least 15 minutes (or any time/temperature equivalent that has been demonstrated to inactivate YHV1);~~

   ~~c) pasteurised crustacean products that have been subjected to heat treatment at 90°C for at least ten minutes (or any time/temperature equivalent that has been demonstrated to inactivate YHV1);~~

   ~~d)~~b) crustacean oil;

   ~~e)~~c) crustacean *meal* that has been heat treated at a core temperature of at least 60°C for at least 15 minutes (or a time/temperature equivalent that has been demonstrated to inactivate YHV1);

   ~~f)~~d) chemically extracted chitin.

~~2) When authorising the importation or transit of *aquatic animal products* derived from a species referred to in Article 9.9.2., other than those referred to in point 1 of Article 9.9.3., *Competent Authorities* should require the conditions prescribed in Articles 9.9.7. to 9.9.12. relevant to the infection with YHV1 status of the *exporting country*, *zone* or *compartment*.~~

~~3) When considering the importation or transit of *aquatic animal products* derived from a species not referred to in Article 9.9.2. but which could reasonably be expected to pose a *risk* of transmission of YHV1, the *Competent Authority* should conduct a *risk analysis* in accordance with the recommendations in Chapter 2.1. The *Competent Authority* of the *exporting country* should be informed of the outcome of this analysis.~~

[…]

CLEAN VERSION

CHAPTER 9.9.

INFECTION WITH YELLOW HEAD VIRUS GENOTYPE 1

[…]

Article 9.9.3.

Measures for the importation or transit of aquatic animal products for any purpose regardless of the infection with YHV1 status of the exporting country, zone or compartment

1) The following *aquatic animal products* have been assessed as meeting the criteria for safety of *aquatic animal products* in accordance with Article 5.4.1. When authorising the importation or transit of the *aquatic animal products* listed below, *Competent Authorities* should not require any *sanitary measures* related to YHV1, regardless of the infection with YHV1 status of the *exporting country*, *zone* or *compartment*:

   a) cooked, pasteurised or retorted *aquatic animal products* that have been subjected to a heat treatment sufficient to attain a core temperature of at least 60°C for at least 15 minutes (or a time/temperature equivalent that has been demonstrated to inactivate YHV1);

   b) crustacean oil;

   c) crustacean *meal* that has been heat treated at a core temperature of at least 60°C for at least 15 minutes (or a time/temperature equivalent that has been demonstrated to inactivate YHV1);

   d) chemically extracted chitin.

[…]

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CHAPTER 11.2.

INFECTION WITH *BONAMIA EXITIOSA*

EU comment

The EU supports the proposed changes to this chapter.

[…]

Article 11.2.1.

For the purposes of the *Aquatic Code*, infection with *Bonamia exitiosa* means *infection* with the *pathogenic agent* ~~B.~~ *Bonamia exitiosa* of the Family Haplosporidiidae.

Information on methods for *diagnosis* is provided in the *Aquatic Manual*.

Article 11.2.2.

Scope

The recommendations in this chapter apply to the following species that meet the criteria for listing as susceptible in accordance with Chapter 1.5.: Argentinean flat oyster (*Ostrea puelchana*), Australian mud oyster *(Ostrea angasi),* ~~and~~ Chilean flat oyster *(Ostrea chilensis),* Dwarf oyster *(Ostrea stentina),* Eastern oyster *(Crassostrea virginica),* European flat oyster *(Ostrea edulis),* Olympia oyster *(Ostrea lurida)* and Suminoe oyster *(Crassostrea ariakensis).* ~~These recommendations also apply to any other *susceptible species* referred to in the *Aquatic Manual* when traded internationally.~~

[…]

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Original: English
November–December 2020

REPORT OF THE OIE *AD HOC* GROUP ON SUSCEPTIBILITY OF MOLLUCS SPECIES TO INFECTION WITH OIE LISTED DISEASES

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[1]: cohabitation with infected hosts; infection by immersion or feeding.	*In vitro* experimental assays (contact between haemocytes and parasites) were not considered appropriate to answer the question of susceptibility or 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

[1] Invasive experimental procedures including injection were only used to demonstrate non-susceptibility.

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 Cochennec *et al*., 2000) OR Species-specific Real-time or conventional PCR (for example Ramilo *et al*., 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 *et al.*, 2008, are expected to be specific to *Bonamia exitiosa* 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 host[2];

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 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*.

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

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: a) Histopathology OR b) Cytology (usually gill or heart imprint or haemolymph smears) OR c) *In-situ* hybridization (ISH) OR 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. *Ostrea chilensis*) species **OR** 2) Demonstration of viability of cells isolated from tissues by: a) Flow cytometry OR a) Vital stains OR b) Successful infection of uninfected animals by inoculation	1) Mortality **OR** 2) Macroscopic lesions such as: a. Discolouration of tissue b. Gill ulceration **OR** 3) Rapid loss of condition **OR** 4) Microscopic lesions 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 *B. exitiosa*, of the *Aquatic Code* and Section 2.2.1 of Chapter 2.4.2, Infection with *B. exitiosa*, 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).
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 *Aquatic Code* or the *Aquatic Manual*. 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 *Aquatic Manual.*
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	*Ostrea edulis*	European flat oyster	YES	YES	YES	ND	YES	YES	1	Abollo *et al*., 2008
			YES	YES	YES	ND	YES	YES	1	Carrasco *et al*., 2012
Ostreidae	*Ostrea chilensis*	Chilean flat oyster	YES	YES	YES	ND	YES	YES	1	Hill *et al*., 2014
			YES	YES	YES	ND	YES	YES	1	Lane *et al*., 2016
Ostreidae	*Ostrea stentina*	Dwarf oyster	YES	YES	YES	ND	YES	YES	1	Hill *et al*., 2014
			YES	YES	YES	ND	ND	YES	1	Hill *et al*., 2010
Ostreidae	*Ostrea puelchana*	Argentinean flat oyster	YES	YES	YES	ND	YES	YES	1	Hill *et al*., 2014
			YES	YES[3]	YES	ND	YES	YES	1	Kroeck, 2010
Ostreidae	*Ostrea angasi*	Australian mud oyster	YES	YES	YES	ND	YES	YES	1	Hill *et al*., 2014
			YES	YES[4]	YES	ND	YES	YES	1	Heasman *et al*., 2004
Ostreidae	*Crassostrea virginica*	Eastern Oyster	YES	YES	YES	ND	YES[5]	YES	1	OIE, 2012 and personal communication (R. Carnegie)
			YES	YES	YES	ND	ND[6]	YES	1	OIE, 2013 and personal communication (R. Carnegie)
			YES	YES	YES	ND	ND	YES	1	Hill *et al*., 2014
			YES	YES	NO	ND	NO	NO	4	Dungan *et al*., 2012

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

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

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

[6] 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	*Crassostrea ariakensis*	Suminoe oyster	YES	YES	YES	ND	YES	YES	1	Burreson *et al*., 2004
			YES	YES	YES	ND	YES	YES	1	Dungan *et al*., 2012
Ostreidae	*Ostrea lurida*	Olympia oyster	YES	YES	YES	ND	YES	YES	1	Hill *et al*., 2014
Score 3										
Ostreidae	*Crassostrea gigas*	Pacific cupped oyster	YES	YES	NO	ND	NO	NO	3	Lynch *et al*., 2010
Ostreidae	*Saccostrea glomerata*	Sydney rock oyster	YES	YES	ND	ND	YES	YES[7]	3	Hill *et al*., 2014
			YES	YES	NO	ND	NO	NO	3	Carnegie *et al*., 2014
			YES	YES	NO	ND	NO	NO	3	Spiers *et al*., 2014
Not scored (NS) because pathogen ID was inconclusive										
Mytilidae	*Geukensia demissa*	Ribbed mussel	YES	NO[8]	NO	ND	NO	NO	NS	Laramore *et al*., 2017
Mytilidae	*Brachidontes exustus*	Scorched mussel	YES	NO	NO	ND	NO	NO	NS	Laramore *et al*., 2017
Mytilidae	*Ischadium recurvum*	Hooked mussel	YES	NO	ND	ND	ND	ND	NS	Laramore *et al.*, 2017
Isognomonid	*Isognomon bicolor*	Bicolor purse-oyster	YES	NO	NO	ND	NO	NO	NS	Laramore *et al.*, 2017
Isognomonid	*Isognomon alatus*	Flat tree-oyster	YES	NO	NO	ND	NO	NO	NS	Laramore *et al.*, 2017

[7] 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*.

[8] 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 MOLLUCS SPECIES TO INFECTION WITH OIE LISTED DISEASES

November–December 2020

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

November–December 2020

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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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Back to Agenda

ASSESSMENT OF THE INFECTION WITH INFECTIOUS HYPODERMAL AND HEMATOPOIETIC NECROSIS VIRUS (IHHNV) FOR DELISTING IN THE *AQUATIC CODE*

Overall assessment

The Aquatic Animal Health Standards Commission (hereinafter referred to as the Aquatic Animals Commission) assessed infection with infectious hypodermal and haematopoietic necrosis virus (IHHNV) against the criteria for listing aquatic animal diseases in Article 1.2.2. of the *Aquatic Code*, and agreed that infection with IHHNV meets the listing criteria 1, 2, 3, and 4b (see Table 1 below), and should, therefore, remain listed in Article 1.3.3.

Table 1. Summary of assessment of infection with IHHNV

	Listing criteria						Conclusion
	1	2	3	4a	4b	4c	
IHHN	+	+	+	NA	+	-	The disease meets the criteria for listing

NA = not applicable.

The criteria for the inclusion of a disease in the OIE list are as follows:

1. International spread of the pathogenic agent (via aquatic animals, aquatic animal products, vectors or fomites) is likely.

AND

2. At least one country may demonstrate country or zone freedom from the disease in susceptible aquatic animals, based on provisions of Chapter 1.4.

AND

3. A precise case definition is available and a reliable means of detection and diagnosis exists.

AND

4a. Natural transmission to humans has been proven, and human infection is associated with severe consequences.

OR

4b. The disease has been shown to affect the health of cultured aquatic animals at the level of a country or a zone resulting in significant consequences, e.g. production losses, morbidity or mortality at a zone or country level.

OR

4c. The disease has been shown to, or scientific evidence indicates that it would affect the health of wild resulting in significant consequences, e.g. morbidity or mortality at a population level, reduced productivity or ecological impacts.

Note

In this assessment the term ‘shrimp’ is used for both marine and freshwater species, however, where the term prawn is used in common names of species, e.g. giant tiger prawn, it has been retained.

Background

The first case of hypodermal and haematopoietic necrosis was reported in Hawaii in 1981, where it had caused mass mortalities in blue shrimp (*Penaeus stylirostris*) farmed in super-intensive raceways (Lightner *et al.*, 1983).

Later it was discovered in *P. stylirostris* and white leg shrimp (*Penaeus vannamei)* in America and the Gulf of California (Morales-Covarrubias *et al.*, 1999; Pantoja *et al.*, 1999). Some reports suggested that it might have contributed to the collapse of the *P. stylirostris* fishery in the Gulf of California. IHHNV has also been identified as the cause of 'runt deformity syndrome' (RDS) in *P. vannamei*.

IHHNV is classified with the subfamily Densovirinae of the virus family Parvoviridae. It was listed by the OIE in 1995. IHHNV is the smallest of the known penaeid shrimp viruses (the virion is a 20–22 nm, non-enveloped icosahedron). At least two distinct genotypes of IHHNV have been identified: type 1 from the Americas and East Asia (principally the Philippines) and type 2 from South-East Asia. Two sequences homologous to part of the IHHNV genome are found embedded in the genome of penaeids. The virus is widespread in shrimp production in Asia and Latin America.

Susceptible species listed by the OIE are: yellowleg shrimp (*Penaeus californiensis*), giant tiger prawn (*Penaeus monodon*), northern white shrimp (*Penaeus setiferus*), blue shrimp (*P. stylirostris*), and white leg shrimp (*P. vannamei*). Northern brown shrimp (*Penaeus aztecus*) has incomplete evidence for susceptibility. Several other species have tested PCR positive, but an active infection has not been demonstrated.

Criterion No. 1 International spread of the pathogenic agent (via aquatic animals, aquatic animal products, vectors or fomites) is likely.

Assessment

Marine and freshwater shrimp farming is currently carried out around the globe in at least 60 countries with production about 4,496,775 metric tons (MT) in 2018. The production is mostly concentrated in 15 nations in Asia and Latin America, including China (People's Rep. of), Indonesia, Vietnam, India, Ecuador, Thailand, Mexico, Bangladesh, Philippines, Brazil, Saudi Arabia, Iran (Islamic Republic of), Malaysia, Honduras and Peru (FAO, 2020; GAA, www.aquaculturealliance.org). In 2018, shrimp exports accounted for approximately 15 percent of the total global trade in aquatic animal products by value. Shrimps have historically been one of the most heavily traded aquatic animal products, with major markets located in the United States of America, the European Union and Japan. The China (People's Rep. of) is becoming a new rapid growing market (FAO, 2020).

Transmission of IHHNV can be horizontal or vertical. Horizontal transmission via ingestion of infected tissues or by contaminated water has been demonstrated, as has vertical transmission via contaminated eggs (OIE, 2019).

International trade in species susceptible to IHHNV includes live animals such as shrimp larvae and broodstock, and frozen shrimp products. Trade in these products provides pathways for international spread of IHHNV. Some examples demonstrating international spread, or presence of IHHNV in traded commodities are summarised below.

In 2019, the UK found IHHNV positive cases in imported *P. vannamei* broodstock at two indoor shrimp farms. At one site, no clinical signs or mortality were observed, but at the other site variable growth rates and stunting were observed. The detections were reported to the OIE. The affected animals were imported as free from IHHNV and other pathogens, i.e. they were sold as specific pathogen-free (SPF) post larval shrimp.

In 2019, Canada detected IHHNV in four premises in imported *P. vannamei* without clinical signs and mortality. The detections were reported to the OIE.

In 2015, 329 samples of *P. monodon* imported to China were tested, and 36.8% samples tested positive for IHHNV (Yu *et al.*, 2016). In 2019, samples of frozen *P. vannamei* imported to South Korea were tested and 40% of batches tested positive for IHHNV (Park *et al.*, 2020).

Conclusion

The criterion is met.

Criterion No. 2 At least one country may demonstrate country or *zone* freedom from the *disease* in susceptible *aquatic animals*, based on provisions of Chapter 1.4.

Assessment

New Caledonia self-declared freedom from IHHNV in 2016. The UK has two shrimp farms both of which became infected with IHHNV in 2019 but which have re-established with IHHNV free stock, and the UK is in a position to demonstrate freedom.

OIE-WAHIS data demonstrates that IHHNV occurs in most shrimp producing countries, as shown in the following table. However, countries in the Middle East that are currently producing shrimp (e.g. Saudi Arabia and Iran), or commencing shrimp production (e.g. Oman) may be in a position to claim freedom from IHHNV. Other important shrimp producers, such as Madagascar and Bangladesh have not reported the occurrence of IHHNV.

Table 1. Reporting of IHHNV by country and year (taken from WAHIS)

Region or Country	2016	2017	2018	2019	2020
Africa					
Europe					
UK				**2**	
America					
Brazil	**+..**	**+..**	**+..**	**+..**	
Canada				**1**	
Costa Rica	**3**	**5**			
Ecuador	**38**	**96**	**111**	**31**	
Guatemala	**2**	**2**			
Honduras	**34**	**72**			
Mexico	**346**	**176**	**237**	**516**	
Nicaragua	**37**	**21**	**31**	**37**	
Peru	**5**	**15**			
El Salvador				**6**	
USA				**4**	
Asia					
China (People's Rep. of)		**64**	**69**	**40**	
Chinese Taipei	**26**	**7**	**1**		
India		**12**	**3**	**3**	
Indonesia	**14**	**7**	**4**		
Thailand	**4**	**8**	**2**	**6**	
Philippines	**+..**	**+..**	**+..**	**+..**	
Oceania					
Australia		**2**	**3**	**6**	

Note: the top 15 shrimp producing countries are China (People's Rep. of), Indonesia, Vietnam, India, Ecuador, Thailand, Mexico, Bangladesh, Philippines, Brazil, Saudi Arabia, Iran (Islamic Republic of), Malaysia, Honduras and Peru.

Conclusion

The criterion is met.

Criterion No. 3 A precise case definition is available and a reliable means of detection and diagnosis exists

Assessment

Case definitions for suspicion and confirmation of infection with IHHNV have been developed by the OIE. Reliable conventional PCR (Tang *et al.*, 2007) and real-time PCR assays have been developed for the detection of IHHNV (Dhar *et al.*, 2001).

In recent years, some rapid tests have been developed, such as loop-mediated isothermal amplification (LAMP), modified PCR, recombinase polymerase amplification (RPA) and real-time PCR with higher sensitivity (Cowley *et al.*, 2018; Qian *et al.*, 2018; Xia *et al.*, 2015; Arunrut *et al.*, 2011). These tests have demonstrated utility and could be recommended in the OIE *Aquatic Manual* pending further validation in accordance with the OIE standards.

Conclusion

Criterion is met.

Criterion No. 4a Natural transmission to humans has been proven, and human infection is associated with severe consequences.

Assessment

There is no evidence of transmission to humans.

Conclusion

Criterion not applicable.

Criterion No. 4b The disease has been shown to affect the health of cultured aquatic animals at the level of a country or a zone resulting in significant consequences, e.g. production losses, morbidity or mortality at a zone or country level.

Assessment

Infection with IHHNV is known to have most severe impact in penaeids native to the Americas, *P. stylirostris* and *P. vannamei*. The disease has been reported to be most severe in *P. stylirostris* resulting in high mortality. In *P. vannamei*, infection with IHHNV is known to cause runting and deformities, resulting in significantly reduced crop value (Lightner *et al.*, 1996; Lightner *et al.*, 2011). Of the major commercial species, the disease has been considered to have least impact on *P. monodon* (Withyachumnarknkul *et al.*, 2006).

IHHNV was first described by Lightner *et al.* (1983) who reported mortalities of up to 90% in *P. stylirostris* post-larvae and juveniles. Subsequently, other studies have shown that in populations of *P. stylirostris*, infection with IHHNV results in an acute disease with high mortalities approaching 100% (Lightner *et al.* 1996). IHHNV outbreaks in farmed *P. stylirostris* caused such severe levels of mortality that some farms in Mexico closed permanently while others shifted to cultivating *P. vannamei* (Pantoja *et al.*, 1999). Although the impacts of IHHNV on *P. stylirostris* production are known to have been historically severe, domesticated populations of *P. stylirostris* have been developed which are considered to be tolerant to infection (Tang *et al.*, 2000).

Infection with IHHNV in populations of *P. vannamei* have resulted in a more subtle, chronic disease in which mortalities may not be significant, but where animals show cuticular deformities and reduced, highly disparate growth – a condition known as runt deformity syndrome (RDS) (Kalagayan *et al.*, 1991). Growth retardation has been reported to be greater than 30% (Wyban *et al.*, 1992, cited by Hsieh *et al.*, 2006) and runted animals have lower economic value resulting in significant economic loss (Kalagayan *et al.*, 1991). Infection with IHHNV also interferes with normal egg, larval, and post-larval development (Motte *et al.*, 2003).

The impacts of IHHNV appear to have declined due to the use of specific (i.e. IHHNV) pathogen free shrimp, changing to cultivation of less susceptible species and the breeding of more IHHNV-tolerant shrimp. However, several recent examples demonstrate that IHHNV continues to affect the health of cultured aquatic animals and results in significant production losses. Some of these examples are highlighted below.

In 2019, IHHNV positive cases were detected in imported *P. vannamei* broodstock at two indoor shrimp farms in the UK. At one of these sites, variable growth rates and stunting were observed. The farms were depopulated and decontaminated.

In surveillance of Indian *P. vannamei* farms from 2013 to 2018, 30 farms were found to be positive for IHHNV (Jagadeesan *et al.*, 2019). Animals at these farms exhibited classical IHHNV cuticular deformities and a wide size variation in growth in the affected farms.

Considerable differences in susceptibility to IHHNV infection were found in three batches of *P. vannamei* from different hatcheries in Northern Mexico. The results indicate varying levels of IHHNV resistance in farmed populations, although possible impacts on productivity were not explored (Escobedo-Bonilla *et al.*, 2014).

A recent study in Australia found an association between sustained presence of high level IHHNV infection with reduced growth performance and survival of *P. monodon* reared under simulated commercial conditions (Sellars *et al.*, 2019).

Conclusion

Criterion is met.

Criterion No. 4c The disease has been shown to, or scientific evidence indicates that it would affect the health of wild resulting in significant consequences, e.g. morbidity or mortality at a population level, reduced productivity or ecological impacts.

Assessment

IHHNV was detected in farmed *P. stylirostris* and *P. vannamei* in Mexico in the late 1980s and was later detected in wild *P. stylirostris* populations in the Gulf of California (Morales- Covarrubias *et al.*, 1999). The detection of IHHNV in wild *P. stylirostris* coincided with declines in fishery landings of up to 50% and it has been suggested that IHHNV contributed to the collapse of the fishery (Morales-Covarrubias *et al.*, 1999; Pantoja *et al.*, 1999). Further sampling in 1996 demonstrated high IHHNV prevalence; however, wild populations were recovering (Morales Covarrubias *et al.*, 1999).

IHHNV has been detected in wild populations of other crustacean species. High prevalence of IHHNV was found in wild *P. vannamei* from the Pacific coast of Panama, Ecuador, Colombia and Panama (Nunan *et al.*, 2001; Motte *et al.*, 2003). In the Pacific coast of Mexico, IHHNV was detected in wild shrimp and crabs with 19.5% prevalence rate (Macías-rodríguez *et al.*, 2014). In the East China Sea, IHHNV was detected in wild *P. penicillatus* and at a prevalence of 19.2% in wild *P. vannamei* (Hu, 2015).

Although IHHNV is thought to have impacted wild populations of *P. stylirostris*, definitive evidence of a causative role is not available. However, it is well known that demonstrating the impact of diseases on wild populations of aquatic animals is difficult, except in the most extreme examples where observable mortality occurs (Miller *et al.*, 2014).

Conclusion

Criterion is not met.

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CONSIDERATION OF EMERGING DISEASES – INFECTION WITH CARP EDEMA VIRUS (CEV)

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THE USE OF ENVIRONMENTAL DNA METHODS FOR DETECTION OF OIE LISTED AQUATIC ANIMAL DISEASES

EU comment

We thank the OIE AAHSC for this very useful paper which explores the potential use of eDNA methods with respect to the standards of the Code and the Manual and which outlines their benefits and limitations. Each of the conclusions which are listed in Point 11, have been explored in the paper, are concise and well founded, and can therefore be fully supported by the EU.

A discussion paper developed by the OIE Aquatic Animal Health Standards Commission (Aquatic Animals Commission) for Member comments.

Version: 6 May 2021

1. Summary

The monitoring of aquatic systems using environmental DNA (eDNA) is a rapidly advancing research field that will provide opportunities for cost-effective, non-destructive methods to screen for pathogenic agents, including those of wild aquatic populations where samples may be difficult or undesirable to obtain.

The Aquatic Animals Commission is aware that eDNA methods are being applied for detecting the causative agents of several OIE listed diseases. As these methods are available and currently in use, the Commission has agreed that it would be advisable for guidance to be provided on appropriate application of eDNA methods and potential limitations.

The Commission notes that, as accurate estimates of diagnostic performance are not available for designing surveillance programmes using eDNA assays, data obtained from eDNA methods are unlikely to be suitable to support declarations of freedom from listed diseases. Confirmation of infection with listed diseases could also not be made using eDNA methods because a positive result does not demonstrate that a susceptible host animal(s) is infected.

Positive eDNA results could, however, provide evidence amounting to suspicion of infection. This application of eDNA methods may be particularly useful for the monitoring of high-value or rare animals as an alternative to collection of tissue samples. It has a potential role in early detection of disease incursion in wild populations or under circumstances when infection is not likely to result in observable clinical signs. However, following suspicion, based on positive eDNA, samples obtained directly from aquatic animals need to be tested—as described in the relevant disease-specific chapters of the *Manual of Diagnostic Tests for Aquatic Animals* (*Aquatic Manual*) to confirm or exclude the case.

This document is intended to explore the potential use of eDNA methods with respect to the standards of the OIE *Aquatic Animal Health Code* (*Aquatic Code*) and *Aquatic Manual* and to outline benefits and limitations.

The use of an eDNA method for the detection of *Gyrodactylus salaris* has been proposed for inclusion in the *Aquatic Manual* chapter for Infection with *Gyrodactylus salaris* (see February 2021 Aquatic Animals Commission report, Part A, Annex 9). The inclusion of this method conforms with the conclusions of this discussion paper.

2. Definitions for eDNA

Numerous definitions for eDNA exist (e.g. Díaz-Ferguson and Moyer, 2014; Bass *et al*., 2015; Thomsen and Willerslev, 2015). Most definitions regard eDNA as detectable short DNA fragments from a living organism derived from cellular components or fluids secreted into the abiotic components of surrounding environment (i.e. water, air, sediments).

For the purposes of this document we define eDNA as: "nucleic acids extracted from 'true' environmental samples (such as water, soil, sediment, biofilm)". Directly host-derived material such as faeces, sloughed cells, and mucous, are excluded from this definition. Once extracted from the environmental sample, target eDNA fragments can be detected using a variety of molecular methods (Díaz-Ferguson and Moyer, 2014). Furthermore, eDNA can be sequenced directly as metagenetic libraries or after PCR amplification of specific target gene regions (Bass *et al*., 2015).

The actual performance of eDNA based detection depends on the sample collection and processing methodology (e.g. volume filtered, presence and removal of PCR inhibitors), biological processes (e.g. rates of shedding, temporal variation) and abiotic factors (analyte degradation, hydrodynamic factors). It is important to evaluate these factors empirically so that the results can be properly interpreted. It is only with a clear understanding of how these factors influence the probability of pathogenic agent detection that eDNA-based detection can be used reliably in a variety of settings (Brunner, 2020).

3. Objectives

This paper considers i) the benefits and ii) limitations of eDNA pathogenic agent detection methods, iii) validation of eDNA methods, iv) the conditions for inclusion of an eDNA method in a disease-specific chapter of the *Aquatic Manual* and v) use of eDNA evidence as diagnostic criteria.

4. Review of published eDNA methods for the detection of aquatic animal pathogenic agents

A literature review was undertaken to assess the application of eDNA methods for the detection and study of pathogens and parasites of aquatic animals. Thirty-three publications reporting the use of eDNA to detect thirteen OIE listed pathogenic agents were identified (see Appendix 1, Table 1 for details). Methods have been developed for the detection of the causative agents of OIE listed pathogenic agents of amphibians, crustaceans, fish and molluscs. The majority of publications concern the detection of the listed pathogenic agents in wild aquatic animal populations, notably infection with *Aphanomyces astaci*, infection with *Batrachochytrium dendrobatidis*, infection with *B. salamandrivorans*, infection with *Ranavirus* species, infection with *G. salaris*.

A further thirteen publications were found that targeted other specific pathogenic agents (e.g. *Microcytos mackini*), groups of pathogenic agents (e.g. of ornamental fish) or applied eDNA methods to broader areas of study (e.g. water-borne transmission of viruses) (see Appendix 1, Table 2 for details).

5. Benefits eDNA methods for the detection of aquatic animal pathogenic agents

eDNA detection is a promising tool that can be used to complement direct sampling of aquatic animals for surveillance. eDNA methods offer some benefits compared to direct sampling and testing of aquatic animals, including, but not limited, to the following:

1. eDNA methods do not require destructive sampling of aquatic animal hosts. They may be particularly useful for rare or valuable aquatic animals, or difficult to collect wild animals (e.g. Rusch *et al*., 2018).

2. eDNA methods do not require handling of animals, avoiding the stress that associated with obtaining non-destructive tissue samples (Brunner, 2020).

3. Sample collection and sample processing time and associated costs may be reduced substantially compared to collection and processing of individual animal samples (Rusch *et al*., 2018).

4. As environmental samples may contain analyte from the entire, or a large percentage of a target captive population, many fewer samples may be required to detect a pathogenic agent (compared to individual animal samples), even when diagnostic sensitivity of the eDNA method is low (Brunner, 2020).

5. The same environmental sample can be analysed for the presence of hosts (e.g. see Rusch *et al*., 2018) and multiple pathogens.

6. Limitations of eDNA methods

Limitations to the application of eDNA based pathogenic agent detection include, but are not limited to, the following:

1. Very little target DNA may be available in the environmental sample due to dilution in the environment and degradation of nucleic acids. This may negatively impact the sensitivity of the method (Brunner, 2020).

2. The concentration of target DNA in an environmental sample will vary due a range of factors such as host density, prevalence and intensity of infection, sampling method (e.g. for water—volume sampled, filter pore size, storage conditions) and environmental conditions (e.g. amount of organic matter). Sensitivity of eDNA methods may, therefore, vary more between localities, surveys undertaken at different time points and target taxa than direct sampling and testing of animal tissues (Brunner, 2020).

3. There are formal frameworks to assess diagnostic performance of tests using animal-derived samples, but these have not been developed for eDNA methods. This means that the design of surveys to demonstrate freedom from infection using eDNA methods is problematic.

4. A positive detection of target pathogen DNA in an environmental sample may be more likely to result from a source of contamination compared to animal-derived samples. Similarly, it may not indicate infection of a host animal with the target pathogenic agent.

7. Validation of eDNA methods

There is an increasing likelihood that disease management decisions will be made based on results from eDNA studies. It is thus imperative that data generated by eDNA studies is reliable, defendable and executed with high quality assurance standards (Klymus *et al*., 2019). Empirical validation of eDNA based pathogen detection should focus on understanding the causes and consequences of variation in test characteristics across sampling conditions.

Chapter 1.1.2. of the *Aquatic Manual* describes the principles and methods of validation of diagnostic assays for infectious diseases. The recommendations of this chapter are intended for diagnostic testing of animal-derived samples; however, the principles and many of the methods are applicable to eDNA methods. It is recommended that the principles and methods of Chapter 1.1.2. be applied to the validation of eDNA detection methods for OIE listed diseases in all cases where they are applicable.

Design and reporting standards are available for diagnostic accuracy studies for methods utilising aquatic animal-derived samples (e.g. Laurin *et al*., 2018). Many of the design and reporting considerations are also applicable to eDNA methods and it is recommended that these standards be applied for eDNA diagnostic accuracy studies.

Additional to the guidance described above, design and reporting considerations have been published specifically for eDNA methods (e.g. Doyle and Uthicke, 2020; Goldberg *et al*., 2016; Klymus *et al*., 2019). Many of these studies report on considerations for detection of macro-organisms rather than pathogenic agents; however, the considerations are generally relevant for eDNA detection methods for pathogenic agents. This guidance will be of particular use for the field collection, processing and preservation of eDNA samples.

8. Minimum requirements for inclusion of an eDNA method in the *Aquatic Manual*

It is recognised that the validation pathway described in Chapter 1.1.2. of the *Aquatic Manual* and the design and reporting standards described by Laurin *et al*., 2018 (see above) are not met by many diagnostic methods currently included in the *Aquatic Manual*. Indeed, many assays included in the *Aquatic Manual* may be validated only to level 1 or 2 of the validation pathway described in Chapter 1.1.2. of the *Aquatic Manual*. For this reason, the Commission proposes that the following minimum reporting requirements be met for an eDNA method to be considered for inclusion in the *Aquatic Manual* [Adapted from Goldberg *et al*., (2016)]:

1. The intended purpose or application of the assay or protocol need to be clearly defined (note that appropriate purposes of use for eDNA methods in the context of OIE standards are discussed further in section 9).

2. Description of sample collection methods and precautions taken to eliminate contamination, including collection volume, container material, negative controls, number of replicates and sampling locations/depth.

3. Description of the methods used to concentrate the target DNA (precipitation / filtration), filter type (if applicable) and filtering location (e.g. in the field).

4. Description of sample preservation and storage (method, temperature, duration).

5. Description of the DNA extraction process including protocol adjustments, contamination precautions, negative controls, and internal positive controls.

6. Description of the qPCR design and optimisation according to (Bustin *et al*., 2009). Furthermore, real time PCR assays should be validated (Level 1) in an environmental matrix according to its purpose of use.

9. Potential application of eDNA detection methods in the disease-specific chapters of the *Aquatic Manual*

The disease-specific chapters of the *Aquatic Manual* recommend tests to identify suspect cases and to confirm suspicion for apparently healthy (or those of unknown health status) and clinically affected animals. Apparently healthy populations may fall under suspicion, and therefore be sampled, if there is an epidemiological link(s) to an infected population. Geographic proximity to, or movement of aquatic animals or aquatic animal products or equipment, etc., from a known infected population equate to an epidemiological link. Alternatively, healthy populations are sampled in surveys to demonstrate freedom.

The following points describe the suitability of evidence from eDNA detection methods for inclusion as case definition criteria in section 6 of the disease-specific chapters of the *Aquatic Manual.*

a) Apparently healthy animals

i) Definition of suspect case in a population of apparently healthy animals

Suitable as a criterion. A positive result obtained from an eDNA method recommended in the *Aquatic Manual* is considered to provide adequate evidence to be included as a criterion for a suspect case.

ii) Definition of confirmed case in apparently healthy animals

Not suitable as a criterion. A positive result obtained from an eDNA method recommended in the *Aquatic Manual* is not considered to provide appropriate evidence to confirm a case in apparently healthy animals. Methods utilising animal derived samples are considered more appropriate for criteria to confirm a case. Evidence to confirm a case in apparently healthy animals must meet the requirements of Section 6.1.2. of the relevant disease-specific chapter of the *Aquatic Manual*. eDNA evidence will not be included as a criterion within this section.

b) Clinically affected animals

i) Definition of a suspect case in clinically affected animals

Suitable as a criterion. Taking an environmental sample to investigate the cause of disease in a population of clinically affected animals is not generally recommended as samples from clinically affected animals are more likely to lead to pathogenic agent detection and are more suitable for disease investigation. However, under some circumstances, an eDNA method may detect a pathogenic agent and lead to the recognition of previously unobserved clinical signs of disease. In these circumstances,

a positive result obtained from an eDNA method recommended in the *Aquatic Manual* is considered to provide adequate evidence to be included as a criterion for a suspect case.

ii) Definition of confirmed case

Not suitable as a criterion. A positive result from an eDNA method recommended in the *Aquatic Manual* would not be included as a criterion for the confirmation of a pathogenic agent in clinically affected animals (or apparently healthy animals, see point (a)(ii) above). Any positive eDNA test would require further investigation involving the collection and testing of animal tissues as stipulated in the relevant disease-specific chapter of the *Aquatic Manual*. Evidence to confirm a case in clinically affected animals must meet the requirements of Section 6.2.2. of the relevant disease-specific chapter of the *Aquatic Manual*. eDNA evidence will not be included as a criterion within this section.

10. Discussion

A country or zone claiming freedom from a specified pathogenic agent(s) are required to have in place an early detection system for disease incursion. Farmer reporting of morbidity and mortality is a key component of an early detection system. Farmed populations can act as sentinels for wild populations only if they are epidemiologically connected (i.e. through shared water). Otherwise active surveillance in wild populations is required as morbidity or mortality is unlikely to be reported (especially as dead or dying animals are likely to be quickly scavenged or predated). Animal sampling of wild populations can present considerable logistical challenges, especially if populations are remote, sparse or if low numbers make destructive sampling undesirable. eDNA based pathogenic agent detection methods overcome many of the challenges of sampling wild aquatic animals (Kamoroff and Goldberg, 2017; Trebitz *et al*., 2017).

Infection with some listed pathogenic agents, under certain conditions or in some host species, will not invariably cause detectable clinical signs. Early detection systems that rely on observations by farmers (or others) of mortality or morbidity are ineffective in these circumstances and active surveillance would be required. Sampling farmed animals on a frequent basis, and at a level to detect a low prevalence, presents considerable logistical challenges and the cost is likely to be unacceptable. eDNA methods can offer a viable alternative (Trujillo-González *et al*., 2019a) for active surveillance for pathogens which may not reliably cause observable clinical signs. They have the additional advantage that the sample will contain analyte from a large percentage, if not the entire, captive population. Thus relatively few environmental, compared with animal samples, are needed (provided sufficient DNA can be extracted).

Farms may be routinely left fallow at the end of a production cycle, or following destocking as part of a disease control programme. The results from testing eDNA taken during the fallow period can support decisions about when to restock. eDNA, therefore, provides a more economical and practical alternative to the stocking of sentinel animals to demonstrate elimination of the pathogenic agent.

The key limitations of eDNA is the lack of validation and diagnostic performance data, meaning that negative results cannot be used to demonstrate disease freedom and positive results always require confirmation using animal samples (Brunner, 2020). Nevertheless, there are circumstances where the advantages of environmental, over animal, sampling means that eDNA approaches can be usefully integrated into a surveillance programme.

11. Conclusions

1. eDNA methods may have utility for enhancing passive surveillance systems for early detection; particularly in cases where conditions are not conducive to clinical expression of disease, or populations are not under sufficient observation to detect clinical disease should it occur.

2. eDNA methods may have utility for rare, valuable or difficult to collect wild aquatic animals, where direct sampling of animals is undesirable or cost prohibitive. They may also provide cost advantages for disease monitoring programs in production environments.

3. There are currently no frameworks to allow evaluation of diagnostic performance of eDNA methods in a manner similar to animal-derived samples. For this reason, evidence from eDNA detection methods cannot be utilised as evidence for self-declaration of freedom from disease.

4. eDNA methods will be considered for inclusion in disease-specific chapters of the *Aquatic Manual*, if minimum disease and reporting standards as described in this paper are met.

5. Positive results from eDNA methods that has been included in the *Aquatic Manual* will be considered as an appropriate criterion for a suspect case of a disease.

6. Positive results from an eDNA methods that has been included in the *Aquatic Manual* will not be considered as an appropriate criterion for a confirmed case of a disease in either apparently healthy or clinically affected animals.

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Appendix 1. Publications describing eDNA methods for aquatic animal pathogenic agents

Table 1. Published applications of eDNA methods for the detection of OIE listed pathogenic agents of aquatic animals.

OIE LISTED DISEASE	PUBLICATION
Amphibian Diseases	
Infection with *Batrachochytrium dendrobatidis*	Walker *et al*., 2007; Pierson and Horner, 2016, Kamoroff and Goldberg, 2017; Mosher *et al*., 2017, Julian *et al*., 2019; Brannelly *et al*., 2020
Infection with *Batrachochytrium salamandrivorans*	Spitzen - van der Sluijs *et al*., 2020; Brunner, 2020
Infection with Ranavirus species	Hall *et al*., 2016; Pierson and Horner, 2016; Julian *et al*., 2019; Miaud *et al*., 2019; Vilaça *et al*., 2020
Fish Diseases	
Infection with *Gyrodactylus salaris*	Rusch *et al*., 2018; Fossøy *et al*., 2020
Infection with HPR-deleted or HPRO infectious salmon anaemia virus	Gregory *et al*., 2009
Infection with koi herpesvirus	Haramoto *et al*., 2007; Honjo *et al*., 2010 and 2012
Infection with salmonid alphavirus	Bernhardt *et al*., 2020; Weli *et al*., 2021
Crustacean Diseases	
Acute hepatopancreatic necrosis disease	Kongrueng *et al*., 2015
Infection with *Aphanomyces astaci* (crayfish plague)	Strand *et al*., 2011 and 2014; Vrålstad *et al*., 2016; Robinson *et al*., 2018; Wittwer *et al*., 2018a and 2018b; Rusch *et al*, 2020
Infection with white spot syndrome virus	Natividad *et al*., 2008; Quang *et al*., 2009
Mollusc Diseases	
Infection with *Bonamia ostreae*	Jørgensen *et al*., 2020
Infection with *Perkinsus marinus*	Audemard *et al*., 2004
Infection with *Xenohaliotis californiensis*	Lafferty & Ben-Horin, 2013

Table 2. Published eDNA studies of pathogenic agents of aquatic animals not listed by the OIE

SUBJECT	PUBLICATION
Ornamental fish parasite detection	Trujillo-González *et al*., 2019b and 2019a
Parasitology	Bass *et al*., 2015
Protozoan parasite outbreaks in fish farms	Bastos Gomes *et al*. 2017 and 2019
Disease transmission in open water Salmon cages	Salama and Rabe, 2013
Emerging aquatic parasites	Sana *et al*., 2018
Pathogenic microbes in bait	Mahon *et al*., 2018
Waterborne virus detection	Oidtmann *et al*., 2018

Halioticida noduliformans in lobsters	Holt *et al*., 2018
Microcytos mackini	Polinski *et al*., 2017
Trematode parasite *Ribieroia ondatrae*	Huver *et al*., 2015
Schistosoma species	Alzaylaee *et al*., 2020

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CHAPTER 2.4.2.

INFECTION WITH *BONAMIA EXITIOSA*

EU comment

The EU supports the proposed changes to this chapter.

[…]

2.2. Host factors

2.2.1. Susceptible host species

~~Oyster species *Ostrea chilensis* (= *Tiostrea chilensis* = *T. lutaria*) (Dinamani et al., 1987), *O. angasi* (Corbeil et al., 2006b; Hine, 1996; Hine & Jones, 1994), *O. edulis* (Abollo et al., 2008; Narcisi et al., 2010) and *O. stentina* (Hill et al., 2010).~~

Species that fulfil the criteria for listing as susceptible to infection with *Bonamia exitiosa* according to Chapter 1.5. of the *Aquatic Animal Health Code* (*Aquatic Code*) are: Argentinean flat oyster (*Ostrea puelchana*), Australian mud oyster *(Ostrea angasi),* Chilean flat oyster *(Ostrea chilensis),* Dwarf oyster *(Ostrea stentina),* Eastern oyster *(Crassostrea virginica),* European flat oyster *(Ostrea edulis),* Olympia oyster *(Ostrea lurida)* and Suminoe oyster *(Crassostrea ariakensis)*

2.2.2. ~~Susceptible stages of the host~~ Species with incomplete evidence for susceptibility

~~In *O. chilensis*, recruit-sized oysters (oysters greater than or equal to 58 mm in length) are known to be susceptible (Dinamani et al., 1987). In *O. edulis*, the parasite was detected in market sized (>60 mm) oysters (Abollo et al., 2008). There are no data concerning the other oyster stages, including spat.~~

~~DNA of *B. exitiosa* has recently been detected in larvae of flat oysters *Ostrea edulis* (Arzul et al., 2011).~~

Species for which there is incomplete evidence to fulfil the criteria for listing as susceptible to infection with *B. exitiosa* according to Chapter 1.5 of the *Aquatic Code* are: none known

In addition, pathogen-specific positive polymerase chain reaction (PCR) results have been reported in the following species, but no active infection has been demonstrated: Pacific cupped oyster (*Crassostrea gigas*) and Sydney rock oyster (*Saccostrea glomerata*).

[…]

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