Ref. Ares(2024)3681938 - 22/05/2024

Report of the Meeting of WOAH Aquatic Animal Health Standards Commission

Annex 2

Original: English (EN)

14 to 21 February 2024

EU position

The EU would like to commend WOAH for its work and thank in particular the Aquatic Animals Commission for having taken into consideration EU comments on the Aquatic Code and the Aquatic Manual submitted previously.

A number of general comments on this report of the February 2024 meeting of the Aquatic Animals Commission as well as the intended positions of the EU on the draft Aquatic Code and Manual chapters proposed for adoption at the 91st WOAH General Session 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 once again its continued commitment to participate in the work of WOAH and to offer all technical support needed by the Aquatic Animals Commission and WOAH ad hoc groups for future work on the Aquatic Code and Manual.

Introduction and Member contribution

This report presents the work of the WOAH Aquatic Animal Health Standards Commission (hereinafter 'the Aquatic Animals Commission') who met in Paris, France from 14 to 21 February 2024.

The Aquatic Animals Commission wished to thank the following Members for providing written comments for the WOAH *Aquatic Animal Health Code* (hereinafter 'the *Aquatic Code*') and WOAH *Manual of Diagnostic Tests for Aquatic Animals* (hereinafter 'the *Aquatic Manual*'): Australia, Canada, Chile, China (People's Rep. of), Chinese Taipei, Japan, Korea (Rep. of), New Caledonia, New Zealand, Norway, Peru, Singapore, Switzerland, Thailand, the United Kingdom (UK), the United States of America (USA), the African Union - Interafrican Bureau for Animal Resources (AU-IBAR), the Members of the WOAH Americas Region (the Americas), and the Member States of the European Union (EU). The Commission also wished to acknowledge the valuable advice and contributions from numerous experts of the WOAH scientific network.

The Aquatic Animals Commission reviewed all comments that were submitted prior to the deadline and were supported by a rationale. Due to the large number of comments, the Commission was not able to provide a detailed explanation of the reasons for accepting or not each of the comments considered and focused its explanations on issued deemed significant. Where amendments were of an editorial nature, no explanatory text has been provided. The Commission wished to note that when texts proposed by Members to improve clarity were not accepted; it considered the text was clear as currently written. The Commission made amendments to draft texts, where relevant, in the usual manner by 'double underline' and 'strikethrough'. In relevant annexes, amendments proposed at this meeting are highlighted in yellow to distinguish them from those made previously.

Annexes

Texts in Annexes 4 to 6, 8 to 39 and 51 to 59 will be proposed for adoption at the 91st General Session in May 2024.

Texts in Annexes 40 to 49 and 60 to 61 are presented for comment.

Texts in Annexes 7 and 50 are presented for information only.

World Organisation for Animal Health
Founded as OIE

Standards Department
[ACC.Secretariat@woah.org]

12, rue de Prony
75017 Paris, France

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

How to submit comments

The Aquatic Animals Commission strongly encourages WOAH Members and International Organisations with a WOAH Cooperation Agreement to participate in the development of WOAH International Standards by submitting comments on relevant annexes of this report.

Engagement of Members and International Organisations in the standard-setting process through the submission of comments is critical to ensure the standards are science based, take into consideration the different contexts among Members and stakeholders, and can be implemented by Members. To ensure that comments are considered, they should be submitted by the deadline and in the format described in the guidance and SOP documents available on the Delegate's website and the WOAH public website.

Comments that are not correctly formatted as described in the guidance document may not be considered by the Commission. Any questions on the requirements for formatting and submission of comments should be sent to AAC.Secretariat@woah.org

The Aquatic Animals Commission wished to highlight that when a Commission discussion is based on the input of an *ad hoc* Group, Members are encouraged to review the relevant *ad hoc* Group report together with the report of the Commission. *Ad hoc* Group reports are available on the dedicated webpages on the WOAH website at https://www.woah.org/en/what-we-do/standards/standards-setting-process/ad-hoc-groups/

Deadline for comments

Comments on relevant texts in this report must be received by 5 July 2024 to be considered by the Aquatic Animals Commission.

Where to send comments

All comments should be sent to AAC.Secretariat@woah.org

Date of the next meeting

The Aquatic Animals Commission noted that the dates for its next meeting will be confirmed following the election of the Members of the Specialist Commissions at the 91st General Session in May 2024.

Table of Contents

List of Annexes

1. Welcome and updates from WOAH Headquarters

1.1. WOAH Director General and Deputy Director General, International Standards and Science

Dr Monique Eloit, WOAH Director General and Dr Montserrat Arroyo, WOAH Deputy Director General, International Standards and Science, met with the Aquatic Animals Commission, Scientific Commission for Animal Diseases and the Terrestrial Animal Health Standards Commission on 14 February 2024, to welcome all Commission members and thank them for their ongoing contributions to the work of WOAH. Dr Eloit thanked the Commission members for their hard work throughout this term and the tremendous amount of work achieved. She acknowledged that this was the last meeting of the current term for each of the Specialist Commissions and wished all well, whether standing for re-election or stepping down.

Dr Eloit provided updates on the selection process for election to the four Specialist Commissions and the review of the WOAH's *Basic Texts* that will be presented to the World Assembly at the 91st General Session in May 2024. Dr Eloit highlighted there will be a global focus on antimicrobial resistance (AMR) throughout 2024, including a UN General Assembly high-level meeting in September 2024 to highlight the global public health threat of AMR, and that WOAH will continue to participate actively in these fora and discussions on AMR.

Dr Arroyo recognised the work of each of the three Commissions present throughout this term, and provided an overview of key accomplishments, and commended them on their commitment to this work.

Dr Arroyo provided a brief update on a number of horizontal topics, including the WOAH Standards Online Navigation Tool project, the decision to freeze Diagnostic Kit Register activities, General Session kiosk topics, work to coordinate the WOAH standard-setting processes, and the publication of comments.

Dr Arroyo thanked the Commission Presidents for agreeing to deliver pre-General Session webinars again this year and emphasised that they are an important contribution to the engagement of Members and partners in the standard-setting process. Dr Arroyo noted that the pre-General Session webinars will be held on 16 April, 17 April and 18 April from 12:00 – 14:00 (CEST) for the Biological Code Commission, the Terrestrial Animal Health Standards Code Commission and the Aquatic Animals Commission, respectively. The webinars will have simultaneous interpretation into French and Spanish and will be recorded and uploaded onto the WOAH website.

Commission members thanked Dr Eloit and Dr Arroyo for their appreciation and these updates, and for their leadership and support throughout the current term. Members also acknowledged the important work of the Secretariats in support of their work.

1.2. Updates from WOAH Headquarters

1.2.1. Transparency of the WOAH process for the elaboration of Standards

The Secretariat updated the Aquatic Animals Commission on progress that had been made to improve the transparency of the WOAH process for the elaboration of Standards, in particular the publication of comments submitted by Members and partners.

The Secretariat informed the Commission that the Director General communicated this initiative to Members in December 2023 and that a Standard Operating Procedure (SOP) had been developed for the submission of comments during the process for the elaboration of WOAH international standards, as well as a guide on how to submit and present comments, and that these documents have been published on the WOAH website and on the Delegates' website.

The Secretariat reminded the Commission that this is a progressive process, that will start in March/April 2024 with the publication on the Delegates' website of comments considered on new and revised standards during February 2024 Commission meetings, at the same time as the publication of the respective February 2024 Commission report. This process takes a step-wise

approach and includes an evolution of the Commission reports towards transparency of comments considered and Commission responses, which will result in better documentation and traceability of the WOAH process for the elaboration of Standards.

1.2.2. WOAH Standards Online navigation tool

The Aquatic Animals Commission was updated on the WOAH Standards Online navigation tool project, which is aimed at providing users with streamlined access and navigation of WOAH Standards.

The project will deliver three user interfaces:

- Navigation and search tool; this interface which will provide a guided navigation experience that will allow users to navigate through the WOAH International Standards.
- Recommendations for safe international trade, by commodity; this interface will enable users to visualise recommendations for safe international trade by commodity through a comprehensive filtering system.
- Management of Standards; this interface will enable WOAH staff to efficiently manage and update standards, following adoption of new or revised text at the WOAH General Assembly.

The tool will be demonstrated at a kiosk at the 91st General Session in May 2024 and projected to go 'live' in July 2024.

This project represents a significant milestone in WOAH's commitment to enhance access and utilisation of WOAH standards and contributes to the objectives of the 7th Strategic Plan to implement digital transformation, respond to Members' needs and improve WOAHs efficiency and agility.

2. Adoption of the agenda

The draft agenda was adopted by the Aquatic Animals Commission. The agenda and the list of participants are attached as Annex 1 and Annex 2 respectively.

3. Cooperation with the Biological Standards Commission

The Bureaux of the Aquatic Animals Commission and the Biological Standards Commission met on 15 February 2024 to discuss areas of common interest which are reported below.

3.1. Reference Centres

The Bureaux of the Commissions discussed several items relevant to reference centres, including:

- A modified curriculum vitae template to promote consistency among nominations of replacement experts and prevent delays caused by a lack of relevant details. The template can be found in Annex 17 of the report of the meeting of the Biological Standards Commission and will be implemented after the General Session in May 2024.
- The Biological Standards Commission proposed a procedure on how it will evaluate the terrestrial Collaborating Centres at the end of their 5-year mandate. The Bureaux agreed to some minor amendments to improve efficiency of the process.
- The Aquatic Animals Commission was considering approaches to enhance communication and engagement with reference centre networks, and would share this work once it was further advanced.

3.2. *Aquatic Manual* and *Terrestrial Manual*

The Bureaux were updated on activities concerning test validation in the *Aquatic Manual* and *Terrestrial Manual*, including:

- The Bureau of the Biological Standards Commission provided an update on the draft chapter concerning the diagnostic validation of point-of-care tests (POCTs) for WOAH-listed viral diseases using field samples. The chapter was reviewed by the Reference Laboratory networks. The networks agree with the principle of publishing information on validation of POCTs, whether as a stand-alone chapter or part of Chapter 1.1.6. or disease-specific chapters, but felt the text needed further development to improve its practicality and applicability. The comments would be submitted to the Collaborating Centre expert who had drafted the text to decide on the best way forward.

- The Bureau of the Biological Standards Commission presented an update on the integration of a new section in the disease-specific chapters, detailing the reasoning behind the selection and scoring of tests in Table 1. 'Test methods available and their purpose'. The Bureau of the Aquatic Animals Commission explained that the information on the intended purpose of use and the extent of validation of Table 4.1 'WOAH recommended diagnostic methods and their level of validation for surveillance of apparently healthy animals and investigation of clinically affected animals' of the disease-specific chapters of the *Aquatic Manual* had also been updated.

- The Aquatic Animals Commission had adapted the template for validation reports initially developed by the Biological Standards Commission. The intention is to use the template to provide validation data for consideration by the Aquatic Animals Commission for tests proposed for inclusion in the *Aquatic Manual* before publication of the data in a peer-reviewed journal. For the Bureau of the Biological Standards Commission the intention is that Reference Laboratory experts contributing to *Terrestrial Manual* chapters will be invited to use it to post their validation data online. In this way a repository of validation reports for recommended tests will be created for anyone seeking the validation data available for a given test. Both Commission Bureaux intend to make the validation report template available on their specific WOAH Specialist Commission webpages.

4. Work plan and priorities

Comments were received from Canada, New Caledonia, Norway, the UK, and the EU.

The Aquatic Animals Commission reviewed comments received and noted support for the work plan.

The Commission acknowledged comments on the structure of the *Aquatic Code*, and the potential for alignment with the *Terrestrial Code*. The Commission noted that under the Aquatic Animal Health Strategy there is a planned activity to review the structure of the *Aquatic Code* to make it more logical for users. Through this review process areas for improvement may be determined.

The Commission noted a comment on the work plan proposing work on Chapter 2.2.4. 'Infection with IHHNV', and they agreed to consider this at its September 2024 meeting.

A Member had asked whether the disease-specific chapters of the *Aquatic Manual* could be translated into French. WOAH will explore with Members the possibility of mobilising resources for these translations.

The Commission reviewed the status of ongoing items on its work plan and agreed on required actions at the next Commission meeting in September 2024. The Commission noted that the new Commission, elected at the May 2024 General Session, would need to consider a forward work plan for its three-year term. The Commission discussed some possible areas of focus, that the new Commission may wish to consider including: completion of existing work (adoption of new *Aquatic Code* chapters on disease outbreak preparedness and response, trade of ornamental aquatic animals, trade of fish genetic materials, and the application of compartmentalisation; finalisation of assessment of susceptible species; and updating the remaining *Aquatic Manual* chapters to reflect the new template) and the accessibility and usability of the Standards.

The Commission noted that the progression of work plan items that were contingent on the convening of *ad hoc* Groups were anticipated to progress as planned for 2024. The list of current and planned *ad hoc* Groups for 2024 are available on the WOAH website.

The updated work plan (to September 2024) is presented as Annex 3 for comments.

5. Aquatic Animal Health Strategy

The Aquatic Animals Commission was informed of the key milestones and achievements of the Aquatic Animal Health Strategy since the last update in September 2024, new activities underway, communication initiatives and key priorities for 2024. The Commission heard proposals for planned activities including reviewing the scientific basis of existing aquatic animal welfare standards, improving the engagement of Reference Laboratories and Collaborative Centres, and increasing the accessibility of standards.

The WOAH Aquatic Animal Health Code

6. Texts that will be proposed for adoption in May 2024

6.1. Usage of glossary definitions: 'Aquatic Animal Health Services', 'Competent Authority', and 'Veterinary Authority'

Comments were received from Canada, Norway, Peru, the UK, AU-IBAR, and the EU.

Background

At the 89th General Session, in May 2022, revised Glossary definitions for 'Aquatic Animal Health Services', 'Competent Authority' and 'Veterinary Authority' in the *Aquatic Code* were adopted. The revision of these definitions was undertaken by the Aquatic Animals Commission in coordination with the Terrestrial Animal Health Standards Commission.

At its September 2022 meeting, the two Commissions agreed to coordinate work to revise the use of these definitions in the *Aquatic Code* and *Terrestrial Code*, respectively, to ensure consistency, where relevant.

At its February 2023 meeting, the Aquatic Animals Commission reviewed every occurrence of 'Aquatic Animal Health Services', 'Competent Authority' and 'Veterinary Authority' in the *Aquatic Code*.

At its September 2023 meeting, the Commission reviewed comments received and noted that Members were generally supportive of the proposed changes with some edits.

The proposed amendments have been circulated twice for comments.

Previous Commission reports where this item was discussed

September 2022 report (Item 6.1., page 12); February 2023 report (Item 8.1., page 17); September 2023 report (Item 6.1., page 9).

February 2024 meeting

The Aquatic Animals Commission noted that Members were supportive of the proposed amendments and did not propose any additional amendments.

In response to a comment the Commission wished to highlight that the 'Veterinary Authority' is a 'Competent Authority'. The definition of a 'Competent Authority' recognises that, in many countries, more than one governmental authority is responsible for implementing standards of the *Aquatic Code*. The term 'Competent Authority' is intended to apply to any governmental authority with some responsibility for the implementation of some WOAH standards. The term 'Veterinary Authority' distinguishes the role

of a single 'Competent Authority' that has responsibility for communicating with WOAH and an overarching responsibility for implementation of WOAH standards. The Commission did not propose any additional amendments.

The proposed amendments to the usage of 'Aquatic Animal Health Services', 'Competent Authority' and 'Veterinary Authority' throughout the *Aquatic Code* are presented as Annex 4 and will be proposed for adoption at the 91st General Session in May 2024.

6.2. Usage of glossary definition: 'aquatic animal products'

Comments were received from Canada, Norway, Peru, the UK, AU-IBAR, and the EU.

Background

At its September 2023 meeting, the Aquatic Animals Commission noted that some occurrences of 'products of aquatic animal origin' should be replaced by the glossary term 'aquatic animal products'. The Commission agreed to revise the relevant text to ensure that the glossary term 'aquatic animal product' is used correctly throughout the *Aquatic Code*.

The proposed amendments have been circulated once for comments.

Previous Commission report where this item was discussed

September 2023 report (Item 6.2., page 9).

February 2024 meeting

The Aquatic Animals Commission noted that Members were supportive of the proposed changes and did not propose any additional amendments.

The proposed amendments for the usage of 'aquatic animal products' are presented as Annex 5 and will be proposed for adoption at the 91st General Session in May 2024.

6.3. Article 1.1.5. of Chapter 1.1. 'Notification of diseases and provision of epidemiological information'

Comments were received from Canada, Norway, Peru, the UK, the USA, AU-IBAR, and the EU.

Background

At its February 2019 meeting, the Terrestrial Animals Commission agreed to remove Article 1.1.5. from the *Terrestrial Code* as it considered that the information was addressed in Chapter 1.6. 'Procedures for official recognition of animal health status'. The amendment of Chapter 1.1. of the *Terrestrial Code,* removing Article 1.1.5., was adopted in May 2021.

At its February 2023 meeting, the Aquatic Animals Commission agreed that the requirements in Article 1.1.5. of Chapter 1.1. 'Notification of diseases and provision of epidemiological information' were now addressed in the revised Chapter 1.4. 'Aquatic animal disease surveillance' which was adopted in May 2022. The Commission therefore agreed to delete Article 1.1.5. to remove duplications within the *Aquatic Code* and to ensure alignment with Chapter 1.1. 'Notification of diseases and provision of epidemiological information' of the *Terrestrial Code*.

At its September 2023 meeting, the Commission reviewed comments and did not propose any additional amendments.

The revised article has been circulated twice for comments.

Previous Commission report where this item was discussed

September 2023 report (Item 6.3., page 10), February 2023 report (Item 8.2., page 19).

February 2024 meeting

The Aquatic Animals Commission noted that Members were supportive of the proposed changes and did not propose any additional amendments.

In response to a comment the Commission did not agree to specify that the notification of listed diseases is to be made to WOAH in point 1 of Article 1.1.5.; this is because the glossary term 'notification' includes informing 'headquarters' which is defined as WOAH.

The revised Article 1.1.5. of Chapter 1.1. 'Notification of diseases and provision of epidemiological information' is presented as Annex 6 and will be proposed for adoption at the 91st General Session in May 2024.

6.4. Article 1.3.1. of Chapter 1.3. 'Diseases listed by WOAH'

Comments were received from Australia, Canada, China (People's Rep. of), Japan, Norway, Peru, Thailand, the UK, the USA, AU-IBAR, Members of the WOAH Americas Region, and the EU.

Background

At its February 2022 meeting, the Aquatic Animals Commission noted that other viruses in the Genus *Megalocytivirus*, in addition to red sea bream iridovirus (RSIV), may cause significant disease in fish. These viruses include two other genogroups of the species infectious spleen and kidney necrosis virus (ISKNV) – the genogroup turbot reddish body iridovirus (TRBIV) and the genogroup ISKNV. The genogroups ISKNV and TRBIV are not included in the scope of the current Chapter 10.8. 'Infection with red sea bream iridovirus' of the *Aquatic Code*.

The Commission noted that if the genogroups ISKNV and TRBIV were to be listed (in addition to RSIV), the viruses would first need to be assessed against the criteria in Article 1.2.2. of Chapter 1.2. 'Criteria for listing aquatic animal diseases'. Given this, the Commission assessed the virus species infectious spleen and kidney necrosis virus (ISKNV species), including its three genogroups RSIV, ISKNV and TRBIV, against criteria in Article 1.2.2. of Chapter 1.2. 'Criteria for listing aquatic animal diseases'. The Commission agreed that the species ISKNV, including the RSIV genogroup (currently a listed disease in Chapter 1.3. of the *Aquatic Code*), as well as the two genogroups ISKNV and TRBIV meet the listing criteria 1, 2, 3, and 4b. Consequently, the Commission proposed that the name of the listed disease should be changed to "infection with infectious spleen and kidney necrosis virus (ISKNV)" and would be defined to include the three genogroups of the species ISKNV (i.e. ISKNV, RSIV and TRBIV genogroups) but would not include scale drop disease virus (SDDV), the other recognised species of *Megalocytivirus*.

At its February 2023 meeting, the Commission reiterated that the proposal was to amend the name of the listed disease in Article 1.3.1. from 'infection with RSIV' to 'infection with the virus species infectious spleen and kidney necrosis virus'. The Commission explained that this proposal maintains the RSIV genogroup as a listed disease and would also include the ISKNV genogroup and the TRBIV genogroup.

At its September 2023 meeting the Commission reviewed comments and noted that the majority of comments were supportive of the proposed change to the listed name of infection with RSIV to infection with the virus species infectious spleen and kidney necrosis virus.

The Commission noted that the classification and nomenclature of the genus *Megalocytivirus* is under review by the International Committee on Taxonomy of Viruses (ICTV) and so it would monitor this potential change and adopt new nomenclature once published by the ICTV.

The revised article has been circulated three times for comments, together with an assessment against the listing criteria of Chapter 1.2.

Previous Commission reports where this item was discussed

February 2022 (Part B, Item 3.1.2.3., page 13); September 2022 (Item 5.1., page 7); February 2023 (Item 8.3., page 19); September 2023 (Item 6.4., page 10).

February 2024 meeting

The Aquatic Animals Commission reviewed comments received and noted there were divergent views on the listing of infection with the virus species infectious spleen and kidney necrosis virus.

The Commission acknowledged the comments supportive for the proposed listing of infection with the virus species infectious spleen and kidney necrosis virus. Members noted that these viruses present a significant risk to food finfish aquaculture and also to native fish populations, with the potential for impacts on food security. Members also noted these viruses had spread with the cross-border trade of ornamental fish and food fish to become an important disease in marine and freshwater fish aquaculture. It was noted that listing would solve confusion about diagnosis and provide guidance on prevention and control for members.

The Commission noted that comments from Members opposed to listing centred on three issues: the widespread nature of the ISKNV genogroup (criterion 2 of Article 1.2.2.), the performance of available diagnostic tests (criterion 3 of Article 1.2.2.), and the potential for listing to have a negative impact on trade (no relevant criterion). The Commission agreed to respond collectively to these three points rather than to individual comments.

The assessment of infection with all genogroups of the virus species infectious spleen and kidney necrosis virus against the criteria in Article 1.2.2. of Chapter 1.2. 'Criteria for listing aquatic animal diseases', which had been provided in its September 2023 report is presented as Annex 7 for information.

Widespread nature of the ISKNV genogroup

The Commission considered several comments that the global distribution of ISKNV genogroups and the nature of ornamental fish trade could mean that no Member could meet listing disease criterion 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.'). The Commission noted that this criterion refers to a country or zone, and that while it may not be possible for some Members to declare freedom at the country level, they may find it advantageous to establish free zones. Further, given that compartmentalisation is a likely means for trading disease-free fish, standards for establishing and maintaining a free compartment may be beneficial. The Commission agreed that the standards in the *Aquatic Code* to guide establishment of compartments and zones free from the species ISKNV may assist Members by providing a harmonised approach.

The Commission noted that there seemed to be a perception among some Members that demonstration of freedom at a country-level requires that all susceptible species in the country be sampled and tested to demonstrate freedom from disease. The Commission noted that this was a misunderstanding and drew attention to the guidance in Article 1.4.16. of Chapter 1.4. 'Aquatic animal disease surveillance', which encourages a risk-based approach to surveillance. Utilizing this guidance, surveys may identify and sample populations that have the greatest likelihood of infection. Within an epidemiological unit, those species most likely to develop infection or display clinical signs would be sampled preferentially.

The Commission observed that surveillance that Members are currently performing for infection with the RSIV genogroup will actually be detecting some presence of the TRBIV and ISKNV genogroups.

The Commission noted that some Members were concerned that listing would result in high surveillance costs for the species ISKNV. The Commission considered the circumstances under which the listing of infection with all genogroups of the virus species ISKNV would require surveillance. For a country where the virus species ISKNV is endemic and not eradicable, surveillance would only be advantageous to declare freedom for zones or compartments, and only for the purpose of accessing markets which are free from infection with the virus species ISKNV, or which are subject to an official control program. For importing countries that are not free from the virus species ISKNV, or which are not subject to an official control program, there would be no justification for requiring ISKNV related sanitary measures from exporting countries.

Potential impacts on trade

The Commission acknowledged the concerns of some Members that listing the virus species ISKNV could have negative impacts on international trade, particularly for Members with large export trade of ornamental fish.

The Commission recognised that listing a disease should be broadly beneficial for Members in the control of the specific disease, and noted three principal benefits to the listing of a disease:

1. Initiation of the development of agreed standards for trade that provide a harmonised approach to sanitary measures (in accordance with sanitary and phytosanitary (SPS) agreement obligations).

2. Initiation of the development of agreed diagnostic standards of the *Aquatic Manual* which include recommendations for particular purposes of use, such as surveillance and diagnosis.

3. Improved transparency on the global sanitary situation through the reporting of presence and absence of the disease.

The Commission agreed that these benefits are on balance positive for the management of risks associated with genogroups of the virus species ISKNV. The Commission highlighted that listing would provide importing and exporting countries with a harmonised approach to sanitary measures and, if implemented appropriately, could provide further opportunities for safe trade rather than threaten trade.

The Commission considered that the trade related concerns of some countries to the listing of all genogroups of the virus species ISKNV, may be due to anticipated inappropriate application of standards. The Commission acknowledged these concerns as it is aware of instances where importing countries have required measures of exporters with little justification, for example when the importing country has not declared freedom from a disease and does not have an official control program for it.

The Commission acknowledged that ornamental fish production occurs mainly in developing countries where this industry is important for economies and livelihoods. Appropriate application of standards for infection with the virus species ISKNV, should it be listed, is essential to ensure that these exporting countries are not unduly affected by possible SPS non-compliant measures of importing countries. Additionally, the benefits of harmonised standards and transparency in the global disease situation is likely to provide opportunities for market access and safe trade between members of differing disease status.

Performance of available diagnostic methods

The Commission noted that the relevant criterion requires that there be a reliable means of detection and diagnosis (criterion 3). The Commission noted that there are a variety of methods that have inclusivity for all three genogroups and these would provide a reliable means of detection (Kawato *et al*., 2021a, Koda *et al*., 2023 and Kim *et al*., 2022). While further validation would be useful for particular purposes of use, for example to determine diagnostic performance for surveillance in specific target populations and species, this does not mean that the methods are unreliable.

The Commission noted that a new *Aquatic Manual* chapter for infection with the virus species ISKNV would include diagnostic methods for the three ISKNV genogroups. The Commission also noted that currently published methods had been used successfully by Members to diagnose outbreaks caused by viruses within each of the three ISKNV genogroups. Further, several studies have been published in recent years to evaluate the performance of assays which include the three ISKNV genogroups. The Commission agreed that this evidence on the performance of diagnostic methods exceeds that available for the methods available for some other listed diseases.

In conclusion, the Commission agreed that there are sufficient diagnostic tools available to detect the species ISKNV, inclusive of its three genogroups, and to construct appropriate case definitions (criterion 3). Further diagnostic accuracy studies are warranted, in particular using TRBIV-infected tissue samples, however this is not an impediment to this criterion being met.

The Commission noted a comment suggesting a mechanism for Members to report separate disease status for infection with each of the ISKNV species genogroups, under the listing of infection with the virus species ISKNV. The Commission agreed to discuss this proposal with the WOAH information department. The Commission suggested that the WOAH Observatory could be utilised to monitor the implementation of Member uptake on the standards on ISKNV if adopted.

The Commission also noted a comment that the large number of susceptible species to infection with the virus species ISKNV could allow for the application of Article 1.5.9. 'Listing susceptible species at a taxonomic ranking of Genus or higher'. The Commission agreed that application of the article should be considered.

The Commission concluded that the information provided in the assessment of infection with the virus species infectious spleen and kidney necrosis virus against the criteria in Chapter 1.2. 'Criteria for listing aquatic animal disease' was robust and reiterated that the assessment supported the listing of all genogroups, including RSIV, ISKNV, and TRBIV.

The Commission wished to remind Members that one of the recommendations based on Member requests at the Global Conference on Aquatic Animal Health held in Chile in April 2019, was additional guidance in the *Aquatic Code* for trade of ornamental aquatic animals. The ISKNV genogroup in particular is a key pathogen of concern associated with international trade in ornamental fish, supporting the proposed listing. The Commission noted, that while the virus does not in all cases cause significant morbidity and mortality in ornamental fish species, there are demonstrated and sometimes severe impacts on other important aquaculture species that are traded internationally (e.g. Nile tilapia (*Oreochromis niloticus*), barramundi (*Lates calcarifer*), largemouth bass (*Micropterus salmoides*), orange-spotted grouper (*Epinephelus coioides*), Malabar grouper (*Epinephelus malabaricus*), mandarin fish (*Siniperca chuatsi*)). There are recent examples of ISKNV genogroup outbreaks resulting in national scale impacts on important industry sectors in developing countries. The Commission agreed that the seriousness of the impacts of this group of viruses warrants collective action by Members to control its impacts.

The revised Article 1.3.1. of Chapter 1.3. 'Diseases listed by WOAH' is presented as Annex 8 and will be proposed for adoption at the 91st General Session in May 2024.

6.5. Safe commodities – Articles X.X.3. for disease-specific chapters

Background

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

Between September 2020 and February 2022, the Commission circulated proposed amendments to Articles X.X.3. in all disease-specific chapters of the *Aquatic Code* to reflect this revised approach. In May 2022, the proposed amendments to Articles 9.X.3. and 10.X.3. were adopted.

At its February 2022 meeting, the Commission noted that the assessments previously undertaken against criteria in Article 5.4.1. of Chapter 5.4. 'Criteria to assess the safety of aquatic animal commodities' needed to be reviewed to take into consideration the new approach and any new evidence on thermal stability, and requested that a subject-matter expert be contracted to undertake this review.

At its February 2023 meeting, the Commission reviewed the revised assessments conducted for all aquatic animal products listed in Article X.X.3. for all the disease-specific chapters and agreed to apply the new approach and new scientific information, where relevant.

At its September 2023 meeting, the Commission agreed to consider adding more than one time/temperature for inactivation for aquatic animal products listed in Articles X.X.3., when supported by the assessment and agreed to further discuss this at its February 2024 meeting.

February 2024 meeting

The Aquatic Animals Commission considered adding more than one time/temperature for inactivation for products listed in Articles X.X.3. as suggested by a Member. The Commission agreed that this may be useful to Members, reviewed the available data for each listed disease and concluded that this approach could not be applied effectively due to the paucity of data. The Commission noted that information on inactivation of listed pathogens should be a focus of further research to provide Members with information that can be applied to aquatic animal products with different manufacturing processes.

The Commission reminded Members that the most recent safe commodities assessments, which informed the revisions of Articles X.X.3. of the disease-specific chapters is available on the WOAH website: https://www.woah.org/en/document/safe-commodity-assessments-for-woah-listed-aquatic-animal-diseases-2023/

6.5.1. Articles 8.X.3. for amphibian disease-specific chapters

Comments were received from Canada, Korea (Rep. of), Norway, Peru, the UK, and the EU.

Background

At its February 2022 meeting, the Aquatic Animals Commission amended Articles 8.X.3. to align with amendments adopted in 2022 in Articles 9.X.3. and 10.X.3. regarding the revised approach to time/temperature treatments.

At its February 2023 meeting, the Commission considered the revised safe commodity assessments for products listed in Articles 8.X.3. and amended these articles accordingly.

At its September 2023 meeting, the Commission noted that Members were supportive of the proposed changes and did not propose any additional amendments.

The revised articles have been circulated three times for comments.

Previous Commission reports where this item was discussed

September 2020 (Item 4.7., page 10); February 2021 (Part B: Item 1.4., page 8); September 2021 (Item 5.1.5., page 24); February 2022 report (Part B: Item 2.1.1.1., page 5); February 2023 report (Item 8.4.1., page 23), September 2023 report (Item 6.9.1., page 14).

February 2024 meeting

The Aquatic Animals Commission reviewed comments received and noted that Members were supportive of the proposed changes and did not propose any additional amendments.

The revised Articles 8.1.3., 8.2.3. and 8.3.3., are presented as Annex 9, Annex 10 and Annex 11, respectively, in track changes and clean versions, and will be proposed for adoption at the 91st General Session in May 2024.

6.5.2. Articles 9.X.3. for crustacean disease-specific chapters

Comments were received from Canada, Korea (Rep. of), Norway, Peru, the UK, and the EU.

Background

At its February 2023 meeting, the Aquatic Animals Commission considered the revised safe commodity assessments for products listed in Articles 9.X.3. and amended Articles 9.3.3., 9.4.3., 9.6.3, 9.7.3. and 9.8.3. accordingly.

At its September 2023 meeting, the Commission reviewed comments received and proposed some amendments.

The revised articles have been circulated twice for comments.

Previous Commission reports where this item was discussed

February 2023 report (Item 8.4.2., page 23); September 2023 report (Item 6.9.2., page 15).

February 2024 meeting

The Aquatic Animals Commission noted that Members were supportive of the proposed changes and did not propose any additional amendments.

The revised Articles 9.3.3., 9.4.3., 9.6.3., 9.7.3. and 9.8.3., are presented as Annex 12, Annex 13, Annex 14, Annex 15 and Annex 16, respectively, and will be proposed for adoption at the 91st General Session in May 2024.

6.5.3. Articles 10.X.3. for fish disease-specific chapters

Comments were received from Canada, China (People's Rep. of). Korea (Rep. of), Norway, Peru, the UK, AU-IBAR, and the EU.

Background

At its February 2023 meeting, the Aquatic Animals Commission considered the revised safe commodity assessments for products listed in Articles 10.X.3. and amended Articles 10.X.3., accordingly.

At its September 2023 meeting, the Commission reviewed comments and agreed to include Article 10.11.3. of the new Chapter 10.11. 'Infection with tilapia lake virus', adopted in May 2023.

The revised articles with the exception of Article 10.11.3. have been circulated twice for comments. Article 10.11.3. has been circulated once for comments.

Previous Commission reports where this item was discussed

February 2023 report (Item 8.4.3., page 23); September 2023 (Item 6.9.3., page 15).

February 2024 meeting

In response to several comments regarding the origin of the time/temperature for inactivation included in Articles 10.3.3., 10.6.3. and 10.9.3. and 10.10.3., the Aquatic Animals Commission noted that the time/temperature for inactivation of *Gyrodactylus salaris*, infectious haematopoietic necrosis virus, spring viraemia of carp virus and viral haemorrhagic septicaemia virus are described in the Safe Commodities Assessments report available on the WOAH website.

The Commission did not agree with a comment to change points 5 and 6 of Article 10.3.3. to include a minimum holding of the animals at 25 ppt for 14 days as *G. salaris* is an external parasite and therefore the Commission deemed that 14 days was not required to further reduce the risk.

The Commission did not agree with a comment to remove point 7 of Article 10.3.3. as this is a separate product than those described in the other points of this article.

The Commission did not agree with a comment to change the time/temperature for inactivation of tilapia lake virus (TiLV) based on the use of a surrogate as there is a publication for TiLV and a surrogate is not necessary for the virus as described in the Safe Commodity Assessments report on the WOAH website.

The Commission agreed to the comments to correct the Spanish translation of point 8 of Article 10.3.3. and the title of Article 10.11.3.

The Commission did not propose any additional amendments to the English texts.

The revised Articles 10.1.3., 10.2.3., 10.3.3., 10.4.3., 10.5.3., 10.6.3., 10.7.3., 10.8.3., 10.9.3. 10.10.3. and 10.11.3., are presented as Annex 17, Annex 18, Annex 19, Annex 20, Annex 21, Annex 22, Annex 23, Annex 24, Annex 25, Annex 26 and Annex 27, respectively, and will be proposed for adoption at the 91st General Session in May 2024.

6.5.4. Articles 11.X.3. for mollusc disease-specific chapters

Comments were received from Canada, Korea (Rep. of), Norway, Peru, the UK, and the EU.

Background

At its February 2022 meeting, the Aquatic Animals Commission amended Articles 11.X.3. to align with amendments adopted in 2022 in Articles 9.X.3. and 10.X.3. regarding the revised approach to time/temperature treatments.

At its February 2023 meeting, the Commission considered the revised Safe commodity assessments for products listed in Articles 11.X.3. and amended these articles accordingly.

At its September 2023 meeting, the Commission noted that Members were supportive of the proposed changes and did not propose any additional amendments.

The revised articles have been circulated three times for comments.

Previous Commission reports where this item was discussed

September 2020 report (Item 4.7., page 10); February 2021 report (Part B: Item 1.4., page 8); September 2021 report (Item 5.1.5., page 24); February 2022 report (Part B: Item 2.1.1.2., page 5), February 2023 report (Item 8.4.4., page 24); September 2023 report (Item 6.9.4., page 16).

February 2024 meeting

In response to several comments regarding the origin of the time/temperature for inactivation included in Articles 11.3.3. and 11.5.3., the Aquatic Animals Commission noted that the

time/temperature for inactivation of *Bonamia ostreae* and *Perkinsus marinus* is described in the Safe Commodities Assessments report available on the WOAH website.

The Commission noted that Members were supportive of the proposed changes and did not propose any additional amendments.

The revised Articles 11.1.3., 11.2.3., 11.3.3., 11.4.3., 11.5.3., 11.6.3., and 11.7.3. are presented as Annex 28, Annex 29, Annex 30, Annex 31, Annex 32, Annex 33 and Annex 34, respectively, in track changes and clean versions, and will be proposed for adoption at the 91st General Session in May 2024.

6.6. Model Articles X.X.5. and X.X.6. for disease-specific chapters

Comments were received from Canada, Norway, Peru, the UK, the USA, AU-IBAR, and the EU.

Background

At its February 2023 meeting, the Aquatic Animals Commission amended the final paragraph of point 4 of Article 10.11.5. for clarity and to describe the actions that should be achieved prior to declaring a new free zone outside the infected and protection zones. The Commission also agreed to add a new final paragraph to Article 10.11.6. using the same wording as point 4 of Article 10.11.5. to ensure consistency between country and zone freedom.

At the General Session in May 2023, Members raised concerns about the proposed changes to the final paragraph of Articles 10.11.5. and 10.11.6. of Chapter 10.11. 'Infection with tilapia lake virus' that were being proposed for adoption in May 2023 because they were inconsistent with point 1 of Article 1.4.14. Chapter 10.11 was adopted but these proposed changes were placed 'under study' and it was agreed that the Commission would review model Articles X.X.5. and X.X.6. for disease-specific chapters at its September 2023 meeting to address the text 'under study'.

At its September 2023 meeting the Commission reviewed the final paragraph of point 4 of model Article X.X.5., and agreed to use the wording proposed in the final paragraph of Article 10.11.5. of Chapter 10.11. 'Infection with tilapia lake virus' but without the part of the sentence that was inconsistent with point 1 of Article 1.4.14. The Commission agreed to also apply this change to model Article X.X.6. by using the same wording as point 4 of Article X.X.5. for the new final paragraph, to ensure consistency between country and zone freedom.

The revised articles have been circulated once for comments.

Previous Commission reports where this item was discussed

September 2023 report (Item 6.10., page 16).

February 2024 meeting

The Aquatic Animals Commission reviewed comments received and did not propose any additional amendments.

Regarding a comment to change the period of basic biosecurity conditions in point 1 of Article X.X.5. and Article X.X.6. from 6 to 12 months, the Commission reminded Members that the default periods of listed diseases are stated in Chapter 1.4. Furthermore, the periods for each listed disease have been assessed (see Item 8.2.).

In point 4 d) ii), the Commission did not agree to decrease the default timeframe for farmed populations as it considered this period to be appropriate.

The Commission agreed to consider adding points i) and ii) from point 4 d) of Article X.X.5. to point 4 d) of Article X.X.6. when the revision of the surveillance periods are being finalised.

The Commission noted that, as these articles are harmonised across all disease-specific chapters, these changes once adopted will be applied to all disease-specific chapters.

The revised model Articles X.X.5. and X.X.6. are presented as Annex 35 and will be proposed for adoption at the 91st General Session in May 2024.

6.7. Article 9.3.2. of Chapter 9.3. 'Infection with decapod iridescent virus 1'

Comments were received from Canada, China (People's Rep. of), Chinese Taipei, Norway, Peru, the UK, and the EU.

Background

At its September 2023 meeting, the Aquatic Animals Commission considered the report of the *ad hoc* Group on the Susceptibility of Crustacean Species to Infection with DIV1. The Commission agreed to amend the list of susceptible species in Article 9.3.2., based on the assessments provided in the *ad hoc* Group report.

The revised article has been circulated once for comments.

Previous Commission reports where this item was discussed

September 2023 report (Item 6.11., page 17).

February 2024 meeting

The Aquatic Animals Commission agreed to amend its convention and to use a table format for the list of susceptible species in Article X.X.2. when there is more than one susceptible species rather than only when there are more than ten species. This change is to improve readability of the list and will be applied as each chapter undergoes review.

Following a comment suggesting there is not enough evidence to list fleshy prawn (*Penaeus chinensis*) and giant river prawn (*Macrobrachium rosenbergii*) as susceptible species, the Aquatic Animals Commission requested that the *ad hoc* Group review its assessments for these two species.

The *ad hoc* Group reviewed the assessment for fleshy prawn (*Penaeus chinensis*) and noted that an error had been made for the Guixiang *et al*., 2022 study in regard to Stage 3A and 3C, which had previously been noted as 'YES', but should be scored as 'ND', and that the outcome of the paper should be '2' rather than '1'. The revised assessment is presented in the table below.

Re-assessment of fleshy prawn (*Penaeus chinensis*):

Stage 1: Route of infection	Stage 2: Pathogen Identification	Stage 3: Evidence of Infection				Outcome	References
		A	B	C	D		
N	nested-PCR and RPA (ATPase gene)	ND	ND	ND	YES	2	Guixiang *et al*., 2022
N	TaqMan real-time PCR	ND	ND	ND	YES	2	Qiu *et al*., 2018a

The *ad hoc* Group concluded that fleshy prawn (*Penaeus chinensis*) did not meet the criteria to be ranked as a susceptible species and should therefore not be included in Article 9.3.2. of Chapter 9.3. 'Infection with DIV1' of the *Aquatic Code*, but should be included in Section 2.2.2. 'Species with incomplete evidence for susceptibility' of Chapter 2.2.X. 'Infection with DIV1' of the *Aquatic Manual*.

The *ad hoc* Group reviewed the assessment for giant river prawn (*Macrobrachium rosenbergii*) and noted that an error had been made for the Guixiang *et al*., 2022 study in regard to Stage 3A and 3C, which had previously been noted as 'YES' but should be scored as 'ND', and that the outcome of the paper should be '2' rather than '1'. The revised assessment is presented in the table below.

Re-assessment of giant river prawn (*Macrobrachium rosenbergii*):

Stage 1: Route of infection	Stage 2: Pathogen Identification	Stage 3: Evidence of Infection				Outcome	References
		A	B	C	D		
N	TaqMan real-time PCR	YES	ND	YES	YES	1	Qui *et al*., 2019
N	nested-PCR and RPA (ATPase gene)	ND	ND	ND	YES	2	Guixiang *et al*., 2022

The *ad hoc* Group concluded that giant river prawn (*Macrobrachium rosenbergii*) continued to meet the criteria to be ranked as a susceptible species and should therefore be included in Article 9.3.2. of Chapter 9.3. 'Infection with DIV1' of the *Aquatic Code*.

The Aquatic Animals Commission reviewed the revised assessments and agreed to remove fleshy prawn (*Penaeus chinensis*) from Article 9.3.2. of Chapter 9.3. 'Infection with DIV1' in the *Aquatic Code* and include this species in Section 2.2.2. 'Species with incomplete evidence for susceptibility' of Chapter 2.2.X. 'Infection with DIV1' in the *Aquatic Manual*. The Commission agreed to keep giant river prawn (*Macrobrachium rosenbergii*) as a susceptible species in Article 9.3.2.

The Commission agreed to change the common name for *Portunus trituberculatus* to swimming crab as it is an accepted common name on FAOTERM. The Commission also agreed to change the scientific name for ridgetail prawn from *Exopalaemon carinicauda* to *Palaemon carinicauda*, which is the accepted name on the World Register of Marine Species (WoRMS).

Relevant sections of Chapter 2.2.X. 'Infection with DIV1' in the *Aquatic Manual* were also amended in line with these revisions (see Item 9.1.5.).

The revised Article 9.3.2. of Chapter 9.3. 'Infection with decapod iridescent virus 1' is presented as Annex 36 and will be proposed for adoption at the 91st General Session in May 2024.

6.8. Article 10.6.2. of Chapter 10.6. 'Infection with infectious haematopoietic necrosis virus'

Comments were received from Canada, Chinese Taipei, Norway, Peru, the UK, and the EU.

Background

At its September 2023 meeting the Aquatics Animal Commission agreed to amend the list of susceptible species in Article 10.6.2., in line with the convention used in Article X.X.2. of the *Aquatic Code*, i.e. to list susceptible species in a table when there are more than ten susceptible species.

The revised article has been circulated once for comments.

Previous Commission reports where this item was discussed

September 2023 report (Item 6.12., page 18).

February 2024 meeting

The Aquatic Animals Commission agreed to amend its convention and to use a table format for the list of susceptible species in Article X.X.2. as mentioned in Item 6.7.

The Commission noted that Members were supportive of the proposed changes and did not propose any additional amendments.

The revised Article 10.6.2. of Chapter 10.6. 'Infection with infectious haematopoietic necrosis virus' is presented as Annex 37 and will be proposed for adoption at the 91st General Session in May 2024.

6.9. Article 10.11.2. of Chapter 10.11. 'Infection with tilapia lake virus'

Comments were received from Canada, Chinese Taipei, Norway, Peru, the UK, and the EU.

Background

At its September 2023 meeting, the Aquatic Animals Commission considered the report of the *ad hoc* Group on the Susceptibility of Fish Species to Infection with TiLV. The Commission agreed to amend the list of susceptible species in Article 10.11.2. in line with recommendations of the *ad hoc* Group.

The revised article has been circulated once for comments.

Previous Commission reports where this item was discussed

September 2023 report (Item 6.13., page 18).

February 2024 meeting

The Aquatic Animals Commission noted that Members were supportive of the proposed changes and did not propose any additional amendments. The Commission agreed to place the list into a table as described above for item 6.8.

The revised Article 10.11.2. of Chapter 10.11. 'Infection with tilapia lake virus' is presented as Annex 38 and will be proposed for adoption at the 91st General Session in May 2024.

6.10. Article 11.5.1. and 11.5.2. of Chapter 11.5. 'Infection with *Perkinsus marinus*'

Comments were received from Canada, Chinese Taipei, Norway, the UK and the EU.

Background

At its February 2023 meeting, the Aquatic Animals Commission considered the report of the *ad hoc* Group on Susceptibility of Mollusc Species to Infection with *Perkinsus marinus*. The Commission agreed to amend the list of susceptible species in Article 11.5.2. in line with the recommendations of the *ad hoc* Group.

At its September 2023 meeting, the Commission agreed to amendments to Article 11.5.2. to align with other disease-specific chapters.

The revised articles have been circulated twice for comments.

Previous Commission reports where this item was discussed

February 2023 report (Item 8.5., page 24); September 2023 report (Item 6.14., page 18).

February 2024 meeting

The Aquatic Animals Commission noted that Members were supportive of the proposed changes and did not propose any additional amendments to these articles or the relevant section of Chapter 2.4.5. 'Infection with *Perkinsus marinus*' in the *Aquatic Manual* (see item 9.2.4.). The Commission agreed to place the list into a table as described above for item 6.8.The revised Articles 11.5.1. and 11.5.2. of

Chapter 11.5. 'Infection with *Perkinsus marinus*' are presented as Annex 39 and will be proposed for adoption at the 91st General Session in May 2024.

7. Items for Member comments

7.1. Draft new Chapter 4.X. 'Emergency disease preparedness' and draft new Chapter 4.Y. 'Disease outbreak management'

Comments were received from Australia, Canada, China (People's Rep. of), New Caledonia, Norway, Peru, Singapore, the UK, the USA, AU-IBAR, and the EU.

Background

At its September 2022 meeting, the Aquatic Animals Commission discussed the work of the *ad hoc* Group on Emergency Disease Preparedness and Disease Outbreak Management for Aquatic Animals, which met twice during 2021-2022, and agreed to continue the work on the development of a draft new Chapter 4.X. 'Emergency disease preparedness' and draft new Chapter 4.Y. 'Disease outbreak management'.

At its September 2023 meeting, the Commission finalised work on these two draft new chapters and noted that these two chapters are closely connected. Chapter 4.X. outlines the essential elements of an emergency disease preparedness framework which encompasses all the elements that will enable the Competent Authority to activate an efficient response to a disease outbreak. Chapter 4.Y. describes the specific actions which are necessary to operationalise the framework in the event of a disease outbreak.

The draft new chapters have been circulated once for comments.

Previous Commission reports where this item was discussed

September 2023 report (Item 6.6., page 12).

February 2024 meeting

The Aquatic Animals Commission reviewed comments received and noted that Members were generally supportive of the draft new Chapter 4.X. 'Emergency disease preparedness', and the draft new Chapter 4.Y. 'Disease outbreak management', while proposing suggestions for clarity and some proposals for content to consider.

The Commission reviewed comments throughout the text of both Chapter 4.X. and 4.Y. proposing changes intended to provide alignment with the *Terrestrial Code* chapters which cover emergency disease preparedness and management. The Commission noted that these chapters in the *Terrestrial Code* are planned for review and revision, and as such the text in the *Terrestrial Code* may change making alignment at this time not possible. The changes were not accepted, unless suggested additions to the text provided information in a more concise manner.

The Commission noted in both Chapter 4.X. and 4.Y. comments were received suggesting the removal of the Competent Authority from certain articles. The Commission wished to remind Members that the Competent Authority is the governmental authority with responsibility for implementation of the relevant standards, and depending on the administrative structures of the member may be a national or regional government authority. It may or may not be the Veterinary Authority.

The Commission considered comments where lists of examples are provided to clarify points in the text, Members suggested examples to be added or removed. As examples provided are not meant to be exhaustive lists, the Commission did not make changes to these points.

Chapter 4.X. Emergency disease preparedness

General Comments

The Commission considered a comment requesting that the terms used in relation to emergency phases in both draft chapters be defined. The Commission noted that these terms and phases are defined in Chapter 4.Y. 'Disease outbreak management' and apply to both chapters.

The Commission acknowledged that in the Spanish translation the glossary term *contingency plan* is translated to 'plan de emergencia', which is not a direct translation of its meaning. The Commission decided to change the term in Chapters 4.X. and 4.Y. to 'plan de contingencia'. Changes to the glossary term usage through the text would be made after adoption of these chapters.

The Commission acknowledged comments to add industry stakeholders as important participants in the emergency disease framework. The Commission noted that the glossary term Aquatic Animal Health Services includes 'the combination of governmental and non-governmental individuals and organisations that perform activities to implement the standards of the *Aquatic Code*' and is used through the text and inclusive of industry members. However, the Commission recognised that individual aquatic animal producers may not be fully reflected in that definition. The Commission agreed to add 'industry stakeholders' in addition to Aquatic Animal Health Services throughout Chapter 4.X.

Article 4.X.1.

The Commission agreed with a comment to add text indicating that development of an emergency disease preparedness plan should be developed in accordance with a Members' priorities and resources.

In response to comments received for both Chapter 4.X. and 4.Y. to define an 'important aquatic animal disease' the Commission added a sentence at the end of the purpose in Article 4.X.1.

Article 4.X.2.

The Commission did not agree with a comment to change language around the elements needed for a disease response in order to provide flexibility in elements considered. Chapter 4.X. contains the essential elements for an emergency disease preparedness framework, and the current wording does not prohibit the Competent Authority from considering other elements that may be relevant to them.

Article 4.X.3.

The Commission received a comment to add 'other national considerations' in addition to a risk analysis to create a list of important disease. The Commission noted that the information in Chapter 4.X. is intended to provide recommendations around elements required for an emergency disease preparedness framework. These recommendations do not exclude the Competent Authority from considering other factors not included in Chapter 4.X. which may be relevant to their national situation.

Article 4.X.4.

The Commission agreed with a comment suggesting where relevant the specific articles in Chapter 4.Y. should be referenced to provide more specificity in the recommendations. The specific articles were added to sub-points a) and b) under point 3.

In point 4, the Commission agreed with comments to clarify the outcomes from simulation exercises, and added text to reflect this.

Article 4.X.5.

The Commission did not agree with a comment to change the language in the first sentence in Article 4.X.5. to 'should have' from 'has' in relation to the Competent Authority. The Commission noted that this article is discussing pre-requisites for an emergency disease preparedness framework, and as pre-requisites are mandatory the usage of 'has' is appropriate in this Article.

The Commission modified point 2 to reference access to resources including funds, to allow for different forms of resources. The Commission did not agree to add an additional point on access to international emergency funds and resources, as the text already allows for different types of resources and specific examples are not required for clarity.

Article 4.X.6.

The Commission considered a comment to expand the title of Article 4.X.6. to be more specific on the purpose of the article. The Commission did not agree to change the article title, however they added a sentence in the first paragraph to clarify that the article elaborate on principles from Chapter 2.1. 'Import risk analysis' in the context of emergency disease preparedness.

The Commission did not agree with a comment that Chapter 1.4. did not provide sufficient information on surveillance and it should be elaborated further in Article 4.X.6. The Commission noted that as stated in Article 1.4.1. the chapter provides guidance on surveillance to be used 'to make and maintain a self-declaration of freedom from disease or confirm the occurrence of a listed disease or an emerging disease'. As such the information provided in Chapter 1.4. is sufficient for surveillance activities to allow for detection and confirmation of an important aquatic disease.

Article 4.X.7.

The Commission did not agree with a comment to include reference to relevant legislation and legal powers under point 1. These requirements are elaborated in Chapter 4.Y. 'Disease outbreak management', and it is redundant to include in Chapter 4.X.

The Commission acknowledged comments noting that both the terms 'emergency management group' and 'emergency task force' were used, and that a single term should be used to minimize confusion. Edits were made to ensure the term 'emergency management group' was used consistently.

In point 3, the Commission agreed with a comment that 'media liaison' should be included to ensure effective communication, and added a new sub-point e) for 'communications and media liaison'.

The Commission did not agree with a comment on point 3 suggesting that the sub-points for point 3 may vary by disease and species impacted, and this information could be included in other disease-specific documents. The Commission highlighted that the glossary definition of 'Contingency Plan' captures this eventuality as it states 'a documented work plan designed to ensure that all needed actions, requirements and resources are provided…'. A Contingency Plan is not necessarily one document and may include other disease-specific documents.

In point 3 a), the Commission did not agree to modify text to 'national disease response structure'. The Commission noted the current text 'central and local disease control centre' is inclusive of the concept of a national disease response structure.

In point 7, the Commission did not agree to reference a 'communication strategy' rather than a 'risk communication strategy'. The Commission noted that risk communication is a glossary term in the *Aquatic Code*, and is the appropriate term to utilise in this context. The Commission added a sentence at the end of point 7, to link to the principles of risk communication in Chapter 2.1.

Article 4.X.8.

The Commission considered a comment suggesting addition of text to the end of paragraph 2 adding detail on when to consider shared waterbodies. The Commission noted that the specificity of the

suggestions are not required, however added ‘where relevant’ to allow for flexibility to consider different factors related to shared waterbodies.

Under point 5, the Commission agreed with a comment to add the creation of an outcome report to ensure outcomes are addressed. Edits were made to point 5 and sub-point c) was added to address the outcome report, and ensure follow-up would occur in a timely manner.

Article 4.X.9.

The Commission did not agree with a comment to remove ‘within an agreed timeframe’ from paragraph 6. The Commission noted it was important to state that the activities should occur within a specified timeframe to ensure they occur and are useful for all stakeholders.

Article 4.X.10.

The Commission acknowledged comments on editorial changes of the French translations of Article 4.X.10. and Article 4.X.11. and made changes to reflect these comments.

Articles 4.X.11.

The Commission noted a comment on point 7 requesting the inclusion of information on protection zones. The Commission did not agree with this addition, as additional information on zoning is provided in Chapter 4.Y.

Chapter 4.Y. Disease outbreak management

General Comments

The Commission acknowledged a comment requesting clarity on the definition of an ‘important aquatic disease’. A definition was added in Article 4.X.1. to provide clarity that an important disease may be listed in Chapter 1.3., an emerging disease or other diseases identified by the Competent Authority.

The Commission noted an editorial comment on the Spanish translation of the chapter title, and agreed to update the Spanish version.

The Commission noted a comment requesting that terms such as alert phase and emergency phase be defined, and added text in the respective sections to clarify the meaning and usage of these terms. The Commission noted that where possible the terms usage is consistent with guidelines from the Food and Agriculture Organization (FAO).

Article 4.Y.3.

In point 2, the Commission acknowledged a comment which questioned whether there needs to be a single Operations Manual as there may be different response depending on the pathogen and species involved. The Commission agreed that the guidance in this point is not meant to be prescriptive for a single document. The Commission added wording to clarify that the Operations Manual can be a series of documents which together provide guidance on the emergency response.

The Commission did not agree with a comment to change Operations Manual to Contingency Plan in points 2 and 4. An Operations Manual as described in Chapter 4.Y. ‘provides guidance on all aspects of an emergency response which includes actions through the alert, emergency and recovery phases. Alternatively, a Contingency Plan is a glossary term defined as ‘a documented work plan designed to ensure that all needed actions, requirements and resources are provided in order to eradicate or bring under control outbreaks of specified diseases of aquatic animals’. Thus an Operations Manual provides guidance on the operational activities required to have a successful emergency response. Whereas the Contingency Plan covers broader aspects to ensure all essential components of a response are in place to ensure effective preparation for a disease emergency.

In point 4, the Commission did not agree to add further text intended to elaborate that the disease should be managed dependent on a countries' resources and priorities. The Commission noted that this is not required as it is outlined in Chapter 4.X. that a disease response will be to a disease that a country has already prioritised as important.

Article 4.Y.4.

The Commission removed the original point 1, and re-numbered the remaining points to avoid duplicative language that was present in points 1 and 2. The Commission acknowledged a comment adding detail on the confirmation and rule out of a pathogen, and noted that changes to the new point 1 reflected these changes.

The Commission agreed with a comment that a working case definition is required when relevant, and added a sub-point b) under point 1 to address this.

For point 2, the Commission considered a comment that epidemiological tracing activities would occur following confirmation and not necessarily during the alert phase. The Commission agreed, and made changes to reflect that tracing activities would be considered during the alert phase.

In point 3 a), in the list of things which movements are controlled the Commission did not agree to add vessels, as the glossary term vehicles includes vehicles. The Commission did agree to add contaminated water to this point.

The Commission noted a comment that communication to the emergency management group is important in the alert phase. To address this comment the Commission made changes to point 4 that communication with the emergency management group should occur during the initial phase of the epidemiological investigation.

The Commission considered a comment that the text was unclear when the epidemiological investigation should be commenced. The Commission added text to point 4 to refer to point 1 which describes the epidemiological investigation starting in the suspicion phase.

In point 4, the Commission agreed to add text indicating that the meeting of the emergency management group is recommended when a suspicion is related to a new case in a previously free country or zone.

In point 5, the Commission agreed with a comment to clarify diagnostic laboratories.

In point 5, the Commission did not agree to change 'should' to 'may' as communication during the alert phase is imperative to ensure relevant personnel are prepared to act if necessary. Point 5 is written referencing 'relevant personnel' which allows flexibility in who should receive these communications.

Article 4.Y.5.

The Commission did not agree to remove reference to the Operations Manual from the first paragraph, as both the Operations Manual and the Contingency Plan are required in the emergency phase.

In point 2, the Commission agreed to add personnel to the list of factors to take into account during the emergency phase.

Article 4.Y.6.

The Commission agreed with a comment that the details on numbers and weights of animals should be removed from point 1 b).

The Commission did not agree to change risk assessment to risk evaluation in point 1 g). The Commission noted that risk evaluation is a glossary term and the definition ('the scientific evaluation of the likelihood and the biological and economic consequences of entry, establishment and spread of a

hazard') is appropriate for its usage in this point. As per the addition in Chapter 4.X. the key principles of Chapter 2.1. apply to risk assessment for a disease outbreak.

The Commission considered a comment adding detail to point 2 to reinforce the importance of biosecurity and containment measures, and point 2 c) was modified to reflect this comment. The Commission also considered a comment about the addition to point 2 on adding text on the safe movement of unaffected stock. Amendments were made to point 2 c) to address this comment.

The Commission acknowledged a comment on point 4 for addition that the review should include details on the legal instruments for roles and responsibilities. The Commission made edits in point 4 to ensure clarity of roles and responsibilities during a disease response.

In point 5, the Commission noted a comment to add consideration of the content of messages, and added in 'agreed messages'. The Commission modified the last sentence of point 5 for clarity.

In point 6, the Commission agreed with a comment that there should be a distribution list for the minutes of meetings during emergency phase and amended the text to reflect this comment.

Article 4.Y.7.

The Commission considered a comment on point 1 a) ii) to include the imposition of disease control measures on other stakeholders, and the text was amended to reflect 'other relevant stakeholders'.

In point 1 a) iv), the Commission noted a comment to add in other locations where disinfection should occur, and added in 'vehicles and other premises' to be more inclusive of where disinfection should occur.

In point 1 b) i), the Commission agreed with a comment that inspections may occur in other locations, and amended the text to allow for other establishments to be included.

The Commission agreed with a comment on point 1 b) ii) to add transportation.

In point 1 b), the Commission agreed with a comment to add two additional points for 'data and record management' and 'human resources management including workplace health and safety'.

The Commission considered a comment to make edits through point 1 to change the heading in point 1 to reference a national disease response structure, remove reference to the Competent Authority, and remove sub-point details on specific details. The Commission did not agree with these suggestions, as there should be consideration of the response on the national, regional and local levels.

In point 3 a), the Commission agreed with a comment to add subpoints that included predatory animal and bird control specialists; telecommunication providers; communication specialists or journalists for media liaison.

The Commission considered a comment that not all Members may be able to recommend specific service providers and edits were made to the last sentence to reflect this situation. The Commission acknowledged the comment that the need to maintain an up-to-date contact list will mean the Operations Manual will have a short lifespan. However, the Commission did not agree to remove maintaining contact points, as the Operations Manual should be reviewed regularly such that all aspects of the manual stay current.

Article 4.Y.8.

The Commission considered a comment that suggested that classification of the health status of establishments should be included as a control measure, and the Commission added a new point 2 to include that information.

The Commission noted a comment for point 5 a), that there the control of movement of additional things should be considered, and added text to include 'vehicles, waste, fomites and vectors'. In addition, examples were added to point 5 c) to provide examples of exemptions from movement controls.

The Commission agreed with a comment to add text on the containment and safe disposal of dead and destroyed stock to point 5 d) iii).

The Commission agreed with a comment to add text to points 5 f) to ensure the disinfectants are approved for use by the Competent Authority.

The Commission agreed to add additional text to point 5 g) and a new point 5 h) for greater clarity in relation to wastewater.

Article 4.Y.9.

The Commission considered a comment that expressed concerns that as a recovery plan may change based on the situation of the outbreak, and it is difficult to include actions for the recovery plan in the Operations Manual. The Commission did not agree as various recovery options will be detailed in the Contingency Plan with potential actions in the Operations Manual. This will allow the Competent Authority to tailor the options utilised to the specific disease situation. Article 4.Y.9. includes flexible terminology indicating considerations in specific cases and scenarios.

The Commission divided Article 4.Y.9. into 4 points, including return to freedom, containment, mitigation, and additional details. Point 1 was edited and divided into sub-points for greater clarity.

The Commission agreed with a comment to add that surveillance is required as per Chapter 1.4. after a new declaration of freedom in made.

The Commission noted a comment to include zoning and compartments as a containment measure in point 2. The Commission agreed that zoning should be included and added to point 2 a) i).

The Commission acknowledged comments on editorial changes on the French translation of Article 4.Y.9. and agreed to the changes.

The Commission agreed to a comment to clarify that surveillance and biosecurity measures in point 4 b) should occur prior to the recommencement of trade.

The draft new Chapter 4.X. 'Emergency disease preparedness' and draft new Chapter 4.Y. 'Disease outbreak management', are presented as Annex 40 and Annex 41, respectively, for comments.

7.2. Draft new Chapter 4.Z. 'Control of pathogenic agents in traded gametes and fertilised eggs of fish'

Comments were received from Australia, Canada, China (People's Rep. of), Chinese Taipei, Norway, Peru, Thailand, the UK, the USA, AU-IBAR, and the EU.

Background

At its September 2023 meeting, the Aquatic Animals Commission reviewed the draft new Chapter 4.Z. 'Control of pathogenic agents in traded gametes and fertilised eggs of fish' which had been developed in collaboration with industry with the purpose to provide recommendations for safe trade in milt and fertilised eggs of fish from areas which have not been declared free from infection with a listed disease.

To take into account the provisions in the draft new Chapter 4.Z., the Commission revised model Articles 10.X.10. and 10.X.15. for Chapter 10.5. 'Infection with SAV', Chapter 10.6. 'Infection with IHNV' and Chapter 10.10. 'Infection with VHSV', and Articles 10.4.15. and 10.4.20. for Chapter 10.4. 'Infection with ISAV'.

The Commission also proposed a new definition for ‘collection and incubation centre’ to the Glossary of the *Aquatic Code* to ensure a common understanding of this term given the importance of its use in the draft new Chapter 4.Z.

The draft new chapter, model articles and new glossary term have been circulated once for comments.

Previous Commission reports where this item was discussed

September 2023 report (Item 6.7., page 12).

February 2024 meeting

General Comments

The Aquatic Animals Commission reviewed comments received and noted that Members were generally supportive of the proposed chapter.

The Commission agreed that it will ask industry experts to draft a new article providing guidance on the biosecurity in the collection and incubation centre to mitigate the risks of cross-contamination during handling, including spawning/stripping until the gametes or fertilised eggs are certified and dispatched from the establishment. The Commission noted that comments regarding adding internal biosecurity measures would be addressed by this new article and did not agree to additions throughout the chapter which would result in repetitions.

The Commission did not agree with comments suggesting that the Competent Authority should not be responsible for approving the collection and incubation centres. The Commission noted that the Competent Authority is the governmental authority with responsibility for implementation of the relevant standards, and depending on the administrative structures of the member may be a national or regional government authority. It may or may not be the Veterinary Authority.

The Commission agreed with several suggested changes that added clarity.

Article 4.Z.1.

The Commission agreed with a comment to replace ‘import’ with ‘trade’ in the introductory paragraph as it allows broader application, including into zones and compartments. The Commission also agreed with a comment to replace ‘risk mitigation’ with ‘risk management’ to better align with the definition of the glossary terms.

The Commission did not agree with a comment to add “research” as a third purpose because research has different contexts and the possibility for different controls including lifelong quarantine.

The Commission agreed with a comment to specify articles rather than referencing ‘Section 10’ in the last paragraph.

Article 4.Z.2.

The Commission did not agree with a comment to list specific measures in Article 4.Z.2., but agreed to add a cross reference to Article 4.Z.3. where these measures are outlined.

Article 4.Z.3.

The Commission agreed with a comment to replace ‘should’ with ‘must’ in point 1 as the use of should is inconsistent with the following points which indicate that only populations of broodstock that test free from the pathogenic agents are suitable.

The Commission agreed with a comment to add a new point 3 to highlight an important risk mitigation measure in the event of a positive detection.

The Commission agreed with a comment to remove the reference to the salmonid disease-specific chapters in the new point 4. However, the Commission did not agree to add that disinfection of the fertilised eggs should be under the supervision of the Aquatic Animal Health Services.

The Commission did not agree with a comment to replace 'parents' with 'broodstock' in the new point 5 as parents implies testing individuals which is intention of this point, whereas broodstock testing could be at a population level.

Article 4.Z.4.

The Commission did not agree with a comment to change the title of the article to remove the reference to 'place of origin' but agreed to change 'place' to 'aquaculture establishment' for clarity.

The Commission agreed with a comment to add 'or other sources of disease that can lower their health status' in point 4 to cover other sources of pathogen introduction such as water or feed.

The Commission did not agree with a comment to change the wording for the demonstration of 95% confidence as the original wording is the wording included in Chapter 1.4. 'Aquatic animal disease surveillance'.

Article 4.Z.5.

The Commission agreed with a comment that an Aquatic Animal Health Professional or veterinarian should have overall responsibility for the operation of a collection and incubation centre.

In point 5, the Commission agreed with a comment to rearrange the points to follow the process flow and add points for holding broodstock and disinfection of fertilised eggs.

Article 4.Z.6.

The Commission agreed with a comment to add a new point 1 to state that stripping and sampling of broodstock should be carried out under the supervision of the Aquatic Animal Health Professional or veterinarian who has responsibility for the collection and incubation centre.

The Commission did not agree with a comment to add details regarding the type of samples taken from the broodstock in the new point 2 as preferred sample types are disease-specific and provided in the disease-specific chapters of the *Aquatic Manual*.

The Commission did not agree with a comment to remove the point regarding disposing of gametes and fish from epidemiological groups that test positive as the chapter's scope is focussed on trade and therefore within that scope, these should be disposed of.

The Commission did not agree with a comment to change the new point 3 regarding the outcome of gametes and fertilised eggs from parents that test positive from 'should not be traded' to 'disposed in a biosecure manner' as this point focusses on trade.

The Commission agreed with a comment to add a new point stating that broodstock testing results should be provided to the Competent Authority of an importing country upon request.

The Commission agreed with a comment to add a new point referring to the disinfection of fertilised eggs as this is a measure that will further reduce the risk of pathogen introduction in collection and incubation centres.

Article 4.Z.7.

The Commission agreed with a comment to remove the reference to 'in the laboratory' in both the title and opening sentence of this article.

The draft new Chapter 4.Z. 'Control of pathogenic agents in traded gametes and fertilised eggs of fish' is presented as Annex 42 for comments.

Model Article 10.X.10. (Article 10.4.15. for infection with ISAV)

The Commission further considered the integration of the new Chapter 4.Z. into the disease-specific chapters. It agreed to retract the proposed changes to this model article from the September 2023 Commission meeting as there may be confusion between the application of Articles 10.X.10 and 10.X.15 (10.4.15 and 10.4.20 for ISAV). The Commission agreed that for those diseases that the protocol of the new Chapter 4.Z. would apply (see below), Article 10.X.10. (and 10.4.15) would exclude application to gametes and fertilised eggs, which would be addressed through Article 10.X.15. (10.4.20. for ISAV).

Model Article 10.X.15. and Article 10.4.20.

The Commission made an assessment of the suitability of the provisions of draft new Chapter 4.Z. for listed diseases of fish. The assessment is intended to determine the suitability of including model article 10.X.15. (10.4.20 for Chapter 10.4. 'Infection with ISAV'), which cross references to the measures of draft new Chapter 4.Z., for inclusion in each of the disease-specific chapters. Considerations to inform the assessment include:

Mode of transmission of the pathogen. For pathogens that do not feature vertical transmission, the measures of Chapter 4.Z. would provide a high degree of risk mitigation. For pathogens that do feature vertical transmission, a lower standard of risk mitigation would be provided by the provisions of chapter 4.Z.

Relevance of trade in gametes and fertilised eggs. For some species, such as salmonids, there is existing trade in fertilised eggs which is suitable due to a relatively long incubation period of eggs and tolerance to mechanical disturbance at some stages of development. For some fish species (e.g. warm-water species), trade of gametes and fertilised eggs is uncommon or does not occur because the incubation period is short (hours or days), and eggs may be fragile. For these species, an alternative trade of fingerlings occurs.

Availability of an egg disinfection protocol. Accepted egg disinfection protocols exist for salmonid eggs and a protocol is included in Chapter 4.5. 'Recommendations for surface disinfection of salmonid eggs' of the *Aquatic Code*. Egg disinfection protocols may be used routinely for other fish species but the protocols may not have been validated for WOAH listed diseases. No protocols are included in the *Aquatic Code* for disinfection of non-salmonid eggs.

Disease	Transmission	Hosts	Accepted egg disinfection protocol available	Comments	Include the proposed model article in the disease-specific chapter
Infection with epizootic haematopoietic necrosis virus	Horizontal. Vertical transmission not known.	Species from 9 families, including one species of Salmonidae	No	Trade in gametes and fertilised eggs may occur for some susceptible species, e.g. *O. mykiss*. Modes of transmission of this disease have not been studied sufficiently to exclude vertical transmission.	**No**

Disease	Transmission	Hosts	Accepted egg disinfection protocol available	Comments	Include the proposed model article in the disease-specific chapter
Infection with *Aphanomyces invadans* (Epizootic ulcerative syndrome)	Horizontal.	Species from 27 families	No	Trade in gametes and fertilised eggs may occur for some susceptible species, e.g. *O. mykiss*. Provisions of Chapter 4.Z. may not be suitable for this disease as diagnostic methods have not been validated sufficiently for surveillance of apparently healthy animals.	**No**
Infection with *Gyrodactylus salaris*	Horizontal.	Several species of Salmonidae	Yes	Trade in gametes and fertilised eggs occurs for susceptible species. Provisions of Chapter 4.Z. are not considered necessary to mitigate transmission risk as there are alternative methods that can be used to adequately manage transmission risks (e.g. sourcing broodstock from seawater, egg disinfection)	**No** (note: Inclusion of an article in the future for egg disinfection is recommended)
Infection with infectious salmon anaemia virus	Horizontal.	Three species of Salmonidae	Yes	Trade in gametes and fertilised eggs occurs for susceptible species. Disease-specific chapter currently includes an article on trade of disinfected eggs (Article 10.4.20.).	**Yes** Include the model article to replace Article 10.4.20.
Infection with salmonid alphavirus	Horizontal.	Three species of Salmonidae and one species of Pleoronectidae	Yes	Trade in gametes and fertilised eggs occurs for susceptible species. Disease-specific chapter currently includes an article on trade disinfected eggs (Article 10.5.15.).	**Yes** Include the model article to replace Article 10.5.15.
Infection with infectious haematopoietic necrosis virus	Horizontal.	Many species of salmonids and one species of Esocidae	Yes	Trade in gametes and fertilised eggs occurs for susceptible species. Disease-specific chapter currently includes an article on trade of disinfected eggs (Article 10.6.15.).	**Yes** Include the model article to replace Article 10.6.15.
Infection with koi herpesvirus	Horizontal.	Common carp (Cyprinus carpio) and hybrids	Yes	Trade in gametes and fertilised eggs is not common for these susceptible species.	**No**

Disease	Transmission	Hosts	Accepted egg disinfection protocol available	Comments	Include the proposed model article in the disease-specific chapter
Infection with red sea bream iridovirus	Horizontal. No evidence for vertical transmission.	Numerous species of warm water fish	No	Trade in gametes and fertilised eggs is not common for these susceptible species. Validated egg disinfection protocols for this pathogen are required. Alternative risk management measures are currently preferable (e.g. trade from free countries, zones or compartments, or the application of provisions of Article 10.11.10.)	**No**
Infection with spring viraemia of carp virus	Horizontal. Note: virus has been detected in ovarian fluid.	Several species of cyprinid, one species of Siluridae	Unknown. Iodophore disinfection is likely to be effective but level of validation is unclear.	Trade in gametes and fertilised eggs is unlikely for susceptible species.	**No**
Infection with viral haemorrhagic septicaemia virus	Horizontal.	Species from 30 families including 11 species of salmonid	Yes	Trade in gametes and fertilised eggs occurs for some susceptible species. Disease-specific chapter currently includes an article on trade of disinfected eggs (Article 10.10.15.).	**Yes** Include the model article to replace Article 10.10.15.
Infection with tilapia lake virus	Vertical transmission	Several species of tilapia	N/A (vertical transmission)	Provisions of chapter 4.Z. not suitable for this disease as there are indications of vertical transmission. Alternative risk management measures are preferable (e.g. trade from free countries, zones or compartments, or the application of provisions of Article 10.11.10.)	**No**

As a result of this assessment, the Commission agreed to only include model Article 10.X.15. to Chapter 10.5. 'Infection with SAV', Chapter 10.6. 'Infection with IHNV' and Chapter 10.10. 'Infection with VHSV', and Article 10.4.20. for Chapter 10.4. 'Infection with ISAV'.

The Commission did not agree to some proposed changes to model Article 10.X.15. as these are covered by the new point 3 in Article 4.Z.3.

The revised model Article 10.X.10. for Chapter 10.5. 'Infection with SAV', Chapter 10.6. 'Infection with IHNV' and Chapter 10.10. 'Infection with VHSV', and Article 10.4.15. for Chapter 10.4. 'Infection with ISAV' are presented as Annex 43 for comments.

The revised model Article 10.X.15. for Chapter 10.5. 'Infection with SAV', Chapter 10.6. 'Infection with IHNV' and Chapter 10.10. 'Infection with VHSV', and Article 10.4.20. for Chapter 10.4. 'Infection with ISAV' are presented as Annex 44 for comments.

Relevant Glossary definitions

The Commission agreed with a comment to add 'holding broodstock' to the definition of 'collection and incubation centre' as the facility may also need to maintain broodstock on site.

The Commission suggested changing the glossary term 'egg' to 'fertilised egg' to remove any confusion between unfertilised and fertilised eggs.

The Commission suggested adding '(contained within seminal fluid or milt)' to the definition of the glossary term 'gametes' to specify what is meant be 'sperm'.

As a result to the suggested revisions to 'fertilised eggs' and 'gametes', the Commission made alignments throughout the draft new Chapter 4.Z., replacing 'milt' with 'gametes'.

The Commission did not agree with a comment to add glossary terms for 'grow-out' and 'harvest'.

The new glossary term and revised glossary terms are presented as Annex 45 for comments.

7.3. Draft new Chapter 5.X. Movement of ornamental aquatic animals

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

Background

At its September 2023, the Aquatic Animals Commission reviewed a draft new Chapter 5.X. 'Movement of ornamental aquatic animals', which it had developed taking into account input from Aquatic Animal Focal Point seminars where the proposed need, purpose and scope had been discussed.

Chapter 5.X. provides recommendations for managing the disease risks associated with movement of ornamental aquatic animals and complements other provisions of the *Aquatic Code*, including the measures specified in the disease-specific chapters.

The Commission added a new definition for 'ornamental aquatic animal' to the glossary of the *Aquatic Code* to ensure a common understanding of this term given the importance of its use in the draft new Chapter 5.X.

The draft new chapter and new glossary term have been circulated once for comments.

Previous Commission reports where this item was discussed

September 2023 report (Item 6.7., page 12).

February 2024 meeting

General Comments

The Aquatic Animals Commission reviewed comments received and noted that Members were generally supportive of the new draft Chapter 5.X. 'Movement of ornamental aquatic animals'.

The Commission acknowledged a comment that trade in ornamental animals can extend to animals outside of those covered in Chapter 5.X. such as coral and echinoderms. The glossary definition of aquatic animals includes fish, crustaceans, molluscs and amphibians, thus while other species may be traded they are outside the current scope of the *Aquatic Code*. However, the Commission noted that the principles in Chapter 5.X. are not limited to taxa for which there are listed diseases and could be applied other aquatic animal taxa.

The Commission noted a comment that expressed concern that a chapter should not be developed for specific markets or end-uses such as trade in ornamental aquatic animals. The Commission noted that the development of a chapter for movement of ornamental aquatic animals was one of the recommendations based on Member requests at the Global Conference on Aquatic Animal Health held in Chile in April 2019, and was subsequently included in the WOAH Aquatic Animal Health Strategy. The development of this chapter was included in the Commission work plan and circulated for Member comment since September 2022. All feedback from Members has been supportive of the development of this chapter.

The Commission acknowledged comments that expressed concern regarding the inclusion of biodiversity and conservation status prior to commencement of a risk analysis. The Commission noted that the import of live species requires a Competent Authority to first consider whether the import is legal under national legislation that may extend beyond biosecurity and disease control. The text references these relevant national regulations because it would be of no benefit to undertake a risk analysis for a species that would not be a legal import under other national regulations. Edits were made through the text to reinforce and clarify this principle.

The Commission considered comments suggesting different descriptive terminology for the level of mortality at which to consider action or requirements. The Commission decided to utilise the word ‘unexplained’ throughout the text. Low levels of mortality may be expected in the daily functioning of an aquaculture establishment and may vary among species and system types. However, ‘unexplained’ mortality should be investigated to determine whether an infectious disease is the cause.

The Commission acknowledged comments suggesting removal of statements that the Competent Authority should select the least restrictive measures to mitigate risk. The Commission noted that the ‘World Trade Organization (WTO) Agreement on the Application of Sanitary and Phytosanitary measures’ (SPS Agreement) states that ‘Members shall ensure that such measures are not more trade-restrictive than required to achieve their appropriate level of sanitary or phytosanitary protection’. These statements in Chapter 5.X. are consistent with the SPS Agreement, and relevant to the discussion on the movement of ornamental animals. This statement was removed from the text in some instances where it was repeated within a specific article.

Article 5.X.1.

In paragraph 1, the Commission did not agree to modify text from ‘address’ risk of disease transmission to ‘prevent’. The text as written reflect the objective of the recommendations in Chapter 5.X. to prevent the entry of a pathogen into a free country, zone or compartment.

Article 5.X.2.

The Commission agreed to suggested amendments in paragraph 1, to clearly state in the scope that information related to listed diseases are in disease-specific chapters.

Article 5.X.3.

The Commission agreed that the examples in point 1 were not needed as they are included in Article 5.X.4.

Article 5.X.4.

The Commission agreed with a comment that Members may have their own list of conservation status for species and this should be considered in addition to consideration of CITES.

The Commission agreed with a comment that public health may be relevant when determining eligibility for import, and amended paragraph 3 to reflect this principle.

Article 5.X.5.

The Commission considered comments that a biosecurity plan should be a requirement for all aquaculture establishments. The Commission did not agree, as there are risk management measures which occur through the import pathway to mitigate risk and a biosecurity plan may not be required. Any requirement for a biosecurity plan should be commensurate with the identified risks and the process for developing risk management measures.

Article 5.X.6.

The Commission noted comments for Article 5.X.6. and Article 5.X.7. that references to surveillance should be added in addition to 'official control programme'. The Commission agreed to review the definition of an 'official control programme' which is referred to in Chapter 5.1. 'General obligations related to certification' and added this to its workplan. The review of this definition and the usage of 'official control programme' will address this issue.

The Commission did not agree to reference a 'free establishment' along with free country, free zone or free compartment in paragraph 2. The Commission noted this status does not exist within the *Aquatic Code*.

Article 5.X.8.

The Commission noted a comment suggesting removal of paragraph 2, the Commission did not agree as this paragraph provides context for this article on risk management.

Article 5.X.9.

The Commission considered a comment proposing the addition of measures to ensure diseases that may impact trade are notifiable. The Commission did not agree to this addition as this may not be a feasible or necessary recommendation for some diseases and specific pathways (e.g. non-listed diseases for which measures are required by few importing countries).

The Commission considered a comment to add a reference to the *WOAH Manual* for point 4. The Commission did not agree as this article can be applied to both listed and non-listed diseases, and the *Aquatic Manual* is only relevant to listed diseases.

Article 5.X.10.

The Commission agreed with a comment to change terminology from 'may' to 'should' in regards to the treatment of effluent water from quarantine facilities.

Article 5.X.11.

The Commission acknowledged comments on point 3 regarding the traceability of ornamental aquatic animals and the feasibility of tracing animals to different end-points. The Commission modified point 3 to refer to traceability to commercial establishments. The Commission noted that all the points presented in Article 5.X.11. are risk management options that can facilitate the safe movement of ornamental aquatic animals dependent on the circumstances of the movement.

The Commission considered a suggestion to add a fourth point on the quarantine of imported ornamental aquatic animals due to the risk of stress of transport leading to disease presentation. The Commission noted that quarantine is referenced in Article 5.X.10.

Article 5.X.12.

The Commission agreed with a comment that increased morbidity and mortality of animals not related to disease should be included as this is also a reflection on animal welfare during transport.

The Commission noted a comment that protocols or transport should be appropriate to the life stage of the aquatic animal, and text was amended to reflect this comment.

In paragraph 3, the Commission considered a comment that live animal regulations for safe transport by air addresses some aspects of animal welfare, and agreed to include reference to this in the text.

The draft new Chapter 5.X. 'Movement of ornamental aquatic animals' is presented as Annex 46 for comments.

The new glossary term is presented as Annex 45 for comments.

7.4. Assessment of default periods in Articles X.X.4. – X.X.8. for disease-specific chapters

Background

The revised Chapter 1.4. 'Aquatic animal disease surveillance' of the *Aquatic Code*, adopted in May 2022, provides guidance for the declaration of disease freedom via four different pathways, including: 1. absence of susceptible species; 2. historical freedom; 3. targeted surveillance; and 4. returning to freedom.

Chapter 1.4. specifies default minimum periods of basic biosecurity conditions (BBC) for all four pathways and targeted surveillance (TS) for pathways 3 and 4. The disease-specific chapters in the *Aquatic Code* provide more specific recommendations for these periods of BBC and TS which may exceed the default periods as determined necessary following assessment against criteria included in Chapter 1.4.

When Chapter 1.4. was adopted in May 2022, the periods of BBC and TS were put under study pending assessment.

February 2024 Meeting

The Aquatic Animals Commission considered the assessment and recommendations for periods of basic biosecurity conditions (BBC) and targeted surveillance (TS) for the disease-specific chapters of the *Aquatic Code* prepared at the request of the Commission by a Collaborating Centre expert. The Commission commended the expert for their comprehensive work.

The assessment to make recommendations for periods for BBC and TS was completed by applying the relevant criteria included in Chapter 1.4. 'Aquatic Animal Disease Surveillance' of the *Aquatic Code*. Relevant pathogen-specific information on the likelihood of pathogen detection by either an early detection system (i.e. passive surveillance) or by TS was extracted from the disease-specific chapters of the *Aquatic Manual*. For each pathway, the relevant information was used to rank pathogens and the rankings were used to recommend periods for BBC for each pathway, and TS for pathways 3 and 4.

The tables below contain the outcomes of the assessment and recommendations for BBC and TS for each disease for pathways 1 to 3.

Recommendations for periods of BBC using Pathway 1. 'Absence of susceptible species':

Period	Diseases of fish	Diseases of crustaceans	Diseases of molluscs	Diseases of amphibians
6 months	EHNV *G. salaris* IHNV ISAV KHV RSIV SVCV TiLV	AHPND *H. penai* IHHNV IMNV MrNV WSSV YHV1	AbHV *B. exitiosa* *B. ostreae* *P. marinus* *M. refringens* *X. californiensis*	*B. dendrobatidis* *B. salmondrivorans* *Ranavirus*
12 months	SAV	Crayfish plague		
Pathway not suitable	EUS VHSV		*P.olseni*	

Recommendations for periods of BBC using Pathway 2. 'Historical freedom':

Period	Diseases of fish	Diseases of crustaceans	Diseases of molluscs	Diseases of amphibians
10 years	EHNV EUS IHNV ISAV RSIV SAV SVCV TiLV VHSV	AHPND Crayfish plague *H. penai* IHHNV IMNV MrNV WSSV YHV1	AbHV *B. exitiosa* *B. ostreae* *P. marinus* *M. refringens* *P. olseni* *X. californiensis*	*B. dendrobatidis* *B. salmondrivorans* *Ranavirus*
15 years	*G. salaris* KHV			

Recommendations for periods of BBC and TS for claims of freedom for countries and zones using Pathway 3. 'Targeted surveillance':

Period	Diseases of fish	Diseases of crustaceans	Diseases of molluscs	Diseases of amphibians
BBC				

Period	Diseases of fish	Diseases of crustaceans	Diseases of molluscs	Diseases of amphibians
1 year	EHNV EUS IHNV ISAV RSIV SAV SVCV VHSV TiLV	AHPND Crayfish plague *H. penai* IHHNV IMNV MrNV WSSV YHV1	AbHV	*B. dendrobatidis* *B. salmondrivorans* *Ranavirus*
2 years	KHV *G. salaris*		*B. exitiosa* *B. ostreae* *P. marinus* *M. refringens* *P. olseni* *X. californiensis*	
TS				
2 years	*A. astacii* EHNV EUS IHNV ISAV RSIV SAV SVCV VHSV	AHPND Crayfish plague *H. penai* IHHNV IMNV MrNV WSSV YHV1	AbHV	*B. dendrobatidis* *B. salmondrivorans* *Ranavirus*
3 years	*G. salaris* KHV		*B. exitiosa* *B. ostreae* *P. marinus* *M. refringens* *P. olseni* *X. californiensis*	

Members are requested to provide comment on the process and outcomes of the assessment, which will be utilised to update the periods of BBC and TS in Articles X.X.5., X.X.6. and X.X.7. of the disease-specific chapters of the *Aquatic Code* (Articles 10.4.5. to 10.4.10. for Chapter 10.4. 'Infection with ISAV') at the Commission's September 2024 meeting. The Commission wished to bring Members' attention to Attachment 1 of Annex 47 which presents the BBC and TS periods that were included in the disease-specific chapters in the 2021 version of the *Aquatic Code*, i.e. prior to adoption of the revised Chapter 1.4. and associated articles of the disease-specific chapters in 2022.

The report on the recommendations for periods of basic biosecurity conditions and targeted surveillance for the disease-specific chapters of the *Aquatic Code* is presented as Annex 47 for comment.

7.5. Article 9.9.2. of Chapter 9.9. 'Infection with white spot syndrome virus'

Background

The *ad hoc* Group on Susceptibility of Crustacean Species to Infection with WOAH Listed Diseases met in November 2023 to continue its work to apply the criteria in Chapter 1.5. 'Criteria for listing species as susceptible to infection with a specific pathogen'. At this meeting the *ad hoc* Group conducted the assessments for susceptibility of crustacean species to infection with white spot syndrome virus (WSSV). This assessment is an update of a previous assessment completed in 2016.

February 2024 meeting

The Aquatic Animals Commission considered the *ad hoc* Group report on Susceptibility of Crustacean Species to Infection with WSSV and commended the members for their comprehensive work.

The Commission agreed to apply Article 1.5.9. 'Listing of susceptible species at a taxonomic ranking of Genus or higher' and to amend the list of susceptible species in Article 9.9.2. in line with the recommendations of the *ad hoc* Group.

Based on Article 1.5.9., to list susceptible species at a ranking of Genus or higher:

- 'more than one species within the taxonomic ranking has been found to be susceptible' AND
- 'no species within the taxonomic ranking has been found to be non-susceptible to infection'

The Commission applied this to the list of species found to be susceptible to infection with WSSV and determined that Penaeidae and Portunidae should be listed at the family level and that *Procambarus*, *Palaemon* and *Panulirus* should be listed at the genus level. This is shown in the table below (highlighted grey):

Family	Genus	Species
Astacidae	Austropotamobius	*Austropotamobius pallipes*
	Pacifastacus	*Pacifastacus leniusculus*
	Pontastacus	*Pontastacus leptodactylus*
Calanidae	Calanus	*Calanus pacificus californicus*
Cambaridae	Faxonius	*Faxonius limosus*
	Procambarus	*Procambarus clarkii*
		Procambarus zonangulus
Cancridae	Cancer	*Cancer pagurus*
Nephropidae	Homarus	*Homarus gammarus*
	Nephrops	*Nephrops norvegicus*
Nereididae	Dendronereis	*Dendronereis sp.*
Paguridae	Pagurus	*Pagurus benedicti*
Palaemonidae	Palaemon	*Palaemon carinicauda*
		Palaemon orientis
		Palaemon ritteri
Palinuridae	Panulirus	*Panulirus penicillatus*
		Panulirus versicolor
Parastacidae	Cherax	*Cherax quadricarinatus*
Penaeidae	Metapenaeus	*Metapenaeus ensis*

Family	Genus	Species
	Penaeus	*Penaeus chinensi*
		Penaeus indicus
		Penaeus japonicus
		Penaeus monodon
		Penaeus paulensis
		Penaeus stylirostris
		Penaeus vannamei
	Trachysalambria	*Trachysalambria curvirostris*
Polybiidae	Liocarcinus	*Liocarcinus depurator*
	Necora	*Necora puber*
Portunidae	Charybdis	*Charybdis (Charybdis) granulata*
	Portunus	*Portunus sanguinolentus*
	Scylla	*Scylla serrata*
Varunidae	Eriocheir	*Eriocheir sinensis*

Relevant sections of Chapter 2.2.8. Infection with white spot syndrome virus, in the *Aquatic Manual* were also amended in line with the recommendations of the *ad hoc* Group (see item 10.1.1.).

The Commission encouraged Members to refer to the *ad hoc* Group's November 2023 report available on the WOAH Website for details of the assessment conducted by the *ad hoc* Group.

The revised Article 9.9.2. of Chapter 9.9. 'Infection with white spot syndrome virus' is presented as Annex 48 for comments.

7.6. Articles 11.6.1. and 11.6.2. of Chapter 11.6. 'Infection with *Perkinsus olseni*'

Background

The *ad hoc* Group on Susceptibility of Mollusc Species to Infection with WOAH Listed Diseases met in June and November/December 2023 to continue its work to apply the criteria in Chapter 1.5. 'Criteria for listing species as susceptible to infection with a specific pathogen'. At these meetings the *ad hoc* Group conducted the assessments for susceptibility of mollusc species to infection with *Perkinsus olseni*.

February 2024 meeting

The Aquatic Animals Commission considered the *ad hoc* Group report on Susceptibility of Mollusc Species to Infection with *Perkinsus olseni* and commended its members for their comprehensive work.

The Commission agreed to amend the list of susceptible species in Article 11.6.2. in line with the recommendations of the *ad hoc* Group, i.e.

- Six species currently listed in Article 11.6.2. as susceptible to infection with *P. olseni*, *Anadara trapezia*, blacklip abalone (*Haliotis rubra*), greenlip abalone (*Haliotis laevigata*), grooved carpet shell (*Ruditapes decussatus*), Japanese carpet clam (*Ruditapes philippinarum*), and Stutchbury's venus clam (*Austrovenus stutchburyi*) were assessed to meet the criteria for listing as susceptible to infection with *P. olseni*, and are therefore proposed to remain in Article 11.6.2.

- Nine new susceptible species, crocus giant clam (*Tridacna crocea*), half-crenated ark cockle (*Anadara kagoshimensis*), Japanese pearl oyster (*Pinctada fucata*), Jedo venus clam (*Leukoma*

jedoensis), Mediterranean mussel (*Mytilus galloprovincialis*), New Zealand mussel (*Perna canaliculus*), *Proteopitar patagonicus*, rooster venus clam (*Protapes gallus*) and undulate venus clam (*Paratapes undulatus*) were assessed to meet the criteria for listing as susceptible to infection with *P. olseni* and are therefore proposed to be added to Article 11.6.2.

- Eight species currently included in Article 11.6.2. as susceptible to infection with *P. olseni*, Ariake cupped oyster (*Magallana ariakensis*) *Barbatia novaezealandiae*, corrugated venus clam (*Venerupis corrugata*), golden carpet shell (*Polititapes aureus*), *Haliotis cyclobates*, *Haliotis scalaris*, *Macomona liliana*, and pipi wedge clam (*Paphies australis*) were assessed to not meet the criteria for listing as susceptible to infection with *P. olseni* and are therefore proposed to be removed from Article 11.6.2.

Relevant sections of Chapter 2.4.6. 'Infection with *Perkinsus olseni*' in the *Aquatic Manual* were also amended in line with the recommendations of the *ad hoc* Group (see item 10.2.1.).

The Commission encouraged Member to refer to the *ad hoc* Group's December 2023 report available on the WOAH Website for details of the assessment conducted by the *ad hoc* Group.

The revised Article 11.6.2. of Chapter 11.6. 'Infection with *Perkinsus olseni*' is presented as Annex 49 for comments.

8. Items for Member Information

8.1. Chapter 4.3. Application of compartmentalisation – discussion paper

Comments and responses were received from Australia, Canada, Chile, China (People's Rep. of), Japan, New Caledonia, Norway, Peru, Singapore, Thailand, the UK, the USA, AU-IBAR, and the EU.

Background

At its September 2023 meeting, the Aquatic Animals Commission agreed to develop a discussion paper to engage Members on issues relevant to the revision of Chapter 4.3. 'Application of Compartmentalisation'. The Commission highlighted that compartmentalisation provides an opportunity to trade disease-free aquatic animal commodities from zones or countries that are not declared free from the diseases of concern.

The discussion paper was informed by Member responses to a short questionnaire provided in the Commission's September 2022 meeting report, as well as feedback from Focal Point workshops. The paper proposed a range of purposes for applying compartments, high-level principles to guide their application and the concept of dependent and independent compartments. Together these proposals are intended to increase clarity on the application of compartments for effective risk management, while also broadening the range of circumstances where they might be applied.

Previous Commission reports where this item was discussed

September 2023 report (Item 6.5., page 11).

February 2024 meeting

The Aquatic Animals Commission reviewed comments and responses received and noted that responses were generally supportive of the proposed approach to the revision of Chapter 4.3.

The Commission reviewed all Member responses to the questions posed in the discussion paper. Member comments were summarised within the revised discussion paper, with the majority views presented together with significant comments or minority views. Based on this consideration of Member comments, the Commission proposed preferred approaches to the drafting of the revised Chapter 4.3.

This final version of the discussion paper will be used to guide the revision of Chapter 4.3., which will be further considered at the Commission's September 2024 meeting.

The discussion paper with summary of responses and proposed approaches on Chapter 4.3. 'Application of compartmentalisation' is presented as Annex 50 for information.

The WOAH Manual of Diagnostic Tests for Aquatic Animals

The Aquatic Animals Commission has commenced the process of progressively reformatting the disease-specific chapters of the *Aquatic Manual* into a new template. As the reformatted and updated chapters have substantial changes, at its meeting in September 2019, the Commission agreed that only clean versions of the revised chapters would be provided in its report. Subsequent changes made to these initial revisions following Member comments would be indicated in the usual style (i.e. ~~strikethrough for deletions~~ and double underline for additions).

A software-generated document that compares the adopted version of a chapter and the proposed new text can be created. This comparison document is not included in the Commission's report, but will be available upon request from the WOAH Standards Department (AAC.Secretariat@WOAH.org).

Some of the discussions of Member comments require horizontal changes to the *Aquatic Manual* template and all chapters. These comments are as follows:

Section/paragraph	Comment	Decision
Section 2.2.1. 'Susceptible host species' and 2.2.2. 'Species with incomplete evidence for susceptibility'	No comment, this resulted from discussions within the Commission	Agree to display all the species in these Section in tabular format with columns for Family, Scientific name and Common name. Previously tables were used only when there were more than 10 species
Section 2.2.2. 'Species with incomplete evidence for susceptibility'	Does not support the listing of species for which there is "incomplete susceptibility" as to date this is subjective since WOAH has not published any criteria for determining that a species meets "incomplete susceptibility" criteria. If a given specifies does not meet the WOAH criteria for listing as susceptible, then it should not be included in the WOAH chapter.	Disagree to remove Section 2.2.2. as this section is part of the template for the chapters in the *Aquatic Manual* and the rationale for its inclusion has been presented previously and agreed by Members.
6.3.1. 'For presumptive diagnosis of clinically affected animals' and 6.3.2. 'For surveillance of apparently healthy animals'	Clarify that in the footnote to the tables, the variable 'n' stands for the number of animals included in the validation study	Agree, the footnote reads: 'n = number of animals used in the validation study', and the change will be applied to the template and to all chapters as they are reviewed

9. Texts that will be proposed for adoption in May 2024

9.1. Section 2.2. Diseases of crustaceans

9.1.1. Chapter 2.2.0. 'General information: diseases of crustaceans'

Comments were received from Canada, Mexico, Norway, the USA, AU-IBAR and the EU.

Background

At its September 2022 meeting, the Aquatic Animals Commission amended Chapter 2.2.0. 'General Information (diseases of crustaceans)' in consultation with the crustacean disease Reference Laboratory experts.

At its February 2023 meeting, the Commission amended the proposed chapter after considering Member comments.

At its September 2023 meeting, the Commission further amended the proposed chapter after considering Member comments.

Previous Commission reports where this item was discussed

September 2022 report (Item 7.1.1., page 15); February 2023 report (Item 11.1.1., page 43); September 2023 report (Item 8.1.1., page 21).

February 2024 meeting

Section/ paragraph	Comment	Decision
General comment	What is the purpose of this chapter? Is it intended to just include diagnostic information that is common across some listed pathogens or if it is for general diagnostics that can be applied to both listed pathogens and guidance for broad screening methods for potentially emerging pathogens?	The purpose of this chapter is to provide general information on sampling and materials/biological products required for the isolation and identification of crustacean pathogens. Specific information concerning listed diseases of crustaceans is found in the relevant disease-specific chapters of the *Aquatic Manual*.
A.1.1. Sample material to be used for tests	Clarify that the method being used may affect which materials should be sampled and the number needed depending on the diagnostic sensitivity, pooling, etc.	Agree, added 'the diagnostic method to be used' to the first sentence
A.1.2. Specifications according to crustacean populations, point i)	Replace 'abnormal' mortality with 'unexplained' mortality	Disagree, the mortality is not just unexplained, it is abnormally high. Also, the term 'abnormal mortality' is used in both the fish and mollusc general information chapters (2.3.0 and 2.4.0, respectively
A.1.2. Specifications according to crustacean populations, point ii)	Replace 'sample' with 'collection' to clarify that the intent is for 'sample' to mean potentially more than 1 sample, rather combining all tissues/animals into a single pooled sample	Partly agree, corrected to 'sample collection'

Section/ paragraph	Comment	Decision
A.1.3. Specifications according to clinical status	In the description of samples to be collected, delete ‘or moribund’ after ‘live crustaceans’ and replace with ‘moribund animals are preferred’ because moribund animals are alive and preferred	Disagree, moribund animals are not necessarily preferred depending on the sampling context. Agree to delete ‘or’ and put ‘(including moribund)’ after ‘live’
	Replace ‘recently dead’ with ‘freshly dead’ samples	Disagree: recently dead is the correct term
A.2.2. Virological examination	Remove the word ‘routinely’ as virus isolation by cell culture is not possible because there are no crustacean cell lines currently available	Agree
B.1.3.2. Virus production for experimental purposes	Clarify that the text is referring to *in-vitro* infection	Agree
B.5. Techniques	Add a sentence regarding the need to increase the number of pools of samples to account for any decrease in sensitivity of test outcomes due to pooling	Disagree, this is standard text and already covers the need for pooling to be thoroughly evaluated and only used where robust supporting data on sensitivity and specificity have been found to be suitable
B.5.3. Histological techniques	Delete ‘or moribund’ between ‘live’ and ‘specimens’ as moribund specimens are alive	Disagree, added ‘(including’ before moribund, and closed the brackets as had been done in Section A.1.3. above
B.5.3.1. Fixation	Clarify if for very large crustaceans, tissues are fixed prior to dissection or can the dissected tissues be placed immediately into fixative	the text in point ii) Fixation procedures with Davidson’s AFA states that ‘For large juveniles and adults: to ensure proper fixation, kill the crustacean using a humane method, then immediately inject fixative (use 5–10% volume:weight).’
B.5.3.1. Fixation, point ii) Fixation procedures with Davidson’s AFA	Delete the clause ‘kill the crustacean using a humane method then’	Disagree, killing using a humane method is necessary.
B.5.3.1. Fixation, point iii) Transport and shipment of preserved samples	Clarify that the samples should be fixed for 24–72 hours before removing them from alcohol	Disagree as the issue it addressed in the point above on fixation procedures. Point ii) covers transport and shipment of already preserved samples
B.5.5.1 Sample preparation and types	For consistency, delete the mention of antibody-based diagnostic test	Agree

Section/ paragraph	Comment	Decision
B.5.5.2 Preservation of RNA and DNA in tissues	Reinstate the recommendation that samples can be stored for up to 1 month at 4°C	Disagree, the recommendations in the chapter must not to affect the sensitivity of the tests, and given that samples may need to be kept for a month if re-testing is required, the recommendation is store for up to 1 week at 4°C or indefinitely at –20°C
	Maintain ethanol concentration within an acceptable range or provide the rationale for specifying a single ethanol concentration of 80%	The recommendation to preserve samples in 80%analytical grade ethanol is standard text based on advice from a WOAH Reference Laboratory

The revised Chapter 2.2.0. 'General information (diseases of crustaceans)' is presented as Annex 51 and will be proposed for adoption at the 91st General Session in May 2024.

9.1.2. Chapter 2.2.2. Infection with '*Aphanomyces astaci* (crayfish plague)'

Comments were received from Canada, China (People's Rep. of), Norway, Switzerland, Thailand, the UK, AU-IBAR and the EU.

Background

At its September 2022 meeting, the Aquatic Animals Commission reviewed Chapter 2.2.2. 'Infection with *Aphanomyces astaci* (crayfish plague)' which had been updated by the WOAH Reference Laboratory experts and reformatted using the new disease chapter template.

At its February 2023 meeting, the Commission amended the proposed chapter after considering Member comments.

At its September 2023 meeting, the Commission the Commission further amended the proposed chapter after considering Member comments.

Previous Commission reports where this item was discussed

September 2022 report (Item 7.1.3., page 17); February 2023 report (Item 11.1.2., page 44); September 2023 report (Item 8.1.2., page 23).

February 2024 meeting

Section/ paragraph	Comment	Decision
2.2.3. Likelihood of infection by species, host life stage, population or sub-populations, paragraph 1	Replace 'mortalities' with 'experience mortality' and clarify that the last sentence refers to susceptible species	Agree

Section/ paragraph	Comment	Decision
2.2.3. Likelihood of infection by species, host life stage, population or sub-populations, paragraph 2	Correct the common name for *Pontastacus leptodactylus*	Agree, added the common name Danube crayfish
2.2.3. Likelihood of infection by species, host life stage, population or sub-populations, paragraph 5	Delete the sentence as the Chinese mitten crab (*Eriocheir sinensis*) is a vector of the crayfish plague pathogen, not a susceptible species	Disagree, the Chinese mitten crab fits the criteria for susceptibility (natural infection, viability in the host, transmissibility, etc.) and as such cannot be considered a vector according to the glossary definition
2.2.5. Aquatic animal reservoirs of infection, paragraph 1	Reinstate European crayfish as carriers and not a reservoir of infection	Disagree, carriers transfer disease without becoming infected. European crayfish perpetuate the infection in wild populations and thus act as a reservoir
2.2.5. Aquatic animal reservoirs of infection, paragraph 2	Add ‘Natural or human-induced’ before ‘colonisation’	Disagree, not sure what is meant by natural colonisation
2.3.2. Clinical signs, including behavioural changes	Replace ‘epizootic’ with ‘outbreak’, and ‘carriers’ with ‘reservoirs’ to align with terminology in the rest of the section	Agree and clarified that the section covers susceptible species
2.3.3. Gross pathology	Not a Member comment but from the Commission. A Member proposed reinstating ‘Susceptible’ species twice in this section.	Agree. This will be discussed to ensure consistency with Section 2.3.3 in other chapters.
2.4.7. General husbandry	Replace ‘animals (crayfish, finfish)’ with ‘susceptible species’ as current listed susceptible species include some species of freshwater crabs in points 1 and 3	Agree and included ‘or vectors’ to keep the original intent of the text
3.2. Selection of organs or tissues	Add ‘and eyestalks’ to the recommended samples as *Aphanomyces astaci* can be found in eyestalks	Agree
	Replace ‘carriers’ with ‘reservoirs’ for consistency	Agree, also removed mention of species that are prone to clinical disease, to clarify that the Section covers both clinically affected and apparently healthy animals

Section/ paragraph	Comment	Decision
Table 4.1 WOAH recommended diagnostic methods and their level of validation for surveillance of apparently healthy animals and investigation of clinically affected animals	Replace 'LAMP' with immunohistochemistry' as the latter is described in the chapter and delete 'other antigen detection methods' as they are included in the row 'Other methods'	Agree
4.2. Histopathology	Add some context on the detection of invasive hyphae	Agree, clarified that *A. astaci* can be visualised in tissues of crayfish species prone to clinical disease
4.4. Nucleic acid amplification	Add a statement that commercial machines can be used for chemical destruction of samples	Amended the text to refer to tissue homogenisation using standard physical methods
4.4.1. Real-time PCR	Method 1, the cycling parameters given in the table are not the same as those in the two publications	Agree, deleted one of the references and amended the table to correspond with the conditions given in the remaining cited paper
	Include the amplicon size	Disagree, the amplicon is not important in the real-time PCR and is not in the standard Table template.
		As the Strand *et al.* (2023) reference has now been published, the method has been reinstated in the Table, and the text in Section 4.4.1 and Section 5 has been amended accordingly, along with the tables in Section 6.3
	Add a statement that amplification reduces the sensitivity of the diagnostic method	Disagree, the topic is covered in Section 6. Corroborative diagnostic criteria
6.1.2. Definition of confirmed case in apparently healthy animals	Delete point i) and replace with a statement that there is no method to confirm cases in apparently healthy animals because the sensitivity of the methods is low	Disagree, if confirmation is not possible on the available suspect cases, it is necessary to resample the suspect population to confirm or exclude infection

The revised Chapter 2.2.2. Infection with '*Aphanomyces astaci* (crayfish plague)' is presented as Annex 52 and will be proposed for adoption at the 91st General Session in May 2024.

9.1.3. Chapter 2.2.6. 'Infection with Macrobrachium rosenbergii nodavirus (white tail disease)'

Comments were received from Canada, China (People's Rep. of), Norway, Thailand, the UK and the EU.

Background

At its February 2023 meeting, the Aquatic Animals Commission reviewed Chapter 2.2.6. 'Infection with Macrobrachium rosenbergii nodavirus (white tail disease)' which had been updated by the WOAH Reference Laboratory experts and a Commission member, and reformatted using the new disease chapter template.

At its September 2023 meeting, the Commission amended the proposed chapter after considering Member comments.

Previous Commission reports where this item was discussed

February 2023 report (Item 11.1.3., page 46); September 2023 report (Item 8.1.3., page 24).

February 2024 meeting

Section/ paragraph	Comment	Decision
General comment	The International Committee on Taxonomy of Viruses (ICTV) has named Macrobrachium rosenbergii nodavirus in regular script, and its abbreviation is "MrNV", to be presented without italic script	Agree, MrNV is not an accepted species so should not be in italics: this change will be made throughout the chapter
4.4.2. Conventional RT-PCR	Delete Sri Widada *et al.* (2003) from the text as it is an incorrect citation	Agree, it has already been deleted from the table of PCR parameters
6.3.1. For presumptive diagnosis of clinically affected animals	Delete Sri Widada *et al.* (2003) from the table as it is an incorrect citation	Agree

The revised Chapter 2.2.6. 'Infection with Macrobrachium rosenbergii nodavirus (white tail disease)' is presented as Annex 53 and will be proposed for adoption at the 91st General Session in May 2024.

9.1.4. Chapter 2.2.9. Infection with 'yellow head virus genotype 1'

Comments were received from Canada, Norway, Thailand, the UK and the EU.

Background

At its February 2023 meeting, the Aquatic Animals Commission reviewed Chapter 2.2.9. 'Infection with yellow head virus genotype 1', which had been updated by the WOAH Reference Laboratory expert and reformatted using the new disease chapter template.

At its September 2023 meeting, the Commission amended the proposed chapter after considering Member comments.

Previous Commission reports where this item was discussed

February 2023 report (Item 11.1.4., page 46); September 2023 report (Item 8.1.4., page 26).

February 2024 meeting

Section/ paragraph	Comment	Decision
Table 4.1 WOAH recommended diagnostic methods and their level of validation for surveillance of apparently healthy animals and investigation of clinically affected animals and Section 4.4.1.	The Reference Laboratory expert had completed the validation report template with the validation data for a real-time PCR.	The Commission accepted the validation report as the basis for including the real-time PCR method in the chapter. The report can be found here.
Table 4.1	Replace 'LAMP' with immunohistochemistry' as the latter is described in the chapter and delete 'other antigen detection methods' as they are included in the row 'Other methods'	Agree
6.1.2. Definition of confirmed case in apparently healthy animals and 6.2.2 Definition of confirmed case in clinically affected animals	Conventional PCR followed by amplicon sequencing is recommended for confirmatory diagnosis, and three RT-PCR protocols are provided in Section 4.4.2. However, these protocols have limitations in detecting virus genotypes. The criteria for confirming YHV1 infection in Sections 6.1.2 and 6.2.2 need to be elaborated, specifically the reference to the use of 'two different RT-PCR methods'. Details on selecting protocols from those listed in Section 4.4.2 should be provided.	A specific real-time RT-PCR method has now been included. Additionally, where conventional RT-PCR is used as a criterion for a confirmed case, the amplicon must be sequenced.
7. References	Include the Walker *et al.* (2021) reference in the list	Agree

The revised Chapter 2.2.9. 'Infection with yellow head virus genotype 1' is presented as Annex 54 and will be proposed for adoption at the 91st General Session in May 2024.

9.1.5. Chapter 2.2.X. 'Infection with decapod iridescent virus 1 (DIV1)'

Comments were received from Canada, China (People's Rep. of), Chinese Taipei, Norway, Peru, Thailand, the UK, the USA and the EU.

Background

At its September 2023 meeting, the Aquatic Animals Commission amended Chapter 2.2.X. 'Infection with decapod iridescent virus 1' which had been developed by the WOAH Reference Laboratory expert and formatted using the new disease chapter template.

Previous Commission reports where this item was discussed

September 2023 report (Item 8.1.5., page 28).

February 2024 meeting

Section/ paragraph	Comment	Decision
2.2.1. Susceptible host species	Remove fleshy prawn (*Penaeus chinensis*) as there is not enough scientific evidence to show that it is a susceptible host species.	Agree, re-evaluated by the *ad hoc* Group on susceptibility of crustacean species to WOAH listed diseases (see item 6.7.)
	Change the scientific name of ridgetail prawn from *Palaemon carinicauda* to *Exopalaemon carinicauda*	Disagree, the accepted name is *Palaemon carinicauda*
2.2.2. Species with incomplete evidence for susceptibility	Delete the Section because it is not appropriate to require testing for a disease in species for which there is incomplete evidence of susceptibility.	Disagree, September 2024 response to this comment: 'Disagree, rationale provided in previous reports and approach agreed by WOAH Members. The scope of trade standards in the Aquatic Code applies only to susceptible species; any measures applied to species with incomplete evidence of susceptibility (which are included in the Aquatic Manual only) would need to be supported by a risk assessment.'
2.2.5. Aquatic animal reservoirs of infection	Change the common name of *Portunus trituberculatus* from gazami crab to swimming crab	Agree, both are given in FAOTERM.
2.3.1. Mortality, morbidity and prevalence	Clarify that mortality after a natural infection with DIV1 has mostly been reported in adult stage shrimp	Agree
2.4.7. General husbandry	Remove the second paragraph because it is experimental and therefore should not be a regular means of disease control	Disagree as the information is useful. Clarified that it concerns an experimental protocol and that DIV1 could rather than can be eliminated from challenged shrimp after 36°C treatment
4.6. *In-situ* hybridisation	The reference Xu *et al.,* 2016 does not cover ISH. It should be replaced with Sanguanrut *et al.,* 2021	Agree

Section/ paragraph	Comment	Decision
6.1.2. Definition of confirmed case in apparently healthy animals	Delete criterion iii) A positive result from each of two different real-time PCR methods because sequencing should be required for the confirmation of this novel pathogen, and two real-time PCR results are not sufficient to confirm detection	Agree, sequencing is preferable for confirmation.
6.2.2. Definition of confirmed case in clinically affected animals	Delete criterion iv) A positive result from each of two different real-time PCR methods (as 6.1.2 above)	Agree (see 6.1.2. above)

The new Chapter 2.2.X. 'Infection with decapod iridescent virus 1' is presented as Annex 55 and will be proposed for adoption at the 91st General Session in May 2024.

9.2. Section 2.4. Diseases of molluscs

9.2.1. Chapter 2.4.0. 'General information: diseases of molluscs'

Comments were received from Canada, China (People's Rep. of), Norway, the UK and the EU.

Background

At its September 2023 meeting, the Aquatic Animals Commission reviewed Chapter 2.4.0. 'General information: diseases of molluscs' which had been updated by the WOAH Reference Laboratory expert. Where relevant, the Commission aligned the text with recommendations in chapters 2.2.0. and 2.3.0., the general information chapters on diseases of crustaceans and fish, respectively.

Previous Commission reports where this item was discussed

September 2023 report (Item 8.2.1., page 29).

February 2024 meeting

Section/ paragraph	Comment	Decision
A.1.2. Specifications according to mollusc populations, point i)	Replace 'abnormal' with 'unexplained', to align with the terminology used in other chapters.	Disagree, 'Abnormal mortality' refers not only to unexplained deaths but also to a mortality rate that is higher than what is typically expected. It should be noted that Chapter 2.3.0. General Information of Fish Disease (adopted in 2022) refers to 'abnormal mortality'
A.1.2. Specifications according to mollusc populations, point ii)	Replace 'weak' by 'moribund'	Disagree, the term 'weak' is more appropriate for molluscs. It should be noted that Chapter 2.3.0. General Information of Fish Disease (adopted in 2022) also refers to 'weak' animals

Section/ paragraph	Comment	Decision
A.1.4. Sampling specifications according to mollusc size	In the previous version of this chapter, specific information on sampling for infection with *Xenohaliotis californiensis* (withering syndrome) and for abalone herpesvirus infections was included in this Section. This information should be reinstated	Agree to reinstate the text as these details are not found elsewhere. Two Sections are added: 1.4.2. For infection with *Xenohaliotis californiensis* and 1.4.3. For abalone herpesvirus infections
B.5. Techniques	Add a sentence regarding the need to increase the number of pools of samples to account for any decrease in sensitivity of test outcomes due to pooling	Disagree, this standard text covers the need for pooling to be thoroughly evaluated and only used where robust supporting data on sensitivity and specificity have been found to be suitable

The revised Chapter 2.4.0. 'General information: diseases of molluscs' is presented as Annex 56 and will be proposed for adoption at the 91st General Session in May 2024.

9.2.2. Chapter 2.4.1. 'Infection with abalone herpesvirus'

Comments were received from Canada, China (People's Rep. of), Norway, Thailand, the UK and the EU.

Background

At its September 2023 meeting the Aquatic Animals Commission amended Chapter 2.4.1. 'Infection with abalone herpesvirus' which had been updated by the WOAH Reference Laboratory expert and reformatted using the new disease chapter template.

Previous Commission reports where this item was discussed

September 2023 report (Item 8.2.2., page 29).

February 2024 meeting

Section/ paragraph	Comment	Decision
General comment	No comment, this resulted from discussions within the Commission	Noting that the pathogenic agent *Aurivirus haliotidmalaco1* agree to use the abbreviation AbHV thoughout the chapter replacing AbHV-1
Table 4.1 WOAH recommended diagnostic methods and their level of validation for surveillance of apparently healthy animals and investigation of clinically affected animals	No comment, this resulted from discussions within the Commission	Deleted histopathology for the purposes of A. Surveillance of apparently healthy animals and C. Confirmatory diagnosis of a suspect result from surveillance or presumptive diagnosis

Section/ paragraph	Comment	Decision
4.3 Histopathology	Delete reference to using 10% formalin and add cross reference to chapter 2.4.0.	Agree
Tables in Section 6.3	No comment, this resulted from discussions within the Commission	Table 6.3.1 was filled in for the conventional PCR and histology and Table 6.3.2 for histology

The revised Chapter 2.4.1. 'Infection with abalone herpesvirus' is presented as Annex 57 and will be proposed for adoption at the 91st General Session in May 2024.

9.2.3. Chapter 2.4.4. 'Infection with *Marteilia refringens*'

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

Background

At its September 2023 meeting the Aquatic Animals Commission amended Chapter 2.4.4. 'Infection with *Marteilia refringens*' which had been updated by the WOAH Reference Laboratory expert and reformatted using the new disease chapter template.

Previous Commission reports where this item was discussed

September 2023 report (Item 8.2.3., page 30).

February 2024 meeting

Section/ paragraph	Comment	Decision
2.2.2. Species with incomplete evidence for susceptibility	Delete the Section because it is not appropriate to require testing for a disease in species for which there is incomplete evidence of susceptibility.	Disagree, September 2023 response to this comment: 'Disagree, rationale provided in previous reports and approach agreed by WOAH Members. The scope of trade standards in the Aquatic Code applies only to susceptible species; any measures applied to species with incomplete evidence of susceptibility (which are included in the *Aquatic Manual* only) would need to be supported by a risk assessment.'
4.1. Wet mounts	Replace 'oysters/mussels' with 'bivalves' as two species of clams are now also listed	Agree
6.3.2. For surveillance of apparently healthy animals	For clarity, replace 'Flat oysters' with '*Ostrea edulis*'	Agree

The revised Chapter 2.4.4. 'Infection with *Marteilia refringens*' is presented as Annex 58 and will be proposed for adoption at the 91st General Session in May 2024.

9.2.4. Section 2.2.1. and 2.2.2. of Chapter 2.4.5. 'Infection with *Perkinsus marinus*'

Comments were received from Canada, Norway, Peru, the UK, the USA, and the EU.

Background

At its February 2023 meeting, the Aquatic Animals Commission amended Sections 2.2.1. and 2.2.2. of Chapter 2.4.5. Infection with *Perkinsus marinus*, in line with the recommendations of the *ad hoc* Group on Susceptibility of Mollusc Species to infection with WOAH listed diseases.

At its September 2023 meeting, the Commission amended the proposed sections after considering Member comments.

Previous Commission reports where this item was discussed

February 2023 report (Item 11.2.1., page 47); September 2023 report (Item 8.2.4., page 30).

February 2024 meeting

The Aquatic Animals Commission changed the scientific name for Gasar cupper oyster in Section 2.2.2. from *Crassostrea tulipa* to *Crassostrea gasar*, in line with the recommendation from the *ad hoc* Group on Susceptibility of Mollusc Species to Infection with WOAH Listed Diseases in their December 2023 report. The original report on the susceptibility of mollusc species to infection with *P. marinus* referenced literature on *C. gasar*, but at the time the *ad hoc* Group changed the species name to *C. tulipa* due to information on WoRMS (now debated information on WoRMS). *C. tulipa* and *C. gasar* continue to be considered distinct species.

The Commission agreed with a comment that the Pacific cupped oyster (*Magallana* [syn. *Crassostrea*] *gigas*) could act as a host carrier for *P. marinus* and noted that it is included in Section 2.2.2. 'Species with incomplete evidence for susceptibility'.

The revised Section 2.2.1. and 2.2.2. of Chapter 2.4.5. 'Infection with *Perkinsus marinus*' is presented as Annex 59 and will be proposed for adoption at the 91st General Session in May 2024.

10. Items for Member comments

10.1. Section 2.2. Diseases of crustaceans

10.1.1. Section 2.2.1. and 2.2.2. of Chapter 2.2.8. 'Infection with white spot syndrome virus'

Background

The *ad hoc* Group on Susceptibility of Crustacean Species to Infection with WOAH Listed Diseases met in November 2023 to continue its work to apply the criteria in Chapter 1.5. 'Criteria for listing species as susceptible to infection with a specific pathogen'. At this meeting the *ad hoc* Group conducted the assessments for susceptibility of crustacean species to infection with white spot syndrome virus (WSSV). This assessment is an update of a previous assessment completed in 2016.

February 2024 meeting

The Aquatic Animals Commission amended Sections 2.2.1. and 2.2.2. of Chapter 2.2.8. 'Infection with WSSV', in line with the recommendations of the *ad hoc* Group on Susceptibility of Crustacean Species to Infection with WOAH Listed Diseases (see Item 7.4.).

The revised Section 2.2.1. and 2.2.2. of Chapter 2.2.8. 'Infection with white spot syndrome virus' is presented as Annex 60 for comments.

10.2. Section 2.4. Diseases of molluscs

10.2.1. Section 2.2.1. and 2.2.2. of Chapter 2.4.6. 'Infection with *Perkinsus olseni*'

Background

The *ad hoc* Group on Susceptibility of Mollusc Species to Infection with WOAH Listed Diseases met in June and November/December 2023 to continue its work to apply the criteria in Chapter 1.5. 'Criteria for listing species as susceptible to infection with a specific pathogen'. At these meetings the *ad hoc* Group conducted the assessments for susceptibility of mollusc species to infection with *Perkinsus olseni*.

February 2024 meeting

The Aquatic Animals Commission amended Sections 2.2.1. and 2.2.2. of Chapter 2.4.6. 'Infection with *P. olseni*', in line with the recommendations of the *ad hoc* Group on Susceptibility of Mollusc Species to Infection with WOAH Listed Diseases (see Item 7.5.).

The revised Section 2.2.1. and 2.2.2. of Chapter 2.4.6. 'Infection with *Perkinsus olseni*' is presented as Annex 61 for comments.

11. Items for Member information

11.1. Develop a mechanism to speed up the process of making updates to diagnostic methods in the *Aquatic Manual*

Background

At its September 2022 meeting, the Aquatic Animals Commission was informed that the Biological Standards Commission was working to develop a template of the validation data that would be requested of applicants wishing to have their tests included in the *Terrestrial Manual*.

At its February 2023 meeting, the Aquatic Animals Commission discussed the template and proposed amendments to streamline it and to make it applicable to the *Aquatic Manual*, for example to replace the seven intended purposes of a diagnostic test in the *Terrestrial Manual* with the three purposes given in the *Aquatic Manual*.

The Aquatic Animals Commission considered that publication of diagnostic accuracy studies in peer-reviewed journals was preferable; however in some instances, this validation report form could provide a mechanism for incorporation of new or revised methods pre-publication.

At its September 2023 meeting, the Aquatic Animals Commission made the final amendments to the template for the validation report form for tests recommended in the *Aquatic Manual*. The intention is to use the template to provide validation data for consideration by the commission for tests proposed for inclusion in the *Aquatic Manual*, before publication of the data in a peer-reviewed journal.

Previous Commission reports where this item was discussed

February 2022 (Item 3.3.1., page 16); September 2022 (Item 8.3., page 25); February 2023 (Item 11.3., page 48); September 2023 (Item 9.1., page 31).

February 2024 meeting

The Reference Laboratory expert on yellow head virus genotype 1 had completed the validation report template with the validation data for a real-time PCR. The Aquatic Animals Commission reviewed the report and agreed that it provided a sound basis for including the method in the *Aquatic Manual*. The validation report is made available on the Commission's page of the WOAH website (here).

12. *Ad hoc* Groups

12.1. *Ad hoc* Group on Susceptibility of Mollusc Species to Infection with WOAH Listed Diseases

The *ad hoc* Group on Susceptibility of Mollusc Species to Infection with WOAH Listed Diseases met in November/December 2023 to complete the assessments for susceptibility of mollusc species to infection with *Perkinsus olseni*.

The Commission was informed that the *ad hoc* Group is planning to meet in June 2024 to progress its work assessing species susceptible to infection with *Xenohaliotis californiensis*.

The report of the *ad hoc* Group on Susceptibility of Mollusc Species to Infection with WOAH Listed Diseases is available on the WOAH Website at: LINK

12.2. *Ad hoc* Group on Susceptibility of fish species to infection with WOAH listed diseases

The *ad hoc* Group on Susceptibility of Fish Species to Infection with WOAH Listed Diseases met in January 2024 to progress its work assessing species susceptible to infection with *Aphanomyces invadans* (epizootic ulcerative syndrome).

The Commission was informed that the *ad hoc* Group is planning to meet in April 2024 to complete its work assessing species susceptible to infection with *Aphanomyces invadans* (epizootic ulcerative syndrome).

12.3. *Ad hoc* Group on Susceptibility of crustacean species to infection with WOAH listed diseases

The *ad hoc* Group on Susceptibility of Crustacean Species to Infection with WOAH Listed Diseases met in November 2023 to complete the assessments for susceptibility of crustacean species to infection with white spot syndrome virus.

The Commission was informed that the *ad hoc* Group is planning to meet in the second half of 2024 to progress its work assessing species susceptible to infection with *Aphanomyces astaci* (crayfish plague).

The report of the *ad hoc* Group on Susceptibility of Crustacean Species to Infection with WOAH Listed Diseases is available on the WOAH Website at: LINK

13. Reference centres or change of experts

13.1. Evaluation of applications for Reference Centres for aquatic animal health issues or change of experts

The Aquatic Animals Commission recommended acceptance of the following application for WOAH Reference Centre status:

WOAH *Collaborating Centre for Fish Health Management in the Middle East Region*
Central Laboratory for Aquaculture Research (CLAR)
Abbassa, Abou Hammad, Sharkia
EGYPT
Tel: +20/553401028 / +20/649201621
E-mail: drmohamedabouelatta@gmail.com; samehabdelazeem7@gmail.com
Website: http://www.arc.sci.eg
http://www.arc.sci.eg/InstsLabs/Default.aspx?OrgID=20&TabId=0&lang
Contact Point: Prof. Mohamed E. Abou El Atta

The Commission extends its gratitude to the applicant for their interest and enthusiasm. Should the application be adopted, this will be the first Collaborating Centre focused on aquatic diseases on the African continent.

WOAH Collaborating Centre for Reference Materials of Molecular Diagnostic Techniques in Aquatic and Terrestrial Animal Diseases
The National Institute of Fisheries Science, NIFS (under the Ministry of Oceans and Fisheries, MOF)
And Animal and Plant Quarantine Agency (under the Ministry of Agriculture, Food and Rural Affairs, MAFRA)
KOREA (REP. OF)
Tel: +82-51 720.24.83
E-mail: hjkim1882@korea.kr; naturelkk@korea.kr
Website: http://www.nifs.go.kr
Contact Point: Dr Hyoung-Jun Kim (for Aquatic Animal Disease); Dr Kyoung-Ki Lee (For Terrestrial Animal Diseases)

The Commission reviewed applications for changes of expert and recommended acceptance of the following:

Infection with abalone herpesvirus
Dr Nick Moody to replace Dr Mark Crane, at the Australian Centre for Disease Preparedness (CSIRO), AUSTRALIA.

In adherence with ethical standards, a Commission Member recused themself from the decision-making process to prevent any potential conflict of interest.

Infection with koi herpesvirus
Dr Heike Schütze to replace Dr Sven Bergmann, at the German Reference Laboratory for Koi Herpesvirus Disease, Friedrich-Loeffler-Institute (FLI), GERMANY.

Infection with spring viraemia of carp virus
Dr Richard Paley to replace Dr David Stone, who has retired from the Centre for Environment, Fisheries and Aquaculture Science (CEFAS) Weymouth Laboratory, UNITED KINGDOM

Infection with koi herpesvirus
Dr Irene Cano Cejas to replace Dr David Stone, who has retired from the Centre for Environment, Fisheries and Aquaculture Science (CEFAS) Weymouth Laboratory, UNITED KINGDOM

13.2. Reference Centre Reporting System Evolution

The Aquatic Animals Commission discussed possible initiatives aimed at enhancing engagement with and among Reference Laboratories. These include inviting experts from Reference Laboratories to attend Commission meetings as observers, thereby fostering deeper collaboration and insight. The proposal of short-term project grants was also discussed, aimed at promoting research aligned with WOAH Standards.

Additionally, the Commission highlighted the necessity of improving communication initiatives, particularly in showcasing the work and expertise of the Reference Centres. This approach aims not only to recognise their contributions but also to promote the sharing of knowledge and best practices within the field.

The Commission anticipates that these strategies would significantly strengthen their collaboration and alignment with the Reference Centres, thereby enhancing the collective efforts in aquatic animal disease management.

14. Any other business

14.1. Registration of Diagnostic Kits

Following the information given to the Aquatic Animals Commission in February 2023 (https://www.woah.org/app/uploads/2023/10/a-aac-sep-2023.pdf, AOB 12.1) about the Future Secretariat for Registration of Diagnostics Kits, the Commission was informed that, in agreement with the Director General and the Deputy Director of General of International Standards and Science, WOAH will proceed with a complete freeze of the Diagnostic Kits Register activities and all related procedures starting after the 91st General Session, for a period of 24 months (i.e. to May 2026). This will mean:

- Validated and approved kits will maintain their certification;
- No renewal processing, even if they arrive to the five-year due date;
- Withdrawal of all incomplete applications, with return of fees to applicants;
- No review of any potential appeal procedure;
- No review or validation of new applications;
- Consideration of exceptional cases, linked to an emergency animal health situation, upon Members request.

_____

…/Annexes

Annex 1. Item 2. – Adopted Agenda

MEETING OF THE WOAH AQUATIC ANIMAL HEALTH STANDARDS COMMISSION

14 to 21 February 2024

_____

1. Welcome from the Deputy Director General
2. Adoption of the agenda
3. Meeting with the Director General
4. Cooperation with other specialist commissions
   4.1. Aquatic Animals Commission and Biological Standards Commission Bureau meeting
5. Work plan of the Aquatic Animals Commission
6. Aquatic Animal Health Strategy
   6.1. Status report on the implementation of the Aquatic Animal Health Strategy
   6.2. Activity 1.3. Review the scientific basis of existing aquatic animal welfare standards
   6.3. Activity 1.6. Increase the accessibility of standards
7. *Aquatic Code*
   7.1. Texts that will be proposed for adoption in May 2024
      7.1.1. Glossary definitions: ‘Competent Authority’, ‘Veterinary Authority’ and ‘Aquatic Animal Health Services’ – review usage in *Aquatic Code*
      7.1.2. Glossary definitions: 'aquatic animal products’ – replacing the use of ‘products of aquatic animal origin' in *Aquatic Code*
      7.1.3. Article 1.1.5. of Chapter 1.1. Notification of diseases and provision of epidemiological information
      7.1.4. Chapter 1.3. Diseases listed by WOAH
         7.1.4.1. Infection with infectious spleen and kidney necrosis virus
      7.1.5. Safe commodities – Articles X.X.3. for disease-specific chapters
         7.1.5.1. Revised Articles 8.X.3. for amphibian disease-specific chapters
         7.1.5.2. Revised Articles 9.X.3. for crustacean disease-specific chapters
         7.1.5.3. Revised Articles 10.X.3. for fish disease-specific chapters
         7.1.5.4. Revised Articles 11.X.3. for mollusc disease-specific chapters
      7.1.6. Model Articles X.X.5.-X.X.6. for disease-specific chapters
      7.1.7. Article 9.3.2. of Chapter 9.3. Infection with decapod iridescent virus 1
      7.1.8. Article 10.6.2. of Chapter 10.6. Infection with infectious haematopoietic necrosis virus
      7.1.9. Article 10.11.2. of Chapter 10.11. Infection with tilapia lake virus
      7.1.10. Articles 11.5.1. and 11.5.2. of Chapter 11.5. Infection with *Perkinsus marinus*
   7.2. Items for Member comment
      7.2.1. Articles 5.1.2. and 5.1.4. of Chapter 5.1. General obligations related to certification
      7.2.2. Draft new Chapter 4.X. Emergency disease preparedness
      7.2.3. Draft new Chapter 4.Y. Disease outbreak management
      7.2.4. Draft new Chapter 4.Z. Control of pathogenic agents in traded milt and fertilised eggs of fish
      7.2.5. Draft new Chapter 5.X. Movement of ornamental aquatic animals
      7.2.6. Article 9.9.2. of Chapter 9.9. Infections with white spot syndrome virus

7.2.7. Articles 11.6.1. and 11.6.2. of Chapter 11.6. Infection with *Perkinsus olseni*

7.3. Items for consideration

7.3.1. Chapter 4.3. Application of compartmentalisation – discussion paper

7.3.2. Assessment of default periods in Articles X.X.4.-X.X.8. for disease-specific chapters

7.3.3. Consideration of emerging diseases

7.3.3.1. Covert mortality nodavirus (CMNV) in zebrafish

7.3.3.2. Infection with *Enterocytozoon hepatopenaei*

7.3.3.3. Other diseases

8. Aquatic Manual

8.1. Texts that will be proposed for adoption in May 2024

8.1.1. Section 2.2. Diseases of crustaceans

8.1.1.1. Chapter 2.2.0. General information: diseases of crustaceans

8.1.1.2. Chapter 2.2.2. Infection with *Aphanomyces astaci* (crayfish plague)

8.1.1.3. Chapter 2.2.6. Infection with *Macrobrachium rosenbergii* nodavirus (white tail disease)

8.1.1.4. Chapter 2.2.9. Infection with yellow head virus genotype 1

8.1.1.5. Chapter 2.2.X. Infection with decapod iridescent virus 1

8.1.2. Section 2.4. Diseases of molluscs

8.1.2.1. Chapter 2.4.0. General information: diseases of molluscs

8.1.2.2. Chapter 2.4.1. Infection with abalone herpesvirus

8.1.2.3. Chapter 2.4.4. Infection with *Marteilia refringens*

8.1.2.4. Section 2.2.1. and 2.2.2. of Chapter 2.4.5. Infection with *Perkinsus marinus*

8.2. Items for Member comment

8.2.1. Section 2.2. Diseases of crustaceans

8.2.1.1. Section 2.2.1. and 2.2.2. of Chapter 2.2.8. Infection with white spot syndrome virus

8.2.2. Section 2.3. Diseases of fish

8.2.2.1. Chapter 2.3.9. Infection with spring viraemia of carp virus

8.2.2.2. Chapter 2.3.4. Infection with HRP-deleted or HPR0 infectious salmon anaemia virus

8.2.3. Section 2.4. Diseases of molluscs

8.2.3.1. Section 2.2.1. and 2.2.2. of Chapter 2.4.6. Infection with *Perkinsus olseni*

8.3. Items for consideration

8.3.1. Develop a mechanism to speed up the process of making updates to diagnostic methods in the *Aquatic Manual* available to Members quicker (arose during the 3rd meeting for the steering committee of the Regional Collaboration Framework for Aquatic Animal Health): feedback on the use of the validation report template

9. *Ad hoc* groups

9.1. Report of the a*d hoc G*roup on Susceptibility of mollusc species to infection with WOAH listed diseases

9.2. Report of the a*d hoc G*roup on Susceptibility of fish species to infection with WOAH listed diseases

9.3. Report of the a*d hoc G*roup on Susceptibility of crustacean species to infection with WOAH listed diseases

10. Reference centres or change of experts

10.1. Evaluation of applications for Reference Centres for aquatic animal health issues or change of experts

10.2. Twinning projects

10.3. Reference Centre Reporting System Evolution

11. Other issues
    11.1. For discussion
        11.1.1. Registration of Diagnostic Kits
    11.2. For information
        11.2.1. Publication of Member comments, including Guidance for commenting
        11.2.2. Standard Online Navigation Tool
        11.2.3. WAHIAD update
        11.2.4. WOAH website update
        11.2.5. Listing/de-listing of diseases SOP
        11.2.6. Draft Chapters 5.4. and 5.6. of the *Terrestrial Code*
    11.3. Review of Commission's progress 2021-2024
12. Meeting review
13. Next meeting

Annex 2. Item 2. – List of Participants

MEETING OF THE AQUATIC ANIMAL HEALTH STANDARDS COMMISSION

14-21 February 2024

_____

MEMBERS OF THE COMMISSION

Dr Ingo Ernst
(President)
Director Aquatic Pest and Health Policy,
Department of Agriculture, Fisheries and Forestry,
Canberra,
AUSTRALIA

Dr Alicia Gallardo Lagno
(Vice-President)
Senior advisor FARMAVET,
University of Chile,
La Pintana,
CHILE

Dr Fiona Geoghegan
(Vice-President)
Legislative Officer,
European Commission,
DG SANTE
Brussels,
BELGIUM

Dr Kevin William Christison
(member)
Specialist Scientist,
Department of Forestry, Fisheries and the Environment,
Vlaeberg,
SOUTH AFRICA

Dr Hong Liu
(member)
Professor in aquatic animal health,
Animal and Plant Inspection and Quarantine Technical Centre,
Shenzhen Customs District,
General Administration of Customs,
CHINA (People's Rep of)

Dr Espen Rimstad
(member)
Professor in Virology,
Norwegian University of Life Sciences
Ås,
NORWAY

OTHER PARTICIPANTS

Dr Mark Crane
CSIRO Honorary Fellow,
Research Group Leader | AAHL Fish Diseases Laboratory
Australian Centre for Disease Preparedness (ACDP) I CSIRO,
Geelong,
AUSTRALIA

WOAH HEADQUARTERS

Dr Gillian Mylrea
Head of Department
Standards Department

Dr Mariana Delgado
Scientific Secretariat Officer
Science Department

Dr Kathleen Frisch
Scientific Coordinator for Aquatic Animal Health
Standards Department

Dr Patricia Kelly
Scientific Coordinator for Aquatic Animal Health
Standards Department

Ms Sara Linnane
Scientific Officer – International Standards
Science Department

Annex 4. Item 6.1. – Usage of glossary definitions: 'Aquatic Animal Health Services', 'Competent Authority' and 'Veterinary Authority'

EU position

The EU supports the adoption of this revised usage of glossary definitions.

Article	Usage
User's guide: B.5.	The standards in the chapters of Section 3 are designed for the establishment, maintenance and evaluation of Aquatic Animal Health Services, including communication. These standards are intended to assist the Aquatic Animal Health Services and the Competent Authorities of Member Countries to meet their objectives of improving aquatic animal health and the welfare of farmed fish, as well as to establish and maintain confidence in their international aquatic animal health certificates.
User's guide: C.8.	International aquatic animal health certificates An international aquatic animal health certificate is an official document that the Competent Authority of the exporting country issues in accordance with Chapter 5.1. and Chapter 5.2. It lists aquatic animal health requirements for the exported commodity. The quality of the exporting country's Aquatic Animal Health Services is essential in providing assurances to trading partners regarding the safety of exported aquatic animal products. This includes the relevant ~~Aquatic Animal Health Services'~~Competent Authority's ethical approach to the provision of international health certificates and the Veterinary Authority's history in meeting their notification obligations.
Glossary	***NOTIFICATION*** means the procedure by which: a) the ~~*Competent Authority*~~*Veterinary Authority* informs the *Headquarters*, b) the *Headquarters* inform ~~*Competent Authority*~~ the *Veterinary Authority* of Member Countries of the occurrence of a *disease* in accordance with the provisions of Chapter 1.1.
Article 1.1.1.	For the purposes of the *Aquatic Code* and in terms of Articles 5, 9 and 10 of the Organic Statutes of the Office International des Epizooties, Member Countries shall recognise the right of the *Headquarters* to communicate directly with the ~~*Competent Authority*~~*Veterinary Authority* of its *territory* or *territories*. All *notifications* and all information sent by WOAH to the ~~*Competent Authority*~~ *Veterinary Authority* shall be regarded as having been sent to the country concerned and all *notifications* and all information sent to WOAH by the ~~*Competent Authority*~~ *Veterinary Authority* shall be regarded as having been sent by the countryconcerned.
Article 1.1.3. paragraph 1	The ~~*Competent Authority*~~*Veterinary Authority* shall, under the responsibility of the Delegate, send to the *Headquarters*:
Article 1.1.4. paragraph 1	~~*Competent Authority*~~*Veterinary Authority* shall, under the responsibility of the Delegate, send to the *Headquarters*:
Article 1.1.5. point 1	The ~~*Competent Authority*~~ *Veterinary Authority* of a country in which an *infected zone* or *compartment* is located shall inform the *Headquarters* when this country, *zone* or *compartment* becomes free from the *disease*.
Article 1.1.5. point 3	The ~~*Competent Authority*~~ *Veterinary Authority* of a Member Country which establishes one or several *free zones* or *free compartments* shall inform the *Headquarters*, giving necessary details, including the criteria on which the free status is based, the requirements for maintaining the status and indicating clearly the location of the *zones* or *compartments* on a map of the territory of the Member Country.

Article	Usage
Article 3.1.2. point 7 paragraph 3	*Competent Authorities*~~*Aquatic Animal Health Services*~~ should define and document the responsibilities and structure of the organisation (in particular the chain of command) in charge of issuing *international aquatic animal health certificates*.
Article 3.1.2. point 10	Information, complaints and appeals The relevant *Competent Authority*~~*Aquatic Animal Health Services*~~ should undertake to reply to requests from ~~*Aquatic Animal Health Services*~~ *the Competent Authority* of other Member Countries ~~or any other authority~~, in particular ensuring that any requests for information, complaints or appeals that are presented are dealt with in a timely manner. A record should be maintained of all complaints and appeals and of the relevant action taken by the *Competent Authority* ~~*Aquatic Animal Health Services*~~.
Article 3.1.5. paragraph 4	The expert(s) facilitate(s) the evaluation of the *Aquatic Animal Health Services* of the Member Country using the WOAH *Performance of* ~~*Veterinary Services and/or*~~ *Aquatic Animal Health Services* (WOAH *PVS Tool*- Aquatic). The expert(s) produce(s) a report in consultation with the ~~Veterinary Services~~*Aquatic Animal Health Services* of the Member Country.
Article 3.2.1. paragraph 2	The recognition of communication as a discipline of the *Aquatic Animal Health Services* and its incorporation within it is critical for their operations. The integration of aquatic animal health and communication expertises is essential for effective communication. ~~Communication between the *Aquatic Animal Health Services,* and Veterinary Services (particularly where *Aquatic Animal Health Services* are separate, and independent of Veterinary Services) is especially important.~~
Article 4.2.3. point 1	The extent of a *zone* should be established by the ~~*Aquatic Animal Health Service*~~*Competent Authority* on the basis of the definition of *zone* and made public through official channels.
Article 4.2.3. point 3	The factors defining a *compartment* should be established by the ~~*Aquatic Animal Health Service*~~*Competent Authority* on the basis of relevant criteria such as management and husbandry practices related to *biosecurity*, and made public through official channels.
Article 4.2.3. point 6	For a *compartment*, the *biosecurity plan* should describe the partnership ~~between~~among the relevant enterprise/industry, ~~and~~ the ~~*Aquatic Animal Health Service*~~ *Competent Authority* and the *Aquatic Animal Health Services*, and their respective responsibilities, including the procedures for oversight of the operation of the *compartment* by the ~~*Aquatic Animal Health Service*~~ *Competent Authority*.
Article 5.3.4. point 2(a)	infrastructure including the legislative base (e.g. *aquatic animal* health law) and administrative systems (e.g. organisation of ~~Veterinary Services or *Aquatic Animal Health Services*~~*Competent Authority*);
Article 5.3.7. point 1(d)(i)	an evaluation of the *exporting country*'s ~~Veterinary Services or~~ *Aquatic Animal Health Services*;
Article 5.3.7. point 2(e)(i)	an evaluation of the *exporting country*'s ~~Veterinary Services or~~ *Aquatic Animal Health Services*;

Annex 5. Item 6.2. – Usage of glossary definition: 'Aquatic Animal Products'

EU position

The EU supports the adoption of this revised usage of glossary definitions.

Article	Usage
4.3.1. paragraph 1	The recommendations in this chapter provide a structured framework for the application and recognition of compartments within countries or zones, based on the provisions of Chapter 4.2. with the objective to facilitate trade in aquatic animals and ~~products of aquatic animal origin~~*aquatic animal products* and as a tool for disease management.
5.9.2. point 2	An *importing country* may require sufficient advance notification regarding the proposed date of entry into its *territory* of a consignment of ~~products of aquatic animal origin~~*aquatic animal products* destined for human consumption, together with information on the nature, quantity and packaging of the products, as well as the name of the *frontier* post.
5.11.1. title	Notes for guidance on the health certificates for international trade in live aquatic animals and ~~products of aquatic animal origin~~*aquatic animal products*
5.11.1. Box I.9.	For ~~products of aquatic animal origin~~*aquatic animal products*: the premises from which the products are to be dispatched.
5.11.1. Box I.22.	Further processing: applies to ~~products of aquatic animal origin~~*aquatic animal products* that have to be further processed before being suitable for end use.
5.11.1. Box I.24.	For ~~products of aquatic animal origin~~*aquatic animal products*: Category (i.e. amphibian, crustacean, fish or mollusc); Wild stocks or cultured stocks; Species (Scientific name); Approval number of establishment(s) (e.g. processing plant; cold store); Lot identification/date code; Number of packages.
5.11.3. title	Model health certificate for international trade in ~~products of aquatic animal origin~~*aquatic animal products*

Annex 6. Item 6.3. – Article 1.1.5. of Chapter 1.1. 'Notification of diseases and provision of epidemiological information'

CHAPTER 1.1.

NOTIFICATION OF DISEASES, AND PROVISION OF EPIDEMIOLOGICAL INFORMATION

EU position

The EU supports the adoption of this revised article.

[…]

~~**Article 1.1.5.**~~

~~1) The Competent Authority of a country in which an infected zone or compartment is located shall inform the Headquarters when this country, zone or compartment becomes free from the disease.~~

~~2) A country, zone or compartment may be considered to have regained freedom from a specific disease when all relevant conditions given in the Aquatic Code have been fulfilled.~~

~~3) The Competent Authority of a Member Country which establishes one or several free zones or free compartments shall inform the Headquarters, giving necessary details, including the criteria on which the free status is based, the requirements for maintaining the status and indicating clearly the location of the zones or compartments on a map of the territory of the Member Country.~~

Article 1.1.~~6~~5.

1) Although Member Countries are only required to notify *listed diseases* and *emerging diseases*, they are encouraged to provide WOAH with other important *aquatic animal* health information.

2) The *Headquarters* shall communicate by email or through the interface of WAHIS to *Competent Authorities* all *notifications* received as provided in Articles 1.1.2. to 1.1.~~5~~4. and other relevant information.

[…]

Annex 8. Item 6.4. – Article 1.3.1. of Chapter 1.3. 'Diseases listed by WOAH'

CHAPTER 1.3.

DISEASES LISTED BY WOAH

EU position

The EU supports the adoption of this revised article.

[…]

Article 1.3.1.

The following *diseases* of fish are *listed diseases*:

- Infection with *Aphanomyces invadans* (epizootic ulcerative syndrome)
- Infection with epizootic haematopoietic necrosis virus
- Infection with *Gyrodactylus salaris*
- Infection with HPR-deleted or HPR0 infectious salmon anaemia virus
- Infection with infectious haematopoietic necrosis virus
- Infection with the~~all~~ genogroups of the virus species *infectious spleen and kidney necrosis virus*
- Infection with koi herpesvirus
- ~~Infection with red sea bream iridovirus~~
- Infection with salmonid alphavirus
- Infection with spring viraemia of carp virus
- Infection with tilapia lake virus
- Infection with viral haemorrhagic septicaemia virus.

[…]

Annex 9. Item 6.5.1. – Article 8.1.3. of Chapter 8.1. 'Infection with *Batrachochytrium dendrobatidis*'

EU position

The EU supports the adoption of this revised article.

(TRACK CHANGES VERSION)

CHAPTER 8.1.

INFECTION WITH *BATRACHOCHYTRIUM DENDROBATIDIS*

- […]

Article 8.1.3.

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

~~1.~~The *aquatic animal products* listed below 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 these *aquatic animal products*, *Competent Authorities* should not require any *sanitary measures* ~~conditions~~ related to *B. dendrobatidis*, regardless of the infection with *B. dendrobatidis* 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 8.1.2. that are intended for any purpose and comply with Article 5.4.1.:~~

1) *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 five minutes, or a time/temperature equivalent that inactivates *B. dendrobatidis*;

   ~~a) heat sterilised hermetically sealed amphibian 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 *B. dendrobatidis*);~~

   ~~b) cooked amphibian 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 *B. dendrobatidis*);~~

   ~~c) pasteurised amphibian 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 *B. dendrobatidis*);~~

   ~~d)~~2) ~~mechanically dried amphibian products that have been subjected to (i.e. a heat treatment sufficient to attain a core temperature of at least 100~~60°C for at least ~~five~~30 minutes, or ~~a~~any time/temperature equivalent that has been ~~demonstrated to inactivates~~ *B. dendrobatidis*~~);~~

~~e)~~~~3~~2) amphibian skin leather.

~~2) When authorising the importation or transit of *aquatic animal products* derived from a species referred to in Article 8.1.2., other than those referred to in point 1 of Article 8.1.3., *Competent Authorities* should require the conditions prescribed in Articles 8.1.7. to 8.1.12. relevant to the infection with *B. dendrobatidis* 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 8.1.2. but which could reasonably be expected to pose a *risk* of transmission of *B. dendrobatidis*, 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 8.1.

INFECTION WITH *BATRACHOCHYTRIUM DENDROBATIDIS*

[…]

Article 8.1.3.

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

The *aquatic animal products* listed below 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 these *aquatic animal products*, *Competent Authorities* should not require any *sanitary measures* related to *B. dendrobatidis*, regardless of the infection with *B. dendrobatidis* status of the *exporting country*, *zone* or *compartment*:

1) *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 five minutes, or a time/temperature equivalent that inactivates *B. dendrobatidis*;

2) amphibian skin leather.

[…]

Annex 10. Item 6.5.1. – Article 8.2.3. of Chapter 8.2. 'Infection with *Batrachochytrium salamandrivorans*'

EU position

The EU supports the adoption of this revised article.

(TRACK CHANGES VERSION)

CHAPTER 8.2

INFECTION WITH *BATRACHOCHYTRIUM SALAMANDRIVORANS*

[…]

Article 8.2.3.

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

~~1)~~The *aquatic animal products* listed below have been assessed as meeting the criteria for safety of *aquatic animal products* in accordance with Article 5.4.1. When authorizing the importation or transit of these *aquatic animal products*, *Competent Authorities* should not require any *sanitary measures* ~~conditions~~ related to *B. salamandrivorans*, regardless of the infection with *B. salamandrivorans* 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 8.2.2. that are intended for any purpose and comply with Article 5.4.1.:~~

1) *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 ~~5~~ five minutes, or a time/temperature equivalent that inactivates *B. salamandrivorans*;

   ~~a) heat sterilised hermetically sealed amphibian 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 *B. salamandrivorans*);~~

   ~~b) cooked amphibian 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 *B. salamandrivorans*);~~

   ~~c) pasteurised amphibian 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 *B. salamandrivorans*);~~

   ~~d)~~2) ~~mechanically dried amphibian products~~ that ~~have been subjected~~ to ~~(i.e. a heat~~ treatment sufficient to attain a core ~~temperature of at least 100~~60°C ~~for at least five~~30 ~~minutes, or a~~any time/temperature equivalent that ~~has been demonstrated to inactivates *B. salamandrivorans;*~~

~~e)~~3) 2) amphibian skin leather.

~~2) When authorising the importation or transit of *aquatic animal products* derived from a species referred to in Article 8.2.2., other than those referred to in point 1 of Article 8.2.3., *Competent Authorities* should require the conditions prescribed in Articles 8.2.7. to 8.2.12. relevant to the infection with *B. salamandrivorans* 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 8.2.2. but which could reasonably be expected to pose a risk of transmission of *B. salamandrivorans*, 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 8.2.

INFECTION WITH *BATRACHOCHYTRIUM SALAMANDRIVORANS*

[…]

Article 8.2.3.

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

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

1) *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 five minutes, or a time/temperature equivalent that inactivates *B. salamandrivorans*;

2) amphibian skin leather.

[…]

Annex 11. Item 6.5.1. – Article 8.3.3. of Chapter 8.3. 'Infection with *Ranavirus* species'

EU position

The EU supports the adoption of this revised article.

(TRACK CHANGES VERSION)

CHAPTER 8.3.

INFECTION WITH *RANAVIRUS* SPECIES

[…]

Article 8.3.3.

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

The *aquatic animal products* listed below 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 these *aquatic animal products*, *Competent Authorities* should not require any *sanitary measures* ~~conditions~~ related to *Ranavirus* species, regardless of the infection with *Ranavirus* species 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 8.3.2. that are intended for any purpose and comply with Article 5.4.1.:~~

1) *aquatic animal products* that have been subjected to a heat treatment sufficient to attain a core temperature of at least ~~65~~60°C for at least 30 minutes, or a time/temperature equivalent that inactivates *Ranavirus* species~~;~~.

   ~~a) heat sterilised hermetically sealed amphibian 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 *Ranavirus* species);~~

   ~~b) cooked amphibian products that have been subjected to heat treatment at 65°C for at least 30 minutes (or any time/temperature equivalent that has been demonstrated to inactivate *Ranavirus* species);~~

   ~~c) pasteurised amphibian 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 *Ranavirus* species);~~

   ~~d~~2) ~~mechanically dried amphibian products that have been subjected to (i.e. a heat treatment sufficient to attain a core temperature of at least 100~~65°C for at least 30 minutes, or ~~a~~any time/temperature equivalent that has been demonstrated to inactivate~~s *Ranavirus* species).~~

~~2) When authorising the importation or transit of *aquatic animal products* derived from a species referred to in Article 8.3.2., other than those referred to in point 1 of Article 8.3.3., *Competent Authorities* should require the conditions prescribed in Articles 8.3.7. to 8.3.12. relevant to the infection with *Ranavirus* species 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 8.3.2. but which could reasonably be expected to pose a *risk* of transmission of *Ranavirus* species, 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 8.3.

INFECTION WITH *RANAVIRUS* SPECIES

[…]

Article 8.3.3.

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

The *aquatic animal products* listed below 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 these *aquatic animal products*, *Competent Authorities* should not require any sanitary measures related to *Ranavirus* species, regardless of the infection with *Ranavirus* species status of the *exporting country*, *zone* or *compartment*:

1) *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 30 minutes, or a time/temperature equivalent that inactivates *Ranavirus* species.

[…]

Annex 12. Item 6.5.2. – Article 9.3.3. of Chapter 9.3. 'Infection with DIV1'

EU position

The EU supports the adoption of this revised article.

CHAPTER 9.3.

INFECTION WITH DECAPOD IRIDESCENT VIRUS 1

[…]

Article 9.3.3.

Measures for the importation or transit of aquatic animal products for any purpose regardless of the infection with DIV1 status of the exporting country, zone or compartment

The *aquatic animal products* listed below 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 these *aquatic animal products*, *Competent Authorities* should not require any *sanitary measures* related to DIV1, regardless of the infection with DIV1 status of the *exporting country*, *zone* or *compartment*:

1) ~~[~~*aquatic animal products* that have been subjected to a heat treatment sufficient to attain a core temperature of at least ~~56~~80°C for at least 30 minutes, or a time/temperature equivalent that inactivates DIV1;

2) crustacean *meal* that has been subjected to a heat treatment sufficient to attain a core temperature of at least ~~56~~80°C for at least 30 minutes, or a time/temperature equivalent that inactivates DIV1;

3) ~~crayfish~~crustacean oil;

4) chemically extracted chitin.~~] (under study).~~

[…]

Annex 13. Item 6.5.2. – Article 9.4.3. of Chapter 9.4. 'Infection with *Hepatobacter penaei*'

EU position

The EU supports the adoption of this revised article.

CHAPTER 9.4.

INFECTION WITH *HEPATOBACTER PENAEI* (NECROTISING HEPATOPANCREATITIS)

[…]

Article 9.4.3.

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

The *aquatic animal products* listed below 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 these *aquatic animal products*, *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*:

1) *aquatic animal products* that have been subjected to a heat treatment sufficient to attain a core temperature of at least ~~63~~95°C for at least ~~30~~five minutes, or a time/temperature equivalent that inactivates *H. penaei*;

2) crustacean *meal* that has been subjected to a heat treatment sufficient to attain a core temperature of at least ~~63~~95°C for at least ~~30~~five minutes, or a time/temperature equivalent that inactivates *H. penaei*;

3) crustacean oil;

4) chemically extracted chitin.

[…]

Annex 14. Item 6.5.2. – Article 9.6.3. of Chapter 9.6. 'Infection with infectious myonecrosis virus'

EU position

The EU supports the adoption of this revised article.

CHAPTER 9.6.

INFECTION WITH INFECTIOUS MYONECROSIS VIRUS

[…]

Article 9.6.3.

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

The *aquatic animal products* listed below 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 these *aquatic animal products*, *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*:

1) *aquatic animal products* that have been subjected to a heat treatment sufficient to attain a core temperature of at least ~~60~~75°C for at least ~~60~~five minutes, or a time/temperature equivalent that inactivates IMNV;

2) crustacean *meal* that has been subjected to a heat treatment sufficient to attain a core temperature of at least ~~60~~75°C for at least ~~60~~five minutes, or a time/temperature equivalent that inactivates IMNV;

3) crustacean oil;

4) chemically extracted chitin.

[…]

Annex 15. Item 6.5.2. – Article 9.7.3. of Chapter 9.7. 'Infection with Macrobrachium rosenbergii nodavirus'

EU position

The EU supports the adoption of this revised article.

CHAPTER 9.7.

INFECTION WITH MACROBRACHIUM ROSENBERGII NODAVIRUS (WHITE TAIL DISEASE)

[…]

Article 9.7.3.

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

The *aquatic animal products* listed below 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 these *aquatic animal products*, *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*:

1) *aquatic animal products* that have been subjected to a heat treatment sufficient to attain a core temperature of at least ~~60~~50°C for at least ~~60~~50five minutes, or a time/temperature equivalent that inactivates MrNV;

2) crustacean *meal* that has been subjected to a heat treatment sufficient to attain a core temperature of at least ~~60~~50°C for at least ~~60~~50five minutes, or a time/temperature equivalent that inactivates MrNV;

3) crustacean oil;

4) chemically extracted chitin.

[…]

Annex 16. Item 6.5.2. – Article 9.8.3. of Chapter 9.8. 'Infection with Taura syndrome virus'

EU position

The EU supports the adoption of this revised article.

CHAPTER 9.8.

INFECTION WITH TAURA SYNDROME VIRUS

[…]

Article 9.8.3.

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

The *aquatic animal products* listed below 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 these *aquatic animal products*, *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*:

1) *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~~108 minutes, or a time/temperature equivalent that inactivates TSV;

2) crustacean *meal* that has been subjected to a heat treatment sufficient to attain a core temperature of at least 70°C for at least ~~30~~108 minutes, or a time/temperature equivalent that inactivates TSV;

3) crustacean oil;

4) chemically extracted chitin.

[…]

Annex 17. Item 6.5.3. – Article 10.1.3. of Chapter 10.1. 'Infection with epizootic haematopoietic necrosis virus'

EU position

The EU supports the adoption of this revised article.

CHAPTER 10.1.

INFECTION WITH EPIZOOTIC HAEMATOPOIETIC NECROSIS VIRUS

[…]

Article 10.1.3.

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

The *aquatic animal products* listed below 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 these *aquatic animal products*, *Competent Authorities* should not require any *sanitary measures* related to EHNV, regardless of the EHNV status of the *exporting country*, *zone* or *compartment*:

1) *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 inactivates EHNV;

~~2) mechanically dried eviscerated fish that has 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 inactivates EHNV;~~

~~3~~2) fish *meal* that has 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 inactivates EHNV;

~~4~~3) fish oil;

~~5~~4) fish skin leather.

[…]

Annex 18. Item 6.5.3. – Article 10.2.3. of Chapter 10.2. 'Infection with *Aphanomyces invadans*'

EU position

The EU supports the adoption of this revised article.

CHAPTER 10.2.

INFECTION WITH *APHANOMYCES INVADANS* (EPIZOOTIC ULCERATIVE SYNDROME)

[…]

Article 10.2.3.

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

The *aquatic animal products* listed below 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 these *aquatic animal products*, *Competent Authorities* should not require any *sanitary measures* related to *A. invadans*, regardless of the infection with *A. invadans* status of the *exporting country*, *zone* or *compartment*:

1) *aquatic animal products* that have been subjected to a heat treatment sufficient to attain a core temperature of at least ~~60~~100°C for at least ~~five~~one minute~~s~~, or a time/temperature equivalent that inactivates *A. invadans*;

~~2) mechanically dried eviscerated fish that have been subjected to a heat treatment sufficient to attain a core temperature of at least 60°C for at least five minutes, or a time/temperature equivalent that inactivates *A. invadans*;~~

~~3~~2) fish *meal* that has been subjected to a heat treatment sufficient to attain a core temperature of at least ~~60~~100°C for at least ~~five~~one minute~~s~~, or a time/temperature equivalent that inactivates *A. invadans*;

~~4~~3) fish oil;

~~5~~4) frozen eviscerated fish;

~~6~~5) frozen fish fillets or steaks.

[…]

Annex 19. Item 6.5.3. – Article 10.3.3. of Chapter 10.3. 'Infection with *Gyrodactylus salaris*'

CHAPTER 10.3.

INFECTION WITH *GYRODACTYLUS SALARIS*

EU position

The EU supports the adoption of this revised article.

[…]

Article 10.3.3.

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

The *aquatic animal products* listed below 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 these *aquatic animal products*, *Competent Authorities* should not require any *sanitary measures* related to *G. salaris*, regardless of the *G. salaris* status of *the exporting country*, *zone* or *compartment*:

1) *aquatic animal products* that have been ~~heat treated and are hermetically sealed~~subjected to a heat treatment sufficient to attain a core temperature of at least 40°C for at least one minute, or a time/temperature equivalent that inactivates *G. salaris*;

~~2) mechanically dried eviscerated fish;~~

~~3~~2) naturally dried eviscerated fish (i.e. sun-dried or wind-dried);

~~4~~3) frozen eviscerated fish that have been subjected to minus 18°C or lower temperatures;

~~5~~4) frozen fish fillets or steaks that have been subjected to minus 18°C or lower temperatures;

~~6~~5) chilled eviscerated fish that have been harvested from seawater with a salinity of at least 25 parts per thousand (ppt) for a continuous period of at least 14 days;

~~7~~6) chilled fish fillets or steaks derived from fish that have been harvested from seawater with a salinity of at least 25 ppt for a continuous period of at least 14 days;

~~8~~7) chilled fish products from which the skin, fins and gills have been removed;

~~9~~8) non-viable fish roe;

~~10~~9) fish oil;

~~11~~10) fish meal;

~~12~~11) fish skin leather.

[…]

Annex 20. Item 6.5.3. – Article 10.4.3. of Chapter 10.4. 'Infection with ISAV'

EU position

The EU supports the adoption of this revised article.

CHAPTER 10.4.

INFECTION WITH INFECTIOUS SALMON ANAEMIA VIRUS

[…]

Article 10.4.3.

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

In this article, all statements referring to ISAV include HPR deleted ISAV and HPR0 ISAV.

The *aquatic animal products* listed below 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 these *aquatic animal products*, *Competent Authorities* should not require any *sanitary measures* related to ISAV, regardless of the ISAV status of the *exporting country*, *zone* or *compartment*:

1) *aquatic animal products* that have been subjected to a heat treatment sufficient to attain a core temperature of at least 56°C for at least five minutes, or a time/temperature equivalent that inactivates ISAV;

~~2)~~ ~~mechanically dried eviscerated fish that have been subjected to a heat treatment sufficient to attain a core temperature of at least 56°C for at least five minutes, or a time/temperature equivalent that inactivates ISAV;~~

~~3~~2) fish *meal* that has been subjected to a heat treatment sufficient to attain a core temperature of at least 56°C for at least five minutes, or a time/temperature equivalent that inactivates ISAV;

~~4~~3) fish oil;

~~5~~4) fish skin leather.

[…]

Annex 21. Item 6.5.3. – Article 10.5.3. of Chapter 10.5. 'Infection with SAV'

EU position

The EU supports the adoption of this revised article.

CHAPTER 10.5.

INFECTION WITH SALMONID ALPHAVIRUS

[…]

Article 10.5.3.

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

The *aquatic animal products* listed below 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 these *aquatic animal products*, *Competent Authorities* should not require any *sanitary measures* related to SAV, regardless of the SAV status of the *exporting country*, *zone* or *compartment*:

1) *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 inactivates SAV;

~~2)~~ ~~mechanically dried eviscerated fish 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 inactivates SAV;~~

~~3~~2) fish *meal* that has 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 inactivates SAV;

~~4~~3) fish oil;

~~5~~4) fish skin leather.

[…]

Annex 22. Item 6.5.3. – Article 10.6.3. of Chapter 10.6. 'Infection with IHNV'

EU position

The EU supports the adoption of this revised article.

CHAPTER 10.6.

INFECTION WITH INFECTIOUS HAEMATOPOIETIC NECROSIS VIRUS

[…]

Article 10.6.3.

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

The *aquatic animal products* listed below 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 these *aquatic animal products*, *Competent Authorities* should not require any *sanitary measures* related to IHNV, regardless of the IHNV status of the *exporting country*, *zone* or *compartment*:

1) *aquatic animal products* that have been subjected to a heat treatment sufficient to attain a core temperature of at least 90°C for at least 30 seconds, or a time/temperature equivalent that inactivates IHNV;

~~2)~~ ~~mechanically dried eviscerated fish that have been subjected to a heat treatment sufficient to attain a core temperature of at least 90°C for at least 30 seconds, or a time/temperature equivalent that inactivates IHNV;~~

~~3~~2) fish *meal* that has been subjected to a heat treatment sufficient to attain a core temperature of at least 90°C for at least 30 seconds, or a time/temperature equivalent that inactivates IHNV;

~~4~~3) fish oil;

~~5~~4) fish skin leather.

[…]

Annex 23. Item 6.5.3. – Article 10.7.3. of Chapter 10.7. 'Infection with KHV'

EU position

The EU supports the adoption of this revised article.

CHAPTER 10.7.

INFECTION WITH KOI HERPESVIRUS

[…]

Article 10.7.3.

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

The *aquatic animal products* listed below 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 these *aquatic animal products*, *Competent Authorities* should not require any *sanitary measures* related to KHV, regardless of the KHV status of the *exporting country*, *zone* or *compartment*:

1) *aquatic animal products* that have been subjected to a heat treatment sufficient to attain a core temperature of at least 50°C for at least ~~three~~one minute~~s~~, or a time/temperature equivalent that inactivates KHV;

~~2)~~ ~~mechanically dried eviscerated fish that have been subjected to a heat treatment sufficient to attain a core temperature of at least 50°C for at least three minutes, or a time/temperature equivalent that inactivates KHV;~~

~~3~~2) fish *meal* that has been subjected to a heat treatment sufficient to attain a core temperature of at least 50°C for at least ~~three~~one minute~~s~~, or a time/temperature equivalent that inactivates KHV;

~~4~~3) fish oil.

[…]

———————

Annex 24. Item 6.5.3. – Article 10.8.3. of Chapter 10.8. 'Infection with RSIV'

EU position

The EU supports the adoption of this revised article.

CHAPTER 10.8.

INFECTION WITH RED SEA BREAM VIRUS

[…]

Article 10.8.3.

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

The *aquatic animal products* listed below 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 these aquatic animal products, *Competent Authorities* should not require any sanitary measures related to RSIV, regardless of the RSIV status of the *exporting country*, *zone* or *compartment*:

1) *aquatic animal products* that have been subjected to a heat treatment sufficient to attain a core temperature of at least 56°C for at least 30 minutes, or a time/temperature equivalent that inactivates RSIV;

~~2)~~ ~~mechanically dried eviscerated fish that have been subjected to a heat treatment sufficient to attain a core temperature of at least 56°C for at least 30 minutes, or a time/temperature equivalent that inactivates RSIV;~~

~~3~~2) fish *meal* that has been subjected to a heat treatment sufficient to attain a core temperature of at least 56°C for at least 30 minutes, or a time/temperature equivalent that inactivates RSIV;

~~4~~3) fish oil;

~~5~~4) fish skin leather.

[…]

Annex 25. Item 6.5.3. – Article 10.9.3. of Chapter 10.9. 'Infection with SVCV'

EU position

The EU supports the adoption of this revised article.

CHAPTER 10.9.

INFECTION WITH SPRING VIRAEMIA OF CARP VIRUS

[…]

Article 10.9.3.

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

The *aquatic animal products* listed below 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 these *aquatic animal products*, *Competent Authorities* should not require any sanitary measures related to SVCV, regardless of the SVCV status of the *exporting country*, *zone* or *compartment*:

1) *aquatic animal products* that have been subjected to a heat treatment sufficient to attain a core temperature of at least ~~90~~60°C for at least 60 ~~seconds~~minutes, or a time/temperature equivalent inactivates SVCV;

~~2) mechanically dried eviscerated fish that have been subjected to a heat treatment sufficient to attain a core temperature of at least 90°C for at least 60 seconds, or a time/temperature equivalent that inactivates SVCV;~~

~~3~~2) fish *meal* that has been subjected to a heat treatment sufficient to attain a core temperature of at least ~~90~~60°C for at least 60 ~~seconds~~minutes, or a time/temperature equivalent that inactivates SVCV;

~~4~~3) fish oil.

[…]

Annex 26. Item 6.5.3. – Article 10.10.3. of Chapter 10.10. 'Infection with VHSV'

EU position

The EU supports the adoption of this revised article.

CHAPTER 10.10.

INFECTION WITH VIRAL HAEMORRHAGIC SEPTICAEMIA VIRUS

[…]

Article 10.10.3.

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

The *aquatic animal products* listed below 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 these *aquatic animal products*, *Competent Authorities* should not require any sanitary measures related to VHSV, regardless of the VHSV status of the *exporting country*, *zone* or *compartment*:

1) *aquatic animal products* that have been subjected to a heat treatment sufficient to attain a core temperature of at least ~~90~~60°C for at least 60 ~~seconds~~minutes, or a time/temperature equivalent that inactivates VHSV;

~~2) mechanically dried eviscerated fish that have been subjected to a heat treatment sufficient to attain a core temperature of at least 90°C for at least 60 seconds, or any a time/temperature equivalent that inactivates VHSV;~~

~~3~~2) fish *meal* that has been subjected to a heat treatment sufficient to attain a core temperature of at least ~~90~~60°C for at least 60 ~~seconds~~minutes, or a time/temperature equivalent that inactivates VHSV;

~~4~~3) naturally dried, eviscerated fish (i.e. sun-dried or wind-dried);

~~5~~4) fish oil;

~~6~~5) fish skin leather.

[…]

Annex 27. Item 6.5.3. – Article 10.11.3. of Chapter 10.11. 'Infection with TiLV'

EU position

The EU supports the adoption of this revised article.

CHAPTER 10.11.

INFECTION WITH TILAPIA LAKE VIRUS

[…]

Article 10.11.3.

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

The *aquatic animal products* listed below 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 these *aquatic animal products*, *Competent Authorities* should not require any sanitary measures related to TiLV, regardless of the TiLV status of *the exporting country*, *zone* or *compartment*:

1) ~~[~~*aquatic animal products* that have been subjected to a heat treatment sufficient to attain a core temperature of at least ~~56~~60°C for at least ~~five~~120 minutes, or a time/temperature equivalent that inactivates TiLV;

2) fish *meal* that has been subjected to a heat treatment sufficient to attain a core temperature of at least ~~56~~60°C for at least ~~five~~120 minutes, or a time/temperature equivalent that inactivates TiLV~~] (under study)~~;

3) fish oil;

4) fish skin leather.

[…]

Annex 28. Item 6.5.4. – Article 11.1.3. of Chapter 11.1. 'Infection with abalone herpesvirus'

EU position

The EU supports the adoption of this revised article.

(TRACK CHANGES VERSION)

CHAPTER 11.1.

INFECTION WITH ABALONE HERPESVIRUS

[…]

Article 11.1.3.

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

~~1)~~The *aquatic animal products* listed below 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 these *aquatic animal products*, *Competent Authorities* should not require any *sanitary measures*~~conditions~~ related to infection with abalone herpesvirus regardless of the infection with abalone herpesvirus status of the *exporting country*, *zone* or *compartment*~~:, when authorising the importation or transit of the following *aquatic animal products* from the species referred to in Article 11.1.2. which are intended for any purpose and which comply with Article 5.4.1.:~~

1) *aquatic animal products* that have been subjected to a heat treatment sufficient to attain a core temperature of at least ~~121~~50°C for at least ~~3~~five minutes ~~and 36 seconds~~, or a time/temperature equivalent that inactivates AbHV~~;~~.

   ~~a) heat sterilised hermetically sealed abalone products (i.e. a heat treatment at 121°C for at least 3.6 minutes or any time/temperature equivalent);~~

   ~~b2) mechanically dried abalone products (i.e. that have been subjected to a heat treatment sufficient to attain a core temperature of at least 100121°C for at least 3 minutes and 36 seconds, 30 minutes or anya time/temperature equivalent which has been demonstrated to that inactivates AbHV).~~

~~2) When authorising the importation or transit of *aquatic animals* and *aquatic animal products* of a species referred to in Article 11.1.2., other than those referred to in point 1 of Article 11.1.3., *Competent Authorities* should require the conditions prescribed in Articles 11.1.7. to 11.1.11. relevant to the infection with abalone herpesvirus status of the *exporting country*, *zone* or *compartment*.~~

~~3) When considering the importation or transit of *aquatic animals* and *aquatic animal products* of a species not covered in Article 11.1.2. but which could reasonably be expected to pose a *risk* of spread of infection with abalone herpesvirus, 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 assessment.~~

[…]

(CLEAN VERSION)

CHAPTER 11.1.

INFECTION WITH ABALONE HERPESVIRUS

[…]

Article 11.1.3.

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

The *aquatic animal products* listed below 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 these *aquatic animal products*, *Competent Authorities* should not require any *sanitary measures* related to infection with abalone herpesvirus regardless of the infection with abalone herpesvirus status of the *exporting country*, *zone* or *compartment*:

1) *aquatic animal products* that have been subjected to a heat treatment sufficient to attain a core temperature of at least 50°C for at least five minutes, or a time/temperature equivalent that inactivates AbHV.

[…]

Annex 29. Item 6.5.4. – Article 11.2.3. of Chapter 11.2. 'Infection with *Bonamia exitiosa*'

EU position

The EU supports the adoption of this revised article.

(TRACK CHANGES VERSION)

CHAPTER 11.2.

INFECTION WITH *BONAMIA EXITIOSA*

[…]

Article 11.2.3.

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

~~1)~~The *aquatic animal products* listed below 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 these *aquatic animal products*, *Competent Authorities* should not require any *sanitary measures*~~conditions~~ related to infection with *B. exitiosa*, regardless of the infection with *B. exitiosa* status of the *exporting country*, *zone* or *compartment*:~~, when authorising the importation or transit of the following *aquatic animals* and *aquatic animal products* from the species referred to in Article 11.2.2. which are intended for any purpose and which comply with Article 5.4.1.:~~

1) *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 15 minutes, or a time/temperature equivalent that inactivates *B. exitiosa;*

~~a)~~~~1~~2) frozen oyster meat; ~~and~~

~~b)~~~~2~~3) frozen half-shell oysters.

~~2) When authorising the importation or transit of *aquatic animals* and *aquatic animal products* of a species referred to in Article 11.2.2., other than those referred to in point 1 of Article 11.2.3., *Competent Authorities* should require the conditions prescribed in Articles 11.2.7. to 11.2.11. relevant to the infection with *B. exitiosa* status of the *exporting country*, *zone* or *compartment*.~~

~~3) When considering the importation or transit of *aquatic animals* and *aquatic animal products* of a species not covered in Article 11.2.2. but which could reasonably be expected to pose a *risk* of spread of infection with *B. exitiosa*, 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 assessment.~~

[…]

———————

(CLEAN VERSION)

CHAPTER 11.2.

INFECTION WITH *BONAMIA EXITIOSA*

[…]

Article 11.2.3.

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

The *aquatic animal products* listed below 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 these *aquatic animal products*, *Competent Authorities* should not require any *sanitary measures* related to infection with *B. exitiosa*, regardless of the infection with *B. exitiosa* status of the *exporting country*, *zone* or *compartment*:

1) *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 15 minutes, or a time/temperature equivalent that inactivates *B. exitiosa*;

2) frozen oyster meat;

3) frozen half-shell oysters.

[…]

Annex 30. Item 6.5.4. – Article 11.3.3. of Chapter 11.3. 'Infection with *Bonamia ostreae*'

EU position

The EU supports the adoption of this revised article.

(TRACK CHANGES VERSION)

CHAPTER 11.3.

INFECTION WITH *BONAMIA OSTREAE*

[…]

Article 11.3.3.

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

~~1)~~The *aquatic animal products* listed below 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 these *aquatic animal products*, *Competent Authorities* should not require any *sanitary measures*~~conditions~~ related to infection with *B. ostreae*, regardless of the infection with *B. ostreae* status of the *exporting country*, *zone* or *compartment*:~~, when authorising the importation or transit of the following *aquatic animals* and *aquatic animal products* from the species referred to in Article 11.3.2. which are intended for any purpose and which comply with Article 5.4.1.:~~

1) *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 15 minutes, or a time/temperature equivalent that inactivates *B. ostreae*;

~~a)~~~~1~~2) frozen oyster meat; ~~and~~

~~b)~~~~2~~3) frozen half-shell oysters.

~~2) When authorising the importation or transit of *aquatic animals* and *aquatic animal products* of a species referred to in Article 11.2.2., other than those referred to in point 1 of Article 11.2.3., *Competent Authorities* should require the conditions prescribed in Articles 11.2.7. to 11.2.11. relevant to the infection with *B. ostreae* status of the *exporting country*, *zone* or *compartment*.~~

~~3) When considering the importation or transit of *aquatic animals* and *aquatic animal products* of a species not covered in Article 11.2.2. but which could reasonably be expected to pose a *risk* of spread of infection with *B. ostreae*, 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 assessment.~~

[…]

———————

(CLEAN VERSION)

CHAPTER 11.3.

INFECTION WITH *BONAMIA OSTREAE*

[…]

Article 11.3.3.

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

The *aquatic animal products* listed below 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 these *aquatic animal products*, *Competent Authorities* should not require any *sanitary measures* related to infection with *B. ostreae*, regardless of the infection with *B. ostreae* status of the *exporting country*, *zone* or *compartment*:

1) *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 15 minutes, or a time/temperature equivalent that inactivates *B. ostreae*;

2) frozen oyster meat;

3) frozen half-shell oysters.

[…]

Annex 31. Item 6.5.4. – Article 11.4.3. of Chapter 11.4. 'Infection with *Marteilia refringens*'

EU position

The EU supports the adoption of this revised article.

(TRACK CHANGES VERSION)

CHAPTER 11.4.

INFECTION WITH *MARTEILIA REFRINGENS*

[…]

Article 11.4.3.

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

~~1)~~The *aquatic animal products* listed below 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 these *aquatic animal products*, *Competent Authorities* should not require any *sanitary measures*~~conditions~~ related to infection with *M. refringens*, regardless of the infection with *M. refringens* status of the *exporting country*, *zone* or *compartment*: ~~, when authorising the importation or transit of heat sterilised hermetically sealed mollusc products (i.e. a heat treatment at 121°C for at least 3.6 minutes or any time/temperature equivalent) from the species referred to in Article 11.4.2. which are intended for any purpose and which comply with Article 5.4.1.~~

1) *aquatic animal products* that have been subjected to a heat treatment sufficient to attain a core temperature of at least 121°C for at least three~~3~~ minutes and 36 seconds, or a time/temperature equivalent that inactivates *M. refringens*.

~~2) When authorising the importation or transit of *aquatic animals* and *aquatic animal products* of a species referred to in Article 11.4.2., other than those referred to in point 1 of Article 11.4.3., *Competent Authorities* should require the conditions prescribed in Articles 11.4.7. to 11.4.11. relevant to the infection with *M. refringens* status of the *exporting country*, *zone* or *compartment*.~~

~~3) When considering the importation or transit of *aquatic animals* and *aquatic animal products* of a species not covered in Article 11.4.2. but which could reasonably be expected to pose a *risk* of spread of infection with *M. refringens*, 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 assessment.~~

[…]

_____

(CLEAN VERSION)

CHAPTER 11.4.

INFECTION WITH *MARTEILIA REFRINGENS*

[…]

Article 11.4.3.

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

The *aquatic animal products* listed below 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 these *aquatic animal products*, *Competent Authorities* should not require any *sanitary measures* related to infection with *M. refringens*, regardless of the infection with *M. refringens* status of the *exporting country*, *zone* or *compartment*:

1) *aquatic animal products* that have been subjected to a heat treatment sufficient to attain a core temperature of at least 121°C for at least three minutes and 36 seconds, or a time/temperature equivalent that inactivates *M. refringens*.

[…]

Annex 32. Item 6.5.4. – Article 11.5.3. of Chapter 11.5. 'Infection with *Perkinsus marinus*'

EU position

The EU supports the adoption of this revised article.

(TRACK CHANGES VERSION)

CHAPTER 11.5.

INFECTION WITH *PERKINSUS MARINUS*

[…]

Article 11.5.3.

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

~~1)~~The *aquatic animal products* listed below 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 these *aquatic animal products*, *Competent Authorities* should not require any *sanitary measures*~~conditions~~ related to infection with *P. marinus*, regardless of the infection with *P. marinus* status of the *exporting country*, *zone* or *compartment*:~~, when authorising the importation or transit of heat sterilised hermetically sealed mollusc products (i.e. a heat treatment at 121°C for at least 3.6 minutes or any time/temperature equivalent) from the species referred to in Article 11.5.2. which are intended for any purpose and which comply with Article 5.4.1.~~

1) *aquatic animal products* that have been subjected to a heat treatment sufficient to attain a core temperature of at least ~~121~~60°C for at least ~~three~~60 minutes ~~and 36 seconds~~, or a time/temperature equivalent that inactivates *P. marinus*.

~~2) When authorising the importation or transit of *aquatic animals* and *aquatic animal products* of a species referred to in Article 11.5.2., other than those referred to in point 1 of Article 11.5.3., *Competent Authorities* should require the conditions prescribed in Articles 11.5.7. to 11.5.11. relevant to the infection with *P. marinus* status of the *exporting country*, *zone* or *compartment*.~~

~~3) When considering the importation or transit of *aquatic animals* and *aquatic animal products* of a species not covered in Article 11.5.2. but which could reasonably be expected to pose a *risk* of spread of infection with *P. marinus*, 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 assessment.~~

[…]

_____

(CLEAN VERSION)

CHAPTER 11.5.

INFECTION WITH *PERKINSUS MARINUS*

[…]

Article 11.5.3.

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

The *aquatic animal products* listed below 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 these *aquatic animal products*, *Competent Authorities* should not require any *sanitary measures* related to infection with *P. marinus*, regardless of the infection with *P. marinus* status of the *exporting country*, *zone* or *compartment*:

1) *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 inactivates *P. marinus*.

[…]

Annex 33. Item 6.5.4. – Article 11.6.3. of Chapter 11.6. 'Infection with *Perkinsus olseni*'

EU position

The EU supports the adoption of this revised article.

(TRACK CHANGES VERSION)

CHAPTER 11.6.

INFECTION WITH *PERKINSUS OLSENI*

[…]

Article 11.6.3.

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

~~1)~~The *aquatic animal products* listed below 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 these *aquatic animal products*, *Competent Authorities* should not require any *sanitary measures*~~conditions~~ related to infection with *P. olseni*, regardless of the infection with *P. olseni* status of the *exporting country*, *zone* or *compartment*:~~, when authorising the importation or transit of heat sterilised hermetically sealed mollusc products (i.e. a heat treatment at 121°C for at least 3.6 minutes or any time/temperature equivalent) from the species referred to in Article 11.6.2. which are intended for any purpose and which comply with Article 5.4.1.~~

1) *aquatic animal products* that have been subjected to a heat treatment sufficient to attain a core temperature of at least ~~121~~60°C for at least ~~three~~60 minutes ~~and 36 seconds~~ minutes, or a time/temperature equivalent that inactivates *P. olseni*.

~~2) When authorising the importation or transit of *aquatic animals* and *aquatic animal products* of a species referred to in Article 11.6.2., other than those referred to in point 1 of Article 11.6.3., *Competent Authorities* should require the conditions prescribed in Articles 11.6.7. to 11.6.11. relevant to the infection with *P. olseni* status of the *exporting country*, *zone* or *compartment*.~~

~~3) When considering the importation or transit of *aquatic animals* and *aquatic animal products* of a species not covered in Article 11.6.2. but which could reasonably be expected to pose a *risk* of spread of infection with *P. olseni*, 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 assessment.~~

[…]

(CLEAN VERSION)

CHAPTER 11.6.

INFECTION WITH *PERKINSUS OLSENI*

[…]

Article 11.6.3.

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

The *aquatic animal products* listed below 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 these *aquatic animal products*, *Competent Authorities* should not require any *sanitary measures* related to infection with *P. olseni*, regardless of the infection *with P. olseni* status of the *exporting country*, *zone* or *compartment*:

1) *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 inactivates *P. olseni*.

[…]

Annex 34. Item 6.5.4. – Article 11.7.3. of Chapter 11.7. 'Infection with *Xenohaliotis californiensis*'

EU position

The EU supports the adoption of this revised article.

(TRACK CHANGES VERSION)

CHAPTER 11.7.

INFECTION WITH *XENOHALIOTIS CALIFORNIENSIS*

[…]

Article 11.7.3.

Measures for the ~~i~~importation or transit of ~~aquatic animals and~~ aquatic animal products for any purpose regardless of the infection with *X. californiensis* status of the exporting country, zone or compartment

~~1)~~The following *aquatic animal products* listed below 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~~ these *aquatic animal products* ~~listed below~~, *Competent Authorities* should not require any *sanitary measures*~~conditions~~ related to infection with *X. californiensis*, regardless of the infection with *X. californiensis* status of the *exporting country*, *zone* or *compartment*:~~, when authorising the importation or transit of heat sterilised hermetically sealed abalone products (i.e. a heat treatment at 121˚C for at least 3.6 minutes or any time/temperature equivalent) from the species referred to in Article 11.7.2. which are intended for any purpose and which comply with Article 5.4.1.~~

~~a~~1) *aquatic animal products* that have been subjected to a heat treatment sufficient to attain a core temperature of at least ~~121~~95˚C for at least ~~3~~five minutes ~~and 36 seconds~~, ~~(~~or a time/temperature equivalent that inactivates *X. californiensis*.~~;~~

~~2) When authorising the importation or transit of *aquatic animals* and *aquatic animal products* of a species referred to in Article 11.7.2., other than those referred to in point 1 of Article 11.7.3., *Competent Authorities* should require the conditions prescribed in Articles 11.7.7. to 11.7.11. relevant to the infection with *X. californiensis* status of the *exporting country*, *zone* or *compartment*.~~

~~3) When considering the importation or transit of *aquatic animals* and *aquatic animal products* of a species not covered in Article 11.7.2. but which could reasonably be expected to pose a *risk* of spread of infection with *X. californiensis*, 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 assessment.~~

[…]

(CLEAN VERSION)

CHAPTER 11.7.

INFECTION WITH *XENOHALIOTIS CALIFORNIENSIS*

[…]

Article 11.7.3.

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

The *aquatic animal products* listed below 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 these *aquatic animal products*, *Competent Authorities* should not require any *sanitary measures* related to infection with *X. californiensis*, regardless of the infection with *X. californiensis* status of the *exporting country*, *zone* or *compartment*:

1) *aquatic animal products* that have been subjected to a heat treatment sufficient to attain a core temperature of at least 95°C for at least five minutes, or a time/temperature equivalent that inactivates *X. californiensis*.

[…]

Annex 35. Item 6.6. – Model Articles X.X.5. and X.X.6. for disease-specific chapters

EU position

The EU supports the adoption of these revised model articles.

Model Articles X.X.5. and X.X.6. for disease-specific chapters

CHAPTER X.X.

INFECTION WITH [PATHOGEN X]

[…]

Article X.X.5.

Country free from infection with [Pathogen X]

If a country shares water bodies with other countries, it can only make a self-declaration of freedom from infection with [Pathogen X] if 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.~~4~~., a Member Country may make a self-declaration of freedom from infection with [Pathogen X] for its entire *territory* if it can demonstrate that:

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 [six] months;

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 *basic biosecurity conditions* have been continuously met and have been in place 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 4.4.) have been completed followed by fallowing as described in Chapter 4.7.; 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 in wild and farmed *susceptible species* without detection of [Pathogen X]; or

ii) at least the last [one] year without detection of [Pathogen X] if affected *aquaculture establishments* were not epidemiologically connected to wild populations of *susceptible species*.

In the meantime, the part of the country outside the *infected zone* and *protection zone* ~~part or all of the country, apart from the *infected* and *protection zones,*~~ may be declared a *free zone* as described in Article 1.4.4.~~provided that such a part meets the conditions in point 2 of Article X.X.6.~~

Article X.X.6.

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 1.4.4., a Member Country may make a self-declaration of freedom from infection with [Pathogen X] for a *zone* within its *territory* if it can demonstrate that:

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 [six] months;

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.; 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 *basic biosecurity conditions* have been continuously met and have been in place 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 4.4.) have been completed followed by fallowing as described in Chapter 4.7.; 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].

In the meantime, a part of the *zone* outside the *infected zone* and *protection zone* may be declared a new *free zone* as described in Article 1.4.4.

[…]

Annex 36. Item 6.7. – Article 9.3.2. of Chapter 9.3. 'Infection with decapod iridescent virus 1'

CHAPTER 9.3.

INFECTION WITH DECAPOD IRIDESCENT VIRUS 1

EU position

The EU supports the adoption of this revised article.

[…]

Article 9.3.2.

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.: ~~[fleshy prawn (*Penaeus chinensis*), gazami crab (*Portunus trituberculatus*), giant river prawn (*Macrobrachium rosenbergii*), kuruma prawn (*Penaeus japonicus*), oriental river prawn (*Macrobrachium nipponense*), red claw crayfish (*Cherax quadricarinatus*), red swamp crawfish (*Procambarus clarkii*), ridgetail prawn (*Exopalaemon carinicauda*) and white leg shrimp (*Penaeus vannamei*), giant tiger prawn (*Penaeus monodon*), red claw crayfish (*Cherax quadricarinatus*), giant freshwater prawn (*Macrobrachium rosenbergii*), red swamp crayfish (*Procambarus clarkii*), oriental river prawn (*Macrobrachium nipponense*) and ridgetail white prawn (*Exopalaemon carinicauda*)] (under study)~~.

Family	Scientific name	Common name
Cambaridae	*Procambarus clarkii*	red swamp crawfish
Palaemonidae	*Macrobrachium nipponense*	oriental river prawn
	Macrobrachium rosenbergii	giant river prawn
	Palaemon carinicauda	ridgetail prawn
Parastacidae	*Cherax quadricarinatus*	red claw crayfish
Penaeidae	*Penaeus japonicus*	kuruma prawn
	Penaeus vannamei	whiteleg shrimp
Portunidae	*Portunus trituberculatus*	swimming crab

[…]

Annex 37. Item 6.8. – Article 10.6.2. of Chapter 10.6. 'Infection with infectious haematopoietic necrosis virus'

EU position

The EU supports the adoption of this revised article.

CHAPTER 10.6.

INFECTION WITH INFECTIOUS HAEMATOPOIETIC NECROSIS VIRUS

[…]

Article 10.6.2.

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.: ~~Arctic charr (*Salvelinus alpinus*), Atlantic salmon (*Salmo salar*), brook trout (*Salvelinus fontinalis*), brown trout (*Salmo trutta*), chinook salmon (*Oncorhynchus tshawytscha*), chum salmon (*Oncorhynchus keta*), coho salmon (*Oncorhynchus kisutch*), cutthroat trout (*Onchorynchus clarkii*), lake trout (*Salvelinus namaycush*), masu salmon (*Oncorhynchus masou*), marble trout (*Salmo marmoratus*), pike (*Esox lucius*), rainbow trout (*Oncorhynchus mykiss*) and sockeye salmon (*Oncorhynchus nerka*).~~

Family	Scientific name	Common name
Esocidae	*Esox lucius*	pike
Salmonidae	*Onchorynchus clarkii*	cutthroat trout
	Oncorhynchus keta	chum salmon
	Oncorhynchus kisutch	coho salmon
	Oncorhynchus masou	masu salmon
	Oncorhynchus mykiss	rainbow trout
	Oncorhynchus nerka	sockeye salmon
	Oncorhynchus tshawytscha	chinook salmon
	Salmo marmoratus	marble trout
	Salmo salar	Atlantic salmon
	Salmo trutta	brown trout
	Salvelinus alpinus	Arctic charr
	Salvelinus fontinalis	brook trout
	Salvelinus namaycush	lake trout

[…]

Annex 38. Item 6.9. – Article 10.11.2. of Chapter 10.11. 'Infection with tilapia lake virus'

EU position

The EU supports the adoption of this revised article.

CHAPTER 10.11.

INFECTION WITH TILAPIA LAKE VIRUS

[…]

Article 10.11.2.

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.: ~~[blue-Nile tilapia hybrid (*Oreochromis aureus* x *Oreochromis niloticus*), mango tilapia (*Sarotherodon galilaeus*), Mozambique tilapia (*Oreochromis mossambicus*), Nile tilapia (*Oreochromis niloticus*) and red hybrid tilapia (*Oreochromis niloticus* x *Oreochromis mossambicus*)~~blue tilapia (*Oreochromis aureus*), Malaysian red hybrid tilapia (*Oreochromis niloticus* x *Oreochromis mossambicus*), Mango tilapia (*Sarotherodon galilaeus*), Mozambique tilapia (*Oreochromis mossambicus*), Nile tilapia (*Oreochromis niloticus*), redbelly tilapia (*Tilapia zilli*), tinfoil barb (*Barbonymus schwanenfeldii*), Tvarnun simon (*Tristramella simonis*) and blue-nile tilapia hybrid (*Oreochromis niloticus* X *Oreochromis aureus*)] (under study).

Family	Scientific name	Common name
Cichlidae	*Oreochromis aureus x O. niloticus*	blue-Nile tilapia hybrid
	Oreochromis mossambicus	Mozambique tilapia
	Oreochromis niloticus	Nile tilapia
	Oreochromis niloticus x O. mossambicus	red hybrid tilapia
	Sarotherodon galilaeus	mango tilapia

[…]

Annex 39. Item 6.10. – Article 11.5.1. and 11.5.2. of Chapter 11.5. 'Infection with *Perkinsus marinus*'

EU position

The EU supports the adoption of this revised chapter.

CHAPTER 11.5.

INFECTION WITH *PERKINSUS MARINUS*

Article 11.5.1.

For the purposes of the *Aquatic Code*, infection with *Perkinsus marinus* means infection with the *pathogenic agent P. marinus* of the Family Perkinsidae.

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

Article 11.5.2.

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.: ~~Eastern oyster~~American cupped oyster ~~*(Crassostrea virginica),* Pacific oyster *(Crassostrea gigas),* Suminoe oyster *(Crassostrea ariakensis),* soft shell clam *(Mya arenaria),* Baltic clam *(Macoma balthica)*~~ Ariake cupped oyster ~~(*Magallana* [Syn. *Crassostrea*] *ariakensis*), Cortez oyster (*Crassostrea corteziensis*)~~ and palmate oyster ~~(*Saccostrea palmula*).hard shell clam *(Mercenaria mercenaria)*. These recommendations also apply to any other *susceptible species* referred to in the *Aquatic Manual* when traded internationally.~~

Family	Scientific name	Common name
Ostreidae	*Crassostrea corteziensis*	Cortez oyster
	Crassostrea virginica	American cupped oyster
	Magallana [syn. *Crassostrea*] *ariakensis*	Ariake cupped oyster
	Saccostrea palmula	palmate oyster

[…]

SECTION 2.2.

DISEASES OF CRUSTACEANS

CHAPTER 2.2.0.

GENERAL INFORMATION

EU position

The EU supports the adoption of this revised chapter.

A. SAMPLING

1. Assessing the health status of the epidemiological unit

1.1. Sample material to be used for tests

Sample material and the number of samples to be collected depend on the specific disease or pathogen, the size of animals, the diagnostic method to be used and the objective of testing (i.e. surveillance of apparently healthy animals, presumptive diagnosis of clinically affected animals or confirmatory diagnosis of a suspect result from surveillance or presumptive diagnosis ~~of overt disease, detection of subclinical infection in apparently healthy animals or sampling for targeted surveillance to demonstrate freedom from infection with a specified pathogen~~). See the individual disease chapters in this *Aquatic Manual* for specific details of sample requirements.

1.2. Specifications according to crustacean populations

For details of animals to sample for a specific listed disease, see the relevant disease chapter in the *Aquatic Manual*. The design of a surveillance system for demonstrating disease-free status for a country, zone or compartment should be in accordance with the recommendations of the WOAH *Aquatic Code* Chapter 1.4. *Aquatic animal disease surveillance.*

Animals to be sampled are selected as follows:

i) Susceptible species should be sampled proportionately or following risk-based criteria for targeted selection of lots, epidemiological units or populations with a history of abnormal mortality or potential exposure events (e.g. replacement with stocks of unknown disease status).

ii) If more than one water source is used for production, animals from all water sources should be included in the sample collection.

iii) ~~For the study of presumptively diseased crustaceans select those animals that are moribund, discoloured, displaying abnormal behaviour, or otherwise abnormal.~~ If weak, abnormally behaving, discoloured or freshly dead (not decomposed) animals are present, such animals should be selected. If such animals are not present, animals should be selected in such a way that all epidemiological units of the farm or waterbody are proportionately represented in the sample.

~~iv) When sampling is aimed at assessing disease occurrence (e.g. estimation of disease prevalence), the preferred selection method is probability sampling.~~

1.3. Specifications according to clinical status

In clinical disease episodes, carefully selected quality specimens with representative lesions should be obtained from live ~~or~~ (including moribund) crustaceans. Collection of dead specimens during disease outbreaks should be avoided when possible, but recently dead samples may be suitable for some diagnostic assays provided they are not decomposed. When cultured or wild crustacean stocks are presenting clinical signs of an active disease that are consistent with, or suggestive of, any one of the WOAH-listed crustacean diseases, care should be taken to ensure that the samples collected are preserved appropriately for the anticipated diagnostic tests (see sample preservation section for recommended methods). In situations other than when clinical disease episodes are investigated, for the WOAH-listed diseases it is highly recommended that the scheduling of sampling be planned (i.e. by farm schedule, season, etc.) so that the particular life-stage(s) are sampled at a time when the pathogen of concern is most likely to be detected. Disease-specific recommendations are provided in Section 3 *Sample selection, sample collection, transportation and handling* of the individual chapters.

~~Recently dead crustaceans may be suitable (depending on their condition) for certain diagnostic assays such as nucleic acid detection techniques.~~

1.4. Specifications according to crustacean size

See the individual disease chapters in this *Aquatic Manual* for specific details of sample requirements.

2. General processing of samples

2.1. Macroscopic examination

See disease-specific chapters in this *Aquatic Manual*.

2.2. Virological examination

Virological examination by virus isolation in cell culture ~~of crustaceans~~ is not ~~routinely~~ used for listed diseases of crustaceans. ~~*Macrobrachium rosenbergii* has been isolated in insect cell lines, but it is not a recommended method.~~

2.2.1. Transportation and antibiotic treatment of samples

~~Culture systems for crustacean viruses are not available; antibiotic treatment of samples is not required. For transportation of samples see Section 3 of disease-specific chapters in this *Aquatic Manual*.~~ Not applicable.

2.2.2. Virus isolation

~~For processing of tissues see Section 3 of disease-specific chapters in this *Aquatic Manual*.~~ Not applicable.

2.2.3. Treatment to neutralise enzootic viruses

Not applicable.

2.3. Bacteriological examination

Bacteriological examination of crustaceans is not routinely used for listed diseases, but it may be used for the strains of *Vibrio parahaemolyticus* (Vp_{AHPND}) that cause acute hepatopancreatic necrosis disease (AHPND). ~~and for can be isolated on standard bacteriological media. *Hepatobacter penaei*, the causative agent of necrotising hepatopancreatitis (NHP) has not been cultured and, because of its very small size, bacteriological examination may be limited to Gram staining.~~ See disease-specific chapters in this *Aquatic Manual* for identification methods.

2.4. Parasitic examination

Not applicable for currently listed diseases.

2.5. Fungal and other protists examination

See Chapter 2.2.2 *Infection with* Aphanomyces astaci (*Crayfish plague*).

B. MATERIALS AND BIOLOGICAL PRODUCTS REQUIRED FOR THE ISOLATION AND IDENTIFICATION OF CRUSTACEAN PATHOGENS

1. Crustacean viruses

1.1. Crustacean cell lines

Not applicable. There are currently no confirmed or documented crustacean cell lines.

1.2. Culture media

Not applicable.

1.3. Virus positive controls and antigen preparation

1.3.1. Virus nomenclature

In general, the virus nomenclature used in the disease-specific chapters follows the most recent taxonomy for viruses as given in the Report of the Committee on Taxonomy of Viruses (see: ICTV [ictvonline.org] for latest information). Also provided in the disease-specific chapters are the disease and virus names that are in common use by the shrimp/prawn farming industries, as well as the more common synonyms that have been used or are in current use.

1.3.2. Virus production for experimental purposes

As no cell lines (crustacean, arthropod, or vertebrate) are known that can be used to produce crustacean virus~~es,~~ stocks *in vitro*, infection of known susceptible host species (which are free ~~of~~ from infection ~~by~~ with the pathogenic agent in question) is the preferred method for virus production for experimental purposes or for the development of positive control material.

1.3.3. Virus preservation and storage

Infectivity of all of the WOAH-listed crustacean viruses can be preserved by freezing infected whole crustaceans or infected target tissues at –20°C for short-term storage, or at –80°C or lower for long-term storage.

2. Crustacean bacteria

2.1. Culture media

See Chapter 2.2.1. *Acute hepatopancreatic necrosis disease* for details.

2.2. Storage of cultures

Lyophilisation or storage at –70°C is recommended for long-term storage of bacterial cultures.

3. Crustacean parasites

3.1. Culture media

Not applicable for currently listed diseases.

3.2. Storage of cultures

Not applicable for currently listed diseases.

4. Crustacean fungi and protists

4.1. Culture media

See Chapter 2.2.2. *Infection with* Aphanomyces astaci (*crayfish plague*)

4.2. Storage of cultures

See Chapter 2.2.2.

5. Techniques

The available diagnostic methods that may be selected for diagnosis of the WOAH-listed crustacean diseases or detection of their aetiological agents are based on:

i) Gross and clinical signs.

ii) Direct bright-field, phase-contrast or dark-field microscopy with whole stained or unstained tissue wet-mounts, tissue squashes, and impression smears; and wet-mounts of faecal strands.

iii) Histology of fixed specimens.

iv) Bioassays of suspect or subclinical carriers using a highly susceptible host (life stage or species) as the indicator for the presence of the pathogen.

v) Antibody-based tests for pathogen detection using specific antisera, polyclonal antibodies (PAbs) or monoclonal antibodies (MAbs).

vi) Molecular methods (including sequencing):

DNA probes or RNA probes for *in-situ* hybridisation (ISH) assays with histological sections of fixed tissues;

Conventional and real-time PCR/RT-PCR and LAMP for direct assay with fresh tissue samples or with extracted DNA or RNA.

Pooling of samples from more than one individual animal for a given purpose ~~should only be~~ is only recommended where robust supporting data on diagnostic sensitivity and diagnostic specificity have been evaluated and found to be suitable. If the effect of pooling on diagnostic sensitivity has not been thoroughly evaluated, larger crustaceans should be processed and tested individually. However, for eggs, larvae and postlarvae, pooling of ~~larger numbers~~ individuals ~~(e.g. ~150 or more eggs or larvae or 50 to 150 postlarvae depending on their size/age)~~ may be necessary to obtain sufficient sample material to run a diagnostic assay.

5.1. Antibody-based tests

See disease-specific chapters in this *Aquatic Manual*.

5.2. Direct microscopy

Samples for direct microscopic examination should be examined as soon as possible after collection. Use live specimens whenever possible, or use fresh, chilled, or fixed specimens when live specimens are not practical. If an adequate field laboratory is available, it should be used to process and examine samples near the site of collection.

5.3. Histological techniques

Only live (including moribund) specimens with clinical signs should be sampled for histology. Collect crustaceans by whatever means are available with a minimum of handling stress. Hold animals in a container appropriate for maintaining suitable water quality and supply adequate aeration to the container if the crustaceans are to be held for a short period of time before actual fixation.

5.3.1. Fixation

A general rule is that a minimum of ten volumes of fixative should be used for one volume of tissue sample (i.e. a 10 g sample of crustacean would require 100 ml of fixative).

i) Davidson's AFA (alcohol, formalin, acetic acid) fixative

Davidson's AFA fixative is recommended for most histological applications. The fixative is rapid, reduces autolytic changes in decapod crustaceans (i.e. especially in crustaceans in tropical and subtropical regions), and its acidic content decalcifies the cuticle. The formulation for Davidson's AFA is (for 1 litre):

330 ml 95% ethyl alcohol
220 ml 100% freshly made formalin (a saturated 37–39% aqueous solution of formaldehyde gas)
115 ml glacial acetic acid
335 ml tap water (for marine crustaceans, seawater may be substituted)
Store the fixative in glass or plastic bottles with secure caps at room temperature.

ii) Fixation procedures with Davidson's AFA

For larvae and postlarvae that are too small to be easily injected with fixative using a tuberculin syringe: Using a fine mesh screen or a Pasteur pipette, select and collect specimens. Immerse crustaceans selected for sampling directly in the fixative. Fix for 12–24 hours in fixative and then transfer to 70% ethyl alcohol for storage.

For juveniles that are too small to be injected: Select and collect specimens. Use a needle or fine-pointed forceps to incise the cuticle and immediately immerse crustaceans selected for sampling directly into the fixative. Fix for 12–24 hours in fixative and then transfer to 70% ethyl alcohol for storage.

For large juveniles and adults: to ensure proper fixation, kill the crustacean using a humane method, then immediately inject fixative (use 5–10% volume:weight). Fix for 12–24 hours in fixative and then transfer to 70% ethyl alcohol for storage.

The hepatopancreas (HP) should be injected first and at two or more sites, with a volume of fixative sufficient to change the HP to a white-to-orange colour (when Davidson's AFA is used); then inject fixative into adjacent regions of the cephalothorax, into the anterior abdominal region, and into the posterior abdominal region.

The fixative should be divided between the different regions, with the cephalothoracic region, specifically the HP, receiving a larger share than the abdominal region.

Immediately following injection, slit the cuticle with dissecting scissors, from the sixth abdominal segment to the base of the rostrum, being particularly careful not to cut deeply into the underlying tissue. The incision in the cephalothoracic region should be just lateral to the dorsal midline, while that in the abdominal region should be approximately mid-lateral.

For crustaceans larger than ~12 g: After injection of fixative, the body should then be transversely bisected, at least once, just posterior to the abdomen/cephalothorax junction, and (optional) again mid-abdominally.

For very large crustaceans (*e.g. lobsters, crabs, adult penaeids, adult Macrobrachium rosenbergii*, *some species and life stages of crabs, crayfish, etc.*): The organs of interest may be excised after injection of fixative. Completion of fixation of these tissue samples is then handled as outlined previously.

Following injection, incisions and bisection/trisection, or excision of key organs, immerse the specimen in the fixative (use 10:1 fixative:tissue ratio).

Allow fixation to proceed at room temperature for 24–72 hours depending on the size of crustacean being preserved. Longer fixation times in Davidson's AFA may be used to thoroughly decalcify the shell of crabs, lobsters, crayfish, etc.

Following fixation, the specimens should be transferred to 70% ethyl alcohol, where they can be stored for an indefinite period.

iii) Transport and shipment of preserved samples

~~As large volumes of alcohol should not be mailed or shipped, the following methods are recommended:~~ Remove the specimens from the 70% ethyl alcohol. For larvae, postlarvae, or small juveniles, use leak-proof, screw-cap plastic vials if available; if glass vials must be used, pack to prevent breakage. For larger specimens, wrap samples with white paper towels to completely cover (do not use raw or processed cotton). Place towel-wrapped specimens in a sealable plastic bag and saturate with 70% ethyl alcohol. Insert the label and seal the bag. Place the bag within a second sealable bag. Multiple small sealable bags can again be placed within a sturdy, crush-proof appropriately labelled container for shipment (for details see *Aquatic Code* Chapter 5.10 *Measures concerning international transport of aquatic animal pathogens and pathological material*).

5.4. Transmission or scanning electron microscopy

Electron microscopy (EM – transmission or scanning) is a valuable research tool for the study of disease in crustaceans. However, EM methods are not routinely used for diagnosis of the diseases listed by WOAH.

5.5. Use of molecular ~~and antibody-based~~ techniques for confirmatory testing and diagnosis

Molecular techniques, including the use of nucleic acid probes for *in-situ* hybridisation, conventional PCR and real-time PCR, have been developed for the identification of many pathogens of aquatic animals. Real-time PCR methods, in general, have high sensitivity and specificity and, following adequate validation, can be used for direct detection of ~~viral~~ nucleic acids ~~in samples prepared~~ extracted from crustacean tissue. ~~The~~ Molecular techniques can be used in direct surveillance of crustacean diseases in apparently healthy populations, if they have a high level of diagnostic sensitivity, as well as in the diagnosis of clinically affected animals.

When using PCR as a diagnostic method, the design of primers and probe, the use of positive and negative controls, as well as validation of the PCR method chosen are important. Real-time PCR is a powerful technique particularly for analysing relatively high numbers of samples (e.g. for surveillance) via high-throughput testing. Several nucleic acid probe and PCR protocols are included in this version of the *Aquatic Manual* as screening, diagnostic or confirmatory methods for crustaceans and can be undertaken as the standard method. Following real-time PCR-positive results, where possible, conventional PCR with sequencing of PCR products should be used for confirmation of pathogen identity.

As with all PCR protocols, optimisation may be necessary depending on the reagents, equipment and the plasticware used. PCR is prone to false-positive and false-negative results. False-positive results (negative samples giving a positive reaction) may arise from either product carryover from positive samples or, more commonly, from cross-contamination by PCR products from previous tests. False-negative results (positive samples giving a negative result) may lead to unwanted transmission of pathogens and biosecurity failure.

Each diagnostic sample~~s~~ should be tested in duplicate, i.e. by testing two aliquots. ~~and~~ Both aliquots must produce positive results for a sample to be deemed positive. In instances where a sample produces one positive and one negative result, these are deemed indeterminate and should be retested. In addition, the following controls should be run with each assay: negative extraction control (e.g. a tissue [or equivalent sample that is under test]) sample from a known uninfected animal; positive control (preferably, one that can be distinguished from the pathogen genomic sequence [e.g. an artificial plasmid], thus allowing detection of any cross-contamination leading to a false positive result); no template control (all reagents with water replacing the template); internal positive control (internal housekeeping gene). All controls should produce their expected results in order for the diagnostic test result to be valid.

To minimise the risk of contamination, aerosol ~~preventing~~ barrier pipette tips should be used for all sample preparation and PCR ~~preparation~~ steps. Additionally, all PCRs should be prepared in a clean area that is separate from the area where ~~the~~ nucleic acid extraction, amplification~~s~~ and gel electrophoresis are performed. Do not share equipment (e.g. pipettes, laboratory coats and consumables) between areas and, where possible, restrict access between areas. Contaminating PCR products can be carried on equipment, clothes, shoes, pens/marker pens and paper (e.g. workbooks). Also, ensure all work-tops and ~~air-flow~~ cabinets/hoods used for the extractions and PCR set-up are regularly cleaned and decontaminated. To ensure sample integrity, always store the samples (e.g. in a freezer or refrigerator) in a location ~~away~~ separate from the molecular biology laboratory and reagents.

Nested PCR involves two rounds of PCR and may be used to achieve increased sensitivity and specificity; however, it increases the risk of contamination. Contaminants from previous reactions can carry over and lead to false-positive results. Strict laboratory practices such as separate workspaces, dedicated equipment, and meticulous pipetting techniques are essential to mitigate this risk. In conclusion, nested PCR is not recommended for surveillance but may sometimes be used for confirmative studies.

5.5.1. Sample preparation and types

Samples should be prepared to preserve the nucleic acid of the pathogen and should be handled and packaged with the greatest care to minimise the potential for cross-contamination among the samples or target degradation before the assay can be performed. ~~Samples selected for nucleic acid based or antibody based diagnostic tests should be handled and packaged (in new plastic sample bags or bottles) with care to minimise the potential for cross-contamination among the sample set taken from different (wild or farmed) stocks, tanks, ponds, farms, etc.~~ A water-resistant label, with the appropriate data filled out, should be placed within each package or container for each sample set.

Some suitable methods for preservation and transport of samples taken for molecular ~~or antibody based~~ tests are:

i) *Live specimens:* these may be processed in the field or shipped to the diagnostic laboratory for testing.

ii) *Haemolymph:* this tissue is the preferred sample for certain molecular ~~and antibody-based diagnostic~~ tests (see disease-specific chapters). Samples may be collected by needle and syringe through cardiac puncture, from the haemocoel (i.e. the ventral sinus in penaeids), or from a severed appendage, and immediately transferred to a tube that is half full with ~~90–95%~~ 80% analytical grade ethanol or suitable nucleic acid preservative.

iii) *Iced or chilled specimens:* these are specimens that can be transported to the laboratory for testing within 24 hours. Pack samples in sample bags surrounded by an adequate quantity of wet ice or freezer bricks around the bagged samples in an insulated box and ship to the laboratory.

iv) *Frozen ~~whole~~ specimens:* select live specimens according to the criteria listed in disease-specific chapters in this *Aquatic Manual*. In situations where it is not possible to get the specimens to the laboratory alive, they may be quick ~~freeze~~ frozen in the field using crushed dry-ice or ~~freeze~~ frozen in ~~the~~ field laboratories using a mechanical freezer at –20°C or lower temperature. Prepare and insert the label into the container with the samples, pack samples with an adequate quantity of dry-ice in an insulated box, and ship to the laboratory.

v) *Alcohol-preserved samples:* in regions where the storage and shipment of frozen samples is problematic, ~~90–95%~~ 80% analytical grade ethanol may be used to preserve, store, and transport certain types of samples for molecular tests. Alcohol-preserved samples are generally not suitable for antibody-based tests. Whole crustaceans (any life stage provided the specimen is no larger than 2–3 g), excised tissues (i.e. pleopods) from large crustaceans, or haemolymph may be preserved in ~~90–95%~~ 80% analytical grade ethanol, and then packed for shipment according to the methods described in Section 5.3.1, paragraph iii (see chapter 5.10 of the *Aquatic Code* for additional details on the international transport of such samples).

vi) *Fixed tissues for* in-situ *hybridisation:* For this purpose, classic methods for preservation of the tissues are adequate. ~~Neutral buffered formalin~~ Davidson's fixative is usually a good choice. Samples should be fixed for 24–48 hours; fixation for ~~over 24~~ more than 48 hours in Davidson's fixative should be avoided. Samples should be transferred to 80% analytical grade ethanol following Davidson's fixation ~~treatment~~.

5.5.2. Preservation of RNA and DNA in tissues

For routine diagnostic testing by PCR or RT-PCR, samples must be prepared to preserve the pathogen's nucleic acid. For most purposes, preservation of samples in analytical grade ethanol ~~alcohol~~ (80~~–90~~%) is the preferred method for subsequent molecular tests. Samples preserved in this way can be stored for up to 1 week at 4°C ~~for 1 month, at or 25°C for 1 week~~ or ~~indefinitely~~ for extended periods at –20°C or below. In addition, other products (e.g. nucleic acid preservatives, various lysis buffers, etc.) are acceptable and are commercially available for the same purpose.

5.5.3. Nucleic acid extraction

To isolate nucleic acids from tissues preserved in ethanol or nucleic acid preservative, simply remove the tissue from the fixative or preservative and treat it as though it was just harvested. Most fresh and preserved or fixed tissues can be homogenised (e.g. with a mortar and pestle or in bead-beating tubes) directly in the lysis or extraction buffer provided with commercially available DNA and RNA extraction kits. Commercial kits should be validated or undergo equivalence testing with current validated extraction procedures prior to routine use.

5.5.4. Preparation of slides for *in-situ* hybridisation

For *in-situ* hybridisation, fixed tissues that have been transferred to ~~70%~~ 80% analytical grade ethanol are embedded in paraffin according to standard histological methods. Sections are cut at a thickness of 5 µm and placed on aminoalkylsilane-coated slides, which are then baked overnight in an oven at 40°C. The sections are de-waxed by immersing in xylene for 10 minutes. This step is repeated once and then the solvent is eliminated by immersion in two successive absolute ethanol baths for 10 minutes each. The sections are then rehydrated by immersion in an ethanol series. The protocol may require a step of membrane permeabilisation enabling access to the target DNA. For this purpose, sections are treated with proteinase K (100 µg ml^{-1}) in TE buffer (Tris [50 mM], EDTA [10 mM]), at 37°C for 30 minutes. For in-situ hybridisation tests (see individual chapters for details), it is essential that both a known positive and a known negative slide be stained to eliminate false positive results due to non-specific staining/stain dropout, and false negative results due to errors in the staining protocol (Qadiri *et al*., 2019; Valverde *et al*., 2017). For further details see disease-specific chapters in this *Aquatic Manual*.

6. Additional information to be collected

Sample information should include the collector's name, organisation, date, time, and description of the geographical location of the place of origin. The geographical location of the place of origin of samples may be described as the name or location of the ~~sampling~~ site from which the sample has originated, or its geographical co-ordinates. There should also be records that provide information to allow trace-backs on the sample movement from the ~~sample~~ site of origin to the storage facility or laboratory and within those facilities.

A history of the specimens should also be collected and should include species, age, weight, details of clinical signs including behavioural changes, as well as observations concerning any gross pathology which has been observed.

Information on the preservation method, storage location, and date and time of storage at each storage locker or freezer along with information on the storage temperature (continuously monitored is preferable) should be collected. This information should be tracked with a unique sample code for all samples. For laboratories, the date of receipt, storage location information, date of analysis, analysis notes, and report date should be maintained for all uniquely coded samples. These data will greatly facilitate the tracking of sample problems and provide assurance that the samples were properly handled.

See disease-specific chapters in this *Aquatic Manual* for recommendations on any additional information that may be required or that may assist the diagnostic laboratory in determining the most appropriate test(s) to be run for submitted samples.

4. KEY REFERENCES FOR FURTHER READING

BELL T.A. & LIGHTNER D.V. (1988). A Handbook of Normal Shrimp Histology. Special Publication No. 1, World Aquaculture Society, Baton Rouge, Louisiana, USA.

BONDAD-REANTASO M.G., MCGLADDERY S.E., EAST I. & SUBASINGHE R.P. (2001). Asian Diagnostic Guide to Aquatic Animal Diseases. *FAO Fisheries Technical Paper*, No. 402, supplement 2. Food and Agriculture Organization of the United Nations (FAO), Rome, Italy, 240 pp.

JOHNSON P.T. (1980). Histology of the Blue Crab, *Callinectes sapidus*. A Model for the Decapoda. Prager, New York, USA, 440 pp.

LIGHTNER D.V. (1996). A Handbook of Shrimp Pathology and Diagnostic Procedures for Diseases of Cultured Penaeid Shrimp. World Aquaculture Society, Baton Rouge, Louisiana, USA. 304 pp.

LIGHTNER D.V., REDMAN R.M., PANTOJA C.R., TANG K.F.J, NOBLE B.L., SCHOFIELD P., MOHNEY L.L., NUNAN L.M. & NAVARRO S.A. (2012). Historic emergence, impact and current status of shrimp pathogens in the Americas. *J. Invert. Pathol.,* **110**, 174–183.

LOTZ J.M. (1997). Special topic review: Viruses, biosecurity and specific pathogen-free stocks in shrimp aquaculture. *World J. Microbiol. Biotechnol*., **13**, 405–413.

MOODY N.J.G. & CRANE M.ST.J. (2016). Validation of diagnostic tests in the OIE manual for aquatic animals. In: Proc. 3rd OIE Global Conference on Aquatic Animal Health – "Riding the Wave of the Future", Ho Chi Minh City, Vietnam, 20–22 January 2015, pp.119–126.

QADIRI S.S.N., SOO-JIN KIM S.-J., KRISHNAN R., KIM J.-O., KOLE S., KIM W.-S. & OH M.-J. (2019). Localization and tissue tropism of viral haemorrhagic septicemia virus (VHSV) in experimentally infected juvenile olive flounder, *Paralichthys olivaceus*: An *in situ* hybridization and immunohistochemical study. *Aquaculture*, **505**, 242–252.

THITAMADEE S, PRACHUMWAT A., SRISALA J., JAROENLAK P., SALACHAN P.V., SRITUNYALUCKSANA K, FLEGEL T.W. & ITSATHITPHAISARN O. (2016). Review of current disease threats for cultivated penaeid shrimp in Asia. *Aquaculture*, **452**, 69–87.

VALVERDE E.J., BORREGO J.J., SARASQUETE M.C., ORTIZ-DELGADO J.B. & CASTRO D. (2017). Target organs for lymphocystis disease virus replication in gilthead seabream (*Sparus aurata*). *Vet. Res*., **48**, 21. doi 10.1186/s13567- 017-0428-3.

WALKER P.J. & MOHAN C.V. (2009). Viral disease emergence in shrimp aquaculture: origins, impact and the effectiveness of health management strategies. *Rev. Aquaculture*, **1**, 125–154.

*
* *

NB: FIRST ADOPTED IN 2000; MOST RECENT UPDATES ADOPTED IN 2012.

CHAPTER 2.2.2.

INFECTION WITH *APHANOMYCES ASTACI* (CRAYFISH PLAGUE)

EU position

The EU supports the adoption of this revised chapter.

1. Scope

Infection with *Aphanomyces astaci* means infection with the pathogenic agent *A. astaci*, Phylum Oomycota. The disease is commonly known as crayfish plague.

2. Disease information

2.1. Agent factors

2.1.1. Aetiological agent

Aphanomyces astaci is a water mould. The Oomycetida or Oomycota, are considered protists and are classified with diatoms and brown algae in a group called the Stramenopiles or Chromista.

Five groups (A–E) of *A. astaci* have been described based on random amplification of polymorphic DNA polymerase chain reaction (RAPD PCR) (Dieguez-Uribeondo *et al.,* 1995; Huang *et al.,* 1994; Kozubikova *et al.,* 2011). Additional geno- or haplotypes are still being detected using molecular methods (Di Domenico *et al*., 2021). Group A (the so called *Astacus* strains) comprises strains isolated from several European crayfish species. These strains are thought to have been in Europe for a long period of time. Group B (*Pacifastacus* strains I) includes isolates from several European crayfish species and from the invasive *Pacifastacus leniusculus* in Europe as well as Lake Tahoe, USA. Imported to Europe, *P. leniusculus* probably introduced this genotype of *A. astaci* and infected the native European crayfish. Group C (*Pacifastacus* strains II) consists of a strain isolated from *P. leniusculus* from Pitt Lake, Canada. Another strain (Pc), isolated from *Procambarus clarkii* in Spain, sits in group D (*Procambarus* strains). This strain shows temperature/growth curves with higher optimum temperatures compared with groups A and B (Dieguez-Uribeondo *et al*., 1995). *Aphanomyces astaci* strains that have been present in Europe for many years (group A strains) appear to be less pathogenic than strains introduced with crayfish imports from North America since the 1960s. North American host species spiny-cheek crayfish (*Orconectes limosus*) has been shown to be a carrier of Group E (Kozubíková *et al.,* 2011).

2.1.2. Survival and stability in processed or stored samples

Aphanomyces astaci is poorly resistant against desiccation and does not survive long in decomposing hosts. Any treatment of the crayfish (freezing, cooking, drying) will affect the survival of the pathogen (Oidtmann *et al.,* 2002). Isolation from processed samples is not possible, however they may be suitable for molecular methods used for pathogen detection.

2.1.3. Survival and stability outside the host

Outside the host *Aphanomyces astaci* is found as zoospores that remain motile for up to 3 days and form cysts that can survive for 2 weeks in distilled water. As *A. astaci* can go through three cycles of encystment and zoospore emergence, the maximum life span outside of a host could be several weeks. Spores remained viable in a spore suspension in clean water kept at 2°C for 2 months (Unestam, 1966). Survival time is probably shorter in natural waters.

For inactivation methods, see Section 2.4.5.

2.2. Host factors

2.2.1. Susceptible host species

The recommendations in this chapter apply to all species of crayfish in all three crayfish families (Cambaridae, Astacidae and Parastacidae).

[Note: an assessment of species that meet the criteria for listing as susceptible to infection with *A. astaci* in accordance with Chapter 1.5. has not yet been completed]

~~All stages of crayfish species native to Europe, including the noble crayfish (*Astacus astacus*) of north-west Europe, the white clawed crayfish (*Austropotamobius pallipes*) of south-west and west Europe, the related *Austropotamobius torrentium* (mountain streams of south-west Europe) and the slender clawed or Turkish crayfish (*Pontastacus leptodactylus*) of eastern Europe and Asia Minor are highly susceptible (e.g. Holdich *et al*., 2009). Australian species of crayfish are also highly susceptible. North American crayfish such as the signal crayfish (*Pacifastacus leniusculus*), Louisiana swamp crayfish (*Procambarus clarkii*) and *Faxonius* spp. are infected by *A. astaci*, but under normal conditions the infection does not cause clinical disease or death. All North American crayfish species investigated to date have been shown to be susceptible to infection, demonstrated by the presence of the pathogen in host cuticle (reviewed by Svoboda *et al*. 2017) and it is therefore currently assumed that this is the case for any other North American species.~~

~~The only other crustacean known to be susceptible to infection by *A. astaci* is the Chinese mitten crab (*Eriocheir sinensis*).~~

2.2.2. Species with incomplete evidence for susceptibility

[Under study]

2.2.3. Likelihood of infection by species, host life stage, population or sub-populations

The host species susceptible to infection with *A. astaci* fall largely into two categories: those ~~highly susceptible to infection with~~ that develop~~ment~~ ~~of~~ clinical disease and experience mortality~~-mortalities~~, and those that are infected ~~without associated~~ but do not display any significant clinical disease or experience mortality~~-mortalities~~. All life stages of susceptible species are considered susceptible to infection with *A. astaci*.

Species that develop clinical disease and experience mortality ~~mortalities~~ include the noble crayfish (*Astacus astacus*) of north-west Europe, the white clawed crayfish (*Austropotamobius pallipes*) of south-west and west Europe, the related *Austropotamobius torrentium* (mountain streams of south-west Europe) and the ~~slender clawed or Turkish~~ Danube crayfish (*Pontastacus leptodactylus*) of eastern Europe and Asia Minor (e.g. Holdich *et al*., 2009). Australian species of freshwater crayfish are also considered vulnerable to clinical disease and mortality ~~mortalities~~.

Species that can be infected but do not normally develop clinical disease include North American crayfish species such as the signal crayfish (*Pacifastacus leniusculus*), Louisiana swamp crayfish (*Procambarus clarkii*) and *Faxonius* spp. All North American crayfish species that have been investigated have been shown to be susceptible to infection, demonstrated by the presence of the pathogen in host cuticle (reviewed by Svoboda *et al*. 2017).

~~*Highly susceptible species:*~~ Clinical disease outbreaks caused by infection with *A. astaci* are generally known as 'crayfish plague' outbreaks. In such outbreaks, moribund and dead crayfish of a range of sizes (and therefore ages) can be found.

The only non-crayfish crustacean species known to be susceptible to infection by *A. astaci* is the Chinese mitten crab (*Eriocheir sinensis*) (Schrimpf *et al.*, 2014).

2.2.4. Distribution of the pathogen in the host

The tissue that becomes initially infected is the exoskeleton cuticle. Soft cuticle, as is found on the ventral abdomen and around joints, is preferentially affected. In ~~the highly susceptible~~ European crayfish species, which are prone to development of clinical disease, the pathogen often manages to penetrate the basal lamina located underneath the epidermis cell layer. From there, *A. astaci* spreads throughout the body primarily by invading connective tissue and haemal sinuses; however, all tissues may be affected.

In North American crayfish species, infection is usually restricted to the cuticle. Based on PCR results, the tailfan (consisting of uropods and telson) and soft abdominal cuticle appear to be frequently infected (Oidtmann *et al.,* 2006; Vralstad *et al.,* 2011).

2.2.5. Aquatic animal reservoirs of infection

North American crayfish species act mostly as reservoirs ~~carriers~~ of the infection without showing clinical signs. However, some strains of *A. astaci,* especially from group A, show lowered virulence, ~~thus~~ enabling ~~normally highly susceptible~~ European crayfish to act as reservoirs ~~carriers~~ as well (see review by Svoboda *et al*., 2017).

Colonisation of habitats~~, initially~~ by North American crayfish species carrying *A. astaci* ~~occupied by highly susceptible~~ is likely to result in an epizootic if crayfish species that are prone to expression of clinical disease are present ~~by North American crayfish species carrying *A. astaci* is likely to result in an epizootic among the highly susceptible animals~~.

2.2.6. Vectors

~~Transportation of finfish may facilitate the spread of *A. astaci* through the presence of spores in the transport water or co-transport of infected crayfish specimens (Alderman *et al*., 1987; Oidtmann *et al*., 2002). There is also circumstantial evidence of spread by contaminated equipment (e.g. nets, boots, clothing, traps) (Alderman *et al*., 1987).~~ None known.

2.3. Disease pattern

2.3.1. Mortality, morbidity, and prevalence

When the infection first reaches a naïve population of ~~highly susceptible~~ crayfish species that are prone to clinical disease, high levels of mortality are usually observed within a short space of time, ~~so that in and~~. In areas with high crayfish densities the bottoms of lakes, rivers and streams ~~are~~ become covered with dead and dying crayfish. A band of mortality will spread quickly from the initial outbreak site downstream, whereas upstream spread is slower. Lower water temperatures are associated with ~~slower~~ a lower rate of mortalities and a greater range of clinical signs in affected animals (Alderman *et al*., 1987). Observations from Finland suggest that at low water temperatures, noble crayfish (*Astacus astacus*) can be infected for several months without ~~the development of~~ any noticeable mortalities (Viljamaa-Dirks *et al*., 2013).

On rare occasions, single specimens of ~~the highly susceptible~~ species that are prone to clinical disease have been found after a wave of infection with *A. astaci* has gone through a river or lake. This is most likely to be due to lack of exposure of these animals during an outbreak (animals may have been present in a tributary of a river or lake or in a part of the affected river/lake that was not reached by spores, or crayfish may have stayed in burrows during the epizootic). However, low virulent strains of *A. astaci* have been described to persist in a waterway, kept alive by a weak infection in the remnant population (Viljamaa-Dirks *et al*., 2011). Although remnant populations of susceptible crayfish species remain in many European watersheds, the dense populations that existed 150 years ago are now heavily diminished (Souty-Grosset *et al*., 2006; Holdich *et al*., 2009). Populations of susceptible crayfish may re-establish, but once population density and geographical distribution is sufficient for susceptible animals to come into contact with spores, new outbreaks of infection with *A. astaci* and large-scale mortalities ~~will~~ may occur.

In ~~the highly susceptible~~ European crayfish species, which are prone to clinical disease, exposure to *A. astaci* spores usually leads to infection and eventually to death. Prevalence of infection within a population in the early stage of an outbreak may be low (few animals in a river population may be affected). However, the pathogen ~~is amplified~~ amplifies in affected animals and is subsequently released into the water; usually leading to 100% mortality in a contiguous population. The rate of spread from initially affected animals depends on several factors, one being water temperature. Therefore, the time from first introduction of the pathogen into a population to noticeable crayfish mortalities can vary greatly and may range from a few weeks to months. Prevalence of infection will gradually increase over this time and usually reach 100%. Data from a noble crayfish population in Finland that experienced an acute mortality event due to infection with *A. astaci* in 2001 suggest that in sparse noble crayfish populations, spread of disease throughout the host population may take several years (Viljamaa-Dirks *et al.,* 2011).

2.3.2. Clinical signs, including behavioural changes

Susceptible ~~species~~ prone to clinical disease

Gross clinical signs are variable and depend on challenge severity and water temperatures. The first sign of an ~~epizootic~~ outbreak may be the appearance of crayfish during daylight (crayfish are normally nocturnal), some of which may show

loss of co-ordination, falling onto their backs and remaining unable to right themselves. Occasionally, the infected animals can be seen trying to scratch or pinch themselves.

Often, however, the first sign of an outbreak may be the presence of large numbers of dead crayfish in a river or lake (Alderman *et al.,* 1987).

Infection with *A. astaci* may cause mass mortality of crayfish. However, investigation of mortality event should consider other causes such as environmental pollution (e.g. insecticides such as cypermethrin have been associated with initial misdiagnoses).

~~*North American crayfish*~~ *Susceptible* ~~-~~*species that do not normally develop clinical disease*

Infected North American crayfish may be subclinical ~~carriers~~ reservoirs. Controlled exposure to a highly virulent strain has resulted in mortality in juvenile stages of *Pacifastacus leniusculus* as well as behavioural alterations in adults (Thomas *et al.,* 2020).

2.3.3 Gross pathology

Susceptible ~~*S*~~*species prone to clinical disease*

Depending on a range of factors, the foci of infection in crayfish may be seen by the naked eye or may not be discernible despite careful examination. Infection foci are best viewed under a low power stereo microscope and are recognisable by localised whitening of the muscle beneath the cuticle. In some cases, a brown colouration of cuticle and muscle may occur, or hyphae may be visible in infected cuticles in the form of fine brown (melanised) tracks in the cuticle. Sites for examination include the intestinal soft ventral cuticle of the abdomen and tail, the cuticle of the perianal region, the cuticle between the carapace and abdomen, the joints of the pereiopods (walking legs), particularly the proximal joint, eyestalks and finally the gills.

~~*North American crayfish*~~ Susceptible ~~*S*~~*species that do not normally develop clinical disease*

Infected North American crayfish do not usually show signs of disease ~~can sometimes show melanised spots in their soft cuticle, for example, the soft abdominal cuticle and joints. These melanisations can be caused by mechanical injuries or infections with other water moulds and are non-specific~~. However, populations with high levels of infection can show abnormally high levels of cuticular damage in individual animals, such as missing legs and claws due to deteriorated joints.

2.3.4. Modes of transmission and life cycle

~~The main routes of spread of the pathogen are through 1) movement of infected crayfish, 2) movement of spores with contaminated water or equipment, as may occur during movements of finfish, or 3) through colonisation of habitats by invasive North American crayfish species.~~

~~The main route of spread of *A. astaci* in Europe between the 1960s and 2000 was through the active stocking of North American crayfish into the wild or escapes from crayfish farms. Subsequent spread occurs through expanding populations of invasive North American crayfish, accidental co-transport of specimens, and release of North American crayfish into the wild by private individuals (Holdich *et al.,* 2009).~~

Transmission from crayfish to crayfish occurs through the release of zoospores from an infected animal and attachment of the zoospores to naïve crayfish. The life cycle of *A. astaci* is simple with vegetative hyphae invading and ramifying through host tissues, eventually producing extramatrical sporangia that release amoeboid primary spores. These initially encyst, but then release a biflagellate zoospore (secondary zoospore). Biflagellate zoospores swim in the water column and, upon encountering a susceptible host, attach and germinate to produce invasive vegetative hyphae. The zoospores of *A. astaci* swim actively in the water column and have been demonstrated to show positive chemotaxis towards crayfish (Cerenius & Söderhäll, 1984). Zoospores are capable of repeated encystment and re-emergence, extending the period of their infectivity (Soderhall & Cerenius 1999). Growth and sporulation capacity is strain-and temperature-dependent (Dieguez-Uribeondo *et al*., 1995).

The main routes of spread of the pathogen are through 1) movement of infected crayfish, 2) movement of spores with contaminated water or equipment, or 3) through colonisation of non-native habitats by invasive North American crayfish species.

The main route of spread of *A. astaci* in Europe between the 1960s and 2000 was through the active stocking of North American crayfish into the wild or escapes from crayfish farms. Subsequent spread occurred through expanding

populations of invasive North American crayfish, accidental co-transport of specimens, and release of North American crayfish into the wild by private individuals (Holdich *et al.,* 2009).

Transportation of finfish may facilitate the spread of *A. astaci* through the presence of spores in the transport water or co-transport of infected crayfish specimens (Alderman *et al*., 1987; Oidtmann *et al*., 2002). There is also circumstantial evidence of spread by contaminated equipment (e.g., nets, boots, clothing, traps) (Alderman *et al*., 1987).

2.3.5. Environmental factors

Under laboratory conditions, the preferred temperature range at which the *A. astaci* mycelium grows varies slightly depending on the strain. In a study, which compared several *A. astaci* strains that had been isolated from a variety of crayfish species, mycelial growth was observed between 4 and 29.5°C, with the strain isolated from *Procambarus clarkii* growing better at higher temperatures compared to the other strains. Sporulation efficiency was similarly high for all strains tested between 4 and 20°C, but it was clearly reduced for the non-*P. clarkii* strains at 25°C and absent at 27°C. In contrast, sporulation still occurred in the *P. clarkii* strain at 27°C. The proportion of motile zoospores (out of all zoospores observed in a zoospore suspension) was almost 100% at temperatures ranging from 4–18°C, reduced to about 60% at 20°C and about 20% at 25°C in all but the *P. clarkii* strain. In the *P. clarkii* strain, 80% of the zoospores were still motile at 25°C, but no motile spores were found at 27°C (Dieguez-Uribeondo *et al*., 1995).

Field observations show that outbreaks of infection with *A. astaci* occur over a wide temperature range, and at least in the temperature range 4–20°C. The rate of spread within a population depends on several factors, including water temperature. In a temperature range between 4 and 16°C, the speed of an epizootic is enhanced by higher water temperatures.

In buffered, redistilled water, sporulation occurs between pH 5 and 8, with the optimal range being pH 5–7. The optimal pH range for swimming of zoospores appears to be pH 6.0–7.5, with a maximum range between pH 4.5 and 9.0 (Unestam, 1966).

Zoospore emergence is influenced by the presence of certain salts in the water. $CaCl_2$ stimulates zoospore emergence from primary cysts, whereas $MgCl_2$ has an inhibitory effect. In general, zoospore emergence is triggered by transferring the vegetative mycelium into a medium where nutrients are absent or low in concentration (Cerenius *et al*., 1988).

2.3.6. Geographical distribution

In Europe the reports of large mortalities of crayfish go back to 1860. The reservoir of the original infections in the 19th century was never established. *Faxonius* (*Orconectes*) spp. were not known to have been introduced into Europe until the 1890s, but the post-1960s extensions are largely linked to more recent introductions of North American crayfish for farming (Alderman, 1996; Holdich *et al*. 2009). *Pacifastacus leniusculus* and *Procambarus clarkii* are now widely naturalised in many parts of Europe.

In recent years, crayfish plague has been reported in Asia and also in North- and South America (see e.g. references in Di Domenico *et al.* 2021). The distribution of *A. astaci* in North America is likely to be much wider than reported (Martín-Torrijos *et al.,* 2021).

~~Any geographical area where North American crayfish species were introduced must be considered as potentially infected if not proven otherwise. Lack of clinical disease in these carrier species may hamper the reliability in reporting the infection. For the highly susceptible species,~~ See WOAH WAHIS (https://wahis.woah.org/#/home) for recent information on distribution at the country level. ~~However, even high mortalities can go unnoticed in wild populations.~~

2.4. Biosecurity and disease control strategies

2.4.1. Vaccination

No vaccines are available.

2.4.2. Chemotherapy including blocking agents

No treatments are currently known~~ that can successfully treat the highly susceptible crayfish species, once infected~~.

2.4.3. Immunostimulation

No immunostimulants are currently known ~~that can successfully protect the highly susceptible crayfish species against infection and consequent disease due to *A. astaci* infection~~.

2.4.4. Breeding resistant strains

A few studies suggest that there might be differences in resistance between populations of ~~highly susceptible species~~ crayfish species that are prone to clinical disease (reviewed by Martín-Torrijos *et al*., 2017; Svoboda *et al.*, 2017). ~~The fact that North American crayfish generally do not develop clinical disease suggests that selection for resistance may be possible and laboratory studies using attenuated strains of *A. astaci* might be successful. However, there are currently no published data from such studies.~~

2.4.5. Inactivation methods

Aphanomyces astaci, both in culture and in infected crayfish, is inactivated by a short exposure to temperatures of 60°C or to temperatures of –20°C (or below) for 48 hours (or more) (Oidtmann *et al*., 2002). Sodium hypochlorite at 100 ppm, free chlorine and iodophors at 100 ppm available iodine, are effective for disinfection of contaminated equipment. Equipment must be cleaned prior to disinfection since organic matter decreases the effectiveness of disinfectants (Alderman & Polglase, 1985). Thorough drying of equipment (>24 hours) is also effective as *A. astaci* is not resistant to desiccation (Rennerfelt, 1936).

2.4.6. Disinfection of eggs and larvae

No information available.

2.4.7. General husbandry

If a ~~crayfish~~ farm for ~~highly susceptible~~ crayfish species that are prone to clinical disease is being planned, it should be carefully investigated whether North American crayfish species are in the vicinity of the planned site or present upstream. If North American crayfish are present, there is a high likelihood that susceptible farmed crayfish will eventually become infected.

In an endemic area where ~~the highly susceptible~~ species prone to expression of clinical disease are being farmed, the following biosecurity recommendations should be followed to avoid an introduction of *A. astaci* onto the site:

1. General biosecurity should be in place (e.g. controlled access to premises; disinfection of boots when entering the site; investigation of mortalities if they occur; introduction of live susceptible species or vectors ~~animals (crayfish, finfish)~~ only from sources known to be free from infection with *A. astaci*).

2. Prevent movements of potentially infected live or dead crayfish, potentially contaminated water, equipment or any other items that might carry the pathogen from an infected to an uninfected site holding susceptible species ~~should be prevented~~.

3. ~~If~~ Do not transfers ~~of finfish or crayfish are being planned, these should not come~~ susceptible species or vectors from streams or other waters that harbour potentially infected crayfish (either susceptible crayfish populations that are going through a current outbreak of infection with *A. astaci* or North American ~~carrier~~ crayfish species).

4. North American crayfish should not be brought onto the site.

5. Finfish obtained from unknown freshwater sources or from sources, where North American crayfish may be present or a current outbreak of infection with *A. astaci* may be taking place, must not be used as bait or feed for crayfish, unless they have been subject to a temperature treatment to kill *A. astaci* (see Section 2.4.5. *Inactivation methods*).

6. Any equipment that is brought onto site should be disinfected.

3. Specimen selection, sample collection, transportation and handling

3.1. Selection of populations and individual specimens

For a suspected outbreak of infection with *A. astaci* in a population of ~~highly susceptible~~ crayfish species ~~that are prone to clinical disease,~~ sampled crayfish should ideally consist of: a) live crayfish showing signs of disease, and b) live, apparently healthy crayfish. Freshly dead crayfish may also be suitable, although this will depend on their condition.

Live crayfish should be transported using insulated containers equipped with small holes to allow aeration. The temperature in the container should not exceed 16°C.

Crayfish should be transported in a moist environment ~~atmosphere~~, for example using moistened wood shavings/wood wool, newspaper, or grass/hay. Unless transport water is sufficiently oxygenated, live crayfish should not be transported in water, as they may suffocate.

Should only dead animals be found at the site of a suspected outbreak, freshly dead animals should be selected for diagnosis. Dead animals can either be: a) transported chilled (if they appear to have died only very recently), or, b) placed in non-methylated ethanol (minimum concentration 70%; see 3.5. *Preservation of samples for submission*), or c) placed in freezer at –20°C to avoid further decay and transported frozen.

When testing any population outside an acute mortality event for the presence of crayfish plague, as many individuals as possible should be inspected visually for signs of cuticular damage. Crayfish that have melanized spots or missing limbs should be selected in the first place for further analysis.

3.2. Selection of organs or tissues

~~In highly susceptible species that are prone to clinical disease,~~ The tissue recommended for sampling is the soft abdominal cuticle, which can be found on the ventral side of the abdomen. Any other soft part of the exoskeleton and eyestalks can be included as well.

If any melanised spots or whitened areas are detected, these should be included in the sampling. From diseased animals, samples should be aseptically collected from the soft abdominal cuticle. For identification of ~~carriers~~ reservoirs, samples should be aseptically collected from soft abdominal cuticle, and telson and uropods, separately.

In the North American crayfish species, sampling of soft abdominal cuticle, uropods and telson are recommended. Any other soft part of the exoskeleton can be included as well. If any melanized spots are detected, these should be included in the sampling.

3.3. Samples or tissues not suitable for pathogen detection

Autolysed material is not suitable for analysis.

3.4. Non-lethal sampling

A non-destructive sampling method that detects *A. astaci* DNA in the microbial biofilm associated with the cuticle of individual crayfish through vigorous scrubbing has been described (Pavic *et al.,* 2020), and could be considered in case of testing vulnerable populations.

If non-lethal tissue sample types differ from recommended tissues (see Section 3.2.), or from the tissue samples used in validation studies, the effect on diagnostic performance should be considered.

3.5. Preservation of samples for submission

The use of non-preserved crayfish is preferred, as described above. If transport of recently dead or moribund crayfish cannot be arranged, crayfish may be frozen or fixed in ethanol (minimum 70%). However, fixation may reduce test sensitivity. The crayfish:ethanol ratio should ideally be 1:10 (1 part crayfish, 10 parts ethanol).

For guidance on sample preservation methods for the intended test methods, see Chapter 2.2.0 *General information* (diseases of crustaceans)

3.5.1. Samples for pathogen isolation

The success of pathogen isolation depends strongly on the quality of samples (time since collection and time in storage). Isolation is best attempted from crayfish with clinical signs delivered alive (see Section 3.1.). Fresh specimens should be kept chilled and preferably sent to the laboratory within 24 hours of collection.

3.5.2. Preservation of samples for molecular detection

Tissue samples for PCR testing should be preserved in 70–90% (v/v) analytical/reagent-grade (undenatured) ethanol. The recommended ratio of ethanol to tissue is 10:1 based on studies in terrestrial animal and human health. The use of lower grade (laboratory or industrial grade) ethanol is not recommended. If material cannot be fixed it may be frozen.

Standard sample collection, preservation and processing methods for molecular techniques can be found in Section B.5.5. of Chapter 2.2.0 *General information* (diseases of crustaceans).

3.5.3. Samples for histopathology

Standard sample collection, preservation and processing methods for histological techniques can be found in Section 2.2 of Chapter 2.2.0 *General information* (diseases of crustaceans).

3.5.4. Samples for other tests

Sensitive molecular methods can be used to detect *A. astaci* DNA directly from water samples (Strand *et al*. 2011, 2012). These methods require validation for diagnostic use.

3.6. Pooling of samples

Pooling of samples from more than one individual animal for a given purpose ~~should only be~~ is only recommended where robust supporting data on diagnostic sensitivity and diagnostic specificity have been evaluated and found to be suitable. The effect of pooling on diagnostic sensitivity has not been thoroughly evaluated, therefore, larger animals should be processed and tested individually. Small life stages such as PL, can be pooled to obtain the minimum amount of material for molecular detection.

4. Diagnostic methods

The methods currently available for pathogen detection that can be used in i) surveillance of apparently healthy animals, ii) presumptive diagnosis in clinically affected animals and iii) confirmatory diagnostic purposes are listed in Table 4.1. by animal life stage.

Ratings for purposes of use. For each recommended assay a qualitative rating for the purpose of use is provided. The ratings are determined based on multiple performance and operational factors relevant to application of an assay for a defined purpose. These factors include appropriate diagnostic performance characteristics, level of assay validation, availability cost, timeliness, and sample throughput and operability. For a specific purpose of use, assays are rated as:

+++ =	Methods are most suitable with desirable performance and operational characteristics.
++ =	Methods are suitable with acceptable performance and operational characteristics under most circumstances.
+ =	Methods are suitable, but performance or operational characteristics may limit application under some circumstances.
Shaded boxes =	Not appropriate for this purpose.

Validation stage. The validation stage corresponds to the assay development and validation pathway in chapter 1.1.2. The validation stage is specific to each purpose of use. Where available, information on the diagnostic performance of recommended assays is provided in Section 6.3.

WOAH Reference Laboratories welcome feedback on diagnostic performance of recommended assays, in particular PCR methods. Of particular interest are any factors affecting expected assay sensitivity (e.g. tissue components inhibiting amplification) or expected specificity (e.g. failure to detect particular genotypes, detection of homologous sequences within the host genome). These issues should be communicated to the WOAH Reference Laboratories so that advice can be provided to diagnostic laboratories and the standards amended if necessary.

Table 4.1. *WOAH recommended diagnostic methods and their level of validation for surveillance of apparently healthy animals and investigation of clinically affected animals*

Method	A. Surveillance of apparently healthy animals				B. Presumptive diagnosis of clinically affected animals				C. Confirmatory diagnosis[1] of a suspect result from surveillance or presumptive diagnosis			
	Early life stages[2]	Juveniles[2]	Adults	LV	Early life stages[2]	Juveniles[2]	Adults	LV	Early life stages[2]	Juveniles[2]	Adults	LV
Wet mounts						+	+	NA				
Histopathology						+	+	NA				
~~Cell~~ Culture						+	+	NA				
Real-time PCR	++	++	++	1	++	++	++	1	++	++	++	1
Conventional PCR	+	+	+	1	++	++	++	1				
Conventional PCR followed by amplicon sequencing									+++	+++	+++	1
In-situ hybridisation												
Bioassay												
~~LAMP~~ Immunohistochemistry												
Ab-ELISA												
Ag-ELISA												
~~Other antigen detection methods~~[3]												
Other methods[3]												

LV = level of validation, refers to the stage of validation in the WOAH Pathway (chapter 1.1.2); NA = not available. PCR = polymerase chain reaction; ~~LAMP = loop-mediated isothermal amplification;~~ Ab- or Ag-ELISA = antibody or antigen enzyme-linked immunosorbent assay, respectively; IFAT = indirect fluorescent antibody test.

[1]For confirmatory diagnoses, methods need to be carried out in combination (see Section 6). [2]Susceptibility of early and juvenile life stages is described in Section 2.2.3.

[3]Specify the test used. Shading indicates the test is inappropriate or should not be used for this purpose

4.1. Wet mounts

Small pieces of soft cuticle excised from the regions mentioned above (Section 2.3.3 *Gross pathology*) and examined under a compound microscope using low-to-medium power will confirm the presence of aseptate fungal-like hyphae 7–9 µm wide. The hyphae can usually be found pervading the whole thickness of the cuticle, forming a three-dimensional network of hyphae in heavily affected areas of the cuticle. The presence of host haemocytes and possibly some melanisation closely associated with and encapsulating the hyphae give good presumptive evidence that the hyphae represent a pathogen rather than a secondary opportunist invader. In some cases, examination of the surface of such mounted cuticles will demonstrate the presence of characteristic *A. astaci* sporangia with clusters of encysted primary spores ~~(see Section 4.3 *Culture for isolation*)~~.

4.2. Histopathology

Unless the selection of tissue for fixation has been well chosen, *A. astaci* hyphae can be difficult to find in stained preparations. A histological staining technique, such as the Grocott silver stain counterstained with conventional haematoxylin and eosin, can be used to visualise *A. astaci* in tissues of crayfish species prone to clinical disease. However, such material does not prove that any hyphae observed are those of *A. astaci*, especially when the material comes from animals already dead by sampling.

See also Section 4.1 *Wet mounts*.

4.3. Culture for isolation

Isolation is not recommended as a routine diagnostic method (Alderman & Polglase, 1986; Cerenius *et al*., 1987; Viljamaa-Dirks, 2006). Test sensitivity and specificity of the cultivation method can be very variable depending on the experience of the examiner, but in general will be lower than the PCR. Isolation of *A. astaci* by culture from apparently healthy crayfish is challenging and molecular methods are recommended. A detailed description of this test is available from the Reference Laboratory[1].

4.4. Nucleic acid amplification

PCR assays should always be run with the controls specified in Section 5.5 '*Use of molecular and antibody-based techniques for confirmatory testing' and diagnosis* of Chapter 2.2.0 *General information* (diseases of crustaceans). Each sample should be tested in duplicate. Shrimp tissues may be used as negative controls.

Live crayfish can be killed using chloroform, electric current or by mechanical destroying the nerve cords. If live or moribund animals are not available, only recently dead animals should be used for DNA extraction. The soft abdominal cuticle is the preferred sample tissue for DNA extraction. Any superficial contamination should first be removed by thoroughly wiping the soft abdominal cuticle with wet (using autoclaved H_2O) clean disposable swabs. The soft abdominal cuticle is then excised and 30–50 mg of tissue is homogenised using standard physical methods ~~ground using a pestle and mortar.~~

Several PCR assays have been developed with varying levels of sensitivity and specificity. Two assays are described here. Both assays target the ITS (internal transcribed spacer) region of the nuclear ribosomal gene cluster within the *A. astaci* genome.

Extraction of nucleic acids

~~Numerous~~ Different kits and procedures can be used for nucleic acid extraction. The quality and concentration of the extracted nucleic acid is important and ~~should~~ can be checked using a suitable method as appropriate to the circumstances ~~using optical density or running a gel~~.

4.4.1. Real-time PCR

The following controls should be run with each assay: negative extraction control; positive control; no template control; internal PCR control.

Pathogen/ target gene	Primer/probe (5'–3')	Concentration	Cycling conditions[(a)]
Method 1*: Vralstad *et al.,* 2009, ~~Strand, 2013~~; GenBank Accession No.: AM947024			

1 https://www.woah.org/en/what-we-offer/expertise-network/reference-laboratories/#ui-id-3

Aphanomyces ~~astacus~~ astaci & *A. fennicus*/ ITS	Fwd: AAG-GCT-TGT-GCT-GGG-ATG-TT Rev: CTT-CTT-GCG-AAA-CCT-TCT-GCT-A Probe: 6-FAM-TTC-GGG-ACG-ACC-C-MGBNFQ	500 nM 500 nM 200 nM	50 cycles of: 95°C/15 sec and ~~60~~58°C/~~30~~ 60 sec
Method 2: Strand *et al.*, 2023; GenBank Accession No.: AM947024			
Aphanomyces astacus astaci/ITS	Fwd: TAT-CCA-CGT-GAA-TGT-ATT-CTT-TAT Rev: GCT-AAG-TTT-ATC-AGT-ATG-TTA-TTT-A Probe: FAM-AAG-AAC-ATC-CCA-GCA-C-MGBNFQ	500 nM 500 nM 100 nM	50 cycles of: 95°C/15 sec and 60°C/30 sec

*These ITS-based methods have been found to give positive results for the species *Aphanomyces fennicus* (Viljamaa-Dirks & Heinikainen 2019).
[a]A denaturation step prior to cycling has not been included.

The absolute limit of detection of method 1 was reported as approximately 5 PCR forming units (= target template copies)~~, which is equivalent to less than one *A. astaci* genome~~ (Vralstad *et al*., 2009). Another study reported consistent detection down to 50 fg DNA using this assay (Tuffs & Oidtmann, 2011).

Analytical test specificity has been investigated (Tuffs & Oidtmann, 2011; Vralstad *et al*., 2009) and no cross-reaction was observed in these studies. However, a novel species, *Aphanomyces fennicus*, isolated from noble crayfish was reported in 2019 (Viljamaa-Dirks & Heinikainen, 2019) that gave a positive reaction in this test at the same level as *A. astaci*. Due to this problem in specificity, ~~A modified alternative method for~~ the assay ~~will be included once it has been published~~ has been modified according to the alternative method 2 (Strand *et al*., 2023~~ manuscript in preparation~~).

Owing to the repeated discovery of new *Aphanomyces* strains, sequencing is required to determine the species of *Aphanomyces*~~.~~ in the case of ~~the non-negative~~ an amplification product in the real-time PCR assay result. This requires separate amplification of a PCR product ~~using primers ITS-1 and ITS-4~~ (see Section 4.5 *Amplicon sequencing*).

4.4.2. Conventional PCR

Pathogen/ target gene	Primer~~/probe~~ (5'–3')	Concentration	Cycling conditions[a]
Method 1*: Oidtmann *et al.,* 2006; GenBank Accession No.: AY310499; ~~Product~~ amplicon size: 569 bp			
Aphanomyces ~~astacus~~ astaci & *A. fennicus*/ ITS	Fwd: GCT-TGT-GCT-GAG-GAT-GTT-CT Rev: CTA-TCC-GAC-TCC-GCA-TTC-TG-	500 nM 500 nM	40 cycles of: 1 min/96°C, 1 min/59°C and 1 min/72°C

*This ITS-based method has been found to give positive results for the species *Aphanomyces fennicus* (Viljamaa-Dirks & Heinikainen 2019).
[a]A denaturation step prior to cycling has not been included.

Confirmation of the identity of the PCR product by sequencing is required as a novel species, *A. fennicus*, isolated from noble crayfish was reported in 2019 (Viljamaa-Dirks & Heinikainen, 2019) that gave a positive reaction in this assay.

The assay consistently detects down to 500 fg of genomic target DNA or the equivalent amount of ten zoospores submitted to the PCR reaction (Tuffs & Oidtmann, 2011).

4.4.3. Other nucleic acid amplification methods

Several genotype-specific molecular methods have been developed that, instead of requiring a pure growth as sample material like the RAPD-PCR assay, can be used to analyse crayfish tissue directly (Di Domenico *et al*., 2021; Grandjean *et al*., 2014; Makkonen *et al.,* 2018; Minardi *et al*., 2018; 2019). Detection of a known genotype group combined with a positive result by a recommended conventional or real-time PCR can be used as a confirmative test in geographical areas where crayfish plague is known to be present. However, the current knowledge of the genotype variation is mostly limited to a few original host species and new genotypes or subtypes are expected to be found. Thus, the suitability of these methods is limited for initial excluding diagnosis or as confirmative tests in geographical areas not known to be infected.

PCR targeting mitochondrial DNA with *A. astaci* genotype specific primers have been shown to detect the known genotypes of *A. astaci*, but these assays may also provide positive results for some other oomycete genera (Casabella-Herrero *et al.,* 2021).

4.5. Amplicon sequencing

~~, and purified by excision from this gel.~~ Both DNA strands of the PCR product must be sequenced and analysed ~~and compared~~ in comparison with ~~published~~ reference sequences.

4.6. *In-situ* hybridisation

Not available.

4.7. Immunohistochemistry

Not available

4.8. Bioassay

No longer used for diagnostic purposes (see Cerenius *et al*., 1988).

4.9. Antibody- or antigen-based detection methods

Not available.

5. Test(s) recommended for surveillance to demonstrate freedom in apparently healthy populations

The recommended method for surveillance is real-time PCR utilising the modified assay by Strand *et al*. (2023 ~~manuscript in preparation~~).

6. Corroborative diagnostic criteria

This section only addresses the diagnostic test results for detection of infection in the absence (Section 6.1.) or in the presence of clinical signs (Section 6.2.) but does not evaluate whether the infectious agent is the cause of the clinical event.

The case definitions for suspect and confirmed cases have been developed to support decision-making related to trade and confirmation of disease status at the country, zone or compartment level. Case definitions for disease confirmation in endemically affected areas may be less stringent. ~~It is recommended that all samples that yield suspect positive test results in an otherwise pathogen-free country or zone or compartment should be referred immediately to the WOAH Reference Laboratory for confirmation, whether or not clinical signs are associated with the case.~~ If a ~~laboratory~~ Competent Authority does not have the ~~capacity~~ capability to undertake the necessary diagnostic tests it should seek advice from the appropriate WOAH Reference Laboratory, and if necessary, refer samples to that laboratory for confirmatory testing of samples from the index case in a country, zone or compartment considered free.

6.1. Apparently healthy animals or animals of unknown health status [2]

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 animals or animal products or equipment, etc., from a known infected population equate to an epidemiological link. Alternatively, healthy populations are sampled in surveys to demonstrate disease freedom.

6.1.1. Definition of suspect case in apparently healthy animals

The presence of infection with *Aphanomyces astaci* shall be suspected if at least one of the following ~~criterion~~ criteria is met:

2 For example transboundary commodities.

i) Positive result by real-time PCR

ii) Positive result by conventional PCR

6.1.2. Definition of confirmed case in apparently healthy animals

The presence of infection with *Aphanomyces astaci* is considered to be confirmed if the following criterion is met:

i) Positive result by real-time PCR and positive result by conventional PCR followed by amplicon sequencing

6.2 Clinically affected animals

Clinical signs are not pathognomonic for a single disease; however, they may narrow the range of possible diagnoses.

6.2.1. Definition of suspect case in clinically affected animals

The presence of infection with *Aphanomyces astaci* shall be suspected if at least one of the following criteria is met:

i) Gross pathology or clinical signs associated with the disease as described in this chapter, with or without elevated mortality

ii) Visual observation of hyphae indicative of *A. astaci* in wet mounts

iii) Observation of hyphae indicative of *A. astaci* in stained histological sections

iv) Culture and isolation of the pathogen

v) Positive result by real-time PCR

vi) Positive result by conventional PCR

6.2.2. Definition of confirmed case in clinically affected animals

The presence of infection with *Aphanomyces astaci* is confirmed if the following criterion is met:

i) Positive result by real-time PCR and positive result by conventional PCR and amplicon sequencing

6.3. Diagnostic sensitivity and specificity for diagnostic tests

The diagnostic performance of tests recommended for surveillance or diagnosis of infection with *Aphanomyces astaci* are provided in Tables 6.3.1. and 6.3.2 (~~none~~ no data are currently available for either). ~~This information can be used for the design of surveys for infection with *Aphanomyces astaci*, however, it should be noted that diagnostic performance is specific to the circumstances of each diagnostic accuracy study (including the test purpose, source population, tissue sample types and host species) and diagnostic performance may vary under different conditions.~~ Data are only presented where tests are validated to at least level 2 of the validation pathway described in Chapter 1.1.2. and the information is available within published diagnostic accuracy studies.

6.3.1. For presumptive diagnosis of clinically affected animals

Test type	Test purpose	Source populations	Tissue or sample types	Species	DSe (*n*)	DSp (*n*)	Reference test	Citation
Real-time PCR	Distinguish between *A. astaci* and *A. finnicus*		Mycelium, tissue samples	*Astacus astacus*		Only detected *A. astacus*		Strand *et al.*, 2023

DSe = diagnostic sensitivity, DSp = diagnostic specificity, *n* = ~~samples~~ number of animals used in the validation study.

6.3.2. For surveillance of apparently healthy animals

Test type	Test purpose	Source populations	Tissue or sample types	Species	DSe (*n*)	DSp (*n*)	Reference test	Citation

Real-time PCR	Distinguish between *A. astaci* and *A. finnicus*		Tissue samples, environmental DNA	*Astacus astacus*		Only detected *A. astacus*		Strand *et al.*, 2023

DSe = diagnostic sensitivity, DSp = diagnostic specificity, *n* = ~~samples~~ number of animals used in the validation study.

7. References

ALDERMAN D.J. (1996). Geographical spread of bacterial and fungal diseases of crustaceans. *Rev sci. tech. Off. int. Epiz.,* **15**, 603–632.

ALDERMAN D.J. & POLGLASE J.L. (1985). Disinfection for crayfish plague. *Aquacult. Fish. Manage.,* **16**, 203–205.

ALDERMAN D.J. & POLGLASE J.L. (1986). *Aphanomyces astaci:* isolation and culture. *J. Fish Dis*., **9**, 367–379.

ALDERMAN D.J., POLGLASE J.L. & FRAYLING M. (1987). *Aphanomyces astaci* pathogenicity under laboratory and field conditions. *J. Fish Dis*., **10**, 385–393.

CASABELLA-HERRERO G., MARTÍNEZ-RÍOS M., VILJAMAA-DIRKS S., MARTÍN-TORRIJOS L. & DIÉGUEZ-URIBEONDO J. (2021). *Aphanomyces astaci* mtDNA: insights into the pathogen's differentiation and its genetic diversity from other closely related oomycetes. *Fungal Biol.,* **125**, 316–325. doi: 10.1016/j.funbio.2020.11.010. Epub 2020 Dec 2.

CERENIUS L. & SÖDERHÄLL K. (1984). Chemotaxis in *Aphanomyces astaci*, an arthropodparasitic fungus. *J. Invertabr. Pathol.,* **43**, 278–281.

CERENIUS L., SÖDERHÄLL K. & FULLER M.S. (1987). *Aphanomyces astaci* and *Aphanomyces* spp. *In:* Zoosporic fungi in teaching and research, Fuller M.S. & Jaworski A., eds. South-Eastern Publishing Corp., Athens, Georgia, USA. pp 64–65.

CERENIUS L., SÖDERHÄLL K., PERSSON M. & AJAXON R. (1988). The crayfish plague fungus, *Aphanomyces astaci* – diagnosis, isolation and pathobiology. *Freshwater Crayfish*, **7**, 131–144.

DI DOMENICO M., CURINI V., CAPRIOLI R., GIANSANTE C., MRUGAŁA A., MOJŽIŠOVÁ M., CAMMA C. & PETRUSEK A. (2021). Real-Time PCR assays for rapid Identification of common *Aphanomyces astaci* genotypes. *Front. Ecol. Evol.*, **9**, art 597585 doi:10.3389/fevo.2021.597585.

DIEGUEZ-URIBEONDO J., HUANG T.-S., CERENIUS L. & SÖDERHÄLL K. (1995). Physiological adaptation of an *Aphanomyces astaci* strain isolated from the freshwater crayfish *Procambarus clarkii. Mycol. Res*., **99**, 574–578.

GRANDJEAN F., VRÅLSTAD T., DIÉGUEZ-URIBEONDO J., JELIĆ M., MANGOMBI J., DELAUNAY C., FILIPOVÁ L., REZINCIUC S., KOZUBÍKOVÁ-BALCAROVA E., GYONNET D., VILJAMAA-DIRKS S. & PETRUSEK A. (2014). Microsatellite markers for direct genotyping of the crayfish plague pathogen *Aphanomyces astaci* (Oomycetes) from infected host tissues. *Vet. Microbiol*., **170**, 317–324.

HOLDICH D.M., REYNOLDS J.D., SOUTY-GROSSET C. & SIBLEY P.J. (2009). A review of the ever increasing threat to European crayfish from non-indigenous crayfish species. *Knowl. Manag. Aquat. Ec.*, **394–395**, 1–46.

HUANG T.S., CERENIUS L. & SÖDERHÄLL K. (1994). Analysis of genetic diversity in the crayfish plague fungus, *Aphanomyces astaci*, by random amplification of polymorphic DNA. *Aquaculture*, **126**, 1–10.

KOZUBÍKOVÁ E., VILJAMAA-DIRKS S., HEINIKAINEN S. & PETRUSEK A. (2011). Spiny-cheek crayfish *Orconectes limosus* carry a novel genotype of the crayfish plague agent *Aphanomyces astaci*. *J. Invertebr. Pathol.,* **108**, 214–216.

MAKKONEN J., JUSSILA J., PANTELEIT J., KELLER N.S., SCHRIMPF A., THEISSINGER K., KORTET R., MARTÍN-TORRIJOS L., SANDOVAL-SIERRA J.V., DIÉGUEZ-URIBEONDO J. & KOKKO H. (2018). MtDNA allows the sensitive detection and haplotyping of the crayfish plague disease agent *Aphanomyces astaci* showing clues about its origin and migration. *Parasitology,* **145**, 1210–1218. https://doi.org/10.1017/S0031182018000227.

MARTÍN-TORRIJOS L., CAMPOS LACH M., POU ROVIRA Q. & DIÉGUEZ-URIBEONDO J. (2017). Resistance to the crayfish plague, *Aphanomyces astaci* (Oomycota) in the endangered freshwater crayfish species, *Austropotamobius pallipes*. *PLoS ONE*, **12** (7), e0181226. https://doi.org/10.1371/journal. pone.0181226

MARTÍN-TORRIJOS L., MARTÍNEZ-RÍOS M., CASABELLA-HERRERO G., ADAMS S.B., JACKSON C.R. & DIÉGUEZ-URIBEONDO J. (2021). Tracing the origin of the crayfish plague pathogen, *Aphanomyces astaci,* to the Southeastern United States. *Sci. Rep.,* **11**, 9332.doi: 10.1038/s41598-021-88704-8.

MINARDI D., STUDHOLME D.J., OIDTMANN B., PRETTO T. & VAN DER GIEZEN M. (2019). Improved method for genotyping the causative agent of crayfish plague (*Aphanomyces astaci*) based on mitochondrial DNA. *Parasitology*, 146, 1022–1029, doi:10.1017/S0031182019000283

MINARDI D., STUDHOLME D.J., VAN DER GIEZEN M., PRETTO T. & OIDTMANN B. (2018). New genotyping method for the causative agent of crayfish plague (*Aphanomyces astaci*) based on whole genome data. *J. Invertebr. Pathol.*, **156**, 6–13.

OIDTMANN B., GEIGER S., STEINBAUER P., CULAS A. & HOFFMANN R.W. (2006). Detection of *Aphanomyces astaci* in North American crayfish by polymerase chain reaction. *Dis. Aquat. Org.,* **72**, 53–64.

OIDTMANN B., HEITZ E., ROGERS D. & HOFFMANN R.W. (2002). Transmission of crayfish plague. *Dis. Aquat. Org.,* **52**, 159–167.

PAVIC D., ČANKOVIĆ M., PETRIĆ I., MAKKONEN J., HUDINA S., MAGUIRE I., VLADUŠIĆA T., ŠVER L., HRAŠĆANA R., ORLIĆ K., DRAGIČEVIĆ P. & BIELEN A. (2020) Non-destructive method for detecting *Aphanomyces astaci*, the causative agent of crayfish plague, on the individual level. *J. Invertebr. Pathol.*, **169**, 107274. https://doi.org/10.1016/j.jip.2019.107274

RENNERFELT E. (1936). Untersuchungen uber die Entwicklung und Biologie des Krebspestpilzes Aphanomyces astaci Schikora. Report of the Institute of Freshwater Research (Drottningholm, Sweden), **10**, 1–21.

SCHRIMPF A., SCHMIDT T. & SCHULZ R. (2014). Invasive Chinese mitten crab (*Eriocheir sinensis*) transmits crayfish plague pathogen (*Aphanomyces astaci*). *Aquatic Invasions*, **9**, 203–209. 10.3391/ai.2014.9.2.09.

SOUTY-GROSSET C., HOLDICH D.M., NOEL P.Y., REYNOLDS J.D. & HAFFNER P. (eds) (2006). Atlas of Crayfish in Europe. Muséum National d'Histoire Naturelle, Paris (Patrimoines naturels, 64), 188 p.

~~STRAND D.A. (2013) Environmental DNA monitoring of the alien crayfish plague pathogen *Aphanomyces astaci* in freshwater systems – Sporulation dynamics, alternative hosts and improved management tools. *Dissertation book, University of Oslo Faculty of Mathematics and Natural Sciences Department of Biosciences, Oslo*, ISSN 1501 7710, 73 p.~~

STRAND D.A., HOLST-JENSEN A., VILJUGREIN H., EDVARDSEN B., KLAVENESS D., JUSSILA J. & VRÅLSTAD T. (2011). Detection and quantification of the crayfish plague agent in natural waters: direct monitoring approach for aquatic environments. *Dis. Aquat. Org*., **95**, 9–17.

STRAND D.A., JINNEROT T., ASPÁN A., VILJAMAA-DIRKS S., HEINIKAINEN S., ROLÉN E. & VRÅLSTAD T. (2023). Molecular detection of *Aphanomyces astaci* – An improved species specific qPCR assay. *J. Invertebr. Pathol.,* **201**, 108008. doi: 10.1016/j.jip.2023.108008. Epub 2023 Oct 18. PMID: 37863282.

STRAND D.A., JUSSILA J., VILJAMAA-DIRKS S., KOKKO H., MAKKONEN J., HOLST-JENSEN A., VILJUGREIN H. & VRÅLSTAD T. (2012). Monitoring the spore dynamics of *Aphanomyces astaci* in the ambient water of latent carrier crayfish. *Vet. Microbiol.,* **160**, 99–107.

SVOBODA J., MRUGAŁA A., KOZUBÍKOVÁ-BALCAROVÁ E. & PETRUSEK A. (2017). Hosts and transmission of the crayfish plague pathogen *Aphanomyces astaci*: a review. *J. Fish Dis*., **40,**127–140. https://doi.org/10.1111/jfd.12472

SODERHALL K. & CERENIUS L. (1999) The crayfish plague fungus: history and recent advances. *Freshwater Crayfish*, **12**, 11-34.

THOMAS J.R., ROBINSON C.V., MRUGAŁA A., ELLISON A.R., MATTHEWS E., GRIFFITHS S.W., CONSUEGRA S. & CABLE J. (2020). Crayfish plague affects juvenile survival and adult behaviour of invasive signal crayfish. *Parasitology*, **1–9**, https://doi.org/10.1017/S0031182020000165

TUFFS S & OIDTMANN B (2011). A comparative study of molecular diagnostic methods designed to detect the crayfish plague pathogen, *Aphanomyces astaci. Vet. Microbiol.,* **153**, 345–353.

UNESTAM T. (1966). Studies on the crayfish plague fungus *Aphanomyces astaci*. II. Factors affecting zoospores and zoospore production. *Physiol. Plant.*, **19**, 1110–1119.

~~UNESTAM T. & SODERHALL K. (1977). Specialisation in crayfish defence and fungal aggressiveness upon crayfish plague infection. *Freshwater Crayfish*, **3**, 321–331.~~

VILJAMAA-DIRKS S. (2006). Improved detection of crayfish plague with a modified isolation method. *Freshwater Crayfish*, **15**, 376–382.

VILJAMAA-DIRKS S. & HEINIKAINEN S. (2019) A tentative new species *Aphanomyces fennicus* sp. nov. interferes with molecular diagnostic methods for crayfish plague. *J. Fish Dis*., **42**, 413–422. https://doi.org/10.1111/jfd.12955

VILJAMAA-DIRKS S., HEINIKAINEN S., NIEMINEN M., VENNERSTRÖM P. & PELKONEN S. (2011). Persistent infection by crayfish plague *Aphanomyces astaci* in a noble crayfish population – a case report. *Bull. Eur. Assoc. Fish Pathol*., **31**, 182–188.

VILJAMAA-DIRKS S., HEINIKAINEN S., TORSSONEN H., PURSIAINEN M., MATTILA J. & PELKONEN S. (2013). Distribution and epidemiology of genotypes of the crayfish plague *Aphanomyces astaci* from noble crayfish *Astacus astacus* in Finland. *Dis. Aquat. Org*., **103**, 199–208.

VRALSTAD T., JOHNSEN S.I., FRISTAD R., EDSMAN L. & STRAND D.A. (2011). Potent infection reservoir of crayfish plague now permanently established in Norway. *Dis. Aquat. Org.,* **97**, 75–83.

VRALSTAD T., KNUTSEN A.K., TENGS T. & HOLST-JENSEN A. (2009). A quantitative TaqMan® MGB real-time polymerase chain reaction based assay for detection of the causative agent of crayfish plague *Aphanomyces astaci*. *Vet. Microbiol.,* **137**, 146–155.

~~WHITE T.J., BRUNS T., LEE S. & TAYLOR J. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *In:* PCR Protocols: A Guide to Methods and Applications, Innis M.A., Gelfand D.H., Sninsky J.J., White T.J., eds. Academic Press, San Diego, California, USA, pp. 315–322.~~

*
* *

NB: There is a WOAH Reference Laboratory for infection with *Aphanomyces astaci* (crayfish plague)
(please consult the WOAH web site for the most up-to-date list:
https://www.woah.org/en/what-we-offer/expertise-network/reference-laboratories/#ui-id-3).
Please contact the WOAH Reference Laboratories for any further information on
infection with *Aphanomyces astaci* (crayfish plague)

NB: FIRST ADOPTED IN 1995; MOST RECENT UPDATES ADOPTED IN 2017.

CHAPTER 2.2.6.

INFECTION WITH MACROBRACHIUM ROSENBERGII NODAVIRUS (WHITE TAIL DISEASE)

EU position

The EU supports the adoption of this revised chapter.

1. Scope

Infection with Macrobrachium rosenbergii nodavirus means infection with the pathogenic agent Macrobrachium rosenbergii nodavirus (MrNV) in the Family *Nodaviridae*. The disease is commonly known as white tail disease (WTD).

Extra small virus (XSV) is associated with disease but its role has not been determined.

2. Disease information

2.1. Agent factors

2.1.1. Aetiological agent

Two viruses are associated with WTD, namely MrNV (primary) and extra small virus (XSV) (associate) (Qian *et al.,* 2003; Romestand & Bonami, 2003). MrNV is a necessary cause of WTD in prawns, however, the role of XSV in pathogenicity remains unclear.

MrNV belongs in the family *Nodaviridae* (Bonami *et al.,* 2005). While the physico-chemical properties of MrNV are consistent with those of other members of the *Nodaviridae*, it differs structurally and genetically from other nodaviruses within the two recognised genera, *Alphanodavirus* and *Betanodavirus* (Ho *et al*., 2017, 2018; Naveenkumar *et al*., 2013). Consequently, a third genus, *Gammanodavirus*, has been proposed for nodaviruses that infect crustaceans, including MrNV and *Penaeus vannamei* nodavirus (*Pv*NV) (Naveenkumar *et al*., 2013).

XSV is the first sequenced satellite virus in aquatic animals and it is also the first record of a satellite-nodavirus association (Bonami *et al.,* 2005). XSV has been classified by the ICTV as *Macrobrachium satellite virus 1* of the family *Sarthroviridae.*

2.1.2. Survival and stability in processed or stored samples

Both viral pathogens (MrNV and XSV) are stable in processed or stored samples stored at –20 or –80°C. Storing the samples at –80°C is recommended for long-time storage and maintenance of pathogen virulence (Sahul Hameed & Bonami, 2012). The infected samples should be processed at low temperature to maintain the stability of the viruses. Viral inoculum prepared from infected prawn stored at –20°C caused 100% mortality in postlarvae (PL) of *M. rosenbergii* by immersion challenge (Qian *et al*., 2003; Sahul Hameed *et al*., 2004a). Ravi & Sahul Hameed (2016) found that MrNV in tissue suspensions was inactivated after exposure to 50°C for at least 5 min.

2.1.3. Survival and stability outside the host

Survival outside the host is not known.

2.2. Host factors

2.2.1. Susceptible host species

Species that fulfil the criteria for listing as susceptible to infection with MrNV according to Chapter 1.5. of the *Aquatic Animal Health Code* (*Aquatic Code*) are: giant river prawn (*Macrobrachium rosenbergii*).

2.2.2. Species with incomplete evidence for susceptibility

Species for which there is incomplete evidence to fulfil the criteria for listing as susceptible to infection with MrNV according to Chapter 1.5. of the *Aquatic Code* are: white leg shrimp (*Penaeus vannamei*).

In addition, pathogen-specific positive polymerase chain reaction (PCR) results (but not active infection) have been reported in the following species:

Family	*Scientific name*	*Common name*
~~*Aeshnidae*~~	~~*Aeshna* sp.~~	~~dragonfly~~
Artemiidae	*Artemia* sp.	brine shrimps
~~*Belostomatidae*~~	~~*Belostoma* sp.~~	~~giant water bug~~
~~*Dytiscidae*~~	~~*Cybister* sp.~~	~~beetle~~
~~*Notonectidae*~~	~~*Notonecta* sp.~~	~~backswimmer~~
Palaemonidae	*Macrobrachium rude*	hairy river prawn
	Macrobrachium malcolmsonii	monsoon river prawn
Parastacidae	*Cherax quadricarinatus*	red claw crayfish
Penaeidae	*Penaeus japonicus*	kuruma prawn
	Penaeus indicus	Indian white prawn
	Penaeus monodon	giant tiger prawn

2.2.3. Likelihood of infection by species, host life stage, population or sub-populations

Experimental pathogenicity studies revealed that larvae, PL and early juveniles of *M. rosenbergii* are susceptible to MrNV/XSV, whereas adults are resistant (Gangnonngiwa *et al.,* 2020; Qian *et al*., 2003; Sahul Hameed *et al*., 2004a).

No mortality was observed either in naturally or experimentally ~~(~~MrNV/XSV~~)~~ infected subadult and adult prawns. Experimental studies confirmed vertical transmission from infected broodstock to PL (Sudhakaran *et al*., 2007a).

2.2.4. Distribution of the pathogen in the host

MrNV and XSV have been demonstrated in gill tissue, head muscle, heart, abdominal muscle, ovaries, pleopods and tail muscle, but not the hepatopancreas or eyestalk (Sahul Hameed *et al*., 2004a; Sri Widada *et al*., 2003).

2.2.5. Aquatic animal reservoirs of infection

~~One study has~~ Studies have indicated ~~the possibility~~ that marine shrimp may act as ~~a~~ reservoirs for MrNV and XSV and that these viruses maintain virulence in the shrimp tissue system (Senapin *et al.,* 2012; Sudhakaran *et al*., 2006).

2.2.6. Vectors

Aquatic insects such as dragonfly (*Aeshna* sp.), giant water bug (*Belostoma* sp.), beetle (*Cybister* sp.) and backswimmer (*Notonecta* sp.) may act as mechanical carriers for MrNV/XSV and are a potential transmission risk to cultivated *Macrobrachium rosenbergii* (Sudhakaran *et al.,* 2008). It is recommended to remove these insects from freshwater prawn culture systems, especially at larval-rearing centres. Sudhakaran *et al*. (2008) demonstrated RT-PCR positives from insects, and infected C6/36 insect cell line with tissue homogenates from the insects. Viral replication was confirmed through EM and RT-PCR, but transmission from insects to naïve shrimp was not demonstrated.

2.3. Disease pattern

2.3.1. Mortality, morbidity and prevalence

Larvae, PL and juveniles of *M. rosenbergii* are susceptible to infection with MrNV, which often causes high mortalities in these life stages. Mortality may reach a maximum in about 5 or 6 days after the appearance of the first clinical signs. Very few PL with infection with MrNV survive beyond 15 days in an outbreak, but PL that survive may grow to market size. Adults are resistant to infection with MrNV, but act as carriers (Qian *et al*., 2003; Sahul Hameed *et al*., 2004a). Prevalence is variable from 10% to 100% in hatchery, nursery and grow-out systems (Arcier *et al*., 1999; Qian *et al*., 2003; Sahul Hameed *et al*., 2004a; 2004b).

2.3.2. Clinical signs, including behavioural changes

Infected PL become opaque and develop a whitish appearance, particularly in the abdominal region. The whitish discoloration appears first in the second or third abdominal segment and gradually diffuses both anteriorly and posteriorly. In severe cases, degeneration of telson and uropods may occur. Floating exuviae (moults) in the tanks appear abnormal and resemble 'mica flakes' (Arcier *et al*., 1999). The infected PL show progressive weakening of their feeding and swimming ability (Sahul Hameed *et al*., 2004a).

2.3.3. Gross pathology

Infection with MrNV is indicated by the whitish coloration of abdominal muscle.

2.3.4. Modes of transmission and life cycle

Transmission is vertical by trans-ovum and horizontal by the waterborne route (Qian *et al*., 2003; Sahul Hameed *et al*., 2004a; Sudhakaran *et al*., 2007a).

2.3.5. Environmental factors

Not available.

2.3.6. Geographical distribution

The disease was first reported in the ~~French West Indies~~ Caribbean (Arcier *et al*., 1999), and later in Asia-Pacific (Murwantoko *et al*., 2016; Owens *et al*., 2009; Qian *et al*., 2003; Saedi *et al*., 2012; Sahul Hameed *et al*., 2004b; Wang *et al*., 2008; Yoganandhan *et al*., 2006).

See WOAH-WAHIS (https://wahis.woah.org/#/home) for recent information on distribution at the country level.

2.4. Biosecurity and disease control strategies

Preventive measures, such as screening of broodstock and PL, and good management practices may help to prevent infection with MrNV in culture systems. As the life cycle of *M. rosenbergii* is completed under controlled conditions, specific pathogen-free (SPF) broodstock and PL can be produced (Romestand & Bonami, 2003; Sri Widada *et al.,* 2003; Yoganandhan *et al.,* 2005).

2.4.1. Vaccination

Not available

2.4.2. Chemotherapy including blocking agents

No known chemotherapeutic agents are reported to treat MrNV-infected prawn.

2.4.3. Immunostimulation

The immunomodulatory effect of recombinant capsid protein and recombinant RNA-dependent RNA polymerase (RdRp) protein of MrNV has been studied and the protection of viral challenged post-larvae from MrNV infection has been demonstrated (Farook *et al*., 2014; NaveenKumar *et al*., 2021).

2.4.4. Breeding resistant strains

None reported.

2.4.5. Inactivation methods

A viral suspension treated with heat at 65°C for 2 hours destroyed infectivity of MrNV and XSV in challenge experiments (Qian *et al*., 2003). The viral inoculum exposed to UV irradiation for a period of 5 minutes and more was totally inactivated and failed to cause mortality in prawn PL ~~of prawn~~ (Ravi & Sahul Hameed, 2016).

2.4.6. Disinfection of eggs and larvae

Routine disinfection procedures followed for crustacean viral disease control are suggested.

2.4.7. General husbandry

MrNV is transmitted both horizontally and vertically in culture systems (Qian *et al*., 2003; Sahul Hameed *et al*., 2004a; Sudhakaran *et al*., 2007a). Good husbandry practices, such as proper disinfection of tanks and water may help to prevent infection. It is recommended to remove insects from freshwater prawn culture systems, especially at larval-rearing centres. Specific pathogen-free (SPF) broodstock and PL can be obtained from disease free populations or by RT-PCR screening and selection of negative broodstock (Romestand & Bonami, 2003; Sri Widada *et al.,* 2003; Yoganandhan *et al.,* 2005).

3. Specimen selection, sample collection, transportation and handling

3.1. Selection of populations and individual specimens

PLs are most suitable for detection of MrNV. PL showing clinical signs of disease can be sampled preferentially. Adults and juveniles can be sampled for MrNV however prevalence in these lifestages may be lower (see Section 2.3.1).

3.2. Selection of organs or tissues

The tissues most affected in moribund PLs/early juveniles are striated muscles of the abdomen, cephalothorax and tail. The whole PL body is preferred for detection of MrNV (Sahul Hameed *et al.,* 2004b; Sri Widada *et al.,* 2003; Yoganandhan *et al*., 2005). All organs of adult *M. rosenbergii* except eyestalks and the hepatopancreas, are best for screening the viruses by RT-PCR.

3.3. Samples or tissues not suitable for pathogen detection

Eyestalks and the hepatopancreas of adult prawns are not suitable (Sri Widada *et al.,* 2003; Sahul Hameed *et al.,* 2004a).

3.4. Non-lethal sampling

Pleopods (swimming legs) are a convenient source of RNA for non-destructive screening of MrNV in adult prawn (Sahul Hameed *et al.,* 2004a).

If non-lethal tissue sample types differ from recommended tissues (see Section 3.2.), or from the tissue samples used in validation studies, the effect on diagnostic performance should be considered.

3.5. Preservation of samples for submission

Infected larvae or PL with prominent signs of whitish muscle in the abdominal region are collected from disease outbreak areas. Samples are washed in sterile saline, transferred to sterile tubes, and transported to the laboratory. For general guidance on sample preservation methods for the intended test methods, see Chapter 2.2.0 *General information* (diseases of crustaceans).

3.5.1. Samples for pathogen isolation

Moribund or frozen PL samples can be used for isolation of viral pathogens using cell lines (C6/36 mosquito cell line (Sudhakaran et al., 2007b).

3.5.2. Preservation of samples for molecular detection

Infected samples stored at –80°C or samples preserved in 80% (v/v) analytical/reagent-grade (undenatured) ethanol should be used for RT-PCR for detection of MrNV (Sri Widada *et al.,* 2003; Sahul Hameed *et al.,* 2004b; Yoganandhan *et al*., 2005).

Standard sample collection, preservation and processing methods for molecular techniques can be found in Section B.5.5. of Chapter 2.2.0 *General information* (diseases of crustaceans).

3.5.3. Samples for histopathology, immunohistochemistry or *in-situ* hybridisation

Tissue samples for histopathology, immunohistochemistry or *in-situ* hybridisation should be fixed immediately after collection in neutral-buffered formalin or modified Davidson's fixative (Sri Widada *et al.,* 2003). The recommended ratio of fixative to tissue is 10:1. Standard sample collection, preservation and processing methods for histological techniques can be found in Section 5.3. of Chapter 2.2.0 *General information* (diseases of crustaceans).

3.5.4. Samples for other tests

Not applicable

3.6. Pooling of samples

Pooling of samples from more than one individual animal for a given purpose ~~should only be~~ is only recommended where robust supporting data on diagnostic sensitivity and diagnostic specificity have been evaluated and found to be suitable. If the effect of pooling on diagnostic sensitivity has not been thoroughly evaluated, larger animals should be processed and tested individually. Small life stages such as PL, can be pooled to obtain the minimum amount of material for virus isolation or molecular detection.

4. Diagnostic methods

The methods currently available for identifying infection that can be used in i) surveillance of apparently healthy animals, ii) presumptive diagnosis in clinically affected animals and iii) confirmatory diagnostic purposes are listed in Table 4.1. by life stage.

Ratings against purposes of use. For each recommended assay a qualitative rating against the purpose of use is provided. The ratings are determined based on multiple performance and operational factors relevant to application of an assay for a defined purpose. These factors include appropriate diagnostic performance characteristics, level of assay validation, successful application by diagnostic laboratories, cost, timeliness, and sample throughput. For a specific purpose of use, assays are rated as:

+++ =	Most suitable methods – desirable performance and operational characteristics;
++ =	Suitable method(s) acceptable performance and operational characteristics under most circumstances;
+ =	Less suitable methods – performance or operational characteristics may significantly limit application;
Shaded boxes =	Not appropriate for this purpose.

Level of validation. The validation stage corresponds to the assay development and validation pathway in chapter 1.1.2. The validation stage is specific to each purpose of use. Where available, information on the diagnostic performance of recommended assays is provided in Section 6.3.

WOAH Reference Laboratories welcome feedback on diagnostic performance of recommended assays, in particular PCR methods. Of particular interest are any factors affecting expected assay sensitivity (e.g. tissue components inhibiting amplification) or expected specificity (e.g. failure to detect particular genotypes). These issues should be communicated to the WOAH Reference Laboratories so that advice can be provided to diagnostic laboratories and the standards amended if necessary.

Table 4.1. *WOAH recommended diagnostic methods for MrNV and their level of validation for surveillance of apparently healthy animals and investigation of clinically affected animals*

Method	A. Surveillance of apparently healthy animals				B. Presumptive diagnosis of clinically affected animals				C. Confirmatory diagnosis[1] of a suspect result from surveillance or presumptive diagnosis			
	Early life stages[2]	Juveniles[2]	Adults	LV	Early life stages[2]	Juveniles[2]	Adults	LV	Early life stages[2]	Juveniles[2]	Adults	LV
Wet mounts												
Histopathology						++	++	NA				
Cell culture												
Real-time RT-PCR	+++	+++	+++	1	+++	+++	+++	1	+++	+++	+++	1
Conventional RT-PCR	++	++	++	1	+++	+++	+++	2				
Conventional RT-PCR followed by amplicon sequencing									+++	+++	+++	2
In-situ hybridisation						++	++	1		++	++	1
Bioassay												
LAMP	++	++	++	1	++	++	++	1				
Ab-ELISA												
Ag-ELISA					++	++	++	1				
Lateral flow assay					++	++	++	2				
Other methods[3]												

LV = level of validation, refers to the stage of validation in the WOAH Pathway (chapter 1.1.2); NA = not available; PCR = polymerase chain reaction; LAMP = loop-mediated isothermal amplification; Ab- or Ag-ELISA = antibody or antigen enzyme-linked immunosorbent assay, respectively; IFAT = indirect fluorescent antibody test.

[1]For confirmatory diagnoses, methods need to be carried out in combination (see Section 6). [2]Susceptibility of early and juvenile life stages is described in Section 2.2.3.

[3]Specify the test used. Shading indicates the test is inappropriate or should not be used for this purpose.

4.1. Wet mounts

None to date

4.2. Histopathology and cytopathology

The most affected tissue in infected PL is striated muscle of the cephalothorax, abdomen and tail. Histological features include the presence of acute Zenker's necrosis of striated muscles, characterised by severe hyaline degeneration, necrosis and muscular lysis. Moderate oedema and abnormal open spaces among the affected muscle cells are also observed, as is the presence of large oval or irregular basophilic cytoplasmic inclusion bodies in infected muscles (Arcier *et al.,* 1999; Hsieh *et al.,* 2006).

4.3. Cell culture for isolation

MrNV has been isolated in insect cell lines, but this is not a recommended method (Hernandez-Herrera *et al.,* 2007; Sudhakaran *et al.,* 2007b).

4.4. Nucleic acid amplification

PCR methods for MrNV and XSV are included in this section for completeness. However, the case definitions in Section 6 are based on detection methods for MrNV only.

PCR assays should always be run with the controls specified in Section 5.5. *Use of molecular techniques for confirmatory testing and diagnosis* of Chapter 2.2.0 *General information* (diseases of crustaceans). Each sample should be tested in duplicate.

Extraction of nucleic acids

Different kits and procedures can be used for nucleic acid extraction. The quality and concentration of the extracted nucleic acid is important and can be checked using a suitable method as appropriate to the circumstances.

4.4.1. Real-time RT-PCR

Real-time RT-PCR assay can be performed using the SYBR Green dye based on the method described by Hernandez-Herrera *et al*. (2007) or the TaqMan assay described by Zhang *et al*. (2006).

Pathogen / target gene	Primer/probe (5'–3')	Concentration	Cycling parameters[(a)]
Method 1: Hernandez-Herrera *et al.* (2007); GenBank Accession No.: AY222839)			
MrNv/RNA1	Fwd: AGG-ATC-CAC-TAA-GAA-CGT-GG Rev: CAC-GGT-CAC-AAT-CCT-TGC-G	500 nM 500 nM	40 cycles of: 95°C/15 sec, 60°C/5 sec and 72°C/10 sec
Method 2: Zhang *et al.* (2006); GenBank Accession No.: AY231436)			
MrNv/RNA1	Fwd: CAA-CTC-GGT-ATG-GAA-CTC-AAG-GT Rev: AGG-AAA-TAC-ACG-AGC-AAG-AAA-AGT-C Probe: FAM-ACC-CTT-CGA-CCC-CAG-CAA-TGG-TG-TAMARA	1000 nM 1000 nM 400 nM	50 cycles of: 94°C/30 sec and 58°C/30 sec
Method 3: Zhang *et al.* (2006); GenBank Accession No.: DQ174318)			
XSV	Fwd: AGC-CAC-ACT-CTC-GCA-TCT-GA Rev: CTC-CAG-CAA-AGT-GCG-ATA-CG Probe: FAM-CAT-GCC-CCA-TGA-TCC-TCG-CA-TAMARA	1000 nM 1000 nM 400 nM	50 cycles of: 94°C/30 sec and 58°C/30 sec

[(a)] A denaturation step prior to cycling has not been included.

4.4.2. Conventional RT-PCR

The protocol for the conventional RT-PCR for detection of MrNV/XSV developed by ~~Sri Widada *et al.* (2003),~~ Sahul Hameed *et al.* (2004a; 2004b) and Sudhakaran *et al*. (2007a) is recommended. MrNV and XSV can be detected by conventional RT-PCR separately using a specific set of primers or these two viruses can be detected simultaneously using a single-tube one-step multiplex RT-PCR (Yoganandhan *et al*., 2005). ~~Conventional real-time RT-PCR is recommended in situations where high sensitivity is required.~~

Pathogen / target gene	Primer (5'–3')	Concentration	Cycling parameters[a]
Method 1: One step RT-PCR (~~Sri Widada *et al*., 2003;~~ Sahul Hameed *et al*., 2004a, b; Sudhakaran *et al*., 2007a) GenBank Accession No.: AY222840 (MrNV) and AY247793 (XSV); amplicon size: 425 bp (MrNv) and 546 bp (XSV)			
MrNv	Fwd: GCG-TTA-TAG-ATG-GCA-CAA-GG Rev: AGC-TGT-GAA-ACT-TCC-ACT-GG	~~0.02 nM~~ 400 nM ~~0.02 nM~~ 400 nM	30 cycles of: 94°C/40 sec, 55°C/40sec and 68°C/60 sec
XSV	Fwd: CGC-GGA-TCC-GAT-GAA-TAA-GCG-CAT-TAA-TAA Rev: CCG-GAA-TTC-CGT-TAC-TGT-TCG-GAG-TCC-CAA	~~0.02 nM~~ 400 nM ~~0.02 nM~~ 400 nM	30 cycles of: 94°C/40 sec, 55°C/40 sec and 68°C/60 sec
Method 2: nested RT-PCR using above-mentioned primers as external primers (Sudhakaran *et al*., 2007a); amplicon size: 205 bp (MrNv) and 236 bp (XSV)			
MrNv	External primers: as for Method 1. Internal primers: Fwd: GAT-GAC-CCC-AAC-GTT-ATC-CT Rev: GTG-TAG-TCA-CTT-GCA-AGA-GG	~~0.02 nM~~ 1000 nM ~~0.02 nM~~ 1000 nM	30 cycles of: 94°C/60 sec, 55°C/60 sec and 72°C/60 sec
XSV	External primers: as for Method 1. Internal primers: Fwd: ACA-TTG-GCG-GTT-GGG-TCA-TA Rev: GTG-CCT-GTT-GCT-GAA-ATA-CC-3	~~0.02 nM~~ 1000 nM ~~0.02 nM~~ 1000 nM	30 cycles of: 94°C/60 sec, 55°C/60 sec and 72°C/60 sec
Method 3: Multiplex RT-PCR (Yoganandhan *et al*., 2005); GenBank Accession No.: AY222840 (MrNV) and AY247793 (XSV); amplicon size: 681 bp (MrNv) and 500 bp (XSV)			
MrNV	Fwd: GAT-ACA-GAT-CCA-CTA-GAT-GAC-C Rev: GAC-GAT-AGC-TCT-GAT-AAT-CC	~~0.02 nM~~ 400 nM ~~0.02 nM~~ 400 nM	30 cycles of: 94°C/40 sec, 55°C/40 sec and 68°C/60 sec
XSV	Fwd: GGA-GAA-CCA-TGA-GAT-CAC-G Rev: CTG-CTC-ATT-ACT-GTT-CGG-AGT-C	~~0.02 nM~~ 400 nM ~~0.02 nM~~ 400 nM	30 cycles of: 94°C/40 sec, 55°C/40 sec and 68°C/60 sec

[a] A denaturation step prior to cycling has not been included.

4.4.3. Other nucleic acid amplification methods: Loop-mediated isothermal amplification (LAMP)

Haridas *et al*. (2010) have applied loop-mediated isothermal amplification (LAMP) for rapid detection of MrNV and XSV in the freshwater prawn. A set of four primers, two outer primers and two inner primers, have been designed separately for detection of MrNV and XSV.

4.5. Amplicon sequencing

The size of the PCR amplicon should be verified, for example by agarose gel electrophoresis. Both DNA strands of the PCR product must be sequenced and analysed in comparison with reference sequences.

4.6. *In-situ* hybridisation

The presence of MrNV in infected cells can be demonstrated in histological sections using a DIG-labelled DNA *in-situ* hybridisation probe specific for MrNV (Sri Widada *et al.,* 2003).

4.7. Immunohistochemistry

None developed.

4.8. Bioassay

Not used for diagnostic purposes.

4.9. Antibody- or antigen-based detection methods

4.9.1. ELISA

Antibody-based diagnostic methods for MrNV include the ELISA described by Romestand & Bonami (2003) or the triple-antibody sandwich (TAS) ELISA based on a monoclonal antibody (Qian *et al.,* 2006).

4.9.2. Lateral flow assay (LFA)

An antibody-based lateral flow assay (LFA) has been developed for the early detection of MrNV in the PL stage (Jamalpure *et al*., 2021).

4.10. Other methods

None

5. Test(s) recommended for surveillance to demonstrate freedom in apparently healthy populations

Real-time RT-PCR is recommended for targeted surveillance to declare freedom from infection with MrNV.

6. Corroborative diagnostic criteria

This section only addresses the diagnostic test results for detection of infection in the absence (Section 6.1.) or in the presence of clinical signs (Section 6.2.) but does not evaluate whether the infectious agent is the cause of the clinical event.

The case definitions for suspect and confirmed cases have been developed to support decision-making related to trade and confirmation of disease status at the country, zone or compartment level. Case definitions for disease confirmation in endemically affected areas may be less stringent. If a Competent Authority does not have the capability to undertake the necessary diagnostic tests it should seek advice from the appropriate WOAH Reference Laboratory, and if necessary, refer samples to that laboratory for confirmatory testing of samples from the index case in a country, zone or compartment considered free.

6.1. Apparently healthy animals or animals of unknown health status[1]

Apparently healthy populations may fall under suspicion, and therefore be sampled, if there is an epidemiological link(s) to an infected population. Hydrographical proximity to, or movement of animals or animal products or equipment, etc., from a known infected population equate to an epidemiological link. Alternatively, healthy populations are sampled in surveys to demonstrate disease freedom.

6.1.1. Definition of suspect case in apparently healthy animals

1 For example transboundary commodities.

The presence of infection with MrNV shall be suspected if at least one of the following criteria is met:

i) Positive result by real-time RT-PCR

ii) Positive result by conventional RT-PCR

iii) Positive result by LAMP

6.1.2. Definition of confirmed case in apparently healthy animals

The presence of infection with MrNV is considered to be confirmed if the following criterion is met:

i) Positive result by real-time RT-PCR result and positive result by conventional RT-PCR and sequence analysis

6.2 Clinically affected animals

6.2.1. Definition of suspect case in clinically affected animals

The presence of infection with MrNV shall be suspected if at least one of the following criteria is met:

i) Clinical signs consistent with infection by MrNV

ii) Histopathology consistent with infection by MrNV

iii) Positive result by real-time RT-PCR

iv) Positive result by conventional RT-PCR

v) Positive result by *in situ* hybridisation

vi) Positive result by LAMP

vii) Positive result by Ag ELISA

viii) Positive result by lateral flow assay

6.2.2. Definition of confirmed case in clinically affected animals

The presence of infection with MrNV is considered to be confirmed if at least one of the following ~~criterion~~ criteria is met:

i) Positive result by real time RT-PCR and positive result by conventional RT-PCR with sequence analysis

ii) Positive result by ISH followed by positive result by conventional RT-PCR with sequence analysis

iii) Positive result by ISH followed by positive result by real-time RT-PCR

6.3. Diagnostic sensitivity and specificity for diagnostic tests

The diagnostic performance of tests recommended for surveillance or diagnosis of infection with MrNV are provided in Tables 6.3.1. and 6.3.2 (no data are currently available). This information can be used for the design of surveys for infection with MrNV, however, it should be noted that diagnostic performance is specific to the circumstances of each diagnostic accuracy study (including the test purpose, source population, tissue sample types and host species) and diagnostic performance may vary under different conditions. Data are only presented where tests are validated to at least level 2 of the validation pathway described in Chapter 1.1.2. and the information is available within published diagnostic accuracy studies.

6.3.1. For presumptive diagnosis of clinically affected animals

Test type	Test purpose	Source populations	Tissue or sample types	Species	DSe (*n*)	DSp (*n*)	Reference test	Citation
RT-PCR	Diagnosis	Clinically affected PL from hatchery and nursery	Whole post-larvae	*Macrobrachium rosenbergii*	100 (*n*=20)	100 (*n*=20)	Western blot or ELISA	~~Sri Widada *et al.* (2003);~~ Sahul Hameed *et al*. (2011)
Lateral flow	Surveillance	PL from prawn hatcheries	Whole post-larvae	*Macrobrachium rosenbergii*	100 (*n*=80)	90 (*n*=80)	RT-PCR	Jamalpure *et al*. (2021)

immune-assay								

DSe = diagnostic sensitivity, DSp = diagnostic specificity, *n* = ~~samples~~ number of animals used in the validation study,
RT-PCR: = reverse transcription polymerase chain reaction.

6.3.2. For surveillance of apparently healthy animals

Test type	Test purpose	Source populations	Tissue or sample types	Species	DSe (*n*)	DSp (*n*)	Reference test	Citation

DSe = diagnostic sensitivity, DSp = diagnostic specificity, *n* = *n* = ~~samples~~ number of animals used in the validation study,
RT-PCR: = reverse transcription polymerase chain reaction.

7. References

ARCIER J.-M., HERMAN F., LIGHTNER D.V., REDMAN R.M., MARI. J. & BONAMI J.-R. (1999). A viral disease associated with mortalities in hatchery-reared postlarvae of the giant freshwater prawn *Macrobrachium rosenbergii*. *Dis. Aquat. Org.,* **38**, 177–181.

BONAMI J.R., SHI Z., QIAN D. & SRI WIDADA J. (2005). White tail disease of the giant freshwater prawn, *Macrobrachium rosenbergii*: separation of the associated virions and characterization of MrNV as a new type of nodavirus. *J. Fish Dis*., **28**, 23–31.

FAROOK M.A., SUNDAR RAJ N., MADAN N., VIMAL S., ABDUL MAJEED S., TAJU G., RAJKUMAR T., SANTHOSHKUMAR S., SIVAKUMAR S. & SAHUL HAMEED A.S. (2014). Immunomodulatory effect of recombinant *Macrobrachium rosenbergii* nodavirus capsid protein (r-MCP) against white tail disease of giant freshwater prawn, *Macrobrachium rosenbergii* (de Man, 1879). *Aquaculture,* **433**, 395–403.

GANGNONNGIWA W., BUNNONTAEA M., PHIWSAIYAA K., SENAPINA S. & DHAR A.K. (2020). In experimental challenge with infectious clones of *Macrobrachium rosenbergii* nodavirus (MrNV) and extra small virus (XSV), MrNV alone can cause mortality in freshwater prawn (*Macrobrachium rosenbergii*). *Virology,* **540** 30–37. https://doi.org/10.1016/j.virol.2019.11.004

HARIDAS D.V., PILLAI D., MANOJKUMAR B., NAIR C.M. & SHERIEF P.M. (2010). Optimisation of reverse transcriptase loop-mediated isothermal amplification assay for rapid detection of *Macrobrachium rosenbergii* nodavirus and extra small virus in *Macrobrachium rosenbergii. J. Virol. Methods*, **167**, 61–67.

HERNANDEZ-HERRERA R.I., CHAPPE-BONNICHON V., ROCH P., SRI WIDADA J. & BONAMI J.R. (2007). Partial susceptibility of the SSN-1 fish cell line to a crustacean virus: a defective replication study. *J. Fish Dis*., **30**, 673–679.

HO K.L., GABRIELSEN M., BEH P.L., KUEH C.L., THONG Q.X. & STREETLEY J. (2018). Structure of the *Macrobrachium rosenbergii* nodavirus: a new genus within the nodaviridae? *PLOS Biology*, **16**, e3000038.

HO K.L., KUEH C.L., BEH P.L., TAN W.S. & BHELLA D. (2017). Cryo-Electron microscopy structure of the *Macrobrachium rosenbergii* nodavirus capsid at 7 angstroms resolution. *Scientific Reports*, **7**, 2083.

HSIEH C.-Y., WU Z.-B., TUNG M.-C., TU C., LO S.-P., CHANG T.-C., CHANG C.-D., CHEN S.-C., HSIEH Y.-C. & TSAI S.-S. (2006). *In situ* hybridization and RT-PCR detection of *Macrobrachium rosenbergii* nodavirus in giant freshwater prawn, *Macrobrachium rosenbergii* (de Man), in Taiwan. *J. Fish Dis*., **29**, 665–671. https://doi.org/10.1111/j.1365-2761.2006.00762.x

JAMALPURE S., VIMAL S., NAFEEZ AHMED A., SAHUL HAMEED A.S., PAKNIKAR K.M. & JYTIKA M.R. (2021). On-site detection of nodavirus in post larval (PL) stage of the giant prawn, *Macrobrachium rosenbergii*: A test to nip the problem in the bud. *Aquaculture*, **534**, 736292; https://doi.org/10.1016/j.aquaculture.2020.736292

MURWANTOKO M., ARIF B., ROOSMANTO R & MASASHI K. (2016). *Macrobrachium rosenbergii* nodavirus infection in a giant freshwater prawn hatchery in Indonesia. *Springer Plus*, **5**, 1729.

NaveenKumar S., Shekar M., Karunasagar I. & Karunas I. (2013). Genetic analysis of RNA1 and RNA2 of *Macrobrachium rosenbergii* nodavirus (MrNV) isolated from India. *Virus Res.,* **173**, 377–385. https://doi.org/10.1016/j.virusres.2013.01.003https://doi.org/10.1016/j.virusres.2013.01.003

Naveen Kumar S., Praveen R., Indrani K. & Karunasagar I. (2021). Recombinant viral proteins delivered orally through inactivated bacterial cells induce protection in *Macrobrachium rosenbergii* (de Man) against White Tail Disease. *J. Fish Dis*., **44**, 601–612.

Owens L., La Fauce K., Juntunen K., Hayakijkosol O. & Zeng C. (2009). *Macrobrachium rosenbergii* nodavirus disease (white tail disease) in Australia. *Dis. Aquat. Org.,* **85**, 175–180.

Qian D., Liu W., Jianxiang W. & Yu L. (2006). Preparation of monoclonal antibody against *Macrobrachium rosenbergii* Nodavirus and application of TAS-ELISA for virus diagnosis in post-larvae hatcheries in east China during 2000–2004. *Aquaculture*, **261**, 1144–1150.

Qian D., Shi Z., Zhang S., Cao Z., Liu W. Li L., Xie Y., Cambournac I. & Bonami J.R. (2003). Extra small virus-like particles (XSV) and nodavirus associated with whitish muscle disease in the giant freshwater prawn, *Macrobrachium rosenbergii. J. Fish Dis*., **26**, 521–527.

Ravi N. & Sahul Hameed A.S. (2016). Effect of chemical and physical treatments on the inactivation of *Macrobrachium rosenbergii* nodavirus (MrNV) and extra small virus (XSV). *Aquaculture Res.,* **47**, 1231–1237.

Romestand B. & Bonami J.R. (2003). A sandwich enzyme linked immunosorbent assay (S-ELISA) for detection of MrNV in the giant freshwater prawn, *Macrobrachium rosenbergii* (de Man). *J. Fish Dis*., **26**, 71–75.

Saedi T. A., Hassan M., Wen S. T., Khatijah Y., Hassan M.D., Kua B.C., Soon G.T. & Subha B. (2012). Detection and phylogenetic profiling of nodavirus associated with white tail disease in Malaysian *Macrobrachium rosenbergii* de Man. *Mol. Biol. Rep*., **39**, 5785–5790.

Sahul Hameed A.S. & Bonami J.R. (2012). White Tail Disease of Freshwater Prawn, *Macrobrachium rosenbergii*. *Indian J. Virol.,* **23**, 134–140.

Sahul Hameed A.S., Ravi M., Farook M.A., Taju G., Hernandez-Herrera R.I. & Bonami J.R. (2011). Screening the post-larvae of *Macrobrachium rosenbergii* for early detection of *Macrobrachium rosenbergii* nodavirus (MrNV) and extra small virus (XSV) by RT–PCR and immunological techniques. *Aquaculture*, **317**, 42–47. http://dx.doi.org/10.1016/j.aquaculture.2011.04.022

Sahul Hameed A.S., Yoganandhan K., Sri Widada J. & Bonami J.R. (2004a). Experimental transmission and tissue tropism of *Macrobrachium rosenbergii* nodavirus (MrNV) and its associated small virus (XSV). *Dis. Aquat. Org.,* **62**, 191–196.

Sahul Hameed A.S., Yoganandhan K., Sri Widada J. & Bonami J.R. (2004b). Studies on the occurrence of *Macrobrachium rosenbergii* nodavirus and extra small virus-like particles associated with white tail disease of *M. rosenbergii* in India by RT-PCR detection. *Aquaculture*, **238**, 127–133.

Senapin S., Jaengsanong C., Phiwsaiya K., Prasertsri S., Laisutisan K., Chuchird N. & Flegel T.W. (2012). Infections of MrNV (*Macrobrachium rosenbergii* nodavirus) in cultivated whiteleg shrimp *Penaeus vannamei* in Asia. *Aquaculture*, **338–341**, 41–46. 10.1016/j.aquaculture.2012.01.019

Sri Widada J., Durand S., Cambournac I., Qian D., Shi Z., Dejonghe E., Richard V. & Bonami J.R. (2003). Genome-based detection methods of *Macrobrachium rosenbergii* nodavirus, a pathogen of the giant freshwater prawn, *Macrobrachium rosenbergii*: dot-blot, *in situ* hybridization and RT-PCR. *J. Fish Dis*., **26**, 583–590.

Sri Widada J., Richard V., Shi Z., Qian D. & Bonami J.R. (2004). Dot-Blot hybridization and RT-PCR detection of extra small virus (XSV) associated with white tail disease of prawn *Macrobrachium rosenbergii. Dis. Aquat. Org.,* **58**, 83–87.

Sudhakaran R., Haribabu P., Kumar S.R., Sarathi M., Ahmed V.P., Babu V.S., Venkatesan C. & Hameedl A.S. (2008). Natural aquatic insect carriers of *Macrobrachium rosenbergii* nodavirus (MrNV) and extra small virus (XSV). *Dis. Aquat. Org.*, **79**, 141–145. doi: 10.3354/dao01886.

Sudhakaran R., Ishaq Ahmed V.P., Haribabu P., Mukherjee S.C., Sri Widada J., Bonami J.R. & Sahul Hameed A.S. (2007a). Experimental vertical transmission of *Macrobrachium rosenbergii* nodavirus (MrNV) and extra small virus (XSV) from brooders to progeny in *Macrobrachium rosenbergii* and *Artemia*. *J. Fish Dis*., **30**, 27–35.

SUDHAKARAN R., PARAMESWARAN V. & SAHUL HAMEED A.S. (2007b). *In vitro* replication of *Macrobrachium rosenbergii*) nodavirus and extra small virus (XSV) in C6/36 mosquito cell line. *J. Virol. Methods*, **146**, 112–118.

SUDHAKARAN R., SYED MUSTHAQ S., HARIBABU P., MUKHERJEE S.C., GOPAL C. & SAHUL HAMEED A.S. (2006). Experimental transmission of *Macrobrachium rosenbergii* nodavirus (MrNV) and extra small virus (XSV) in three species of marine shrimp (*Penaeus indicus*, *Penaeus japonicus* and *Penaeus monodon*). *Aquaculture*, **257**, 136–141.

WANG C.S., CHANG J.S., WEN C.M., SHIH H.H., & CHEN S.N. (2008). *Macrobrachium rosenbergii nodavirus* infection in *M. rosenbergii* (de Man) with white tail disease cultured in Taiwan. *J. Fish Dis*., **31**, 415–422.

YOGANANDHAN K., LEARTVIBHAS M., SRIWONGPUK S. & LIMSUWAN C. (2006). White tail disease of the giant freshwater prawn *Macrobrachium rosenbergii* in Thailand. *Dis. Aquatic. Org.*, **69**, 255–258.

YOGANANDHAN K., SRI WIDADA J., BONAMI J.R. & SAHUL HAMEED A.S. (2005). Simultaneous detection of *Macrobrachium rosenbergii* nodavirus and extra small virus by a single tube, one-step multiplex RT-PCR assay. *J. Fish Dis*., **28**, 65-69.

ZHANG H., WANG J., YUAN J., LI L., ZHANG J., BONAMI J.-R. & SHI Z. (2006). Quantitative relationship of two viruses (MrNV and XSV) in white tail disease of *Macrobrachium rosenbergii*. *Dis. Aquat. Org.*, **71**, 11–17.

*
* *

NB: There is a WOAH Reference Laboratory for infection with *Macrobrachium rosenbergii* nodavirus (white tail disease) (please consult the WOAH web site: https://www.woah.org/en/what-we-offer/expertise-network/reference-laboratories/#ui-id-3) any further information on infection with *Macrobrachium rosenbergii* nodavirus (white tail disease)

NB: FIRST ADOPTED IN 2009. MOST RECENT UPDATES ADOPTED IN 2017.

CHAPTER 2.2.9

INFECTION WITH YELLOW HEAD VIRUS GENOTYPE 1

EU position

The EU supports the adoption of this revised chapter.

1. Scope

Infection with yellow head virus genotype 1 means infection with the pathogenic agent yellow head virus genotype 1 (YHV1) of the Genus *Okavirus* and Family *Roniviridae*.

2. Disease information

2.1. Agent factors

2.1.1. Aetiological agent

Yellow head virus genotype 1 (YHV1; species *Yellow head virus*) is one of eight known genotypes in the yellow head complex of viruses and is the only known genotype that causes yellow head disease. YHV1 forms enveloped, rod-shaped particles 40–50 nm × 150–180 nm (Chantanachookin *et al.,* 1993; Wongteerasupaya *et al*., 1995). Envelopes are studded with prominent peplomers projecting approximately 11 nm from the surface. Nucleocapsids appear as rods (diameter 20–30 nm) and possess a helical symmetry with a periodicity of 5–7 nm. Virions comprise three structural proteins (nucleoprotein p20 and envelope glycoproteins gp64 and gp116) and a ~26 kb positive-sense single-stranded RNA genome. The nucleotide sequence of the ORF1b region of the viral genome has been used to determine the phylogenetic relationships of YHV1 and other yellow head virus genotypes (Dong *et al*., 2017; Mohr *et al*., 2015; Wijegoonawardane *et al.,* 2008a).

YHV1, yellow head virus genotype 2 (YHV2; species *Gill-associated virus*) and yellow head virus genotype 8 (YHV8; species *Okavirus 1*) have been formally classified by the International Committee on Taxonomy of Viruses (Walker *et al*., 2021). Four other genotypes in the complex (YHV3–YHV6) occur commonly in healthy *Penaeus monodon* in East Africa, Asia and Australia and are rarely or never associated with disease (Walker *et al.,* 2001; Wijegoonawardane *et al.,* 2008a). Of the remaining two yellow head virus genotypes, YHV7 was detected in diseased *P. monodon* in Australia (Mohr *et al*., 2015) and YHV8 was detected in *P. chinensis* suspected of suffering from acute hepatopancreatic necrosis disease (Liu *et al*., 2014). There is evidence of genetic recombination between genotypes (Wijegoonawardane *et al.*, 2009).

2.1.2. Survival and stability in processed or stored samples

YHV1, identified by either transmission electron microscopy (TEM) (Nunan *et al.,* 1998), or molecular methods (Durand *et al.,* 2000; McColl *et al.,* 2004), has been detected in frozen commodity prawns with infectivity demonstrated by bioassay.

2.1.3. Survival and stability outside the host

YHV1 remains viable in aerated seawater for up to 72 hours (Flegel *et al.*, 1995b).

For inactivation methods, see Section 2.4.5.

2.2. Host factors

2.2.1. Susceptible host species

Species that fulfil the criteria for listing as susceptible to infection with YHV1 according to Chapter 1.5 of the *Aquatic Animal Health Code* (*Aquatic Code*) are: ~~blue shrimp (*Penaeus stylirostris*), dagger blade grass shrimp (*Palaemonetes pugio*), giant tiger prawn (*Penaeus monodon*), jinga shrimp (*Metapenaeus affinis*) and whiteleg shrimp (*Penaeus vannamei*).~~

Family	Scientific name	Common name
Palaemonidae	*Palaemonetes pugio*	dagger blade grass shrimp
Penaeidae	*Metapenaeus affinis*	jinga shrimp
	Penaeus monodon	giant tiger prawn
	Penaeus stylirostris	blue shrimp
	Penaeus vannamei	whiteleg shrimp

2.2.2. Species with incomplete evidence for susceptibility

Species for which there is incomplete evidence to fulfil the criteria for listing as susceptible to infection with YHV1 according to Chapter 1.5 of the *Aquatic Code* are:

Family	Scientific name	Common name
Palaemonidae	*Palaemon serrifer*	carpenter prawn
	Palaemon styliferus	Pacific blue prawn
	Macrobrachium sintangense	Sunda river prawn
Parastacidae	*Cherax quadricarinatus*	red claw crayfish
Penaeidae	*Metapenaeus brevicornis*	yellow shrimp
	Penaeus aztecus	northern brown shrimp
	Penaeus duorarum	northern pink shrimp
	Penaeus japonicus	kuruma prawn
	Penaeus merguiensis	banana prawn
	Penaeus setiferus	northern white shrimp

In addition, pathogen-specific positive polymerase chain reaction (PCR) results have been reported in the

following species, but an active infection has not been demonstrated: ~~acorn barnacle (*Chelonibia patula*), blue crab (*Callinectes sapidus*), cyclopoid copepod (*Ergasilus manicatus*), gooseneck barnacle (*Octolasmis muelleri*), Gulf killifish (*Fundulus grandis*) and paste shrimp (*Acetes* sp.).~~

Family	Scientific name	Common name
Chelonibiidae	*Chelonibia patula*	acorn barnacle
Ergasilidae	*Ergasilus manicatus*	cyclopoid copepod
Fundulidae	*Fundulus grandis*	gulf killifish
Poecilasmatidae	*Octolasmis muelleri*	gooseneck barnacle
Portunidae	*Callinectes sapidus*	blue crab
Sergestidae	*Acetes sp.*	paste shrimp

2.2.3. Likelihood of infection by species, host life stage, population or sub-populations

Penaeus monodon are susceptible to YHV1 infection beyond PL15 (Khongpradit *et al.*, 1995). Lightner *et al*. (1998) YHV1 challenge caused disease in juveniles of *Penaeus aztecus, P. duorarum, P. setiferus,* and *P. vannamei* but postlarvae appeared resistant (Lightner *et al*. 1998). YHV1 infections are usually detected only when disease is evident, however infections have been detected in healthy wild populations of *P. stylirostris* (Castro-Longoria *et al.,* 2008). Natural YHV1 infections have been detected in *P. japonicus*, *P. merguiensis*, *P. setiferus*, *M. ensis*, and *P. styliferus* (Cowley *et al.,* 2002; Flegel *et al.,* 1995a; 1995b).

2.2.4. Distribution of the pathogen in the host

YHV1 targets tissues of ectodermal and mesodermal origin including lymphoid organ, haemocytes, haematopoietic tissue, gill lamellae and spongy connective tissue of the subcutis, gut, antennal gland, gonads, nerve tracts and ganglia (Chantanachookin *et al*., 1993; Lightner, 1996).

2.2.5. Aquatic animal reservoirs of infection

YHV1 was detected by reverse-transcription polymerase chain reaction (RT-PCR) in clinically normal wild *P. stylirostris* collected for surveillance purposes in the Gulf of California in 2003 (Castro-Longoria *et al*., 2008). ~~The infectious nature of the YHV1 detected was confirmed by experimental infections. There is also evidence that YHV1 can persist in survivors of experimental infection (Longyant *et al.*, 2005; 2006).~~

2.2.6. Vectors

There are no known vectors of YHV1.

2.3. Disease pattern

2.3.1. Mortality, morbidity and prevalence

In farmed *P. monodon*, YHV disease can cause up to 100% mortality within 3–5 days of the first appearance of clinical signs (Chantanachookin *et al.,* 1993). Mortalities can be induced by experimental exposure of *P. monodon* to YHV1 (Oanh *et al.,* 2011).

2.3.2. Clinical signs, including behavioural changes

Shrimp from late postlarvae (PL) stages onwards can be infected experimentally with YHV1. In cultured shrimp, infection can result in mass mortality occurring, usually in early to late juvenile stages. Moribund shrimp may exhibit a bleached overall appearance and a yellowish discoloration of the cephalothorax. However, these disease features are not particularly distinctive, and gross signs are not reliable even for preliminary diagnosis of YHV1.

Exceptionally high feeding activity followed by an abrupt cessation of feeding may occur within 2–4 days of the appearance of gross clinical signs of disease and mortality. Moribund shrimp may congregate at pond edges near the surface (Chantanachookin *et al.,* 1993).

2.3.3 Gross pathology

The yellow hepatopancreas of diseased shrimp may be exceptionally soft when compared with the brown hepatopancreas of a healthy shrimp (Chantanachookin *et al.,* 1993).

2.3.4. Modes of transmission and life cycle

YHV1 can be transmitted horizontally by ingestion of infected tissue, immersion in membrane-filtered tissue extracts, or by cohabitation with infected shrimp (Walker & Sittidilokratna, 2008). YHV1 replicates in the cytoplasm of infected cells in which long filamentous pre-nucleocapsids are abundant and virions bud into cytoplasmic vesicles in densely packed paracrystalline arrays for egress at the cytoplasmic membrane (Chantanachookin *et al.,* 1993).

2.3.5. Environmental factors

Elevated virus infection levels accompanied by disease can be precipitated by physiological stress induced by sudden changes in pH or dissolved oxygen levels, or other environmental factors (Flegel *et al*., 1997).

2.3.6. Geographical distribution

YHV1 has been reported in South-East Asia (Walker *et al.*, 2001). YHV1 has also been detected in *P. stylirostris* and *P. vannamei* in the Americas (Castro-Longoria *et al.,* 2008; Sanchez-Barajas *et al.*, 2009).

See WAHIS (https://wahis.woah.org/#/home) for recent information on distribution at the country level.

2.4. Biosecurity and disease control strategies

2.4.1. Vaccination

None available.

2.4.2. Chemotherapy including blocking agents

No effective commercial anti-viral product is yet available.

2.4.3. Immunostimulation

A multi-target dsRNA for simultaneous inhibition of YHV1 and white spot syndrome virus demonstrated inhibition of the two viruses when administered to shrimp by injection (Chaimongkon *et al.,* 2020)

2.4.4. Breeding resistant strains

Not reported.

2.4.5. Inactivation methods

YHV1 can be inactivated by heating at 60°C for 15 minutes (Flegel *et al.*, 1995b). Little information is available on other inactivation methods but the virus appears to be susceptible to treatment with chlorine at 30 parts per million (0.03 mg ml^{-1}) (Flegel *et al.,* 1997).

2.4.6. Disinfection of eggs and larvae

Not reported.

2.4.7. General husbandry

The focus is to exclude YHV1 from entering production systems; for example, by using specific pathogen-free (SPF) stock, batch testing stock and biosecurity measures to reduce entry into culture systems.

3. Specimen selection, sample collection, transportation and handling

This section draws on information in Sections 2.2, 2.3 and 2.4 to identify populations, individuals and samples that are most likely to be infected.

3.1. Selection of populations and individual specimens

For diagnosis during a disease outbreak, moribund shrimp collected from pond edges are the preferred source of material for examination. Apparently healthy shrimp should also be collected from the same ponds. For surveillance in populations of apparently healthy shrimp, life stages from PL stage 15 onwards can provide tissue sources useful for testing.

3.2. Selection of organs or tissues

In moribund shrimp suspected to be infected with YHV1, pleopods, gill and lymphoid organ are the most suitable sample tissues. For screening or surveillance of juvenile or adult shrimp that appear grossly normal, pleopods or gills are preferred.

3.3. Samples or tissues not suitable for pathogen detection

Not determined.

3.4. Non-lethal sampling

Haemolymph can be used for non-lethal sampling.

If non-lethal tissue sample types differ from recommended tissues (see Section 3.2.), or from the tissue samples used in validation studies, the effect on diagnostic performance should be considered.

3.5. Preservation of samples for submission

For guidance on sample preservation methods for the intended test methods, see Chapter 2.2.0 *General information* (diseases of crustaceans).

3.5.1. Samples for bioassay

The success of bioassay depends strongly on the quality of samples (time since collection and time in storage). Fresh specimens should be kept on ice and preferably sent to the laboratory within 24 hours of collection. To avoid degradation of samples, use alternative storage methods only after consultation with the receiving laboratory.

3.5.2. Preservation of samples for molecular detection

Tissue samples for PCR testing should be preserved in 70–90% (v/v) analytical/reagent-grade (absolute) ethanol. The recommended ratio of ethanol to tissue is 10:1 based on studies in terrestrial animal and human health. The use of lower grade (laboratory or industrial grade) ethanol is not recommended. If material cannot be fixed it can be frozen at –20°C or below for 1 month or less; for long-term storage. –80°C is recommended.

Standard sample collection, preservation and processing methods for molecular techniques can be found in Section B.5.5. of Chapter 2.2.0 *General information* (diseases of crustaceans).

3.5.3. Samples for histopathology, immunohistochemistry or *in-situ* hybridisation

Standard sample collection, preservation and processing methods for histological techniques can be found in Section B.2.2 of Chapter 2.2.0 *General information* (diseases of crustaceans).

3.5.4. Samples for other tests

Not applicable.

3.6. Pooling of samples

Pooling of samples from more than one individual animal for a given purpose is only recommended where robust supporting data on diagnostic sensitivity and diagnostic specificity have been evaluated and found to be suitable. If the effect of pooling on diagnostic sensitivity has not been thoroughly evaluated, larger specimens should be processed and tested individually. Small life stages can be pooled to obtain the minimum amount of material for molecular detection.

4. Diagnostic methods

The methods currently available for pathogen detection that can be used in i) surveillance of apparently healthy animals, ii) presumptive diagnosis in clinically affected animals and iii) confirmatory diagnostic purposes are listed in Table 4.1. by animal life stage.

Ratings for purposes of use. For each recommended assay a qualitative rating for the purpose of use is provided. The ratings are determined based on multiple performance and operational factors relevant to application of an assay for a defined purpose. These factors include appropriate diagnostic performance characteristics, level of assay validation, availability cost, timeliness, and sample throughput and operability. For a specific purpose of use, assays are rated as:

+++ =	Methods are most suitable with desirable performance and operational characteristics.
++ =	Methods are suitable with acceptable performance and operational characteristics under most circumstances.
+ =	Methods are suitable, but performance or operational characteristics may limit application under some circumstances.
Shaded boxes =	Not appropriate for this purpose.

Validation stage. The validation stage corresponds to the assay development and validation pathway in chapter 1.1.2. The validation stage is specific to each purpose of use. Where available, information on the diagnostic performance of recommended assays is provided in Section 6.3.

WOAH Reference Laboratories welcome feedback on diagnostic performance of recommended assays, in particular PCR methods. Of particular interest are any factors affecting expected assay sensitivity (e.g. tissue components inhibiting amplification) or expected specificity (e.g. failure to detect particular genotypes, detection of homologous sequences within the host genome). These issues should be communicated to the WOAH Reference Laboratories so that advice can be provided to diagnostic laboratories and the standards amended if necessary.

Table 4.1. *WOAH recommended diagnostic methods and their level of validation for surveillance of apparently healthy animals and investigation of clinically affected animals*

Method	A. Surveillance of apparently healthy animals				B. Presumptive diagnosis of clinically affected animals				C. Confirmatory diagnosis[1] of a suspect result from surveillance or presumptive diagnosis			
	Early life stages[2]	Juveniles[2]	Adults	LV	Early life stages[2]	Juveniles[2]	Adults	LV	Early life stages[2]	Juveniles[2]	Adults	LV
Wet mounts												
Histopathology						++	++	1				
Cell culture												
Real-time RT-PCR	++	+++	+++	1	++	+++	+++	4	++	+++	+++	4
Conventional RT-PCR	++	++	++	1	++	++	++	1				
Conventional PCR followed by amplicon sequencing									++	+++	+++	1
In-situ hybridisation						++	++	1				
Bioassay					+	+	+	1				
~~LAMP~~ Immunohistochemistry												
Ab-ELISA												
Ag-ELISA												
~~Other antigen detection methods[3]~~												
Other methods[3]												

LV = level of validation, refers to the stage of validation in the WOAH Pathway (chapter 1.1.2); PCR = polymerase chain reaction; ~~LAMP = loop-mediated isothermal amplification;~~
Ab- or Ag-ELISA = antibody or antigen enzyme-linked immunosorbent assay, respectively; IFAT = indirect fluorescent antibody test.
[1]For confirmatory diagnoses, methods need to be carried out in combination (see Section 6). [2]Susceptibility of early and juvenile life stages is described in Section 2.2.3.
[3]Specify the test used. Shading indicates the test is inappropriate or should not be used for this purpose.

4.1. Wet mounts

Not applicable.

4.2. Histopathology and cytopathology

Fix the cephalothorax tissues of moribund shrimp suspected to be affected by YHV1 in Davidson's fixative, prepare tissue sections and stain with Meyer's haematoxylin and eosin (H&E) using standard histological procedures (Lightner, 1996). Examine tissues of ectodermal and mesodermal origin by light microscopy for the presence of moderate to large numbers of deeply basophilic, evenly stained, spherical, cytoplasmic inclusions approximately 2 µm in diameter or smaller (Chantanachookin *et al.,* 1993). Tissues of the lymphoid organ, stomach subcuticulum and gills are particularly informative.

Lymphoid organ spheroids are commonly observed in healthy *P. monodon* chronically infected with YHV1 or GAV and lymphoid organ necrosis often accompanies disease (Spann *et al.*, 1997). However, spheroid formation and structural degeneration of lymphoid organ tissue also result from infection by other shrimp viruses (Lightner, 1996).

4.3. Cell culture for isolation

Although primary shrimp cell culture methods are available, they are not recommended to isolate and identify YHV1 as a routine diagnostic method. No continuous cell lines suitable for YHV1 culture are available.

4.4. Nucleic acid amplification

PCR assays should always be run with the controls specified in Section 5.5 *Use of molecular and antibody-based techniques for confirmatory testing and diagnosis* of Chapter 2.2.0 *General information* (diseases of crustaceans). Each sample should be tested in duplicate.

Extraction of nucleic acids

Different kits and procedures can be used for nucleic acid extraction. The quality and concentration of the extracted nucleic acid is important and can be checked using a suitable method as appropriate to the circumstances.

4.4.1. Real-time PCR

~~Not available.~~

The protocol for the real-time RT-PCR for detection of YHV1 has been developed by the WOAH Reference Laboratory for YHV1. This assay is specific for genotype 1. Validation data are provided in the submitted validation report for diagnostic tests and confirm suitability to be recommended for inclusion in the *Aquatic Manual* (ADD LINK OR AGREED REFERENCE).

Pathogen/ target gene	Primer/probe (5'–3')	Concentration	Cycling conditions[(a)]
YHV1 /ORF1	YHV1-12-qF: AGT-CTA-CAG-TGC-TCT-GAT-CT YHV1-12-qR: GAT-TCT-TGA-AGC-GCA-TGA-GT YHV1-12-qPr: FAM-TCT-CAT-GTG/ZEN/TCA-TGA-TAT-TCT-CAA-GCG-AGT-IABkFQ	900 nM of each primer 250 nM of probe	Reverse transcription at 48°C/30 min 1 cycle 95°C/10 min 45 cycles of 95°C/15 sec and 60°C/60 sec

[(a)]A denaturation step prior to cycling has not been included.

4.4.2. Conventional RT-PCR

Three RT-PCR protocols are described. For all conventional RT-PCR protocols, assignment to YHV genotype 1 can be achieved by nucleotide sequence analysis of the RT-PCR amplicon. Reference sequences for YHV1 include:

Protocol 1 is a 1-step RT-PCR that can be used to detect YHV1 in affected shrimp. The protocol is as described by Mohr *et al.* (2015) and adapted from Wongteerasupaya *et al.* (1997). This protocol will detect YHV1 but not GAV or any of the other genotypes currently recognised.

Protocol 2 is a more sensitive multiplex nested RT-PCR that can be used to differentiate YHV1 from GAV. The protocol is as described by Mohr *et al.* (2015) and adapted from Cowley *et al.* (2004). The first stage of the multiplex nested RT-PCR (primary RT-PCR) was designed to detect YHV1 and GAV but has been reported to also detect YHV7 (Mohr *et al*., 2015). Both the primary RT-PCR and the nested PCR detected the novel YHV genotype from China (People's Rep. of) (Liu *et al*., 2014). In the second PCR step, a 277 bp product indicates detection of YHV and a 406 bp product indicates detection of GAV. The presence of both 406 bp and 277 bp products indicates a dual infection with GAV and YHV1. The nested PCR can be run as two separate assays specific for YHV1 or GAV by omitting either the G6 or Y3 primer, respectively. **NOTE:** Due to reported problems with primer specificity for some emerging strains, all PCR products generated using protocol 2 should be sequenced to confirm the virus genotype.

Protocol 3 is a multiplex nested RT-PCR protocol that can be used for screening shrimp for any of the seven genotypes of the yellow head complex of viruses. The protocol is as described by Mohr *et al*. (2015) and adapted from Wijegoonawardane *et al.* (2008b). Two primers were designed to each site, one accommodating sequence variations amongst YHV1 isolates and the other variations amongst isolates of the other genotypes (Wijegoonawardane *et al.*, 2008b). It is not known whether this assay will detect ~~the~~ YHV8 ~~genotype recently detected in China (People's Rep. of)~~ (Liu *et al*., 2014).

Primer sequences

Pathogen / target gene	Primer (5'–3')	Concentration	Cycling parameters[(a)]
Protocol 1 (Wongteerasupaya *et al.,* 1997; GenBank Accession No.: FJ848675.1; amplicon size: 135 bp)			
YHV1 / ORF1b	10F: CCG-CTA-ATT-TCA-AAA-ACT-ACG 144R: AAG-GTG-TTA-TGT-CGA-GGA-AGT	180 nM 180 nM	40 cycles of 94°C/30sec, 58°C/45 sec, 68°C/45 sec,
Protocol 2 (Cowley *et al.,* 2004; GenBank Accession No.: FJ848675.1)			
YHV1 and GAV / ORF1b	Primary (Amplicon size: 794 bp) GY1: ~~5~~GAC-ATC-ACT-CCA-GAC-AAC-ATC-TG GY4: GTG-AAG-TCC-ATG-TGT-GTG-AGA-CG Nested for detection of YHV1 (Amplicon size: 277 bp) GY2: CAT-CTG-TCC-AGA-AGG-CGT-CTA-TGA Y3: ACG-CTC-TGT-GAC-AAG-CAT-GAA-GTT Nested for detection of GAV (Amplicon size: 406 bp) GY2: CAT-CTG-TCC-AGA-AGG-CGT-CTA-TGA G6: GTA-GTA-GAG-ACG-AGT-GAC-ACC-TAT	 180 nM 180 nM 360 nM 360 nM 360 nM 360 nM	35 cycles of 95°C/30 sec, 66°C/30 sec, and 68°C/45 sec
Protocol 3 (Wijegoonawardane *et al.,* 2008b; GenBank Accession No.: FJ848675.1)			
YHV1 to YHV7 / ORF1b	Primary (amplicon size: 359 bp) YC-F1ab pool: ATC-GTC-GTC-AGC-TAC-CGC-AAT-ACT-GC ATC-GTC-GTC-AGY-TAY-CGT-AAC-ACC-GC YC-R1ab pool: TCT-TCR-CGT-GTG-AAC-ACY-TTC-TTR-GC TCT-GCG-TGG-GTG-AAC-ACC-TTC-TTG-GC Nested (amplicon size: 147 bp) YC-F2ab pool: CGC-TTC-CAA-TGT-ATC-TGY-ATG-CAC-CA CGC-TTY-CAR-TGT-ATC-TGC-ATG-CAC-CA YC-R2ab pool: RTC-DGT-GTA-CAT-GTT-TGA-GAG-TTT-GTT GTC-AGT-GTA-CAT-ATT-GGA-GAG-TTT-RTT Mixed base codes: R(AG), Y(CT), M(AC), K(GT), S(GC), W(AT), H(ACT), B(GCT), V(AGC), D(AGT), N(AGCT).	 180 nM 180 nM 180 nM 180 nM 180 nM 180 nM 180 nM 180 nM	35 cycles of 94°C/45 sec, 60°C/45 sec, 68°C/45 sec, 35 cycles of 94°C/45 sec, 60°C/45 sec, 72°C/45 sec;

[(a)]A denaturation step prior to cycling has not been included.

The Protocol 2 Y3 primer contains a mismatch for GAV but is specific for YHV1. The mismatch can cause false-positives with GAV in the nested PCR of protocol 2 where GAV generates an amplicon of very similar size to the expected size of the YHV1 amplicon. ~~For GAV, the 7th base from left (T) is substituted for C so that the primer sequence for GAV should be 5'-CAT-CTG-CCC-AGA-AGG-CGT-CTA-TGA-3', according to the sequence data of the GAV genome (database accession numbers: NC_010306.1 and AF227196.2).~~

The following controls should be run with each assay: negative extraction control; positive control; no template control; internal PCR control.

4.4.3. Other nucleic acid amplification methods

Not available.

4.5. Amplicon sequencing

The size of the PCR amplicon should be verified, for example by agarose gel electrophoresis. Both DNA strands of the PCR product must be sequenced and analysed in comparison with reference sequences.

4.6. *In-situ* hybridisation

The *in-situ* hybridisation (ISH) protocol of Tang *et al.* (2002) is suitable for detecting YHV1 or GAV (Tang & Lightner, 1999). To preserve viral RNA accessibility, fix tissues sampled from live shrimp in neutral-buffered, modified Davidson's fixative without acetic acid (RF-fixative) (Hasson *et al.*, 1997). To achieve good tissue preservation whilst also preserving RNA accessibility, normal Davidson's fixative can be used as long as the fixation time is limited to 24 hours (maximum of 48 hours). Detailed methods can be found in Tang *et al.* (2002) YHV-infected cells give a blue to purple-black colour against the brown counter stain. Positive controls of YHV-infected tissue and negative controls of uninfected shrimp tissue should be included. The digoxigenin-labelled DNA probe can be prepared by PCR labelling using the following primers:

YHV1051F: 5'-ACA-TCT-GTC-CAG-AAG-GCG-TC-3'

YHV1051R: 5'-GGG-GGT-GTA-GAG-GGA-GAG-AG-3'

4.7. Immunohistochemistry

Not applicable.

4.8. Bioassay

The bioassay procedure is based on that described by Spann *et al.* (1997), but similar procedures have been described by several other authors (e.g. Lu *et al.,* 1994). The bioassay should be conducted in susceptible shrimp that have been determined to be free from YHV complex viruses.

Suspect YHV1-infected samples should be maintained at 4°C or on ice. If necessary, the whole shrimp or the retained cephalothorax may be snap-frozen and stored at –80°C or in liquid nitrogen until required. Viral inoculum should be prepared as described by Spann *et al.* (1997).

Juvenile shrimp of a known susceptible species are injected with viral inoculum. Negative controls (buffer injected) and positive controls (known YHV1 positive material) treatment groups are required. Shrimp should be maintained separately to prevent cross-contamination between treatments. Observe the shrimp and record mortalities for at least 21 days or until the test and positive control groups reach 100% mortality.

Dead shrimp can be processed for PCR and sequence analysis. The surviving shrimp are processed for gross signs, histopathology, PCR and sequence analysis. A positive result is indicated by the detection of gross signs and characteristic histological lesions, and by PCR and amplicon sequence analysis. The negative control shrimp must remain negative for at least 21 days for gross or histological signs of infection with YHV1.

4.9. Antibody- or antigen-based detection methods (ELISA, etc.)

None has been successfully developed.

4.10. Other methods

None at present.

5. Test(s) recommended for surveillance to demonstrate freedom in apparently healthy populations

~~Nested~~ Real-time RT-PCR ~~(Protocol 3)~~ is recommended for demonstrating freedom from YHV1 in ~~an~~ apparently healthy populations. ~~Sequencing of any amplified PCR products is required to determine the YHV genotype. Two-step PCR negative results are required for YHV1.~~

6. Corroborative diagnostic criteria

This section only addresses the diagnostic test results for detection of infection in the absence (Section 6.1.) or in the presence of clinical signs (Section 6.2.) but does not evaluate whether the infectious agent is the cause of the clinical event.

The case definitions for a suspect and confirmed case have been developed to support decision making related to trade and confirmation of disease status at the country, zone or compartment level. Case definitions for disease confirmation in endemically affected areas may be less stringent. If a Competent Authority does not have the capability to undertake the necessary diagnostic tests it should seek advice from the appropriate WOAH Reference Laboratory, and if necessary, refer samples to that laboratory for confirmatory testing of samples from the index case in a country, zone or compartment considered free.

6.1. Apparently healthy animals or animals of unknown health status[1]

Apparently healthy populations may fall under suspicion, and therefore be sampled, if there is an epidemiological link(s) to an infected population. Hydrographical proximity to, or movement of animals or animal products or equipment, etc., from a known infected population equate to an epidemiological link. Alternatively, healthy populations are sampled in surveys to demonstrate disease freedom.

6.1.1. Definition of suspect case in apparently healthy animals

The presence of infection with YHV1 shall be suspected if the following criterion is met:

i) Positive result by ~~a recommended~~ conventional RT-PCR ~~detection test~~

ii) Positive result by real-time RT-PCR

6.1.2. Definition of confirmed case in apparently healthy animals

The presence of infection with YHV1 is considered to be confirmed if the following criterion is met:

i) Positive result by real-time RT-PCR and positive result by conventional RT-PCR followed by amplicon sequencing

ii) ~~A positive result by conventional RT-PCR and identification of YHV1 by sequence analysis of the amplicon from each of two different RT-PCR methods followed by sequence analysis of the amplicons to identify YHV1~~

6.2 Clinically affected animals

Clinical signs are not pathognomonic for a single disease; however they may narrow the range of possible diagnoses.

6.2.1. Definition of suspect case in clinically affected animals

The presence of infection with YHV1 shall be suspected if at least one of the following criteria is met:

i) Gross pathology or clinical signs consistent with YHV1 infection

ii) Histopathology consistent with YHV1 infection

iii) Positive result by real-time RT-PCR

iv) Positive result by conventional RT-PCR

1 For example transboundary commodities.

v) Positive result by ISH

vi) Positive result by bioassay

6.2.2. Definition of confirmed case in clinically affected animals

The presence of infection with YHV1 is considered to be confirmed if the following criterion is met:

i) Positive result by real-time RT-PCR and positive result by conventional RT-PCR followed by amplicon sequencing

~~i) A positive result from each of two different RT-PCR methods targeting non-overlapping parts of the genome followed by sequence analysis of the amplicons to identify YHV1~~

6.3. Diagnostic sensitivity and specificity for diagnostic tests

The diagnostic performance of tests recommended for surveillance or diagnosis of infection with YHV1 are provided in Tables 6.3.1. and 6.3.2 (no data are currently available for either). ~~This information can be used for the design of surveys for infection with YHV1, however, it should be noted that diagnostic performance is specific to the circumstances of each diagnostic accuracy study (including the test purpose, source population, tissue sample types and host species) and diagnostic performance may vary under different conditions.~~ Data are only presented where tests are validated to at least level 2 of the validation pathway described in Chapter 1.1.2. and the information is available within published diagnostic accuracy studies.

6.3.1. For presumptive diagnosis of clinically affected animals

Test type	Test purpose	Source populations	Tissue or sample types	Species	DSe (*n*)	DSp (*n*)	Reference test	Citation
YHV1 RT-qPCR	Diagnosis	Infected by co-habitation or feeding	Pleopods	*Penaeus monodon, P. merguiensis*	100% (n=130)	100% (n=130)	Real-time PCR	Validation report

DSe = diagnostic sensitivity, DSp = diagnostic specificity, *n* = ~~samples~~ number of animals used in the validation study,
PCR: = polymerase chain reaction.

6.3.2. For surveillance of apparently healthy animals

Test type	Test purpose	Source populations	Tissue or sample types	Species	DSe (*n*)	DSp (*n*)	Reference test	Citation

DSe = diagnostic sensitivity, DSp = diagnostic specificity, *n* = ~~samples~~ number of animals used in the validation study,
PCR: = polymerase chain reaction.

7. References

CASTRO-LONGORIA R., QUINTERO-ARREDONDO N., GRIJALVA-CHON J.M. & RAMOS-PAREDES J. (2008). Detection of the yellow-head virus (YHV) in wild blue shrimp, *Penaeus stylirostris*, from the Gulf of California and its experimental transmission to the Pacific white shrimp, *Penaeus vannamei*. *J. Fish Dis.*, **31**, 953–956.

CHAIMONGKON D., ASSAVALAPSAKUL W., PANYIM S. & ATTASART P. (2020). A multi-target dsRNA for simultaneous inhibition of yellow head virus and white spot syndrome virus in shrimp. *J. Biotechnol.*, **321**, 48–56. doi: 10.1016/j.jbiotec.2020.06.022. Epub 2020 Jun 29. PMID: 32615142.

CHANTANACHOOKIN C., BOONYARATPALIN S., KASORNCHANDRA J., DIREKBUSARAKOM S., AEKPANITHANPONG U., SUPAMATTAYA K., SRIURAITANA S. & FLEGEL T.W. (1993). Histology and ultrastructure reveal a new granulosis-like virus in *Penaeus monodon* affected by yellow-head disease. *Dis. Aquat. Org.*, **17**, 145–157.

COWLEY J.A., CADOGAN L.C., WONGTEERASUPAYA C., HODGSON R.A.J., BOONSAENG V. & WALKER P.J. (2004). Multiplex RT-nested PCR differentiation of gill-associated virus (Australia) from yellow head virus (Thailand) of Penaeus monodon. J. *Virol. Methods*, **117**, 49–59. doi: 10.1016/j.jviromet.2003.11.018.

COWLEY J.A., HALL M.R., CADOGAN L.C., SPANN K.M. & WALKER P.J. (2002). Vertical transmission of gill-associated virus (GAV) in the black tiger prawn *Penaeus monodon. Dis. Aquat. Org*., **50**, 95–104.

DONG X., LIU S., ZHU L., WAN X., LIU Q., QIU L., ZOU P., ZHANG Q. & HUANG J. (2017) Complete genome sequence of an isolate of a novel genotype of yellow head virus from *Fenneropenaeus chinensis* indigenous in China. *Arch Virol* **162**, 1149-1152.

DURAND S.V., TANG K.F.J. & LIGHTNER D.V. (2000). Frozen commodity shrimp: potential avenue for introduction of white spot syndrome virus and yellow head virus*. J. Aquatic Anim. Health,* **12**, 128–135.

FLEGEL T.W., BOONYARATPALIN S. & WITHYACHUMNARNKUL B. (1997). Current status of research on yellow-head virus and white-spot virus in Thailand. *In:* Diseases in Asian Aquaculture III, Flegel T.W. & MacRae I.H., eds. Fish Health Section, Asian Fisheries Society, Manila, the Philippines, 285–296.

FLEGEL T.W., FEGAN D.F. & SRIURAIRATANA S. (1995a). Environmental control of infectious shrimp diseases in Thailand. *In:* Diseases in Asian Aquaculture II, Shariff M., Subasinghe R.P. & Arthur J.R., eds. Asian Fisheries Society, Manila, the Philippines, 65–79.

FLEGEL T.W., SRIURAIRATANA S., WONGTERRASUPAYA C., BOONSAENG V., PANYIM S. & WITHYACHUMNARNKUL B. (1995b). Progress in characterization and control of yellow-head virus of *Penaeus monodon*. *In:* Swimming Through Troubled Water, Proceedings of the Special Session on Shrimp Farming, Aquaculture '95, Browdy C.L. & Hopkins J.S., eds. World Aquaculture Society, Baton Rouge, USA, 76–83.

HASSON K.W., HASSON J., AUBERT H., REDMAN R.M. & LIGHTNER D.V. (1997). A new RNA-friendly fixative for the preservation of penaeid shrimp samples for virological assay using cDNA probes. *J. Virol. Methods,* **66**, 227–236.

KHONGPRADIT R., KASORNCHANDRA J. & BOONYARATALIN S. (1995). Susceptibility of the postlarval stages of black tiger shrimp (*Penaeus monodon*) to yellow-head baculovirus (YBV). *In:* Diseases in Asian Aquaculture II, Shariff M., Subasinghe R.P. & Arthur J.R., eds. Asian Fisheries Society, Manila, the Philippines, p. 6.

LIGHTNER D.V. (ED.) (1996). Handbook of Pathology and Diagnostic Procedures for Diseases of Penaeid Shrimp. World Aquaculture Society, Baton Rouge, USA.

LIGHTNER D.V., HASSON K. W., WHITE B. L & REMAN R. M. (1998) Experimental infection of western hemisphere penaeid shrimp with Asian white spot syndrome virus and Asian yellow head virus *J. Aquatic Anim. Health*, **10,** 271-281

LIU Q., HUANG J., YANG H.-L., YANG B., WANG H.-L., WANG Q.-T., LIU F. & ZHANG Q.-L. (2014) Detection of a new genotype of yellow-head virus in farmed shrimp suspicious of EMS/AHPNS infection. *Oceanol. Limnol. Sin*., **45**, 703–709.

~~LONGYANT S., SATTAMAN S., CHAIVISUTHANGKURA P., RUKPRATANPORN S., SITHIGORNGUL W. & SITHIGORNGUL P. (2006). Experimental infection of some penaeid shrimps and crabs by yellow head virus (YHV). *Aquaculture,* **257**, 83–91.~~

~~LONGYANT S., SITHIGORNGUL P., CHAIVISUTHANGKURA P., RUKPRATANPORN S., SITHIGORNGUL W. & MENASVETA P. (2005). Differences in the susceptibility of palaemonid shrimp species to yellow head virus (YHV) infection. *Dis. Aquat. Org*., **64**, 5–12.~~

LU Y., TAPAY L.M., BROCK J.A. & LOH P.C. (1994). Infection of the yellow head baculo-like virus (YBV) in two species of penaeid shrimp *Penaeus stylirostris* (Stimpson) and *Penaeus vannamei* (Boone). *J. Fish Dis.*, **17**, 649–656.

MCCOLL K.A., SLATER J., JEYASEKARAN G., HYATT A.D. & CRANE M.ST.J. (2004). Detection of white spot syndrome virus and yellowhead virus in prawns imported into Australia. *Aust. Vet. J.,* **82**, 69–74.

MOHR P.G., MOODY N.J.G, HOAD J., WILLIAMS L.M., BOWATER R.O., CUMMINS D.M., COWLEY J.A. & CRANE M.ST.J. (2015). New yellow head virus genotype (YHV7) in giant tiger shrimp *Penaeus monodon* indigenous to northern Australia. *Dis. Aquat. Org*., **115**, 263–268.

MOODY N. ET AL (IN PREPARATION). Development of a real-time and conventional PCR assays for the detection of yellow head virus genotype 1.

NUNAN L.M., POULOS B.T. & LIGHTNER D.V. (1998). The detection of white spot syndrome virus (WSSV) and yellow head virus (YHV) in imported commodity shrimp. *Aquaculture,* **160**, 19–30.

OANH D.T., VAN HULTEN M.C., COWLEY J.A. & WALKER P.J. (2011). Pathogenicity of gill-associated virus and Mourilyan virus during mixed infections of black tiger shrimp (*Penaeus monodon*). *J. Gen. Virol*., **92**, 893–901.

SANCHEZ-BARAJAS M., LINAN-CABELLO M.A. & MENA-HERRERA A. (2009). Detection of yellow-head disease in intensive freshwater production systems of *Litopenaeus vannamei*. *Aquacult. Int.,* **17,** 101–112.

SPANN K.M., COWLEY J.A., WALKER P.J. & LESTER R.J.G. (1997). A yellow-head-like virus from *Penaeus monodon* cultured in Australia. *Dis. Aquat. Org.*, **31**, 169–179.

TANG K.F.J. & LIGHTNER D.V. (1999). A yellow head virus gene probe: nucleotide sequence and application for *in situ* hybridization. *Dis. Aquat. Org*., **35**, 165–173.

TANG K.F.J., SPANN K.M., OWENS L. & LIGHTNER D.V. (2002). *In situ* detection of Australian gill-associated virus with a yellow head virus gene probe. *Aquaculture,* **205**, 1–5.

WALKER P.J., COWLEY J.A., DONG X., HUANG J., MOODY N., ZIEBUHR J., CONSORTIUM I.R. (2021). ICTV virus taxonomy profile: Roniviridae. *J. Gen. Virol.*, **102**, jgv001514. doi: 10.1099/jgv. 0.001514

WALKER P.J., COWLEY J.A. SPANN K.M., HODGSON R.A.J. HALL M.R & WITHYACHUMNARNKUL B. (2001). Yellow head complex viruses: Transmission cycles and topographical distribution in the Asia-Pacific Region. *In:* The New Wave, Proceedings of the Special Session on Sustainable Shrimp Culture, Aquaculture 2001, Browdy C.L. & Jory D.E., eds. World Aquaculture Society, Baton Rouge, LA, USA, 292–302.

Walker P.J. & Sittidilokratna N. (2008). Yellow Head Virus. *In:* Encyclopedia of Virology, third edition. Academic Press, 476–483. https://doi.org/10.1016/B978-012374410-4.00779-2

WIJEGOONAWARDANE P.K.M., COWLEY J.A., SITTIDILOKRATNA, N., PHETCHAMPAI, N., COWLEY, J.A., GUDKOVS, N. & WALKER P.J. (2009). Homologous genetic recombination in the yellow head complex of nidoviruses infecting *Penaeus monodon shrimp*. *Virology* doi: 1016/j.virol.2009.04.015.

WIJEGOONAWARDANE P.K.M., COWLEY J.A., PHAN T., HODGSON R.A.J., NIELSEN L., KIATPATHOMCHAI W. & WALKER P.J. (2008a). Genetic diversity in the yellow head nidovirus complex. *Virology* **380**, 213–225.

WIJEGOONAWARDANE P.K.M., COWLEY J.A. & WALKER P.J. (2008b). Consensus RT-nested PCR to detect yellow head virus genotypes in penaeid shrimp. *J. Virol. Methods*, **153**, 168–175.

WONGTEERASUPAYA C., BOONSAENG V., PANYIM S., TASSANAKAJON A., WITHYACHUMNARNKUL B. & FLEGEL T.W. (1997). Detection of yellow-head virus (YHV) of *Penaeus monodon* by RT-PCR amplification. *Dis. Aquat. Org.,* **31**, 181–186.

WONGTEERASUPAYA C., SRIURAIRATANA S., VICKERS J.E., AKRAJAMORN A., BOONSAENG V., PANYIM S., TASSANAKAJON A., WITHYACHUMNARNKUL B. & FLEGEL T.W. (1995). Yellow-head virus of P*enaeus monodon* is an RNA virus. *Dis. Aquat. Org.,* **22**, 45–50.

*
* *

NB: There is a WOAH Reference Laboratory for infection with yellow head virus genotype 1
(please consult the WOAH web site:
https://www.woah.org/en/what-we-offer/expertise-network/reference-laboratories/#ui-id-3).
Please contact the WOAH Reference Laboratories for any further information on
infection with yellow head virus genotype 1

NB: FIRST ADOPTED IN 1995 AS YELLOWHEAD DISEASE. MOST RECENT UPDATES ADOPTED IN 2019.

Annex 55. Item 9.1.5. – Chapter 2.2.X. Infection with decapod iridescent virus 1

CHAPTER 2.2.X.

INFECTION WITH DECAPOD IRIDESCENT VIRUS 1

EU position

The EU supports the adoption of this revised chapter.

1. Scope

Infection with decapod iridescent virus 1 means infection with the pathogenic agent decapod iridescent virus 1 (DIV1), Genus *Decapodiridovirus*, Subfamily *Betairidovirinae*, Family *Iridoviridae*.

2. Disease information

2.1. Agent factors

2.1.1. Aetiological agent

DIV1 is the only species of the genus *Decapodiridovirus* assigned to the subfamily *Betairidovirinae*, family *Iridovirus* (ICTV, 2023). DIV1 is a 150–158 nm, enveloped icosahedral double-stranded DNA virus, with a linear genome of 165 kb composed of 34.6% G + C content and 170–178 putative open reading frames (ORFs) (Li *et al*., 2017; Qiu *et al*., 2017; 2018a; Xu *et al*., 2016). Although *Cherax quadricarinatus* iridovirus (CQIV) (Xu *et al*., 2016) and shrimp haemocyte iridescent virus (SHIV) (Qiu *et al*., 2017) have been reported from the redclaw crayfish (*C. quadricarinatus*), and the whiteleg shrimp (*L. vannamei*), respectively, they are classified as different isolates (strains) within the DIV1 species.

2.1.2. Survival and stability in processed or stored samples

DIV1-infected cephalothoraxes are infectious after homogenisation, centrifugation, filtration and storage at –80°C (Qiu *et al*., 2022a; Xu *et al.,* 2016).

2.1.3. Survival and stability outside the host

Not available.

For inactivation methods, see Section 2.4.5.

2.2. Host factors

2.2.1. Susceptible host species

Species that fulfil the criteria for listing as susceptible to infection with DIV1 according to chapter 1.5. *Aquatic Animal Health Code* (*Aquatic Code*) are: ~~fleshy prawn (*Penaeus chinensis*), gazami crab (*Portunus trituberculatus)*, giant river prawn (*Macrobrachium rosenbergii*), kuruma prawn (*Penaeus japonicus*), Oriental river prawn (*Macrobrachium nipponense*), red claw crayfish (*Cherax quadricarinatus*), red swamp crawfish (*Procambarus clarkii*), ridgetail prawn (*Palaemon carinicauda*), and whiteleg shrimp (*Penaeus vannamei*).~~

Family	Scientific name	Common name
Cambaridae	*Procambarus clarkii*	red swamp crawfish
Palaemonidae	*Macrobrachium nipponense*	Oriental river prawn
	Macrobrachium rosenbergii	giant river prawn
	Palaemon carinicauda	ridgetail prawn

Family	Scientific name	Common name
Parastacidae	*Cherax quadricarinatus*	red claw crayfish
Penaeidae	*Penaeus japonicus*	kuruma prawn
	Penaeus vannamei	whiteleg shrimp
Portunidae	*Portunus trituberculatus*	swimming crab

2.2.2. Species with incomplete evidence for susceptibility

Species for which there is incomplete evidence to fulfil the criteria for listing as susceptible to infection with DIV1 according to Chapter 1.5 of the *Aquatic Code* are: ~~giant tiger prawn (*Penaeus monodon*).~~

Family	Scientific name	Common name
Penaeidae	*Penaeus chinensis*	fleshy prawn
	Penaeus monodon	giant tiger prawn

In addition, pathogen-specific positive polymerase chain reaction (PCR) results have been reported in the following species, but an active infection has not been demonstrated: ~~channeled applesnail (*Pomacea canaliculata*), *Helice tientsinensis*, Japanese shore crab (*Hemigrapsus penicillatus*), *Macrobrachium superbum* and *Plexippus paykulli*.~~

Family	Scientific name	Common name
Ampullariidae	*Pomacea canaliculata*	channeled applesnail
Palaemonidae	*Macrobrachium superbum*	no common name
Salticidae	*Plexippus paykulli*	no common name
Varunidae	*Helice tientsinensis*	no common name
	Hemigrapsus penicillatus	Japanese shore crab

2.2.3. Likelihood of infection by species, host life stage, population or sub-populations

All live stages are potentially susceptible to infection; DIV1 has been detected in post-larvae (PL), juvenile and sub-adult stages of shrimp (*Penaeus vannamei*, *P. chinensis*, *Exopalaemon carinicauda*, *Macrobrachium nipponense, M. rosenbergii*, crayfish [*Cherax quadricarinatus*, *Procambarus clarkia*] and crab [*Portunus trituberculatus*]) as natural infection or by experimental (*per os*) exposure (Chen *et al*., 2019; Qiu *et al*.,-2018; 2019b; 2020b; 2021b; 2022b). Species with a positive DIV1 polymerase chain reaction (PCR) result, without an active infection include: *Penaeus monodon*, *Pomacea canaliculata, Macrobrachium superbum, Plexippus paykulli and Hemigrapsus penicillatus (*Qiu *et al.,* 2021; 2019a; 2022b; Srisala *et al.,* 2021).

2.2.4. Distribution of the pathogen in the host

The principal target tissues for DIV1 include lymphoid organ, haematopoietic tissues, as well as epithelia and haemocytes in gills, muscle, hepatopancreas, pereiopods, pleopods, uropods, and antenna (Qiu *et al*., 2017; 2019a; 2021a; Sanguanrut *et al*., 2021).

2.2.5. Aquatic animal reservoirs of infection

There is evidence that crustacean species may become reservoirs of DIV1 infection. DIV1 was detected in non-clinical adult wild giant tiger prawn (*P. monodon*) (Srisala *et al*., 2021), wild crabs (*Helis tientsinensis*, *Hemigrapsus penicillatus*) in drainage ditches (Qiu *et al*., 2022a), and *Macrobrachium superbum* in affected shrimp ponds (Qiu *et al*., 2019a).

Subclinical infection has been reported in gazami crab, *Portunus trituberculatus*, which is widely distributed in environmental waters in Asia and could be a potential source of DIV1 infection on shrimp farms (Qiu *et al.,* 2022a).

2.2.6. Vectors

There are no confirmed vectors of DIV1.

2.3. Disease pattern

2.3.1. Mortality, morbidity and prevalence

Mortality can be high (80–100%) after a natural infection with DIV1 and mostly reported in the adult stage of shrimp (Liao *et al.,* 2022) ~~in shrimp~~ and crayfish species, which has been confirmed by experimental infection through intramuscular injection or oral administration in *P. vannamei*, *Cherax quadricarinatus*, *Procambarus clarkii* and *Macrobrachium rosenbergii* (Qiu *et al*., 2017; 2019a; Xu *et al*., 2016). Experimental infection with DIV1 administered orally or by intramuscular injection resulted in 50% and 100% mortality, respectively, in the gazami crab (*Portunus trituberculatus*) (Qiu *et al*., 2022a).

In pathogenicity studies of crustacean species, mortalities rose more rapidly in *Litopenaeus vannamei* compared with *Cherax quadricarinatus* or *Procambarus clarkii* in experimental infections (Xu *et al*., 2016).

The prevalence of DIV1 infection was 15.5, 15.2, and 50% in *P. vannamei, P. chinensis*, and *M. rosenbergii,* respectively, in a survey of shrimp farms tested in the period 2014 to 2016 (Qiu *et al*., 2017).

2.3.2. Clinical signs, including behavioural changes

Clinical signs in affected whiteleg shrimp (*P. vannamei*) are reddish bodies, white atrophied hepatopancreas, soft shells and empty stomachs and intestines, while giant freshwater shrimp (*M. rosenbergii*) showed a white discoloration at the base of the rostrum (white head) and hepatopancreatic atrophy (Qiu *et al.,* 2017; 2019a). However, these disease signs are not always distinctive because the course of the disease varies in affected animals.

2.3.3 Gross pathology

See Section 2.3.2.

2.3.4. Modes of transmission and life cycle

Based on experimental and natural infections, DIV1 is thought to be transmitted horizontally by oral routes and contaminated water (Qiu *et al*., 2017; 2019a; 2022a; Xu *et al*., 2016).

2.3.5. Environmental factors

Temperature and co-culture play an important role in DIV1 infection. DIV1 has been detected in shrimp and crayfish reared at 16–32°C, but not at temperatures above 32°C in a 2017–2018 survey (Qiu *et al.,* 2018b; 2019b; 2020b; 2021b 2022b). In shrimp farm management, polyculture with different species of crustaceans increases the risk of DIV1 infection in farmed shrimp due to cross-species transmission (Qiu *et al*., 2019a; 2022a).

2.3.6. Geographical distribution

DIV1 has been reported in farmed shrimp and crayfish in the Asia-Pacific region (Qiu *et al*., 2017; Xu *et al*., 2016).

See WAHIS (https://wahis.woah.org/#/home) for recent information on distribution at the country level.

2.4. Biosecurity and disease control strategies

2.4.1. Vaccination

Not available.

2.4.2. Chemotherapy including blocking agents

Not available.

2.4.3. Immunostimulation

Not available.

2.4.4. Breeding resistant strains

Not available.

2.4.5. Inactivation methods

Not known.

2.4.6. Disinfection of eggs and larvae

Not available

2.4.7. General husbandry

Biosecurity practices can be used to reduce the risk of DIV1 infection. These includes PCR pre-screening of broodstock and larvae, PCR pre-screening of polychaetes and food organisms for broodstock and larvae, disinfection of rearing water and farming equipment, controlled stocking density, and avoidance of polyculture with different crustacean species.

Using an experimental protocol of 15-day thermal treatment at 36°C combined with 15-day restoration treatment at 28°C, *P. vannamei* infected by intramuscular injection of DIV1 showed no clinical signs, no DNA replication, no histopathology and *in-situ* DIG-labelling, loop-mediated DNA amplification (ISDL) results, indicating DIV1 could ~~can~~ be eliminated from challenged shrimp after 36°C treatment (Guo *et al*., 2022).

3. Specimen selection, sample collection, transportation and handling

This section draws on information in Sections 2.2, 2.3 and 2.4 to identify populations, individuals and samples that are most likely to be infected.

3.1. Selection of populations and individual specimens

For diagnosis during a disease outbreak, moribund and apparently healthy crustacean specimens of susceptible species (see Section 2.2.3) from the same ponds, especially in polyculture mode, are selected as samples for identification testing. Apparently healthy or even dead and dried samples from crustacean farms next to the affected farms can be used as sources of materials for examination (Qiu *et al*., 2019a). For surveillance in apparently healthy populations, all life stages of samples reared at 16–32°C should be suitable for testing (see Section 2.3.5)

Shrimp and crayfish that are 4–7 cm in body length provide the highest detection rate of DIV1 when used for examination (Qiu *et al*., 2018b ;2019b ;2020b; 2021b ;2022b).

3.2. Selection of organs or tissues

Suitable tissues for testing are lymphoid organ, haematopoietic tissues, muscle, gills, hepatopancreas, pereiopods, pleopods, uropods, and antennae (Qiu *et al*., 2017; 2019a; 2021a; Srisala *et al*., 2021). Quantitative virus analysis from different tissues of naturally infected *Macrobrachium rosenbergii* showed that muscle and hepatopancreas had lower virus load compared with that of the lymphoid organ, haematopoietic tissues, gills, pereiopods, pleopods, uropods and antennae (Qiu *et al*., 2019a).

3.3. Samples or tissues not suitable for pathogen detection

Autolytic and compound eyes samples are not suitable for PCR-based pathogen detection.

3.4. Non-lethal sampling

If non-lethal tissue sample types differ from recommended tissues (see Section 3.2.), or from the tissue samples used in validation studies, the effect on diagnostic performance should be considered.

3.5. Preservation of samples for submission

For guidance on sample preservation methods for the intended test methods, see Chapter 2.2.0 *General information* (diseases of crustaceans).

3.5.1. Samples for pathogen isolation

The success of pathogen isolation and results of bioassay depend strongly on the quality of samples (time since collection and time in storage). Fresh specimens should be kept on ice and preferably sent to the laboratory within

24 hours of collection. To avoid degradation of samples, use alternative storage methods only after consultation with the receiving laboratory.

3.5.2. Preservation of samples for molecular detection

Tissue samples for PCR testing should be preserved in 70–90% (v/v) analytical/reagent-grade (undenatured) ethanol. The recommended ratio of ethanol to tissue is 10:1 based on studies in terrestrial animal and human health. The use of lower grade (laboratory or industrial grade) ethanol is not recommended. If material cannot be fixed, it may be frozen.

Standard sample collection, preservation and processing methods for molecular techniques can be found in Section B.5.5 of Chapter 2.2.0 *General information* (diseases of crustaceans).

3.5.3. Samples for histopathology, immunohistochemistry or *in-situ* hybridisation

Standard sample collection, preservation and processing methods for histological techniques can be found in Section B.5.3 of Chapter 2.2.0 *General information* (diseases of crustaceans).

3.5.4. Samples for other tests

Not available

3.6. Pooling of samples

Pooling of samples from more than one individual animal for a given purpose is only recommended where robust supporting data on diagnostic sensitivity and diagnostic specificity have been evaluated and found to be suitable. If the effect of pooling on diagnostic sensitivity has not been thoroughly evaluated, larger shrimp (or other decapod crustaceans) should be processed and tested individually. Small life stages such as larvae or PLs can be pooled to obtain the minimum amount of material for virus isolation or molecular detection.

4. Diagnostic methods

The methods currently available for pathogen detection that can be used in i) surveillance of apparently healthy animals, ii) presumptive diagnosis in clinically affected animals and iii) confirmatory diagnostic purposes are listed in Table 4.1. by animal life stage.

Ratings for purposes of use. For each recommended assay a qualitative rating for the purpose of use is provided. The ratings are determined based on multiple performance and operational factors relevant to application of an assay for a defined purpose. These factors include appropriate diagnostic performance characteristics, level of assay validation, availability cost, timeliness, and sample throughput and operability. For a specific purpose of use, assays are rated as:

+++ = Methods are most suitable with desirable performance and operational characteristics.

++ = Methods are suitable with acceptable performance and operational characteristics under most circumstances.

\+ = Methods are suitable, but performance or operational characteristics may limit application under some circumstances.

Shaded boxes = Not appropriate for this purpose.

Validation stage. The validation stage corresponds to the assay development and validation pathway in chapter 1.1.2. The validation stage is specific to each purpose of use. Where available, information on the diagnostic performance of recommended assays is provided in Section 6.3.

WOAH Reference Laboratories welcome feedback on diagnostic performance of recommended assays, in particular PCR methods. Of particular interest are any factors affecting expected assay sensitivity (e.g. tissue components inhibiting amplification) or expected specificity (e.g. failure to detect particular genotypes, detection of homologous sequences within the host genome). These issues should be communicated to the WOAH Reference Laboratories so that advice can be provided to diagnostic laboratories and the standards amended if necessary.

Table 4.1. *WOAH recommended diagnostic methods and their level of validation for surveillance of apparently healthy animals and investigation of clinically affected animals*

Method [amend or shade in as relevant]	**Surveillance of apparently healthy animals**				**Presumptive diagnosis of clinically affected animals**				**Confirmatory diagnosis1 of a suspect result from surveillance or presumptive diagnosis**			
	Early life stages[2]	Juveniles[2]	Adults	LV	Early life stages[2]	Juveniles[2]	Adults	LV	Early life stages[2]	Juveniles[2]	Adults	LV
Wet mounts												
Histopathology						++	++	1				
Cell culture												
Real-time PCR	++	+++	+++	NA	+++	+++	+++	1	+++	+++	+++	1
Conventional PCR	++	++	++	NA	++	++	++	NA				
Conventional nested PCR followed by amplicon sequencing									+	+	+	1
Conventional PCR followed by amplicon sequencing									+++	+++	+++	1
In-situ hybridisation						++	++	1		+++	+++	1
Bioassay					+	+	+	NA				
LAMP	+	+	+	NA	+	+	+	NA				
Quantitative LAMP	++	++	++	NA	++	++	++	1				
Ag-ELISA												
RPA	++	++	++	NA	++	++	++	1				
Other methods[3]												

LV = level of validation, refers to the stage of validation in the WOAH Pathway (chapter 1.1.2); NA = not available;
PCR = polymerase chain reaction; LAMP = loop-mediated isothermal amplification
Ag-ELISA = antigen enzyme-linked immunosorbent assay; RPA = recombinase polymerase amplification
[1]For confirmatory diagnoses, methods need to be carried out in combination (see Section 6). [2]Susceptibility of early and juvenile life stages is described in Section 2.2.3.
[3]Specify the test used. Shading indicates the test is inappropriate or should not be used for this purpose.

4.1. Wet mounts

Not relevant

4.2. Histopathology and cytopathology

Histopathological examination revealed pathognomonic dark eosinophilic cytoplasmic inclusion bodies in the karyopyknotic cells of haemopoietic tissues and lymphoid organs, and in the haemocytes of gills, pereopods and sinus of the hepatopancreas (Qiu *et al.,* 2017; 2019a), as well as cuticular epithelium under the cuticles (Chen *et al.,* 2019).

4.3. Cell culture for isolation

Not available.

4.4. Nucleic acid amplification

PCR assays should always be run with the controls specified in Section 5.5 '*Use of molecular and antibody-based techniques for confirmatory testing and diagnosis'* of Chapter 2.2.0 *General information* (diseases of crustaceans). Each sample should be tested in duplicate.

Extraction of nucleic acids

Different kits and procedures can be used for nucleic acid extraction. The quality and concentration of the extracted nucleic acid is important and can be checked using a suitable method as appropriate to the circumstances.

4.4.1. Real-time PCR

Table 4.4.1.1. Primers and probes (sequences) and cycling conditions for DIV1 real-time PCR

Target gene	Primer/probe (5'–3')	Concentration	Cycling parameters[(a)]
Method 1: Qiu *et al.,* 2018a; GenBank Accession No.: MF599468.1			
ATPase	SHIV-F: AGG-AGA-GGG-AAA-TAA-CGG-GAA-AAC SHIV-R: CGT-CAG-CAT-TTG-GTT-CAT-CCA-TG Probe: FAM-CTG-CCC-ATC-TAA-CAC-CAT-CTC-CCG-CCC-TAMRA	500 nM 200 nM	40 cycles of 95°C/100 sec and 60°C/30 sec
Method 2: Qiu *et al.,* 2020a; GenBank Accession No.: MF599468.1			
MCP	142F: AAT-CCA-TGC-AAG-GTT-CCT-CAG-G 142R: CAA-TCA-ACA-TGT-CGC-GGT-GAA-C Probe: FAM- CCA-TAC-GTG-CTC-GCT-CGG-CTT-CGG-TAMRA	500 nM 200 nM	40 cycles of 95°C/10 sec and 60°C/30 sec
Method 3: Gong *et al.,* 2021; GenBank Accession No.: MF599468.1			
ATPase	DIV1-F: AGG-AAA-GGA-AAC-GAA-AGA-AAT-TAT-ACC DIV1-R: GCT-TGA-TCG-GCA-TCC-TTG-A Probe: FAM-CAC-ATG-ATT-TGC-AAC-AAG-CTT-CCA-GCA-TAMRA	400 nM 200 nM	40 cycles of: 95°C/10 sec and 60°C/30 sec

[(a)]A denaturation step prior to cycling has not been included.

4.4.2. Conventional PCR/nested PCR

Table 4.4.2.1. Primer sequences and cycling conditions for DIV1 PCR and nested PCR

Target gene	Primer (5'–3')	Concentration	Cycling parameters[a]
Method 1: Xu *et al.,* 2016; GenBank Accession No.: ; amplicon size: 103 bp			
MCP	CQIV-MCP-F: GAA-ACT-TTA-TGC-ACA-ATC-TTA-T CQIV-MCP-R: CCA-ATC-ATG-TTG-TCG-TAT-CC	NA	25 cycles of: 94°C/30 sec, 55°C/30 sec and 72°C/30 sec
Method 2: Qiu *et al.,* 2017; GenBank Accession No.: KY618040; amplicon size: 457 and 129 bp			
ATPase	Primary step: SHIV-F1: GGG-CGG-GAG-ATG-GTG-TTA-GAT SHIV-R1: TCG-TTT-CGG-TAC-GAA-GAT-GTA Nested PCR: SHIV-F2: CGG-GAA-ACG-ATT-CGT-ATT-GGG SHIV-R2: TTG-CTT-GAT-CGG-CAT-CCT-TGA	400 nM 400 nM	Primary and nested steps: 95°C/3 min; 35 cycles of 95°C/30 sec, 59°C/30 sec and 72°C/30 sec

[a]A denaturation step prior to cycling has not been included.

4.4.3. Other nucleic acid amplification methods

Table 4.4.3 Primers and probes (sequences) for DIV1 LAMP, RPA and qLAMP

Method / Target gene	Primer (5'–3')	Concentration	Cycling parameters[a]/ method
Method 1: Chen *et al.,* 2019; GenBank Accession No.:xxx			
LAMP/ DNA-directed RNA polymerase II	SHIV-FIP (F1C + F2): TGG-GGT-TTC-ATA-TGG-GCA-AA T-GAT-TTT-AAG-AAT-GGA-AAG-ATC-CTA-TCA-GC SHIV-BIP (B1C + B2): AGG-AGA-AAA-GGT-TGG-ATT-GGT-TAC-TTT-TAC-TTC-TGT-TAC-TGC-GAT-GG SHIV-LF: GAG-AGG-CGT-GCA-ACT-TTC-TG SHIV-LB: TTT-GGC-ATT-GTC-TGC-TAC-AAT-TTC-C SHIV-F3: GAT-GGC-CAT-TCC-TTC-AAA-C SHIV-B3: AAA-ATA-GTC-ATC-CTG-AAA-TCC-T	1600 nM 1600 nM 800 nM 800 nM 200 nM 200 nM	60 cycles of: 60°C 85°C/5 min:
Method 2: Chen *et al.,* 2020; GenBank Accession No.:xxx			
RPA/ MCP	RPA-F : CAG-ATC-AGA-GCG-CAT-TCG-ATC-CCA-TAG-GCA-CCG-C RPA-R: CGT-AAG-AGA-ACA-TGT-GGT-ATC-CGG-TGA-GTT-CGG-G RPA- Probe: ATA-CGA-ATC-TTC-AGA-TCG-TAT-TCC-CGT-GA(FAM-dT)G(THF)C(BHQ1-dT)GCC-GAT-TAC-TTC-TC (phosphorylation)	400 nM 400 nM 120 nM	40 cycles of: 39°C/45 sec, and 39°C/15 sec:
Method 3: Gong *et al.,* 2021; GenBank Accession No.:xxx			
qLAMP/ ATPase	F3: GGC-TTG-GTA-TCT-TAT-TCA-GAG-AT B3: ATT-CAC-AAC-ATC-GTC-ACC-AT FIP: CTC-TTG-ATG-GAT-ACA-CTG-ATC-TTC-GGA-GCC-AGA-GAT-TGT-AAC-GG BIP: ATT-CAG-TAT-TCA-AGG-ATT-GGT-TCA-AAA-GTT-CTT-CCA-TCT-ACC-TCT-C LF: TTC-GGT-ACG-AAG-ATG-TAG-C LB: GAA-GAG-TAT-CCT-AAT-ATG-ACC-ATC-C	200 nM 200 nM 1600 nM 1600 nM 800 nM 800 nM	63°C/30 sec 40 cycles of: 63°C/60 sec:

[a]A denaturation step prior to cycling has not been included.

4.5. Amplicon sequencing

The size of the PCR amplicon should be verified, for example, by agarose gel electrophoresis. Both DNA strands of the PCR product must be sequenced and analysed in comparison with reference sequences.

4.6. *In-situ* hybridisation

In-situ hybridisation has been applied to paraffin sections to determine the specific location of DIV1 in target tissues by either DIG-labelled oligonucleotide probe or DIG-labelling-loop-mediated DNA amplification (ISDL) (Chen *et al*., 2019; ~~Xu *et al*., 2016~~ Sanguanrut *et al.,* 2021). ISDL is the preferred method to use because it is highly sensitive through simultaneous pathogen DNA amplification and labelling techniques, compared with routine probe-based *in-situ* hybridisation.

4.7. Immunohistochemistry

Not available.

4.8. Bioassay

Bioassay has application in presumptive diagnosis, but cost, accuracy, labour, timing, or other factors limit its application (Qiu *et al*., 2017; Xu *et al*., 2016).

4.9. Antibody- or antigen-based detection methods (ELISA, etc.)

Not available.

4.10. Other methods

Not available.

5. Test(s) recommended for surveillance to demonstrate freedom in apparently healthy populations

Any of the real-time PCR assays is recommended for surveillance to demonstrate freedom in apparently health populations.

6. Corroborative diagnostic criteria

This section only addresses the diagnostic test results for detection of infection in the absence (Section 6.1.) or in the presence of clinical signs (Section 6.2.) but does not evaluate whether the infectious agent is the cause of the clinical event.

The case definitions for a suspect and confirmed case have been developed to support decision making related to trade and confirmation of disease status at the country, zone or compartment level. Case definitions for disease confirmation in endemically affected areas may be less stringent. If a Competent Authority does not have the capability to undertake the necessary diagnostic tests it should seek advice from the appropriate WOAH Reference Laboratory, and if necessary, refer samples to that laboratory for confirmatory testing of samples from the index case in a country, zone or compartment considered free.

6.1. Apparently healthy animals or animals of unknown health status[1]

Apparently healthy populations may fall under suspicion, and therefore be sampled, if there is an epidemiological link(s) to an infected population. Hydrographical proximity to, or movement of animals or animal products or equipment, etc., from a known infected population equate to an epidemiological link. Alternatively, healthy populations are sampled in surveys to demonstrate disease freedom.

6.1.1. Definition of suspect case in apparently healthy animals

The presence of infection with DIV1 shall be suspected if at least one of the following criteria is met:

i) Positive result by real-time PCR

ii) Positive result by conventional PCR,

1 For example transboundary commodities.

iii) Positive result by LAMP

iv) Positive result by RPA

6.1.2. Definition of confirmed case in apparently healthy animals

The presence of infection with DIV1 is considered to be confirmed if at least one of the following criteria is met:

i) Positive result by real-time PCR followed by conventional PCR and amplicon sequencing.

ii) Positive result by real-time PCR followed by conventional nested PCR and amplicon sequencing.

~~iii) A positive result from each of two different real-time PCR methods~~

6.2 Clinically affected animals

Clinical signs are not pathognomonic for a single disease; however, they may narrow the range of possible diagnoses.

6.2.1. Definition of suspect case in clinically affected animals

The presence of infection with DIV1 shall be suspected if at least one of the following criteria is met:

i) Gross pathology or clinical signs associated with the disease as described in this chapter, with or without elevated mortality

ii) Positive result by real-time PCR

iii) Positive result by conventional PCR

iv) Positive result by LAMP

v) Positive result by RPA

vi) Histopathological changes consistent with the presence of the pathogen or the disease

vii) Positive result by *in-situ* hybridisation

6.2.2. Definition of confirmed case in clinically affected animals

The presence of infection with DIV1 is considered to be confirmed if at least at least one of the following criteria is met:

i) Positive result by real-time PCR and positive result by conventional PCR followed by amplicon sequencing

ii) Positive result by real-time PCR and positive result by conventional nested PCR and amplicon sequencing

iii) Positive result by real-time PCR and positive result by *in-situ* hybridisation

~~iv) A positive result from each of two different real-time PCR methods~~

6.3. Diagnostic sensitivity and specificity for diagnostic tests

The diagnostic performance of tests recommended for surveillance or diagnosis of infection with DIV1 are provided in Tables 6.3.1. and 6.3.2 (no data are currently available for either). Data are only presented where tests are validated to at least level 2 of the validation pathway described in Chapter 1.1.2. and the information is available within published diagnostic accuracy studies.

6.3.1. For presumptive diagnosis of clinically affected animals

Test type	Test purpose	Source populations	Tissue or sample types	Species	DSe (*n*)	DSp (*n*)	Reference test	Citation

DSe = diagnostic sensitivity, DSp = diagnostic specificity, *n* = number of ~~samples~~ animals used in the validation study,
PCR: = polymerase chain reaction.

6.3.2. For surveillance of apparently healthy animals

Test type	Test purpose	Source populations	Tissue or sample types	Species	DSe (*n*)	DSp (*n*)	Reference test	Citation

DSe = diagnostic sensitivity, DSp = diagnostic specificity, *n* = number of ~~samples~~ animals used in the validation study
PCR: = polymerase chain reaction.

7. References

CHEN Z., HUANG J., ZHANG F., ZHOU Y. & HUANG H. (2020). Detection of shrimp hemocyte iridescent virus by recombinase polymerase amplification assay. *Mol. Cell. Probes*, **49**, 101475.

CHEN X., QIU L., WANG H., ZOU P., DONG X., LI F. & HUANG J. (2019). Susceptibility of *Exopalaemon carinicauda* to the infection with shrimp hemocyte iridescent virus (SHIV 20141215), a strain of decapod iridescent virus 1 (DIV1). *Viruses*, **11**, 387.

GONG H.Y., LI Q.Y., ZHANG H., YE L., SHI L. & FENG Y.H. (2021). Development and comparison of qPCR and qLAMP for rapid detection of the decapod iridescent virus 1 (DIV1). *J. Invert. Pathol.*, **182**, 107567

GUO X.M., QIU L., GAO W, WANG G.H., CHEN X. & HUANG J. (2022). Radical thermal therapy against infection with decapod iridescent virus 1 (DIV1). *Aquaculture*, **561,** 738636.

INTERNATIONAL COMMITTEE OF TAXONOMY ON VIRUSES (ICTV) (2023). Genus: Decapodiridovirus. https://ictv.global/report/chapter/iridoviridae/iridoviridae/decapodiridovirus

LI F., XU L. & YANG F. (2017). Genomic characterization of a novel iridovirus from redclaw crayfish *Cherax quadricarinatus*: evidence for a new genus within the family *Iridoviridae*. *J. Gen. Virol.*, **98**, 2589–2595.

LIAO X., HE J. & LI C. (2022). Decapod iridescent virus 1: An emerging viral pathogen in aquaculture. *Rev. Aquaculture*, **14**, 1779–1789.

QIU L., CHEN X., GAO W, GUO X.M., XIE G.S., GONG M. & HUANG J. (2022a). Confirmation of susceptibility of swimming crab to infection with decapod iridescent virus 1. *Aquaculture*, **548**, 737607.

QIU L., CHEN, X., GAO W., LI C., GUO X.M., ZHANG Q.L. & HUANG J. (2021a). Molecular epidemiology and histopathological study of a natural infection with decapod iridescent virus 1 in farmed white leg shrimp, *Penaeus vannamei*. *Aquaculture,* **533**, 736105.

QIU L., CHEN X., GUO X.M., GAO W., ZHAO R.H., ZHANG Q.L., YANG B. & HUANG J. (2020a). A TaqMan probe based real-time PCR for the detection of Decapod iridescent virus 1. *J. Invertebr. Pathol.*, **173**, 107367. doi: 10.1016/j.jip.2020.107367.

QIU L., CHEN M.M., WAN X.Y., LI C., ZHANG Q.L., WANG R.Y. & HUANG J. (2017). Characterization of a new member of Iridoviridae, shrimp hemocyte iridescent virus (SHIV), found in white leg shrimp (*Litopenaeus vannamei*). *Sci. Rep.*, **7**, 11834.

QIU L., CHEN M.M., WAN X.Y., ZHANG Q.L., LI C., DONG X. & HUANG J. (2018a). Detection and quantification of shrimp hemocyte iridescent virus by TaqMan probe based real-time PCR. *J. Invert. Pathol.*, **154**, 95–101.

QIU L., CHEN X., ZHAO R.H., LI C., GAO W., ZHANG Q.L. & HUANG J. (2019a). Description of a natural infection with decapod iridescent virus 1 in farmed giant freshwater prawn, *Macrobrachium rosenbergii. Viruses,* **11**, 354.

QIU L., DONG X., WAN X.Y. & HUANG J. (2018b). Analysis of iridescent viral disease of shrimp (SHID). *In:* Analysis of Important Diseases of Aquatic Animals in China in 2017 (in Chinese). Fishery and Fishery Administration Bureau under the Ministry of Agriculture and Rural Affairs, National Fishery Technical Extension Center, Eds., China Agriculture Press, Beijing, 187–204, ISBN 978-7-109-24522-8.

QIU L., DONG X., WAN X.Y. & HUANG J. (2019b). Analysis of iridescent viral disease of shrimp (SHID) in 2018. *In:* Analysis of Important Diseases of Aquatic Animals in China in 2018. Fishery and Fishery Administration Bureau under the Ministry of Agriculture and Rural Affairs, National Fishery Technical Extension Center, Eds., (in press) (in Chinese).

QIU L., DONG X., WAN X. Y., ZHANG Q.-L. & HUANG J. (2020b). Analysis of iridescent viral disease of shrimp (SHID) in 2019. *In:* Bureau of Fisheries, Ministry of Agriculture and Rural Affairs, National Fisheries Technology Extension Center (Ed.), 2020 Analysis of Important Diseases of Aquatic Animals in China. China Agriculture Press, Beijing, pp. 185–200 (in Chinese).

QIU L., DONG X., WAN X. Y., ZHANG Q.-L. & HUANG J. (2021b). Analysis of iridescent viral disease of shrimp (SHID) in 2020. *In:* Bureau of Fisheries, Ministry of Agriculture and Rural Affairs, National Fisheries Technology Extension Center (Ed.), 2021 Analysis of Important Diseases of Aquatic Animals in China. China Agriculture Press, Beijing, pp. 182–196 (in Chinese).

QIU L., DONG X., WAN X. Y. & ZHANG Q.-L. (2022b). Analysis of infection with Decapod iridescent virus 1 (iDIV1) in 2021. *In:* Bureau of Fisheries, Ministry of Agriculture and Rural Affairs, National Fisheries Technology Extension Center (Ed.), 2022 Analysis of Important Diseases of Aquatic Animals in China. China Agriculture Press, Beijing, pp. 161–174 (in Chinese).

SANGUANRUT P., THAIUE D., THAWONSUWAN J., ALDAMA-CANO D.J., FLEGEL T.W. & SRITUNYALUCKSANA K. (2021). The lymphoid organ (LO) is an additional, prime target for decapod iridescent virus 1 (DIV1) in *Penaeus vannamei*. *Aquaculture*, **547**, 737482.

RISALA J., SANGUANRUT P., THAIUE D., LAIPHROM S., SIRIWATTANO J., KHUDET J., THAIUE D., POWTONGSAK S., FLEGEL T.W. & SRITUNYALUCKSANA K. (2021). Infectious myonecrosis virus (IMNV) and decapod iridescent virus 1 (DIV1) detected in captured, wild *Penaeus monodon*. *Aquaculture,* **545**, 737262.

XU L., WANG T., LI F. & YANG F. (2016) Isolation and preliminary characterization of a new pathogenic iridovirus from redclaw crayfish *Cherax quadricarinatus*. *Dis. Aquat. Org*., **120**, 17–26.

*
* *

NB: There is a WOAH Reference Laboratory for infection with decapod iridescent virus 1
(please consult the WOAH web site:
https://www.woah.org/en/what-we-offer/expertise-network/reference-laboratories/#ui-id-3).
Please contact the WOAH Reference Laboratories for any further information on
infection with decapod iridescent virus 1

NB: FIRST ADOPTED IN 20XX.

SECTION 2.4.

DISEASES OF MOLLUSCS

EU position

The EU supports the adoption of this revised chapter.

CHAPTER 2.4.0.

GENERAL INFORMATION

A. SAMPLING

1. Assessing the health status of the epidemiological unit

1.1. Sample material to be used for tests

Sample material and the number of samples to be collected depend on the specific disease or pathogen, the size of animals and the objective of testing (i.e. surveillance of apparently healthy animals, presumptive diagnosis of clinically affected animals or confirmatory diagnosis of a suspect result from surveillance or presumptive diagnosis). See individual disease chapters in this *Aquatic Manual* for specific details of sample requirements.

1.2. Specifications according to mollusc populations

For details of animals to sample for a specific listed disease, see the relevant disease chapter in this *Aquatic Manual.* The design of a surveillance system for demonstrating disease-free status for a country, zone or compartment should be in accordance with the recommendations of the WOAH *Aquatic Code* Chapter 1.4. *Aquatic animal disease surveillance*.

The following factors should be considered when selecting animals to be sampled:

i) for apparently healthy populations, susceptible species should be sampled proportionately or following risk-based criteria for targeted selection of lots, epidemiological units or populations with a history of abnormal mortality or potential exposure events (e.g. stocking with animals of unknown disease status);

ii) If weak, abnormally behaving or freshly dead (not decomposed) animals are present, such animals should be selected. If such animals are not present, animals should be selected in such a way that all epidemiological units of the farm or waterbody are proportionately represented in the sample;

iii) if more than one water source is used for production, animals from all water sources should be included in the sample.

1.3. Specifications according to clinical status

In addition to sampling of target tissues, other organs showing macroscopic abnormalities or lesions should also be sampled. For disease outbreaks, at least ten diseased or moribund molluscs should be sampled for testing. Parallel samples (n > 10) from apparently normal animals in the same production region should also be collected. Collection of dead specimens during disease outbreaks should be avoided when possible, but recently dead samples may be suitable for some diagnostic assays

provided the animals are not decomposed. Disease-specific recommendations are provided in Section 3 *Sample selection, sample collection, transportation and handling* of the individual chapters.

1.4. Specifications according to mollusc size

For the WOAH-listed diseases it is recommended that the scheduling of sampling be planned (i.e. by farm schedule, season, etc.) so that the particular life-stage(s) are sampled at a time when the pathogen of concern is most likely to be detected.

1.4.1. For the listed parasites

Juveniles below 1.5 cm: sample the entire animal but remove the shell when possible or proceed with a decalcification protocol. When animals are too small for individual analyses, analyses can be performed on pools of several animals.

Juveniles 1.5–3 cm: sample the entire mollusc and cut in half sagittally. Keep one half of the animal for histological analyses and the other half for molecular analyses.

Molluscs over 3 cm: take a cross-section of the body, passing through the mantle, gills, digestive gland and gonads for histological analyses. Keep the remaining tissues for molecular analyses.

1.4.2. For infection with *Xenohaliotis californiensis*

For abalone ≥20 mm, excise several 3–5 mm cross sections containing posterior oesophagus (postoesophagus), digestive gland, and foot muscle.

1.4.3. For abalone herpesvirus infections

Sample as outlined in Section 1.4.2 above with the addition of a cross section of the head to obtain the cerebral ganglion and removal of several sections of the foot and adductor muscle complex including one section 0.25–1.0 cm (distance depends on abalone maximum length) posterior to the head to obtain the pedal ganglion. In addition, a longitudinal section from the anterior pedal ganglion to the posterior portion of the pedal musculature should be taken.

2. General processing of samples

Sampled molluscs should be delivered alive to the diagnostic laboratory. The laboratory should be informed of the estimated time of arrival of the sample so the required materials to process the molluscs can be prepared before receipt of the samples.

Mollusc samples should be packed appropriately in order to keep them alive. Required samples should be shipped as soon as possible after collection from the water. Unless otherwise specified, moribund animals should be sent on ice (but not frozen) to reduce sample decomposition.

For samples that cannot be delivered live to the diagnostic laboratory, specimens should be fixed on site as recommended in the following sections of this chapter or the relevant disease chapters of this *Aquatic Manual*. While this may be suitable for subsequent histology, transmission electron microscopy examination or PCR analyses for example, other techniques, such as fresh smears, tissue imprints, routine bacteriology, mycology or Ray's fluid thioglycollate culture of *Perkinsus* spp., cannot be performed on such samples. Diagnostic needs and sample requirements should be discussed with the diagnostic laboratory prior to collection of the sample.

2.1. Macroscopic examination

The gross observation of molluscs should target, as far as possible, animal behaviour, shell surface, inner shell and soft tissues.

It is often difficult to observe the behaviour of molluscs in open systems. However, observation of molluscs in certain rearing facilities, such as broodstock in tanks and larvae in hatcheries, can provide useful indications of disease-related behavioural changes. If signs are noted (e.g. pre-settlement of larvae on the bottom, food accumulation in tanks, signs of weakening, etc.), samples may be examined for gross signs, including observation under a dissecting microscope for abnormalities and deformities, fouling organisms, and fixed for further processing as recommended below. For adults and juveniles, signs of weakening may include gaping, accumulation of sand, mud and debris in the mantle and on the gills, mantle retraction away from the edge of the shell, decreased activity (scallop swimming, clam burrowing, abalone grazing), etc. The righting reflex

of abalone after being inverted does not occur in weakened animals, and it is a good indicator of weakness. Mortality in open systems should be monitored for patterns of losses, and samples should be collected for further analysis. Environmental factors, pre- and post-mortality, should be recorded.

Even under culture conditions, the shells of molluscs may not be clean and fouling organisms are normal colonists of mollusc shell surfaces. Organisms such as barnacles, limpets, sponges, polychaete worms, bivalve larvae, tunicates, bryozoans, etc., do not normally threaten the health of molluscs. Culture systems, such as suspension and shallow water culture, can even increase exposure to fouling organisms and shells may become covered by other animals and plants. This can affect health directly by impeding shell opening and closing or indirectly through competition for food resources. Signs of weakening associated with heavy fouling should be a cause for concern rather than fouling itself. Shell damage by boring organisms, such as sponges and polychaete worms, are usually benign, but under certain conditions may reach proportions that make the shell brittle or pierce through to the soft tissues. This degree of shell damage can weaken the mollusc and render it susceptible to pathogen infections. Shell deformities (shape, holes in the surface), fragility, breakage or repair should be noted, but may not be indicative of a disease concern. Burrowing epibionts may cause deformities and weaken the shell(s). Abnormal coloration and smell may indicate a possible soft-tissue infection that may need to be examined at a laboratory.

The molluscs should be opened carefully so as not to damage the soft tissues, in particular the mantle, gills, heart and digestive gland. The presence of fouling organisms on the inner shell surface is a clear indication of weakness. The inner surface of the shell is usually smooth and clean because of mantle and gill action. Perforation of the inner surface may occur but can be sealed off by the deposition of additional conchiolin and nacre. This may result in formation of mud- or water-filled blisters. Blisters may also form over superficial irritants such as foreign bodies. The degree of shell perforation can be determined by holding the shell up to a strong light. Where abnormalities occurring within the matrix of the shell warrant further investigation, freshly collected specimens can be brought intact to the laboratory or fixed for subsequent decalcification, as required. The appearance of the soft tissues is frequently indicative of the physiological condition of the animal. Soft tissues should be examined for the presence of abscess lesions, pustules, tissue discoloration, pearls, oedema, overall transparency or wateriness, gill deformities, etc., and, when found in association with weak or dying animals. Abnormalities and lesions of the tissues should be noted and recorded, as well as any shell deformities, shell-boring organisms and conspicuous mantle inhabitants. Levels of tissue damage should be recorded and samples of affected and unaffected animals collected for laboratory examination as soon as possible.

2.2. Virological examination

See Chapter 2.4.1. Infection with abalone herpesvirus for specific details.

2.3. Bacteriological examination

See Chapter 2.4.7. Infection with *Xenohaliotis californiensis* for specific details.

2.4. Parasitic (protists) examination

See Chapters 2.4.2 to 2.4.6. Infections with listed protists for specific details.

2.5. Fungal examination

Not applicable for currently listed diseases.

B. MATERIALS AND BIOLOGICAL PRODUCTS REQUIRED FOR THE ISOLATION AND IDENTIFICATION OF MOLLUSC PATHOGENS

1. Mollusc viruses

1.1. Mollusc cell lines

Not applicable. There are currently no confirmed or documented mollusc cell lines suitable for virus isolation.

1.2. Culture media

Not applicable.

1.3. Virus positive controls and antigen preparation

1.3.1. Virus nomenclature

In general, the virus nomenclature used in the disease-specific chapters follows the most recent taxonomy for viruses as given in the Report of the Committee on Taxonomy of Viruses (see: ICTV [ictvonline.org] for latest information).

1.3.2. Virus production for experimental purposes

As no cell lines are known that can be used to produce mollusc virus stocks, infection of known susceptible host species (which are free of infection with the pathogenic agent in question) is the preferred method for virus production for experimental purposes or for the production of positive control material.

1.3.3. Virus preservation and storage

Infectivity of all of the WOAH-listed mollusc viruses can be preserved by freezing infected whole molluscs or infected target tissues at –20°C for short-term storage, or at –80°C or lower for long-term storage.

2. Mollusc bacteria

Not applicable. There is currently no developed procedure to cultivate *Xenohaliotis californiensis*.

3. Mollusc parasites (protists)

3.1. Culture media

See Chapters 2.4.5 Infection with *Perkinsus marinus* and 2.4.6 Infection with *Perkinsus olseni* for details.

3.2. Storage of cultures

Perkinsus spp. cultures in the exponential phase of growth can be pelleted by centrifugation and cryopreserved by resuspending the pellet in 40% DMEM Ham's F-12 (1:1) culture medium with 10% glycerol and 50% FBS and freezing them using standard procedures.

4. Mollusc fungi

4.1. Culture media

Not applicable for currently listed diseases.

4.2. Storage of cultures

Not applicable for currently listed diseases.

5. Techniques

The available diagnostic methods that may be selected for diagnosis of the WOAH-listed mollusc diseases or detection of their aetiological agents are based on:

i) Gross and clinical signs.

ii) Direct bright-field, phase-contrast or dark-field microscopy with whole stained or unstained tissue wet-mounts, tissue squashes, and impression smears.

iii) Histology, *in-situ* hybridisation and electron microscopy of fixed specimens.

iv) Culture methods where applicable.

v) Molecular methods (including sequencing): Conventional and real-time PCR and LAMP for direct assay with fresh, frozen or ethanol fixed-tissue samples or with extracted DNA.

Bioassays of suspect or subclinical carriers using a highly susceptible host (life stage or species) may also be used as an indicator for the presence of the pathogen.

Pooling of samples from more than one individual animal for a given purpose is only recommended where robust supporting data on diagnostic sensitivity and diagnostic specificity have been evaluated and found to be suitable. If the effect of pooling on diagnostic sensitivity has not been thoroughly evaluated, larger molluscs should be processed and tested individually. However, for eggs, larvae and postlarvae, pooling of individuals may be necessary to obtain sufficient sample material to run a diagnostic assay.

5.1. Gross and clinical signs

Macroscopic examination of gross and clinical signs reveals non-specific signs only (e.g. gaping in bivalves or general weakness of the foot muscle in abalone), and mortality may be caused by several disease agents or physiological problems, such as loss of condition following spawning. To obtain a definitive diagnosis further investigation is required and this can only be determined using a range of other techniques including histology/electron microscopy and molecular techniques such as PCR and gene sequence analysis.

5.2. Direct microscopy

Samples for direct microscopic examination should be examined as soon as possible after collection. Use live specimens whenever possible, or use fresh, chilled, or fixed specimens when live specimens are not practical. If an adequate field laboratory is available, it should be used to process and examine samples near the site of collection.

5.3. Histological techniques

Live moribund animals or freshly dead (within minutes) animals provide the optimal tissues for examination. Due to tissue lysis that occurs during the freeze-thaw cycle, frozen samples are not appropriate for histology. Should a delay between animal mortality and sampling occur, it is recommended that animals be stored intact on ice or in a refrigerator.

To obtain a sample that includes all the major tissues, a section should be taken to include digestive gland, gills, gonad, mantle and palps, where possible. For large specimens, it may be necessary to take several sections to include all the important tissues. Tissue preparation for examination by light microscopy involves several steps, including tissue fixation, dehydration, impregnation and embedding of samples, preparation of sections, staining and mounting of slides.

5.3.1. Tissue fixation

Tissue fixation is required to maintain the morphology of the tissues and to prevent post-sampling necrosis. Recommended fixatives used for the study of marine molluscs are Davidson's solution, Carson's solution and 10% formalin in filtered sea water. The ratio of fixative to tissue volume should be at least 10:1 to ensure good fixation.

Davidson's solution:	
1 µm filtered sea water	1200 ml
95% Alcohol	1200 ml
35–40% Formaldehyde[1]	800 ml
Glycerol	400 ml
Glacial acetic acid	10% (add just prior to use)

Carson's solution:	
$NaH_2PO_4.2H_2O$	23.8 g
Sodium hydroxide (NaOH)	5.2 g
Distilled water	900 ml
40% Formaldehyde[1]	100 ml

1 A saturated 37–39% aqueous solution of formaldehyde gas.

Adjust the pH to 7.2–7.4

10% formalin in filtered sea water solution:

1 µm filtered sea water	900 ml
35–40% Formaldehyde[1]	100 ml

These solutions allow tissue structure to be preserved and different histochemical methods to be used including for *in-situ* hybridisation with DNA probes. Over-fixation (over 24–48 hours) should be avoided. After fixation, the specimens should be transferred to 70% ethyl alcohol, where they can be stored indefinitely. Davidson's solution is normally used because it provides better preservation of the cell nuclei. Carson's solution or 10% formalin in seawater can be used to examine tissues by electron microscopy. As electron microscopy can be a valuable aid in diagnosing or confirming infections in bivalve molluscs, fixing some samples (particularly the smaller ones) with glutaraldehyde, as described in Section B.5.4.1 of this chapter, may be considered, and will provide electron micrographs of the highest quality. It is recommended that a representative portion of the mollusc is fixed in Davidson's solution, while another representative portion is fixed in Carson's solution for further examination to ensure that all tissues/organs are fixed in both fixatives. If neither is available, 10% formalin buffered with filtered seawater will suffice.

For transport and shipping, see *Aquatic Code* Chapter 5.10 *Measures concerning international transport of aquatic animal pathogens and pathological material*.

5.3.2. Dehydration, impregnation and embedding of the samples

The fixed samples are transferred through a series of graded alcohols (70–95% [v/v]) before final dehydration in absolute ethanol. The alcohol contained in the tissues is next eliminated by immersing them in xylene. The tissues are then impregnated with paraffin, which is soluble in xylene, at 60°C. These steps are often carried out automatically using a tissue processing machine. Should processing be delayed, fixed tissues may be stored in 70% ethanol.

Histological blocks are produced by letting the tissues cool in moulds filled with paraffin on a cooling table.

5.3.3. Preparation of the sections

After the blocks have cooled and the paraffin has solidified, histological sections of about 2–5 µm are cut using a microtome. The sections are recovered on histological slides, drained and dried for up to 1 hour at 40–42°C or overnight at room temperature.

5.3.4. Staining and mounting the slides

Before staining, the paraffin is removed from the sections by immersing them in xylene or equivalent clearing solution for 10–20 minutes. This is repeated once and then the solvent is eliminated by immersion in two successive absolute ethanol baths for 10-minute periods each, and they are then rehydrated through a descending series of ethanol baths (for example 95%, 70%, 50%, 30%, 10 minutes each) with a final immersion in tap water for 10 minutes. Different topographical or histochemical staining techniques can then be performed.

When haematoxylin–eosin (H&E) stain is used (haematoxylin or equivalent), nuclear and basophilic structures stain a blue-to-dark-purple colour, the endoplasmic reticulum stains blue, while the cytoplasm takes on a grey colour. The acid dye eosin stains the other structures pink. This staining technique is simple and reproducible and, although it only allows a limited differentiation of cell structures, it is possible to detect any abnormalities in tissue and cellular structure. Other techniques may be applied to demonstrate particular structures or features as required (e.g. trichrome for connective tissue and cytoplasmic granules).

5.4. Transmission electron microscopy methods

Transmission electron microscopy can be used as part of the diagnostic procedures for diseases of molluscs.

Fixation for electron microscopy should be done immediately after the animal has been killed and before fixation for histology. Only samples taken rapidly from live animals will be of any use. The preparation of samples for electron microscopy involves the following steps: tissue fixation, decalcification of the samples (when necessary), dehydration, impregnation and embedding of the samples, preparation and counterstaining of the sections.

5.4.1. Tissue fixation

For tissues that are to be examined by electron microscopy, it is important that the fixation be performed correctly in order to cause as little damage as possible to the ultrastructure. The specimens are cut such that their dimensions do not exceed 1–2 mm. This small size allows rapid penetration of the various solutions into the tissue sample.

Fixation is carried out directly in 3% glutaraldehyde for 1–4 hours. The samples are washed in buffer three times, then post-fixed in 1% osmic acid (aqueous OsO_4) and washed twice again in buffer. Various formulations of glutaraldehyde fixative and buffers work equally well.

In order to cause as little damage as possible to the ultrastructure, the samples are treated with solutions that have an osmolarity close to that of the tissues. Thus, mollusc tissues are treated with solutions with an osmolarity of approximately 1000 mOsm. The osmolarity of the solutions is adjusted with artificial sea salts or NaCl. Alternatively, the glutaraldehyde can be formulated with 0.22 µm filtered seawater, and filtered seawater used for subsequent washes.

Sodium cacodylate	0.4 M: 8.6 g in 100 ml of distilled water
Sodium chloride	10% in distilled water
Cacodylate buffer, pH 7.4:	
1000 mOsm	
Sodium cacodylate	50 ml from 0.4 M stock solution
NaCl	20 ml from 10% stock solution
Distilled water	30 ml
Adjust the pH to 7.4	
3% Glutaraldehyde:	
1000 mOsm	
25% glutaraldehyde	2.5 ml
0.4 M sodium cacodylate	5 ml
10% NaCl	3.5 ml
Distilled water	9 ml
1% Osmic acid:	
1000 mOsm	
4% Osmic acid	1 volume
0.4 M sodium cacodylate	1 volume
NaCl	1 volume from 10% stock solution
Distilled water	1 volume
5% ethylene diamine tetra-acetic acid (*EDTA*)*:*	
Disodium EDTA	5 g
Cacodylate buffer	100 ml

EDTA dissolves when the pH is above 8. When the solution becomes clear adjust the pH to 7.4 by adding concentrated HCl.

If the samples have been previously fixed and stored in Carson's solution, they should be washed several times in a bath of buffer before fixation with 3% glutaraldehyde.

5.4.2. Dehydration, impregnation and embedding of the samples

The samples are dehydrated in successive baths of ethanol: 70% ethanol once, 95% ethanol twice, absolute ethanol three times. The dehydration is completed by two baths of propylene oxide, which allows subsequent impregnation with Epon.

The samples are impregnated progressively. After a first bath in a mixture of polypropylene oxide–Epon (50/50), the samples are placed in a bath of Epon. The longer the incubation, the better the impregnation of the tissues.

Embedding is carried out by placing the samples in moulds filled with Epon resin. A label identifying the sample is included in each block and the blocks are then placed at 60°C (the temperature at which Epon resin polymerises) for 48 hours.

5.4.3. Preparation of the sections and the counterstaining

The blocks are cut to appropriate sizes with a razor blade and, using an ultra- microtome, semi-thin sections (0.5–1 µm) are cut and placed on glass slides. These will be used to monitor the quality of the samples by light microscopy and to locate the areas of interest on the section.

The semi-thin sections are stained at 90–100°C with 1% toluidine blue solution. After drying, the slides are mounted under cover-slips with a drop of synthetic resin and observed using the light microscope.

Ultra-thin sections 80–100 nm thick are placed on mesh copper grids for electron microscopy. Uranyl acetate and lead citrate are used to counterstain the ultra-thin sections.

5.5. Use of molecular techniques for surveillance, confirmatory testing and diagnosis

Molecular techniques, including the use of nucleic acid probes for *in-situ* hybridisation, conventional PCR and real-time PCR, have been developed for the identification of many pathogens of aquatic animals. Real-time PCR methods, in general, have high sensitivity and specificity and, following adequate validation, can be used for direct detection of pathogen nucleic acids in samples prepared from mollusc tissues. These techniques can be used in direct surveillance of apparently healthy populations, if they have a high level of diagnostic sensitivity, as well as in the diagnosis of clinically affected animals.

When using PCR as a diagnostic method, the design of primers and probe, the use of positive and negative controls, as well as validation of the PCR method chosen are important. Real-time PCR is a powerful technique particularly for analysing relatively high numbers of samples (e.g. for surveillance) via high-throughput testing. Several nucleic acid probe and PCR protocols are included in this version of the *Aquatic Manual* as screening, diagnostic or confirmatory methods for molluscs and can be undertaken as the standard method. Following real-time PCR-positive results, where possible, conventional PCR with sequence analysis of PCR products should be used for confirmation of pathogen identity.

As with all PCR protocols, optimisation may be necessary depending on the reagents, equipment and the plasticware used. PCR is prone to false-positive and false-negative results. False-positive results (negative samples giving a positive reaction) may arise from either product carryover from positive samples or, more commonly, from cross-contamination by PCR products from previous tests. Therefore, each assay and tissue extraction should include a negative control to rule out contamination. False-negative results (positive samples giving a negative result) may lead to unwanted transmission of pathogens and biosecurity failure. Therefore, each assay (and ideally each tissue extraction) should include positive controls to ensure the assay performed correctly. Additionally, mollusc tissues are known to potentially contain PCR inhibitors. It is therefore recommended to check for the presence of PCR inhibitors in DNA extracts to avoid false negative results.

To minimise the risk of contamination, aerosol barrier pipette tips should be used for all sample preparation and PCR steps. Additionally, all PCRs should be prepared in a clean area that is separate from the area where the nucleic acid extraction, amplification and gel electrophoresis are performed. Do not share equipment (e.g. laboratory coats and consumables) between areas and, where possible, restrict access between areas. Contaminating PCR products can be carried on equipment, clothes, shoes and paper (e.g. workbooks). Also, ensure all work-tops and air-flow hoods/cabinets used for the extractions and PCR set-up are regularly cleaned and decontaminated. To ensure sample integrity, always store the samples (e.g. in a freezer or refrigerator) in a location separate from the molecular biology laboratory and reagents.

Nested PCR involves two rounds of PCR and may be used to achieve increased sensitivity and specificity; however, it increases the risk of contamination. Contaminants from previous reactions can carry over and lead to false-positive results. Strict laboratory practices such as separate workspaces, dedicated equipment, and meticulous pipetting techniques are essential to mitigate this risk. In conclusion, nested PCR is not recommended for surveillance but may sometimes be used for confirmative studies.

5.5.1. Sample preparation

Samples should be prepared to preserve the nucleic acid of the pathogen and should be handled and packaged with the greatest care to minimise the potential for cross-contamination amongst the samples or target degradation before the assay can be performed. A water-resistant label, with the appropriate data filled out, should be placed within each package or container for each sample set. Use of household permanent markers should be avoided as

their ink dissolves in ethanol and may result in loss of the sample label. Use pencil or histology pens only to label vials or jars.

Some suitable methods for preservation and transport of samples taken for molecular tests are:

i) *Live, iced specimens or chilled specimens:* for specimens that can be rapidly transported to the laboratory for testing within 24 hours, pack samples in sample bags in an insulated box containing a cold pack and ship to the laboratory. Note: cold packs should not be in direct contact with the animals to avoid freezing some parts of the tissues if histological analyses are also planned on the samples (histology cannot be performed on frozen tissues).

ii) *Frozen whole specimens:* select live specimens according to the purpose of sampling, quick freeze in the field using crushed dry-ice, or freeze in a field laboratory using a mechanical freezer at –20°C or lower temperature. Prepare and insert the label into the container with the samples, pack samples with an adequate quantity of dry-ice in an insulated box, and ship to the laboratory.

iii) *Alcohol-preserved samples:* 80% analytical grade ethanol (i.e. methanol-free ethanol) can be used to preserve, store, and transport mollusc tissues. Tissues should be fully immersed in ethanol. Shipment can be performed at room temperature .

iv) *Fixed tissues for* in-situ *hybridisation:* for this purpose, classic methods for preservation of the tissues for histology are adequate. Davidson's solution is usually a good choice for later use of molecular probes (See Section B.5.3). For DNA, specifically, over-fixation (more than 48 hours) should be avoided.

5.5.2. Preservation of DNA in tissues

For routine diagnostic testing by PCR, samples must be prepared to preserve the pathogen's nucleic acid. For most purposes, preservation of samples in analytical grade ethanol (80%) at room temperature is the preferred method for subsequent molecular tests. Samples preserved in this way can be stored for up to 1 week at 4°C or 25°C for 1 week or for extended periods at –20°C or below. In addition, other products (e.g. nucleic acid preservatives, various lysis buffers, etc.) are acceptable and are commercially available for the same purpose.

5.5.3. Nucleic acid extraction

To isolate nucleic acids from tissues preserved in ethanol or other preservative, simply remove the tissue from the fixative or preservative, press the tissues on absorbent paper to remove the excess of ethanol and let the ethanol evaporate, then treat it as fresh or frozen samples. Most fresh and preserved or fixed tissues can be homogenised (e.g. with a mortar and pestle or in bead-beating tubes) directly in the lysis or extraction buffer provided with commercially available DNA and RNA extraction kits. Commercial kits should be validated or undergo equivalence testing with current validated extraction procedures prior to routine use.

5.5.4. Preparation of slides for *in-situ* hybridisation

For *in-situ* hybridisation, molluscs are fixed and embedded in paraffin, according to the methods described above for histology. Sections are cut at 5 µm thick and placed on aminoalkylsilane-coated slides, which are then dried overnight at room temperature or in an oven at 40°C. The sections are dewaxed by immersing in xylene for 10 minutes. This step is repeated once and then the solvent is eliminated by immersion in two successive absolute ethanol baths for 10 minutes each. The sections could be rehydrated by immersion in a descending ethanol series. The protocol may require a step of membrane permeabilisation enabling access to the target DNA. For this purpose, sections are treated with proteinase K (100 µg ml^{-1}) in TE buffer (Tris [50 mM], EDTA [10 mM]), at 37°C for 10–30 minutes in a humid chamber. Slides are dehydrated by immersion in one or several ethanol series and then air-dried. For *in-situ* hybridisation tests (see individual chapters for details), it is essential that both a known positive and a known negative slide be stained to eliminate false positive results due to non-specific staining/stain dropout, and false negative results due to errors in the staining protocol. It is also recommended to test non-specific ISH probes (e.g. "universal" 18s probes) on tested samples to check if the material is suitable for ISH analyses.

For further details see disease-specific chapters in this *Aquatic Manual*.

6. Additional information to be collected

Sample information should include the collector's name, organisation, date, time, and description of the geographical location of the place of origin. The geographical location of the place of origin of samples may be described as the name or location of the site from

which the sample has originated, or its geographical co-ordinates. There should also be records that provide information to allow trace-backs on the sample movement from the site of origin to the storage facility or laboratory and within those facilities.

Samples should be accompanied with background information, including the reason for submitting the sample (surveillance, abnormal mortality, abnormal growth, etc.), gross observations and associated environmental parameters, approximate prevalence and patterns of mortality, origin and nature of the molluscs (species, age, whether or not the samples are from local mollusc populations or stocks transferred from another site, date of transfer and source location, etc.). This information should identify possible changes in handling or environmental conditions that could be a factor in mortality in association, or not, with the presence of infectious agents

Information on the preservation method, storage location, and date and time of storage at each storage locker or freezer along with information on the storage temperature (continuously monitored is preferable) should be collected. This information should be tracked with a unique sample code for all samples. For laboratories, the date of receipt, storage location information, date of analysis, analysis notes, and report date should be maintained for all uniquely coded samples. These data will greatly facilitate the tracking of sample problems and provide assurance that the samples were properly handled.

See disease-specific chapters in this *Aquatic Manual* for recommendations on any additional information that may be required or that may assist the diagnostic laboratory in determining the most appropriate test(s) to be run for submitted samples.

7. Key references for further reading

ALMEIDA M., BERTHE F., THEBAULT A. & DINIS M.T. (1999). Whole clam culture as a quantitative diagnostic procedure of *Perkinsus atlanticus* (Apicomplexa, Perkinsea) in clams *Ruditapes decussatus. Aquaculture,* **177**, 325–332.

ARZUL I., CORBEIL S., MORGA B. & RENAULT T. (2017). Viruses infecting marine molluscs. *J. Invert. Pathol*., **147**, 118-135.

BERTHE F.C.J., LE ROUX F., ADLARD R.D. & FIGUERAS A.J. (2004). Marteiliosis of molluscs: a review. *Aquat. Living Resour.*, **17**, 433–448.

BONDAD-REANTASO M.G., MCGLADDERY S.E., EAST I. & SUBASINGHE R.P. (2001). Asian Diagnostic Guide to Aquatic Animal Diseases. *FAO Fisheries Technical Paper*, No. 402, supplement 2. Food and Agriculture Organization of the United Nations (FAO), Rome, Italy, 240 pp.

BUSHEK D., FORD S.E. & ALLEN S.K. (1994). Evaluation of methods using Ray's fluid thioglycollate medium for diagnosis of *Perkinsus marinus* infections in the eastern oyster, *Crassostrea virginica. Ann. Rev. Fish Dis.,* **4**, 201–217.

GUO X. & FORD S.E. (2015). Infectious diseases of marine molluscs and host responses as revealed by genetic tools. *Phil. Trans. R. Soc.,* **B 371**, 20150206.

HOWARD D.W. LEWIS E.J., KELLER J. & SMITH C.S. (2004). Histological techniques for marine bivalve molluscs and crustaceans. *NOAA Technical Memorandum NOS NCCOS* 5, 218 pp.

MCDOWELL E. & TRUMP B.F. (1976). Histologic fixatives suitable for diagnostic light and electron microscopy. *Arch. Pathol. Lab. Med*., **100**, 405–414.

MOODY N.J.G. & CRANE M.ST.J. (2016). Validation of diagnostic tests in the OIE manual for aquatic animals. In: Proc. 3rd OIE Global Conference on Aquatic Animal Health – "Riding the Wave of the Future", Ho Chi Minh City, Vietnam, 20–22 January 2015, pp.119–126.

QADIRI S.S.N., SOO-JIN KIM S.-J., KRISHNAN R., KIM J.-O., KOLE S., KIM W.-S. & OH M.-J. (2019). Localization and tissue tropism of viral haemorrhagic septicemia virus (VHSV) in experimentally infected juvenile olive flounder, *Paralichthys olivaceus*: An *in situ* hybridization and immunohistochemical study. *Aquaculture*, **505**, 242–252.

VALVERDE E.J., BORREGO J.J., SARASQUETE M.C., ORTIZ-DELGADO J.B. & CASTRO D. (2017). Target organs for lymphocystis disease virus replication in gilthead seabream (*Sparus aurata*). *Vet. Res*., **48**, 21. doi 10.1186/s13567- 017-0428-3.

VILLALBA A., REECE K.S., ORDÁS M.C., CASAS S.M. & FIGUERAS A.J. (2004). Perkinsosis in molluscs. *Aquat. Living Resour.*, **17**, 411–432.

*

* *

NB: FIRST ADOPTED IN 1997. MOST RECENT UPDATES ADOPTED IN 2012.

Annex 57. Item 8.2.2. – Chapter 2.4.1. Infection with abalone herpesvirus

CHAPTER 2.4.1.

INFECTION WITH ABALONE HERPESVIRUS

EU position

The EU supports the adoption of this revised chapter.

1. Scope

Infection with abalone herpesvirus means infection with the pathogenic agent *Aurivirus haliotidmalaco1* (~~commonly~~ previously known as *Haliotid herpesvirus 1*, and *abalone herpesvirus* [AbHV-~~1~~]) of the genus *Aurivirus* and the Family *Malacoherpesviridae*.

2. Disease information

2.1. Agent factors

2.1.1. Aetiological agent

Aurivirus haliotidmalaco1 ~~AbHV-1~~ is the aetiological agent of abalone viral ganglioneuritis (AVG); for the purpose of this chapter, the agent will be referred to as AbHV. AVG is a contagious disease of abalone species in Australia (Ellard *et al.,* 2009; Hooper *et al.,* 2007), China (People's Rep. of) (Gu *et al*., 2019; Wang *et al.,* 2004) and Chinese Taipei (Chang *et al.,* 2005). Comparison of nucleotide sequences of the Victorian isolate of AbHV-~~1~~ and ostreid herpesvirus-1 (Davison *et al.,* 2009; Le Deuff & Renault, 1999) over common coding regions identified similarities ranging from 19% to 53%, indicating that these viruses share a low level of sequence similarity (Savin *et al.,* 2010). AbHV-~~1~~ has been assigned as a second member of the *Malacoherpesviridae* (ICTV, 2022). Complete genome sequences of isolates demonstrated that there are at least five genetic variants of AbHV-~~1~~ within Australia (Cowley *et al*., 2012; Corbeil *et al*., 2016) and one Chinese Taipei strain (Chang *et al*., 2005). More recent analysis demonstrated that the Chinese strain represents a further variant (Bai *et al*., 2019b).

Purified AbHV-~~1~~ particles (Tan *et al.,* 2008) observed by transmission electron microscopy are enveloped and icosahedral with electron dense cores and 100–110 nm in diameter. The intranuclear location of AbHV-~~1~~ particles, their size and ultrastructure are characteristic of members of the *Herpesviridae*. Isopycnic gradient centrifugation (in potassium tartrate and caesium chloride density gradients) indicated a virus particle buoyant density of 1.17–1.18 g ml^{-1} (Tan *et al.,* 2008).

2.1.2. Survival and stability in processed or stored samples

Virus derived from tissue obtained from experimentally infected abalone that had been homogenised in sterile EMEM Gibco) containing 10% fetal bovine serum, centrifuged (1500 **g** for 20 minutes at 4°C), filtered (0.22 µM) and stored as 250 µl aliquots in liquid nitrogen remains infectious for at least 21 months (Corbeil *et al*., 2012b).

2.1.3. Survival and stability outside the host

Experimental studies (Corbeil *et al*., 2012b) demonstrated that AbHV-~~1~~ remained infectious for up to 5 days when held in seawater at 4°C and for only 1 day at 15°C.

2.2. Host factors

Acute disease was first reported in farmed *Haliotis diversicolor supertexta* in Chinese Taipei (Chang *et al*., 2005). Subsequently, disease outbreaks occurred in both farmed and wild abalone populations in Australia in all age classes of *H. rubra*, *H. laevigata*, and their hybrids (Hooper *et al.,* 2007). AbHV-~~1~~ is also suspected to be the aetiological agent of an epizootic disease that devastated the abalone aquaculture industry in southeastern China (People's Rep. of) starting in 1999

and continuing through the early 2000s (Gu *et al*., 2019; Wei *et al*., 2018; Wu & Zhang, 2016). Interestingly, New Zealand päua (*H. iris*) was highly resistant to experimental infection (Corbeil *et al*., 2017).

2.2.1. Susceptible host species

Species that fulfil the criteria for listing as susceptible to infection with AbHV ~~1~~ according to Chapter 1.5. of the *Aquatic Animal Health Code* (*Aquatic Code*) are: ~~small abalone (*Haliotis diversicolor*), Greenlip abalone (*Haliotis laevigata*), Blacklip abalone (*Haliotis rubra*) and hybrids of Greenlip × Blacklip abalone (*Haliotis laevigata* × *Haliotis rubra*).~~

Family	Scientific name	Common name
Haliotidae	*Haliotis diversicolor*	small abalone
	Haliotis laevigata	greenlip abalone
	Haliotis rubra	blacklip abalone
	Haliotis laevigata × *H. rubra*	hybrid of greenlip × blacklip abalone

2.2.2. Species with incomplete evidence for susceptibility

Species for which there is incomplete evidence to fulfil the criteria for listing as susceptible to infection with AbHV ~~1~~ according to Chapter 1.5 of the *Aquatic Code* are: none ~~Japanese abalone (*Haliotis discus*) and Rainbow abalone (*Haliotis iris*)~~.

In addition, pathogen-specific positive polymerase chain reaction (PCR) results have been reported in the following species, but no active infection has been demonstrated: ~~none.~~

Family	Scientific name	Common name
Haliotidae	*Haliotis discus*	Japanese abalone
	Haliotis iris	rainbow abalone

2.2.3. Likelihood of infection by species, host life stage, population or sub-populations

All age classes of *H. diversicolor, H. rubra*, *H. laevigata*, and hybrids of *H. rubra* × *H. laevigata* appear to be highly susceptible to disease (Corbeil 2020; Gu *et al.,* 2019).

2.2.4. Distribution of the pathogen in the host

The major histopathological lesion identified in abalone affected with AVG is ganglioneuritis: inflammation confined to neural tissue. The cerebral, pleuropedal and buccal ganglia can be affected as well as the cerebral commissure and associated peripheral nerves (Bai *et al*., 2019a; Chang & Handlinger, 2022; Hooper *et al.,* 2007). The Chinese variant is also able to infect and replicate in haemocytes of *H. diversicolor* (Bai *et al.,* 2020)

2.2.5. Aquatic animal reservoirs of infection

No information available.

2.2.6. Vectors

No information available.

2.3. Disease pattern

Outbreaks of AVG in both farmed and wild abalone populations in Australia are associated with the rapid onset of high mortality rates (up to 90%) in all age classes (Corbeil *et al.,* 2010). Similarly, in Chinese Taipei, during the epizootic in cultured abalone (the water temperature was 16–19°C), both adult and juvenile abalone suffered from the disease, with cumulative mortalities of 70–80%. It was reported that death of all of the abalone in a pond could occur within 3 days of the onset of clinical signs (Chang *et al.,* 2005). A similar disease pattern occurred with experimental infections (Chang *et al.,* 2005; Crane *et al.,* 2009).

2.3.1. Mortality, morbidity and prevalence

In on-farm epizootics in Australia cumulative mortality in all age classes can reach >90%. In experimental trials, 100% mortality can occur within 5 days post-exposure. Most abalone that display gross signs are likely to die within 1–2 days.

In Australia, and similarly in Chinese Taipei, an outbreak of AVG is associated with a rapid rise in mortality rate (up to 90% or more). Affected abalone demonstrating clinical signs (e.g. curling of the foot) are likely to die within 1 day of showing these signs. Ganglioneuritis is observed in sections of neural tissue by light microscopy and confirmation of the presence of AbHV-~~1~~ is obtained by real-time PCR or *in-situ* hybridisation (Crane *et al.,* 2016). The precise prevalence of AVG in wild abalone populations in Australian waters is unknown. The first epidemiological study undertaken in China (People's Rep. of), using real-time PCR (Gu *et al*., 2019), revealed a detection rate of 27–30% in abalone (*H. diversicolor* and *H. discus hannai*) farms with both healthy and diseased abalone.

2.3.2. Clinical signs, including behavioural changes

AVG outbreaks in both farmed and wild abalone were associated with high mortality rates (up to 90% on farm). Clinically, abalone may demonstrate one or more of the following signs: irregular peripheral concave elevation of the foot; swollen and protruding mouth parts; eversion of the radula; minimal movement of the pedal muscle; excessive mucus production; absence of the marked extension of the foot shown in the righting reflex when healthy abalone are turned onto their backs; reduced pedal adhesion to the substrate. In Tasmania, abalone affected by AVG in processing plants exhibited 'hard foot' or tetany, excessive mucus production, abnormal spawning and 'bloating' (Ellard *et al.,* 2009). These facilities also experienced much lower morbidity and mortality rates than reported on farms or in wild abalone in Victoria, Australia. Similar signs have been reported for an abalone disease epizootic in Chinese Taipei (Chang *et al.,* 2005).

AVG is normally an acute disease, with abalone dying within 1–2 days of demonstrating gross signs of the disease. Wild harvested abalone held in live-holding facilities in Tasmania have previously exhibited slower onset of clinical signs and mortality. Some Tasmanian wild caught abalone have previously tested positive for AVG using real-time PCR without overt clinical or histological signs.

2.3.3 Gross pathology

Abalone that are loosely attached to the substrate owing to weakness or abnormalities of the pedal muscle should be selected for sampling. If this gross pathology is caused by acute AVG, it is likely that these abalone will die within 1–2 days.

2.3.4. Modes of transmission and life cycle

Horizontal transmission (Bai *et al.,* 2019a; Chang *et al.,* 2005; Crane *et al.,* 2009) has been demonstrated experimentally by:

1. exposing healthy abalone to water containing diseased abalone in the same tank without direct contact between the diseased and healthy abalone;
2. placing healthy abalone in water that was previously inhabited by diseased abalone.

In all cases, 100% mortality was observed with a preclinical period of 1–2 days following exposure and then mortality commenced until 100% mortality occurred within 2–5 days post-infection.

2.3.5. Environmental factors

In Australia, the initial outbreak of AVG occurred on a farm during summer 2005/2006 and subsequently appeared to spread to wild populations, which experienced mortality throughout the following year i.e. during all seasons. All experimental infections to date have been carried out in the temperature range 15–18°C. In Chinese Taipei, during the reported epizootic, the water temperature was 16–19°C, and experimental infections were carried out at 17–20°C. In China (People's Rep. of), natural infections were only detected at water temperatures below 23°C (Gu *et al*., 2019). How temperature affects viral replication and onset of disease has yet to be determined. The possible effects of changes in other environmental factors such as salinity and dissolved oxygen are unknown.

2.3.6. Geographical distribution

Reported in Asia-Pacific.

See WAHIS (https://wahis.woah.org/#/home) for recent information on distribution at the country level.

2.4. Biosecurity and disease control strategies

2.4.1. Vaccination

None.

2.4.2. Chemotherapy including blocking agents

No data available.

2.4.3. Immunostimulation

No data available.

2.4.4. Breeding resistant strains

No data available.

2.4.5. Inactivation methods

AbHV-~~1~~ was inactivated by treatment with 50 ppm of the iodophor Buffodine® as well as a 1% solution of the non-ionic surfactant Impress®. Calcium hypochlorite (1.5 ppm) treatment also inactivated the virus (Corbeil *et al*., 2012b).

2.4.6. Disinfection of eggs and larvae

No data available.

2.4.7. General husbandry

To date, experimental data indicates that AbHV-~~1~~ is highly virulent. Practices that could be implemented to reduce the severity of the disease have not been identified. It is interesting to note that, in contrast to the situation in Victoria, Australia, clinical disease has not been reported in wild abalone populations in Tasmania, Australia. Disease outbreaks in processing plants in Tasmania suggest that stress factors may influence expression of subclinical infection.

3. Specimen selection, sample collection, transportation and handling

This section draws on information in Sections 2.2, 2.3 and 2.4 to identify populations, individuals and samples that are most likely to be infected.

3.1. Selection of populations and individual specimens

At the first signs of increased numbers of abalone appearing to be weak or behaving abnormally, or sudden onsets of unexplained mortality, live moribund individuals should be selected for sampling. If moribund or freshly dead abalone are not available, samples of overtly normal abalone from all parts of the farm, and representing all age classes, should be selected for sampling.

3.2. Selection of organs or tissues

Neural tissue that includes the cerebral, pleuropedal and buccal ganglia.

3.3. Samples or tissues not suitable for pathogen detection

To date, lesions have not been detected consistently in non-neural tissues.

3.4. Non-lethal sampling

Not available.

3.5. Preservation of samples for submission

For guidance on sample preservation methods for the intended test methods, see Chapter 2.4.0 *General information* (*diseases of molluscs*).

3.5.1. Samples for pathogen isolation

The results of bioassay depend strongly on the quality of samples (time since collection and time in storage). Fresh specimens should be kept on ice and preferably sent to the laboratory within 24 hours of collection. To avoid degradation of samples, use alternative storage methods only after consultation with the receiving laboratory.

3.5.2. Preservation of samples for molecular detection

Tissue samples for PCR testing should be preserved in 70–90% (v/v) analytical/reagent-grade (undenatured) ethanol. The recommended ratio of ethanol to tissue is 10:1 based on studies in terrestrial animal and human health. The use of lower grade (laboratory or industrial grade) ethanol is not recommended. If material cannot be fixed it may be frozen.

Standard sample collection, preservation and processing methods for molecular techniques can be found in Section B.5.5 of Chapter 2.4.0 *General information* (diseases of molluscs).

3.5.3. Samples for histopathology, immunohistochemistry or *in-situ* hybridisation

Standard sample collection, preservation and processing methods for histological techniques can be found in Section B.5.3 of Chapter 2.4.0 *General information* (diseases of molluscs).

3.5.4. Samples for other tests

Not applicable.

3.6. Pooling of samples

Pooling of samples from more than one individual animal for a given purpose is only recommended where robust supporting data on diagnostic sensitivity and diagnostic specificity have been evaluated and found to be suitable. If the effect of pooling on diagnostic sensitivity has not been thoroughly evaluated, larger specimens should be processed and tested individually. Small life stages such as larvae can be pooled to obtain the minimum amount of material for molecular detection.

4. Diagnostic methods

The methods currently available for pathogen detection that can be used in i) surveillance of apparently healthy animals, ii) presumptive diagnosis in clinically affected animals and iii) confirmatory diagnostic purposes are listed in Table 4.1. by animal life stage.

Ratings for purposes of use. For each recommended assay a qualitative rating for the purpose of use is provided. The ratings are determined based on multiple performance and operational factors relevant to application of an assay for a defined purpose. These factors include appropriate diagnostic performance characteristics, level of assay validation, availability cost, timeliness, and sample throughput and operability. For a specific purpose of use, assays are rated as:

+++ =	Methods are most suitable with desirable performance and operational characteristics.
++ =	Methods are suitable with acceptable performance and operational characteristics under most circumstances.
+ =	Methods are suitable, but performance or operational characteristics may limit application under some circumstances.
Shaded boxes =	Not appropriate for this purpose.

Validation stage. The validation stage corresponds to the assay development and validation pathway in chapter 1.1.2. The validation stage is specific to each purpose of use. Where available, information on the diagnostic performance of recommended assays is provided in Section 6.3.

WOAH Reference Laboratories welcome feedback on diagnostic performance of recommended assays, in particular PCR methods. Of particular interest are any factors affecting expected assay sensitivity (e.g. tissue components inhibiting amplification) or expected specificity (e.g. failure to detect particular genotypes, detection of homologous sequences within the host genome). These issues should be communicated to the WOAH Reference Laboratories so that advice can be provided to diagnostic laboratories and the standards amended if necessary.

Table 4.1. *WOAH recommended diagnostic methods and their level of validation for surveillance of apparently healthy animals and investigation of clinically affected animals*

Method	A. Surveillance of apparently healthy animals				B. Presumptive diagnosis of clinically affected animals				C. Confirmatory diagnosis[1] of a suspect result from surveillance or presumptive diagnosis			
	Early life stages[2]	Juveniles[2]	Adults	LV	Early life stages[2]	Juveniles[2]	Adults	LV	Early life stages[2]	Juveniles[2]	Adults	LV
Wet mounts												
Imprints												
~~Histopathology~~		~~+~~	~~+~~	~~NA~~		~~++~~	~~++~~	~~2~~		~~++~~	~~++~~	~~2~~
Transmission electron microscopy						+	+	NA		~~+~~	~~+~~	~~NA~~
Real-time PCR		+++	+++	2		+++	+++	2		+++	+++	2
Conventional PCR						++	++	2				
Conventional PCR followed by amplicon sequencing										+++	+++	2
In-situ hybridisation						++	++	NA		++	++	NA
Bioassay						+	+	NA				
LAMP												
Ab-ELISA												
Ag-ELISA												
Other antigen detection methods												
Other methods												

LV = level of validation, refers to the stage of validation in the WOAH Pathway (chapter 1.1.2); PCR = polymerase chain reaction; LAMP = loop-mediated isothermal amplification; Ab- or Ag-ELISA = antibody or antigen enzyme-linked immunosorbent assay, respectively; IFAT = indirect fluorescent antibody test.
[1]For confirmatory diagnoses, methods need to be carried out in combination (see Section 6). [2]Susceptibility of early and juvenile life stages is described in Section 2.2.3.
Shading indicates the test is inappropriate or should not be used for this purpose.

4.1. Wet mounts

Not applicable.

4.2. Electron microscopy/cytopathology

Transmission electron microscopy is not a routine diagnostic method but can be used to confirm the presence of viral particles in infected ganglia. AbHV~~-1~~ particles are icosahedral with electron dense cores and a diameter of 100–110 nm. The intranuclear location of the particles and their ultrastructure are characteristic of members of the *Herpesviridae* (Tan *et al.,* 2008).

Tissue samples (containing pleuropedal ganglion) for examination by electron microscopy should be fixed using 2.5% (v/v) glutaraldehyde and 2–4% (v/v) paraformaldehyde in 0.1 M cacodylate buffer and post-fixed in 1% (w/v) osmium tetroxide, washed in reverse osmosis water (3 × 5 minutes), dehydrated in a graded series of 'analytical grade' ethanol (70%, overnight at 4°C; 95%, 20 minutes; 100%, 3 × 20 minutes), infiltrated in 100% Spurr's resin (overnight) and then embedded in Spurr's resin.

4.3. Histopathology

Neural tissue (cerebral, pleuropedal and buccal ganglia, branches of the pedal nerve and peripheral nerves) is the prime target and should be sampled, fixed ~~(using 10% formalin)~~ and processed using standard procedures, and stained with haematoxylin and eosin for histological examination as specified in Chapter 2.4.0.

Abalone affected with AVG demonstrate inflammation (increased infiltration by haemocytes) and necrosis confined to neural tissue (cerebral, pleuropedal and buccal ganglia, branches of the pedal nerve and peripheral nerves) as observed in histological sections of neural tissue stained with haematoxylin and eosin and examined by light microscopy (Chang & Handlinger, 2022; Ellard *et al.,* 2009; Hooper *et al.,* 2007).

4.4. Nucleic acid amplification

PCR assays should always be run with the controls specified in Section B.5.5 *Molecular methods* of Chapter 2.4.0 *General information* (diseases of molluscs). An 18S rDNA real-time PCR can be used to validate nucleic acid extraction and integrity, and the absence of PCR inhibitors (Crane *et al.,* 2016). Each sample should be tested in duplicate.

Extraction of nucleic acids

Different kits and procedures can be used for nucleic acid extraction. The quality and concentration of the extracted nucleic acid is important and can be checked using a suitable method as appropriate to the circumstances.

4.4.1. Real-time PCR

Following validation of the real-time PCR test targeted to ORF49 (Corbeil *et al.,* 2010), the discovery of genotypic variants in Australia not recognised by this test necessitated other real-time PCR tests to be developed based on more conserved regions of the viral genome. Real-time PCR tests targeted to ORF49 and ORF66 have been used extensively in disease investigations and the accumulated data have been used in test validation (Caraguel *et al*., 2019). For the detection of all genetic variants, the ORF49 and ORF66 real-time PCR tests should be run in parallel, and infection with AbHV can be confirmed by a positive result from either of the two tests. Each of these tests can be multiplexed with an 18S rDNA real-time PCR test, used to validate nucleic acid extraction and integrity, and the absence of PCR inhibitors (Crane et al., 2016).

***Primers and probes* (*sequences*)**

Pathogen/ target gene	**Primer/probe (5'–3')**	**Concentration**	**Cycling parameters[(a)]**
Crane *et al.,* 2016; GenBank Accession No.: MW412419.1			

AbHV ORF49	ORF49F1: AAC-CCA-CAC-CCA-ATT-TTT-GA ORF49R1: CCC-AAG-GCA-AGT-TTG-TTG-TT 49Prb1: 6FAM-CCG-CTT-TCA-ATC-TGA-TCC-GTG-G-TAMRA	300 nM 300 nM 100 nM	50 cycles of: 95°C/3 sec and 62°C/30 sec
Crane *et al.,* 2016; GenBank Accession No.: MW412419.1			
AbHV ORF66	ORF66F1: TCC-CGG-ACA-CCA-GTA-AGA-AC ORF66R1: CAA-GGC-TGC-TAT-GCG-TAT-GA 66Prb1: 6FAM-TGG-CCG-TCG-AGA-TGT-CCA-TG-TAMRA	300 nM 300 nM 100 nM	50 cycles of: 95°C/3 sec and 60°C/30 sec

[(a)]A denaturation step prior to cycling has not been included.

4.4.2. Conventional PCR

Conventional PCR may also be used for detection of AbHV~~-1~~ in tissue samples. Nucleic acid is extracted as described above. The AbHV1617 PCR has been shown to generate amplicons of various length (522bp to 588bp) depending on the AbHV~~-1~~ isolate. Thus, it is potentially useful for epidemiological studies and to confirm positive real-time PCR results (Crane *et al*., 2016). A second PCR targeting the Taiwanese AbHV~~-1~~ DNA polymerase gene has also been developed (Chen *et al*., 2012). The primer sequences for the two tests are detailed below.

Primer sequences

Pathogen / target gene	**Primer (5'–3')**	**Concentration**	**Cycling parameters[(a)]**
Method 1: Crane *et al.,* 2016; GenBank Accession No.:MW412419.1 amplicon size: 522–588 bp (depending on genetic variant)			
AbHV	AbHV-16: GGC-TCG-TTC-GGT-CGT-AGA-ATG AbHV-17: TCA-GCG-TGT-ACA-GAT-CCA-TGT-C	360 nM 360 nM	40 cycles of: 94°C/30 sec and 52°C/30 sec
Method 2: Chen *et al.,* 2012; GenBank Accession No.: HQ317456; amplicon size: 606 bp			
AbHV	40f: TCC-ATC-GAG-ATT-CCC-AGT-TC 146r: ACG-CCA-CCC-TGT-ATA-ACG-AG	400 nM 400 nM	35 cycles of: 94°C/60 sec and 52°C/60 sec

[(a)]A denaturation step prior to cycling has not been included.

4.4.3. Other nucleic acid amplification methods

A loop-mediated isothermal amplification assay for rapid and sensitive detection of AbHV~~-1~~ has been developed that is 100-fold more sensitive than conventional PCR (Chen *et al*., 2014) but is not widely used because of false positive and false negative results.

4.5. Amplicon sequencing

The size of the PCR amplicon should be verified, for example by agarose gel electrophoresis. Both DNA strands of the PCR product must be sequenced and analysed in comparison with reference sequences.

4.6. *In-situ* hybridisation

In-situ hybridisation localises AbHV~~-1~~ infected cells within the neural tissue which, on histological examination, demonstrates ganglioneuritis typified by an inflammatory change with increased cellularity involving mainly haemocytes and glial cells, and cell necrosis in the affected nerves (Mohammad *et al.,* 2011).

The *in-situ* hybridisation (ISH) procedure uses a digoxygenin (DIG)-labelled DNA probe to detect AbHV~~-1~~ in formalin-fixed, paraffin-embedded (FFPE) tissue sections and is described in Crane *et al.* (2016).

4.7. Immunohistochemistry

Not applicable.

4.8. Bioassay

A bioassay is not normally required for routine diagnosis. However, when there is a suspect case due to the presence clinical signs and/or histopathology but molecular tests yield negative results, a bioassay (Corbeil *et al*., 2012a) can be used for confirmation of the presence of a previously unknown genetic variant. Homogenised and clarified neural tissue is used as inoculum and injected (i.m.) into the foot of known uninfected susceptible abalone host species. The inoculated abalone are monitored for clinical signs such as loss of adhesion to the substrate and then samples taken for histology, molecular analyses and electron microscopy. If presence of a herpesvirus is confirmed by electron microscopy further investigation such as whole genome sequencing should be initiated.

4.9. Antibody- or antigen-based detection methods

None currently available.

4.10. Other methods

None.

5. Test(s) recommended for surveillance to demonstrate freedom in apparently healthy populations

The real-time PCR assays targeting ORF49 and ORF66 performed in parallel is recommended for surveillance to demonstrate freedom in apparently health populations (Caraguel *et al.*, 2019).

6. Corroborative diagnostic criteria

This section only addresses the diagnostic test results for detection of infection in the absence (Section 6.1.) or in the presence of clinical signs (Section 6.2.) but does not evaluate whether the infectious agent is the cause of the clinical event.

The case definitions for a suspect and confirmed case have been developed to support decision making related to trade and confirmation of disease status at the country, zone or compartment level. Case definitions for disease confirmation in endemically affected areas may be less stringent. If a Competent Authority does not have the capability to undertake the necessary diagnostic tests it should seek advice from the appropriate WOAH Reference Laboratory, and if necessary, refer samples to that laboratory for confirmatory testing of samples from the index case in a country, zone or compartment considered free.

6.1. Apparently healthy animals or animals of unknown health status[1]

Apparently healthy populations may fall under suspicion, and therefore be sampled, if there is an epidemiological link(s) to an infected population. Hydrographical proximity to, or movement of animals or animal products or equipment, etc., from a known infected population equate to an epidemiological link. Alternatively, healthy populations are sampled in surveys to demonstrate disease freedom.

6.1.1. Definition of suspect case in apparently healthy animals

The presence of infection with abalone herpesvirus shall be suspected if at least one of the following criteria is met:

i) Positive result by a real-time PCR

ii) Positive result by conventional PCR

~~Histopathological changes consistent with the presence of the pathogen~~

6.1.2. Definition of confirmed case in apparently healthy animals

The presence of Infection with abalone herpesvirus is considered to be confirmed if one of the following criteria are met:

1 For example transboundary commodities.

i) Positive results by real-time PCR and positive result by conventional PCR followed by sequence analysis of the amplicon

ii) Positive results by *in-situ* hybridisation and positive result by conventional PCR followed by sequence analysis of the amplicon

6.2 Clinically affected animals

Clinical signs are not pathognomonic for a single disease; however, they may narrow the range of possible diagnoses.

6.2.1. Definition of suspect case in clinically affected animals

The presence of infection with abalone herpesvirus shall be suspected if at least one of the following criteria is met:

i) Gross pathology or clinical signs associated with the disease as described in this chapter, with or without elevated mortality

ii) Positive result by a real-time PCR

iii) Positive result by conventional PCR

iv) Histopathological changes consistent with the presence of the pathogen or the disease

v) Positive result of a bioassay

6.2.2. Definition of confirmed case in clinically affected animals

The presence of infection with abalone herpesvirus is considered to be confirmed if one of the following criteria are met:

i) Positive results by real-time PCR and by conventional PCR followed by sequence analysis of the amplicon

ii) Positive results by *in-situ* hybridisation and by conventional PCR followed by sequence analysis of the amplicon

6.3. Diagnostic sensitivity and specificity for diagnostic tests

The diagnostic performance of tests recommended for surveillance or diagnosis of infection with abalone herpesvirus are provided in Tables 6.3.1. and 6.3.2. This information can be used for the design of surveys for infection with abalone herpesvirus, however, it should be noted that diagnostic performance is specific to the circumstances of each diagnostic accuracy study (including the test purpose, source population, tissue sample types and host species) and diagnostic performance may vary under different conditions. Data are only presented where tests are validated to at least level 2 of the validation pathway described in Chapter 1.1.2. and the information is available within published diagnostic accuracy studies.

6.3.1. For presumptive diagnosis of clinically affected animals

Test type	Test purpose	Source populations	Tissue or sample types	Species	DSe (*n*)	DSp (*n*)	Reference test	Citation
Real-time PCR	Diagnosis	Clinically diseased abalone from the wild and processing plants	Pleuropedal ganglion or pedal nerve cords	*Haliotis rubra*	100 (48)	100 (48)	Histopathology	Corbeil *et al.*, 2010
Conventional PCR								
~~Histopathology~~								

DSe = diagnostic sensitivity, DSp = diagnostic specificity, *n* = ~~samples~~ number of animals used in the validation study,
PCR: = polymerase chain reaction.

6.3.2. For surveillance of apparently healthy animals

Test type	Test purpose	Source populations	Tissue or sample types	Species	DSe (*n*)	DSp (*n*)	Reference test	Citation

Real-time PCR	Surveillance	Naturally AbHV~~-1~~ infected wild and farmed populations; AbHV~~-1~~ free populations	Pleuropedal ganglion or pedal nerve cords	*Haliotis laevigata*; *H. rubra*; *H. laevigata* x *H. rubra* hybrids	90.1 (1452)	97.7 (1452)	Histopathology	Caraguel *et al.,* 2019
Histopathology	Surveillance	Naturally AbHV-1 infected wild and farmed populations; AbHV-1 free populations	Pleuropedal ganglion or pedal nerve cords	*Haliotis laevigata*; *H. rubra*; *H. laevigata* x *H. rubra* hybrids	6.3 (1452)	100 (1452)	real-time PCR	Caraguel *et al.,* 2019

DSe = diagnostic sensitivity, DSp = diagnostic specificity, *n* = ~~samples~~ number of animals used in the validation study, PCR: = polymerase chain reaction.

7. References

BAI C.-M., LI Y.-N., CHANG P.-H., JIANG J.-Z., XIN L.-S., LI C., WANG J.-Y. & WANG C.-M. (2019a). Susceptibility of two abalone species, *Haliotis diversicolor supertexta* and *Haliotis discus hannai*, to *Haliotid herpesvirus* 1 infection. *J. Invertebr. Pathol.,* **160**, 26-32.

BAI C.-M., LI Y.-N., CHANG P.-H., JIANG J.-Z., XIN L.-S., LI C., WANG J.-Y. & WANG C.-M. (2020). *In situ* hybridization revealed wide distribution of Haliotid herpesvirus 1 in infected small abalone, *Haliotis diversicolor supertexta*. *J. Invertebr. Pathol.,* **173**, 107356.

BAI C.-M., ROSANI U., LI Y.-N., ZHANG S.-M., XIN L.-S. & WANG C.-M. (2019b). RNA-seq of HaV-1-infected abalones reveals a common transcriptional signature of Malacoherpesviruses. *Nature Sci. Rep*., **9**, 938.

CARAGUEL C.G.B., ELLARD K., MOODY N.J.G., CORBEIL S., WILLIAMS L.M., MOHR P.G., CUMMINS D.M., HOAD J., SLATER J. & CRANE M.ST.J. (2019). Diagnostic test accuracy when screening for *Haliotid herpesvirus 1* (AbHV) in apparently healthy populations of Australian abalone *Haliotis* spp. *Dis. Aquat. Org*., **136**, 199-207.

CHANG P.H. & HANDLINGER J. (2022). ABALONE HERPESVIRUS. In: Aquaculture Pathophysiology, Vol. II. Crustacean and Mollusks Diseases, (K.S.B. Kibenge, R.S.-M. Chong, B. Baldisserotto (eds.)), Pp. 451-459. Academic Press, Elsevier.

CHANG P.H., KUO S.T., LAI S.H., YANG H.S., TING Y.Y., HSU C.L. & CHEN H.C. (2005). Herpes-like virus infection causing mortality of cultured abalone *Halitotis diversicolor supertexta* in Taiwan. *Dis. Aquat. Org.*, **65**, 23–27.

CHEN M.H., KUO S.T., RENAULT T., FRIEDMAN C.S. & CHANG P.H. (2012). Development of a polymerase chain reaction for the detection of abalone herpesvirus infection based on the DNA polymerase gene. *J. Virol. Meths*., **185**, 1-6.

CHEN M.H., KUO S.T., RENAULT T. & CHANG P.H. (2014). The development of a loop-mediated isothermal amplification assay for rapid and sensitive detection of abalone herpesvirus DNA. *J. Virol. Meths*., **196**, 199-2013.

CORBEIL S. (2020). Abalone Viral Ganglioneuritis. *Pathogens*, **9**, 720.

CORBEIL S., COLLING A., WILLIAMS L.M., WONG F.Y.K., SAVIN K., WARNER S., MURDOCH B., COGAN N.O.I., SAWBRIDGE T.I., FEGAN M., MOHAMMAD I., SUNARTO A., HANDLINGER J., PYECROFT S., DOUGLAS M., CHANG P.H. & CRANE M.ST.J. (2010). Development and validation of a TaqMan® PCR assay for the Australian abalone herpes-like virus. *Dis. Aquat. Org.*, **92**, 1–10.

CORBEIL S., MCCOLL K.A., WILLIAMS L.M., MOHAMMAD I., HYATT A.D., CRAMERI S.G., FEGAN M. & CRANE M.ST.J. (2012a). Abalone viral gamglioneuritis: Establishment and use of an experimental immersion challenge system for the study of abalone herpesvirus infections in Australian abalone. *Virus Res*., **165**, 207-213.

CORBEIL S., MCCOLL K.A., WILLIAMS L.M., SLATER J. & CRANE M.ST.J. (2017). Innate resistance of New Zealand päua to abalone viral ganglioneuritis. *J. Invertebr. Pathol.,* **146**, 31-35.

CORBEIL S., WILLIAMS L.M., BERGFELD J. & CRANE M.ST.J. (2012b). Abalone herpes virus stability in sea water and susceptibility to chemical disinfectants. *Aquaculture*, **326-329**, 20-26.

CORBEIL S., WILLIAMS L.M., MCCOLL K.A. & CRANE M.ST.J. (2016). Australian abalone (*Haliotis laevigata*, *H. rubra* and *H. conicopora*) are susceptible to infection by multiple abalone herpesvirus genotypes. *Dis. Aquat. Org*., **119**, 101-106.

COWLEY J.A., CORBEIL S., BULACH D., MOODY N.J., ELLARD K., FEGAN M., SAVIN K., WARNER S. & CRANE M.STJ. (2012). Complete genome sequences of abalone herpes virus (AbHV) strains from Victoria and Tasmania provide insights into its origins and identity variations useful for epidemiology. In: 8th International Abalone Symposium Hobart, Tasmania, Australia, 6–11 May 2012.

CRANE M.ST.J., CORBEIL S., FEGAN M. & WARNER S. (2009). Aquatic Animal Health Subprogram: Development of molecular diagnostic procedures for the detection and identification of herpes-like virus of abalone (*Haliotis* spp.). ISBN 978 0 643 09835 0. 79 pp.

CRANE M.STJ., MCCOLL K.A., COWLEY J.A., ELLARD K., SAVIN K.W., CORBEIL S., MOODY N.J.G., FEGAN M. & WARNER S. (2016). Abalone Herpesvirus In: Molecular Detection of Animal Viral Pathogens (D. Liu (ed.)) Pp 807-815. CRC Press, Taylor & Francis Group, Boca Raton, USA.

DAVISON A.J., EBERLE R., EHLERS B., HAYWARD G.S., MCGEOCH D.J., MINSON A.C., PELLETT P.E., ROIZMAN B., STUDDERT M.J. & THIRY E. (2009). The order Herpesvirales. *Arch. Virol*., **154**, 171–177.

ELLARD K., PYECROFT S., HANDLINGER J. & ANDREWARTHA R. (2009). Findings of disease investigations following the recent detection of AVG in Tasmania. Proceedings of the Fourth National FRDC Aquatic Animal Health Scientific Conference, Cairns, Australia, 22–24 July 2009.

GU L., QI R.-J., YANG R., HAN T., JIANG J.-Z. & WANG J.-Y. (2019). The prevalence of abalone herpesvirus in two *Haliotis* species in South China during 2002-2013. *Aquaculture*, **505**, 18-29.

HOOPER C., HARDY-SMITH P. & HANDLINGER J. (2007). Ganglioneuritis causing high mortalities in farmed Australian abalone (*Haliotis laevigata* and *Haliotis rubra*). *Aus. Vet. J.*, **85**, 188–193.

INTERNATIONAL COMMITTEE OF TAXONOMY ON VIRUSES (ICTV) (2022). Abolish 6 species and rename 1 family, 4 genera and 124 species in the order *Herpesvirales*. https://ictv.global/filebrowser/download/11652

LE DEUFF R.M. & RENAULT T. (1999). Purification and partial genome characterization of a herpes-like virus infecting the Japanese oyster *Crassostrea gigas. J. Gen. Virol.*, **80**, 1317–1322.

MOHAMMAD I.M., WARNER S., KVALHEIM N., CRANE M.STJ. & FEGAN M. (2011) Development of an *in situ* hybridisation assay for the detection and identification of the abalone herpes-like virus. Proceedings of the First FRDC Australasian Scientific Conference on Aquatic Animal Health, Cairns, Australia, 5–8 July 2011.

SAVIN K.W., COCKS B.G., WONG F., SAWBRIDGE T., COGAN N., SAVAGE D. & WARNER S. (2010). A neurotropic herpesvirus infecting the gastropod, abalone, shares ancestry with oyster herpesvirus and a herpesvirus associated with the amphioxus genome. *Virol. J.*, **7**, 308. http://www.virologyj.com/content/7/1/308.

TAN J., LANCASTER M., HYATT A., VAN DRIEL R., WONG F. & WARNER S. (2008). Purification of a herpes-like virus from abalone (*Haliotis* spp.) with ganglioneuritis and detection by transmission electron microscopy. *J. Virol. Methods*, **149**, 338–341.

WANG J., GUO Z., FENG J., LIU G., XU L., CHEN B. & PAN J. (2004). Virus infection in cultured abalone, *Haliotis diversicolor* Reeve in Guangdong Province, China. *J. Shellfish Res.*, **23**, 1163–1168.

WEI H.Y., HUANG S., YAO T., GAO F., JIANG J.Z. & WANG J.Y. (2018). Detection of viruses in abalone tissue using metagenomics technology. *Aquaculture Res.,* **49**, 2704–2713. doi:10.1111/are.13731.

WU F.C. & ZHANG G.F. (2016). Pacific Abalone Farming in China: Recent Innovations and Challenges. *J. Shellfish. Res.,* **35**, 703–710.

*
* *

NB: There is a WOAH Reference Laboratory for infection with abalone herpesvirus
(please consult the WOAH web site:
https://www.woah.org/en/what-we-offer/expertise-network/reference-laboratories/#ui-id-3).
Please contact WOAH Reference Laboratories for any further information on infection with abalone herpesvirus

NB: FIRST ADOPTED IN 2012.

Annex 58. Item 9.2.3. – Chapter 2.4.4. Infection with *Marteilia refringens*

CHAPTER 2.4.4.

INFECTION WITH *MARTEILIA REFRINGENS*

EU position

The EU supports the adoption of this revised chapter.

1. Scope

Infection with *Marteilia refringens* means infection with the pathogenic agent *M. refringens* (including O and M types) of the Family *Marteiliidae.*

2. Disease information

2.1. Agent factors

2.1.1. Aetiological agent

Marteilia refringens is a protozoan parasite of the Family *Marteiliidae* (Cavalier-Smith & Chao, 2003; Feist *et al.*, 2009) infecting the digestive system of several bivalve species and inducing physiological disorders and eventual death of the animal (Alderman, 1979; Grizel *et al*., 1974). Two types of *M. refringens* (Grizel *et al*., 1974), types O and M, were defined by Le Roux *et al.* (2001). Although more recent results suggest that *M. refringens* should be distinguished from *M. pararefringens* (previously *M. maurini* or *M. refringens* type M) (Kerr *et al.*, 2018), a larger set of samples is required to properly define both species and most available data in the literature do not allow differentiation of *M. refringens* type O (= *M. refringens* in Kerr *et al.*, 2018) or *M. refringens* type M (= *M. pararefringens* in Kerr *et al.*, 2018) to be made.

2.1.2. Survival and stability in processed or stored samples

No information available

2.1.3. Survival and stability outside the host

After its release from the European flat oyster (*Ostrea edulis*), *M. refringens* can survive at least 20 days in seawater and faeces. Parasite survival seems improved in faeces compared with seawater (Mérou *et al.*, 2022).

2.2. Host factors

2.2.1. Susceptible host species

Species that fulfil the criteria for listing as susceptible to infection with *Marteilia refringens* according to Chapter 1.5. of the *Aquatic Animal Health Code* (*Aquatic Code*) are: ~~blue mussel (*Mytilus edulis*), dwarf oyster (*Ostrea stentina*), European flat oyster (*Ostrea edulis*), European razor clam (*Solen marginatus*), golden mussel (*Xenostrobus securis*), Mediterranean mussel (*Mytilus galloprovincialis*) and striped venus clam (*Chamelea gallina*).~~

Family	Scientific name	Common name
Ostreidae	*Ostrea edulis*	European flat oyster
	Ostrea stentina	dwarf oyster
Mytilidae	*Mytilus edulis*	blue mussel
	Mytilus galloprovincialis	Mediterranean mussel
	Xenostrobus securis	golden mussel

Family	Scientific name	Common name
Solenidae	*Solen marginatus*	European razor clam
Veneridae	*Chamelea gallina*	striped venus clam

Additionally, a copepod species (*Paracartia grani*) has been found to meet the criteria for listing as susceptible to infection with *M. refringens* and is considered an intermediate host.

2.2.2. Species with incomplete evidence for susceptibility

Species for which there is incomplete evidence to fulfil the criteria for listing as susceptible to infection with *M. refringens* according to Chapter 1.5 of the *Aquatic Code* are: ~~Chilean flat oyster (*Ostrea chilensis*), a copepod (*Paracartia latisetosa*) and Japanese flat oyster (*Ostrea denselamellosa*).~~

Family	Scientific name	Common name
Acartiidae	*Paracartia latisetosa*	no common name
Ostreidae	*Ostrea chilensis*	Chilean flat oyster
	Ostrea denselamellosa	Japanese flat oyster

In addition, pathogen-specific positive polymerase chain reaction (PCR) results have been reported in the following species, but no active infection has been demonstrated: ~~Cortez oyster (*Crassostrea corteziensis*), grooved carpet shell (*Ruditapes decussatus*), Pacific cupped oyster (*Magallana* [syn. *Crassostrea] gigas*) and zooplankton (*Acartia discaudata, Centropages typicus, Euterpina acutifrons*, unidentified *Oithona* sp., *Penilia avirostris*).~~

Family	Scientific name	Common name
Acartiidae	*Acartia discaudata*	no common name
Achidiidae	*Euterpina acutifrons*	no common name
Centropagidae	*Centropages typicus*	no common name
Oithonidae	*Oithona* sp.	no common name
Ostreidae	*Magallana* [syn. *Crassostrea*] *gigas*	Pacific cupped oyster
	Crassostrea corteziensis	Cortez oyster
Sididae	*Penilia avirostris*	no common name
Veneridae	*Ruditapes decussatus*	grooved carpet shell

2.2.3. Likelihood of infection by species, host life stage, population or sub-populations

Marteilia refringens usually causes clinical infection in the European flat oyster, *O. edulis* (Berthe *et al.*, 2004; Grizel *et al.*, 1974). In flat oysters and mussels, prevalence and infection intensity are generally higher in individuals 2 years old or older (Audemard *et al.*, 2001; Villalba *et al.*, 1993b).

2.2.4. Distribution of the pathogen in the host

Marteilia refringens infects the digestive tract. Young plasmodia are mainly found in the epithelium of labial palps, oesophagus and the stomach (Grizel *et al*., 1974). Sporulation takes place in the digestive gland tubules and ducts. Propagules are released into the lumen of the digestive tract and shed into the environment in faeces (Audemard *et al.,* 2002; Berthe *et al*., 2004; Mérou *et al.*, 2022).

2.2.5. Aquatic animal reservoirs of infection

Infected flat oysters, *O. edulis,* and mussels, *Mytilus edulis* and *M. galloprovincialis,* might not exhibit clinical signs or mortality, however they can release parasite sporangiospores (Arzul *et al.,* 2014; Mérou *et al.,* 2023).

2.2.6. Vectors

None known.

2.3. Disease pattern

2.3.1. Mortality, morbidity and prevalence

Infection is lethal for oysters: a 50–90% mortality rate is usually reported during summer and autumn and is associated with sporulation of the parasite (Grizel, 1985; Grizel *et al*., 1974). Similarly, morbidity is higher during

warmer periods. Mussels are less affected by infection but mortalities up to 40% were reported in impacted areas (Berthe *et al*., 2004; Villalba *et al*., 1993b) and naïve mussels presented 100% mortality after being cultured for 6 months in an infected area (Thébault *et al.,* 1999).

Prevalence is highly variable – up to 98% in *O. edulis*. Higher prevalence is expected depending on farming practices and in areas where potential hosts have had more than 1 year of exposure to infection (Berthe *et al*., 2004; Grizel, 1985). Prevalence usually peaks in summer whereas the parasite is usually absent or found at lower infection intensity in winter and early spring (Audemard *et al.*, 2001; Mérou *et al.*, 2023). An additional prevalence peak in spring has been reported in several studies (Arzul *et al.*, 2014; Boyer *et al.*, 2013; Carrasco *et al.*, 2007; Mérou *et al.*, 2023).

2.3.2. Clinical signs, including behavioural changes

Clinical signs include dead or gaping molluscs (Grizel, 1985; Grizel *et al*., 1974) but are not specific for infection with *M. refringens* and could be indicative of other infections.

2.3.3 Gross pathology

Pale digestive gland, thin watery flesh, mantle retraction and reduced growth rate were reported for infected flat oysters (Berthe *et al*., 2004; Grizel, 1985; Grizel *et al*., 1974), although these gross signs are not specific for infection with *M. refringens*. Reduced growth rate and inhibition of gonad development were reported for infected mussels (Villalba *et al*., 1993a).

2.3.4. Modes of transmission and life cycle

Horizontal transmission of *M. refringens* occurs, probably via an intermediate host (Audemard *et al.,* 2002; Carrasco *et al.,* 2008b). The parasite could be experimentally transmitted from *O. edulis* and *M. galloprovincialis* to the copepod *Paracartia grani* (Audemard *et al.,* 2002; Carrasco *et al.,* 2008b). Transmission from *P. grani* to *O. edulis* or *M. galloprovincialis* has not been demonstrated experimentally (Audemard *et al.,* 2002; Carrasco *et al.,* 2008b). In oysters, the early stages of disease occur in the oesophagus, stomach, palps and even gill epithelia. It is thought that initial infection occurs via feeding currents. In mussels, the early stages have been observed in the epithelium of the gills, mantle, stomach and primary digestive tubules (Carrasco *et al.,* 2008a).

The life cycle of *M. refringens* is suspected to be indirect and may include *P. grani* (Audemard *et al.,* 2001; 2002), at least in pond systems. Other species (see Sections 2.2.5 and 2.2.6) might be involved as reservoirs or vectors in the *M. refringens* life cycle but their role has not been demonstrated).

The detection of *M. refringens* DNA in plankton, particularly nanoplankton, and in the benthos, suggests their involvement in the parasite life-cycle including transmission and storage or possible overwintering, respectively (Mérou *et al.*, 2023).

2.3.5. Environmental factors

The threshold temperature for parasite sporulation and transmission is 17°C. This temperature is common in estuaries or bays where prevalence is usually higher in the upper parts of the water column (Audemard *et al.,* 2001; Berthe *et al*., 2004; Carrasco *et al.,* 2007; Grizel, 1985). Infection with *M. refringens* is seldom observed in open sea waters (Grizel, 1985). High salinity and water renewal could be detrimental to *M. refringens* development and transmission, although these parameters appear to be less significant than temperature (Audemard *et al.,* 2001).

Parasite DNA detection in pelagic compartments was found higher when temperature, salinity and cholorophyll-a were higher (Mérou *et al.*, 2023).

2.3.6. Geographical distribution

Reported in Europe and North Africa.

See WAHIS (https://wahis.woah.org/#/home) for recent information on distribution at the country level.

2.4. Biosecurity and disease control strategies

2.4.1. Vaccination

None.

2.4.2. Chemotherapy including blocking agents

None.

2.4.3. Immunostimulation

None.

2.4.4. Breeding resistant strains

None.

2.4.5. Inactivation methods

No data available.

2.4.6. Disinfection of eggs and larvae

No data available.

2.4.7. General husbandry

Stocking at low density or in association with resistant mollusc species, such as *Crassostrea gigas,* has been shown to be effective (Grizel, 1985). Stocking bivalves in deep zones exposed to currents seems to limit the transmission of the parasite. Considering the possible presence of the parasite in the sediment (Mérou *et al.*, 2023), maintaining bivalves at distance from the bottom should limit the number of infected animals.

3. Specimen selection, sample collection, transportation and handling

This section draws on information in Sections 2.2, 2.3 and 2.4 to identify populations, individuals and samples that are most likely to be infected.

3.1. Selection of populations and individual specimens

Gaping or freshly dead individuals (2 or more years old) of species referred to in Section 2.2.1., should be sampled preferentially, to increase the chances of finding infected bivalves. For histology, only live (including moribund) bivalves should be sampled.

Sampling of bivalves should be organised when prevalence is known to be at a maximum. When such data are not available in a particular ecosystem, sampling should preferably be carried out when temperature reaches the yearly maximum (Audemard *et al.,* 2001; Carrasco *et al.,* 2007).

3.2. Selection of organs or tissues

A 3–5 mm thick section of tissues including gills and digestive mass is used for diagnosis of *M. refringens* infection by histology and PCR. A piece of digestive gland is preferred for imprints.

3.3. Samples or tissues not suitable for pathogen detection

Tissues other than gills and digestive mass are not suitable.

3.4. Non-lethal sampling

Examination of fresh samples of faeces collected from potentially infected bivalves using light microscopy is possible although this approach has not been validated (See Section 4.1)

3.5. Preservation of samples for submission

For guidance on sample preservation methods for the intended test methods, see Chapter 2.4.0 *General information* (*diseases of molluscs*).

3.5.1. Samples for pathogen isolation

The success of pathogen isolation and results of bioassay depend strongly on the quality of samples (time since collection and time in storage). Fresh specimens should be kept on ice and preferably sent to the laboratory within 24 hours of collection. To avoid degradation of samples, use alternative storage methods only after consultation with the receiving laboratory.

3.5.2. Preservation of samples for molecular detection

Tissue samples for PCR testing should be preserved in 80% (v/v) analytical-grade ethanol. The recommended ratio of ethanol to tissue is 10:1 based on studies in terrestrial animal and human health. The use of lower grade (laboratory or industrial grade) ethanol is not recommended. If material cannot be fixed it may be frozen.

Standard sample collection, preservation and processing methods for histological techniques can be found in Section B.5.5 of Chapter 2.4.0 *General information* (diseases of molluscs).

3.5.3. Samples for histopathology, immunohistochemistry or *in-situ* hybridisation

Standard sample collection, preservation and processing methods for histological techniques can be found in Section B.5.3 of Chapter 2.4.0 *General information* (diseases of molluscs).

3.5.4. Samples for other tests

None.

3.6. Pooling of samples

Pooling of samples from more than one individual animal for a given purpose is only recommended where robust supporting data on diagnostic sensitivity and diagnostic specificity have been evaluated and found to be suitable. If the effect of pooling on diagnostic sensitivity has not been thoroughly evaluated, larger specimens should be processed and tested individually. Small life stages such as spat can be pooled to obtain the minimum amount of material for molecular detection.

Performances of diagnostic methods applied on pools have not been evaluated.

4. Diagnostic methods

The methods currently available for pathogen detection that can be used in i) surveillance of apparently healthy animals, ii) presumptive diagnosis in clinically affected animals and iii) confirmatory diagnostic purposes are listed in Table 4.1. by animal life stage.

Ratings for purposes of use. For each recommended assay a qualitative rating for the purpose of use is provided. The ratings are determined based on multiple performance and operational factors relevant to application of an assay for a defined purpose. These factors include appropriate diagnostic performance characteristics, level of assay validation, availability cost, timeliness, and sample throughput and operability. For a specific purpose of use, assays are rated as:

+++ =	Methods are most suitable with desirable performance and operational characteristics.
++ =	Methods are suitable with acceptable performance and operational characteristics under most circumstances.
+ =	Methods are suitable, but performance or operational characteristics may limit application under some circumstances.
Shaded boxes =	Not appropriate for this purpose.

Validation stage. The validation stage corresponds to the assay development and validation pathway in chapter 1.1.2. The validation stage is specific to each purpose of use. Where available, information on the diagnostic performance of recommended assays is provided in Section 6.3.

WOAH Reference Laboratories welcome feedback on diagnostic performance of recommended assays, in particular PCR methods. Of particular interest are any factors affecting expected assay sensitivity (e.g. tissue components inhibiting amplification) or expected specificity (e.g. failure to detect particular genotypes, detection of homologous sequences within the host genome). These issues should be communicated to the WOAH Reference Laboratories so that advice can be provided to diagnostic laboratories and the standards amended if necessary.

Table 4.1. *WOAH recommended diagnostic methods and their level of validation for surveillance of apparently healthy animals and investigation of clinically affected animals*

Method	A. Surveillance of apparently healthy animals				B. Presumptive diagnosis of clinically affected animals				C. Confirmatory diagnosis[1] of a suspect result from surveillance or presumptive diagnosis			
	Early life stages[2]	Juveniles[2]	Adults	LV	Early life stages[2]	Juveniles[2]	Adults	LV	Early life stages[2]	Juveniles[2]	Adults	LV
Wet mounts						+	+	NA				
Tissue imprints		++	++	NA		+++	+++	NA				
Histopathology		++	++	2		+++	+++	NA				
Transmission electron microscopy					+	++	++	NA	~~+~~	~~++~~	~~++~~	~~NA~~
Real-time PCR	+++	+++	+++	3	+++	+++	+++	NA	+++	+++	+++	NA
Conventional PCR	++	++	++	2	+++	+++	+++	NA				
Conventional PCR followed by amplicon sequencing									+++	+++	+++	NA
In-situ hybridisation									+	+++	+++	NA
Bioassay												
LAMP												
Ab-ELISA												
Ag-ELISA												
Other antigen detection methods[3]												
Other methods[3]												

LV = level of validation, refers to the stage of validation in the WOAH Pathway (chapter 1.1.2); PCR = polymerase chain reaction; LAMP = loop-mediated isothermal amplification; Ab- or Ag-ELISA = antibody or antigen enzyme-linked immunosorbent assay, respectively; IFAT = indirect fluorescent antibody test.

[1]For confirmatory diagnoses, methods need to be carried out in combination (see Section 6). [2]Susceptibility of early and juvenile life stages is described in Section 2.2.3.

[3]Specify the test used. Shading indicates the test is inappropriate or should not be used for this purpose.

4.1. Wet mounts

Samples to be taken consist of gaping bivalves ~~oysters/mussels~~ or freshly dead bivalves ~~oysters/mussels~~.

Squash a piece of digestive gland on a glass slide. Observations are then made at ×400 magnification and can potentially show refringent granules in mature sporangia.

Marteilia species are indicated by the presence of large (9–30 µm) spherical bodies containing thick wall structures.

4.2. Imprints

In moderate and advanced infections, digestive gland imprints are prepared.

Samples to be taken consist of fresh, gaping, or freshly dead bivalves.

*A*fter drying tissues on absorbent paper, several imprints are made on a glass slide. Slides are air-dried, fixed and stained using a commercially available blood-staining kit, in accordance with the manufacturer's instructions; fixation can be done using methanol or absolute ethanol. After rinsing in tap water and drying, the slides are mounted with a cover-slip using an appropriate synthetic resin. Slides are observed first at ×200 magnification and then under oil immersion at ×1000 magnification.

The observation of cells with a range in size of 5–8 µm diameter in the early stages of development and up to 30–40 µm during sporulation, may indicate infection with *Marteilia refringens.* The cytoplasm stains basophilic, whereas the nucleus stains eosinophilic. Pale halos around large, strongly stained (refringent) granules and, in larger cells, cell-within-cell arrangements are observed. In advanced stages, eight secondary cells can be observed in the primary cells and four spores in each secondary cell (Berthe *et al*., 2000; 2004; Grizel *et al*., 1974).

4.3. Histopathology

Samples to be taken consist of live or moribund bivalves.

*S*ections of tissues that include gills, digestive gland, mantle and gonad should be fixed for a minimum of 24 hours in a recommended fixative followed by standard processing for histology as described in section 5.3 of Chapter 2.4.0 *General information* (diseases of molluscs). Observations are made at increasing magnifications up to ×1000.

Specificity and sensitivity: values of diagnostic sensitivity and specificity for histology were estimated at 70% and 99%, respectively (Thébault *et al*., 2005).

The observation of cells ranging in size from 4 to 40 µm may be indicative of infection with *Marteilia refringens*. Young stages (uninucleated primary cells) are mainly found in the apical part of the epithelium of labial palps, stomach and sometimes in the digestive tubules. Sporulation involves divisions of cells within cells and generally takes place in the digestive gland tubules and ducts. Refringent granules appear during sporulation but are not observed in early stages. The cytoplasm stains basophilic, whereas the nucleus stains eosinophilic. The granules can range from deep orange to deep red; *M. refringens* can sometimes be observed in other organs including gill and mantle connective tissues (Carrasco *et al.*, 2015; Grizel *et al.*, 1974).

Marteilia refringens is slightly different from other *Marteilia* species including *M. sydneyi* or *M. octospora.* Recognition criteria are mainly based on the number of secondary and tertiary cells (respectively 8 and 4 for *M. refringens*). Although *M. christenseni* and *Eomarteilia granula* display the same number of secondary and tertiary cells as *M. refringens,* they infect different host species in different geographic zones.

4.4. Transmission electron microscopy

A small-sized piece of digestive gland (1–2 mm) should be fixed in an appropriate fixative for at least 1 hour and then processed as described in Section B.5.4 *Transmission electron microscopy methods* of Chapter 2.4.0 *General information* (diseases of molluscs).

The presence of parasites within the epithelia of the digestive gland or the stomach may be indicative of infection with *Marteilia refringens*. Different parasite stages can be observed (Grizel *et al.,* 1974; Longshaw *et al*., 2001). The first stage (=

primary cell) is uninucleated but is often observed presenting a single secondary cell within it. Secondary cells result from a series of divisions within the primary cells and include eight presporangia. These presporangia (=secondary cells) divide and contain four-spore primordia (= tertiary cells). Spore primordia cleave internally to produce mature spores. Mature spores consist of three sporoplasms, one inside the other, the outermost one containing haplosporosomes.

4.5. Nucleic acid amplification

Samples to be taken consist of tissues of digestive gland and gills from live or freshly dead molluscs.

PCR assays should always include the controls specified in Section B.5.5 *Molecular methods* of Chapter 2.4.0 *General information* (diseases of molluscs). Molluscs are known to potentially contain substances that can inhibit PCR reactions. It is recommended to check for the presence of PCR inhibitors in DNA extracts to avoid false negative results. In case PCR inhibitors are present, DNA samples can be diluted prior to PCR analyses (a 1/10 dilution resolves most cases of PCR inhibition).

Extraction of nucleic acids

Different kits and procedures can be used for nucleic acid extraction. The quality and concentration of the extracted nucleic acid is important and can be checked using a suitable method as appropriate to the circumstances.

4.5.1. Real-time PCR

Two multiplex real-time PCR assays targeting the ITS (internal transcribed spacer) gene have been developed for the specific detection and discrimination of *M. refringens* type O and type M (Carrasco *et al*., 2017; EURL, 2023).

Additionally, a multiplex real-time PCR assay targeting the 18S gene allows the concomitant detection of *M. refringens* and *Bonamia* spp. parasites (Canier *et al.*, 2020). However, validation tests showed that this PCR assay is less specific and also amplifies *M. cochillia* and to a lesser extent *M. sydneyi*.

***Primers and probes* (*sequences*)**

Pathogen/ target gene	Primer/probe (5'–3')	Concentration	Cycling parameters[(a)]
Method 1: Carrasco *et al.* (2017); GenBank Accession No.: MH304865.1			
M. refringens types O and M ITS	Fwd Mare-F: YCA-GGC-GAG-TGC-TCT-CGT-T Rev Mare-R: TGA-TCT-GAT-ATT-ATT-CAG-CTG-TTC-GA Probe Mare-O: CCT-TTC-CCC-GAC-GGC (VIC MGB-NFQ) Probe MareM: GCT-TGC-CCT-ACG-GCC (FAM MGB-NFQ)	400 nM 400 nM 80 nM 80 nM	50 cycles of: 95°C/3 sec and 60°C/30 sec
Method 2: EURL (2023); GenBank Accession No.: MH304863.1			
M. refringens types O and M ITS	Fwd TaqMar-F: GTG-TTC-GGC-ACG-GGT-AGT Rev TaqMar-R: TGA-TCT-GAT-ATT-ATT-CAG-CTG-TTC-G TaqProb-O: GCC-CTT-TCC-CCG-ACG-GCC-G (FAM-BHQ-1) TaqProb-M: GCG-CTT-GCC-CTA-CGG-CCG-TGC (HEX-BHQ-1)	100 nM 300 nM 250 nM 250 nM	40 cycles of: 95°C/30 sec and 60°C/1 min
Method 3: Canier *et al.* (2020); GenBank Accession No.: MH342044.1			
M. refringens Also amplifies *M. cochillia* and *M. sydneyi* 18S	Fwd Mar_18S_F: ACG-ATC-AAA-GTG-AGC-TCG-TG Rev Mar_18S_R: CAG-TTC-CCT-CAC-CCC-TGA-T Probe Mar_18S_IN: GCA-TGG-AAT-CGT-GGA-ACG-GG (FAM-BHQ-1)	400 nM 400 nM 300 nM	40 cycles of: 95°C/15 sec and 60°C/1 min

[(a)]A denaturation step prior to cycling has not been included.

4.5.2. Conventional PCR

PCR primers are available that target the ITS1 (internal transcribed spacer) region (Le Roux *et al*., 2001), 18S gene (Le Roux *et al*., 1999) and the IGS (rDNA intergenic spacer) region (López-Flores *et al*., 2004).

Primer sequences

Pathogen/ target gene	Primer (5'–3')	Concentration	Cycling parameters[a]
Method 1: Le Roux *et al.* (2001); GenBank Accession No.: MH329403.1; amplicon size 412 bp			
M. refringens types M and O Also amplifies *M. cochillia* ITS-1	Fwd Pr4 (M2A): CCG-CAC-ACG-TTC-TTC-ACT-CC Rev Pr5 (M3AS): CTC-GCG-AGT-TTC-GAC-AGA-CG	1000 nM 1000 nM	30 cycles of: 95°C/1 min and 55°C/1 min and 72°/1 min
Method 2: Lopez-Flores *et al.* (2004) (nested PCR) ; GenBank Accession No.: MH356753.1; amplicon size [525bp & 358 bp]			
M. refringens types M and O Also amplifies *M. cochillia* and possibly other species IGS	PCR1 Fwd MT1: GCC-AAA-GAC-ACG-CCT-CTA-C Rev MT2: AGC-CTT-GAT-CAC-ACG-CTTT PCR2 Fwd MT-1B: CGC-CAC-TAC-GAC-CGT-AGC-CT Rev MT-2B: CGA-TCG-AGT-AAG-TGC-ATG-CA	1000 nM 1000 nM 1000 nM 1000 nM	PCR 1 I30 cycles of: 95°C/1 min and 55°C/1 min and 72°/1 min PCR2 25 cycles of: 95°C/30 sec and 60°C/30 sec and 72°/30 sec
Method 3: Le Roux *et al.* (1999); GenBank Accession No.: MH342044.1; amplicon size [266bp or 700 bp]			
***Marteilia* spp. amplifies *M. refringens* types M and O, *M. cochillia*, and possibly other species** **18S**	**Fwd SS2: CCG-GTG-CCA-GGT-ATA-TCT-CG** **(Rev SAS1: TTC-GGG-TGG-TCT-TGA-AAG-GC)** **Or** **Rev SAS2: CGA-ACG-CAA-ATT-GCG-CAG-GG**	**1000 nM** **1000 nM** **1000 nM**	**30 cycles of: 95°C/1 min and 55°C/1 min and 72°/1 min**

[a]A denaturation step prior to cycling has not been included.
Note: according to the alignment of available sequences
Rev SAS1 primer sequence should be: TTC-GG-TGG-TCT-TGA-AAG-GC

4.5.3. Other nucleic acid amplification methods

A loop-mediated isothermal amplification (LAMP) assay for the detection of *M. refringens* has been developed, but is not validated (Xie *et al.*, 2012).

4.6. Amplicon sequencing

The size of the PCR amplicon is verified by agarose gel electrophoresis and purified by excision from this gel for sequence analysis. Obtained sequences are compared with published sequences.

Sequencing is recommended as one of the final steps for confirmatory diagnostic. Targeted regions are the SSU rDNA (except 18S PCR SS2/SAS1), ITS1 and IGS (intergenic spacer). Although sequences are available in the public gene banks, it is recommended to refer such cases to the appropriate WOAH Reference Laboratory.

4.7. *In-situ* hybridisation

Le Roux *et al.* (1999) developed an ISH genus-specific method targeting the 18S gene. This method allows the detection of all currently known *Marteilia* species. It has been validated against histology for the detection of *M. refringens* (Thébault *et al.*, 2005).

Two other ISH assays have been developed, one targeting the ITS1 (internal transcribed spacer) region (Le Roux *et al*., 2001) and the other targeting the IGS (intergenic spacer) region (Lopez-Flores *et al*., 2008a; 2008b). These assays allow the detection of *M. refringens* type O and type M.

Samples to be taken consist of live or gaping molluscs.

Technical procedure:

Reference	Pathogen/target gene	ISH probe	Probe size
Le Roux *et al.* (1999)	*Marteilia* spp. 18S	Digoxigenin-labelled PCR product obtained with SS2/SAS1 primers	266 bp
Le Roux *et al.* (2001)	*M. refringens* types M and O ITS1	Digoxigenin-labelled PCR product obtained with Pr4/Pr5 primers	412 bp
Lopez-Flores *et al.* (2004)	*M. refringens* types M and O IGS	Digoxigenin-labelled PCR product obtained with MT-1B/MT-2B primers	358 bp

The first steps follow the recommendations described in Section B.5.5.4. of Chapter 2.4.0 *General information* (diseases of molluscs). For hybridisation, sections are incubated with 100 µl of hybridisation buffer (4 × SSC [standard saline citrate], 50% formamide, 1 × Denhardt's solution, 250 µg ml^{-1} yeast tRNA, 10% dextran sulphate) containing approx. 10 ng (2 to 5µl µl of digoxigenin-labelled probe prepared by conventional PCR as described above (section 4.5.2; Le Roux *et al.*, 1999; 2001, Lopez-Flores *et al*., 2004; 2008a; 2008b). Sections are covered with *in-situ* plastic cover-slips and placed on a heating block at 94°C for 5 minutes. Slides are then cooled on ice for 1 to 5 minutes before overnight hybridisation at 42°C in a humid chamber. Sections are washed twice for 5 minutes in 2 × SSC at room temperature, and once for 10 minutes in 0.4 × SSC at 42°C. The detection steps are performed according to the manufacturer's instructions. The slides are then rinsed with appropriate buffer. The sections are counter-stained with Bismarck Brown Yellow, rinsed in tap water, immersed in 95% and then 100% ethanol, 30 seconds for each, rinsed in Xylene (10–30 seconds), and cover-slips are applied using an appropriate mounting medium.

Positive/negative controls: inclusion of the following controls is compulsory. 1) Infected host positive control; 2) non-specific ISH (18S) on samples as an internal positive control. 3) No probe ISH negative control; 4) Uninfected host negative control. Positive controls are available on request from the WOAH Reference Laboratory.

4.8. Immunohistochemistry

Not available.

4.9. Bioassay

Not available.

4.10. Antibody- or antigen-based detection methods (ELISA, etc.)

Not currently available or used for diagnostic purposes but monoclonal antibodies have been developed (Berthe *et al*., 2004). These antibodies did not cross-react with *M. sydneyi*.

4.11. Other methods

None available.

5. Test(s) recommended for surveillance to demonstrate freedom in apparently healthy populations

Real-time PCR is recommended for targeted surveillance to declare freedom from infection with *M. refringens*.

6. Corroborative diagnostic criteria

This section only addresses the diagnostic test results for detection of infection in the absence (Section 6.1.) or in the presence of clinical signs (Section 6.2.) but does not evaluate whether the infectious agent is the cause of the clinical event.

The case definitions for a suspect and confirmed case have been developed to support decision making related to trade and confirmation of disease status at the country, zone or compartment level. Case definitions for disease confirmation in endemically affected areas may be less stringent. If a Competent Authority does not have the capability to undertake the necessary diagnostic tests it should seek advice from the appropriate WOAH Reference Laboratory, and if necessary, refer samples to that laboratory for confirmatory testing of samples from the index case in a country, zone or compartment considered free.

6.1. Apparently healthy animals or animals of unknown health status[1]

Apparently healthy populations may fall under suspicion, and therefore be sampled, if there is an epidemiological link to an infected population. Hydrographical proximity to, or movement of animals or animal products or equipment, etc., from a known infected population, equate to an epidemiological link. Alternatively, healthy populations are sampled in surveys to demonstrate disease freedom.

6.1.1. Definition of suspect case in apparently healthy animals

The presence of infection with *M. refringens* shall be suspected if at least one of the following criteria is met:

i) Positive result by a recommended molecular detection test

ii) Visual observation of the pathogen by microscopy

6.1.2. Definition of confirmed case in apparently healthy animals

The presence of infection with *M. refringens* is considered to be confirmed if the following criterion is met:

i) positive result by real-time PCR and conventional PCR followed by sequence analysis

6.2 Clinically affected animals

Clinical signs are not pathognomonic for a single disease; however they may narrow the range of possible diagnoses.

6.2.1. Definition of suspect case in clinically affected animals

The presence of infection with *M. refringens* shall be suspected if at least one of the following criteria is met:

i) Positive result by wet mounts

ii) Positive result by tissue imprints

iii) Positive result by histopathology

iv) Positive result by real-time PCR

v) Positive result by conventional PCR

6.2.2. Definition of confirmed case in clinically affected animals

The presence of infection with *M. refringens* is considered to be confirmed if at least one of the following criteria is met:

i) positive result by real-time-PCR and conventional PCR followed by sequence analysis

ii) positive result by species-specific ISH and conventional PCR followed by sequence analysis

iii) Positive result of real-time PCR followed by species-specific ISH

1 For example transboundary commodities.

6.3. Diagnostic sensitivity and specificity for diagnostic tests

The diagnostic performance of tests recommended for surveillance or diagnosis of infection with *M. refringens* are provided in Tables 6.3.1. (no data are currently available) and 6.3.2. This information can be used for the design of surveys for infection with *M. refringens*, however, it should be noted that diagnostic performance is specific to the circumstances of each diagnostic accuracy study (including the test purpose, source population, tissue sample types and host species) and diagnostic performance may vary under different conditions. Data are only presented where tests are validated to at least level 2 of the validation pathway described in Chapter 1.1.2. and the information is available within published diagnostic accuracy studies.

6.3.1. For presumptive diagnosis of clinically affected animals [under study]

Test type	Test purpose	Source populations	Tissue or sample types	Species	DSe (*n*)	DSp (*n*)	Reference test	Citation

DSe = diagnostic sensitivity, DSp = diagnostic specificity, *n* = ~~samples~~ number of animals used in the validation study.

6.3.2. For surveillance of apparently healthy animals

Test type	Test purpose	Source populations	Tissue or sample types	Species	DSe (*n*)	DSp (*n*)	Reference test	Citation
Histology	Surveillance	Field samples from France and Netherlands, representative of three different levels of prevalence (free, mild, high)	Section of tissues including visceral mass	*Ostrea edulis* ~~Flat oysters~~	70% (200)	99% (200)	*In-situ* hybridisation (18S probe) Bayesian analyses	Thébault *et al.*, 2005
In-situ hybridisation (18S probe)	Surveillance	Field samples from France and Netherlands, representative of three different levels of prevalence (free, mild, high)	Section of tissues including visceral mass	*Ostrea edulis* ~~Flat oysters~~	90% (200)	99% (200)	Histology Bayesian analyses	Thébault *et al.*, 2005
Real-time PCR (Canier *et al.*, 2020)	Surveillance	Field samples from the three main producing areas in France, representative of three different levels of prevalence (free, low, high)	Gills and digestive gland tissues	*Ostrea edulis* ~~Flat oysters~~	87,2% (386)	98,4% (386)	Conventional PCR (Le Roux *et al.*, 2001) Bayesian analyses	Canier *et al.*, 2020
Conventional PCR (Le Roux *et al.*, 2001)	Surveillance	Field samples from the three main producing areas in France, representative of three different levels of prevalence (free, low, high)	Gills and digestive gland tissues	*Ostrea edulis* ~~Flat oysters~~	60.7% (386)	99.9% (386)	Real-time PCR (Canier *et al.*, 2020) Bayesian analyses	Canier *et al.*, 2020

DSe = diagnostic sensitivity, DSp = diagnostic specificity, *n* = ~~samples~~ number of animals used in the validation study,
PCR: = polymerase chain reaction.

7. References

ALDERMAN D.J. (1979). Epizootiology of *Marteilia refringens* in Europe. *Mar. Fishery Rev.,* **41**, 67–69.

ARZUL I., CHOLLET B., BOYER S., BONNET D., GAILLARD J., BALDI Y., ROBERT M., JOLY J.-P., GARCIA C. & BOUCHOUCHA M. (2014). Contribution to the understanding of the cycle of the protozoan parasite *Marteilia refringens* . *Parasitology*, **141**, 227–240.

AUDEMARD C., BARNAUD A., COLLINS C.M., LE ROUX F., SAURIAU P.-G., COUSTAU C., BLACHIER P. & BERTHE F.C.J. (2001). Claire ponds as an experimental model for *Marteilia refringens* life-cycle studies: new perspectives. *J. Exp. Mar. Biol. Ecol.,* **257**, 87–108.

AUDEMARD C., LE ROUX F., BARNAUD A. COLLINS C., SAUTOUR B., SAURIAU P.-G., DE MONTAUDOUIN X., COUSTAU C., COMBES C. & BERTHE F.C.J. (2002). Needle in a haystack: involvement of the copepod *Paracartia grani* in the life cycle of the oyster pathogen *Marteilia refringens. Parasitology,* **124**, 315–323.

BERTHE F.C.J., LE ROUX F., PEYRETAILLADE E., PEYRET P., RODRIGUEZ D., GOUY M. & VIVARÈS C.P. (2000). The existence of the phylum Paramyxea Desportes and Perkins, 1990 is validated by the phylogenetic analysis of the *Marteilia refringens* small subunit ribosomal RNA. *J. Euk. Microbiol*., **47**, 288–293.

BERTHE F.C.J., ROUX F., ADLARD R.D. & FIGUERAS A. (2004). Marteiliosis in molluscs: A review. *Aquat. Living Resour.*, **17**, 433–448.

BOYER S., CHOLLET B., BONNET D., ARZUL I. (2013). New evidence for the involvement of *Paracartia grani* (Copepoda, Calanoida) in the life cycle of *Marteilia refringens* (Paramyxea). *Int. J. Parasitol.,* **43**, 1089–1099.

CANIER L., DUBREUIL C., NOYER M., SERPIN D., CHOLLET B., GARCIA C. & ARZUL I. (2020). A new multiplex real-time PCR assay to improve the diagnosis of shellfish regulated parasites of the genus *Marteilia* and *Bonamia*. *Prev. Vet. Med.*, **183**, 105126.

CARRASCO N., ARZUL I., BERTHE F.C.J. & FURONES M.D. (2008a). *In situ* hybridization detection of initial infective stages of *Marteilia refringens* (Paramyxea) in its host *Mytilus galloprovincialis*. *J. Fish Dis.* **31**, 153–157.

CARRASCO N., ARZUL I., CHOLLET B., ROBERT M., JOLY J.-P., FURONES M.D. & BERTHE F. (2008b). Comparative experimental infection of the copepod *Paracartia grani* with *Marteilia refringens* and *M. maurini*. *J. Fish Dis.* **31**, 497–504.

CARRASCO N., GREEN T., ITOH N. (2015). *Marteilia* spp. parasites in bivalves: A revision of recent studies. *J. Invertebr. Pathol.,* **131**, 43–57.

CARRASCO N., LOPEZ-FLORES I., ALCARAZ M., FURONES M.D., BERTHE F.C.J. & ARZUL I. (2007) Dynamics of the parasite *Marteilia refringens* (Paramyxea) in *Mytilus galloprovincialis* and zooplankton populations in Alfacs Bay (Catalonia, Spain). *Parasitology,* **134**, 1541–1550.

CARRASCO N., VOORBERGEN-LAARMAN M., LACUESTA B., FURONES D. & ENGELSMA M.Y. (2017). Application of a competitive real time PCR for detection of *Marteilia refringens* genotype "O" and "M" in two geographical locations: The Ebro Delta, Spain and the Rhine-Meuse Delta, the Netherlands. *J. Invertebr. Pathol.,* **149**, 51–55.

CAVALIER-SMITH T. & CHAO E.E. (2003). Phylogeny and classification of phylum Cercozoa (Protozoa). *Protist.,* **154**, 341–358.

EUROPEAN UNION REFERENCE LABORATORY (EURL) for mollusc diseases (2023). SOP *Marteilia refringens* detection and typing by real-time polymerase chain reaction (PCR) *(*3rd edition, March 2023), https://www.eurl-mollusc.eu/

FEIST S.W., HINE P.M., BATEMAN K.S., STENTIFORD G.E. & LONGSHAW M. (2009). *Paramarteilia canceri* sp. n. (Cercozoa) in the European edible crab (*Cancer pagarus*) with a proposal for the revision of the order Paramyxida Chatton, 1911. *Folia Parasitologica*, **56**, 73–85.

GRIZEL H. (1985). Etude des récentes épizooties de l'huître plate (*Ostrea edulis* Linné) et leur impact sur l'ostréiculture bretonne. Thèse Doctorat es Sciences, Université des Sciences et Techniques du Languedoc, Montpellier, France, 145 p.

GRIZEL H., COMPS M., BONAMI J.R., COUSSERANS F., DUTHOIT J.L., & LE PENNEC M.A. (1974). Recherche sur l'agent de la maladie de la glande digestive de *Ostrea edulis* Linne. *Sci. Pêche. Bull. Inst. Pêches marit.,* **240**, 7–29.

KERR R., WARD G., STENTIFORD G., ALFJORDEN A., MORTENSEN S., BIGNELL J., FEIST S.W., VILLALBA A., CARBALLAL M.J., CAO A., ARZUL I., RYDER D. & BASS D. (2018). *Marteilia refringens* and *Marteilia pararefringens* sp. nov. are distinct parasites of bivalves and have different European distributions. *Parasitology*, **145**, 1483–1492.

LE ROUX F., LORENZO G., PEYRET P., AUDEMARD C., FIGUERAS A., VIVARÈS C., GOUY M. & BERTHE F.C.J. (2001). Molecular evidence for the existence of two species of *Marteilia* in Europe. *J. Euk. Microbiol.*, **48**, 449–454.

LE ROUX F., AUDEMARD C., BARNAUD A. & BERTHE F.C.J. (1999). DNA probes as potential tools for the detection of *Marteilia refringens*. *Mar. Biotechnol.*, **1**, 588–597.

LONGSHAW M., FEIST S.W., MATTHEWS A. & FIGUERAS A. (2001) Ultrastructural characterisation of *Marteilia* species (Paramyxea) from *Ostrea edulis*, *Mytilus edulis* and *Mytilus galloprovincialis*. *Dis. Aquat. Org.,* **44,** 137–142.

LOPEZ-FLORES I., DE LA HERRAN R., GARRIDO-RAMOS, M.A., NAVAS J.I., RUIZ-REJON C., RUIZ-REJON M. (2004). The molecular diagnosis of *Marteilia refringens* and differentiation between *Marteilia* strains infecting oysters and mussels based on the rDNA IGS sequence. *Parasitology,* **129**, 411–419

LOPEZ-FLORES I., GARRIDO-RAMOS M.A., DE LA HERRAN R., RUIZ-REJÓN C., RUIZ-REJÓN M. & NAVAS J.I. (2008a). Identification of *Marteilia refringens* infecting the razor clam *Solen marginatus* by PCR and *in situ* hybridization. *Mol. Cell Probes*, **22**, 151–155.

LOPEZ-FLORES I., ROBLES F., VALENCIA J.M., GRAU A., VILLALBA A., DE LA HERRÁN R., GARRIDO-RAMOS M.A., RUIZ-REJÓN C., RUIZ-REJÓN M. & NAVAS J.I. (2008b). Detection of *Marteilia refringens* using nested PCR and in situ hybridisation in *Chamelea gallina* from the Balearic Islands (Spain). *Dis. Aquat. Org*., **82**, 79–87.

MEROU N., LECADET C., BILLON T., CHOLLET B., POUVREAU S. & ARZUL I. (2022). Investigating the environmental survival of *Marteilia refringens*, a marine protozoan parasite of the flat oyster *Ostrea edulis*, through an environmental DNA and microscopy-based approach. *Front. Mar. Sci*. **9.** doi: 10.3389/fmars.2022.811284

MEROU N., LECADET C., UBERTINI M., POUVREAU S. & ARZUL I. (2023). Environmental distribution and seasonal dynamics of *Marteilia refringens* and *Bonamia ostreae*, two protozoan parasites of the European flat oyster, *Ostrea edulis*. *Front. Cell. Infect. Microbiol*., **13**, 1154484. doi: 10.3389/fcimb.2023.1154484.

THÉBAULT A., BAUD J.P., LE SAUX J.C., LE ROUX F., CHOLLET B., LE COGUIC M.J., FLEURY P.G., BERTHE F. & GÉRARD A. (1999). Compte rendu sur les mortalité de juillet 1999 des moules (*Mytilus edulis*) en poches dans l'Aber Benoît. Rapport IFREMER. 12 p.

THÉBAULT A., BERGMANN S., POUILLOT S., LE ROUX F. & BERTHE F.C.J. (2005). Validation of *in situ* hybridization and histology assays for the detection of the oyster parasite *Marteilia refringens*. *Dis. Aquat. Org.,* **65**, 9–16.

VILLALBA A., MOURELLE S.G., CARBALLAL M.J. & LOPEZ M.C. (1993a). Effects of infection by the protistan parasite *Marteilia refringens* on the reproduction of cultured mussels *Mytilus galloprovincialis* in Galicia (NW Spain). *Dis. Aquat. Org.,* **17**, 205–213.

VILLALBA A., MOURELLE S.G., LOPEZ M.C., CARBALLAL M.J. & AZEVEDO C. (1993b). Marteiliasis affecting cultured mussels *Mytilus galloprovincialis* of Galicia (NW. Spain). I. Etiology, phases of the infection, and temporal and spatial variability in prevalence. *Dis. Aquat. Org.,* **16**, 61–72.

XIE L., XIE Z., PANG Y., DENG X., XIE Z. & LIU J. (2012). Development of a loop-mediated isothermal amplification assay for visual detection of *Marteilia refringens* in shellfish. *Chinese J. Vet. Sci.*, **32**, 993–996.

*
* *

NB: There is a WOAH Reference Laboratory for infection with *Marteilia refringens*
(please consult the WOAH web site:
https://www.woah.org/en/what-we-offer/expertise-network/reference-laboratories/#ui-id-3).
Please contact WOAH Reference Laboratories for any further information on infection with *Marteilia refringens*

NB: FIRST ADOPTED IN 1995 AS MARTEILIOSIS. MOST RECENT UPDATES ADOPTED IN 2012.

Annex 59. Item 9.2.4. – Section 2.2.1. and 2.2.2. of Chapter 2.4.5. 'Infection with *P. marinus*'

CHAPTER 2.4.5.

INFECTION WITH *PERKINSUS MARINUS*

EU position

The EU supports the adoption of these revised sections.

[…]

2.2. Host factors

2.2.1. Susceptible host species

~~Eastern oyster, *Crassostrea virginica*; Pacific oyster, *C. gigas*; suminoe oyster, *C. ariakensis;* mangrove oyster, *C. rhizophorae;* Cortez oyster, *C. corteziensis* (Andrews 1996; Calvo *et al.,* 1999; Calvo *et al.,* 2001; Villalba *et al.,* 2004; Cáceres-Martínez *et al.,* 2008); softshell clam, *Mya arenaria*; Baltic macoma, *Macoma balthica* (Dungan *et al.,* 2007).~~

Species that fulfil the criteria for listing as susceptible to infection with *Perkinsus marinus* according to Chapter 1.5. of the *Aquatic Animal Health Code* (*Aquatic Code*) are: ~~American cupped oyster (*Crassostrea virginica*), Ariake cupped oyster (*Magallana* [Syn. *Crassostrea*] *ariakensis*), Cortez oyster (*Crassostrea corteziensis*) and palmate oyster (*Saccostrea palmula*).~~

Family	Scientific name	Common name
Ostreidae	*Crassostrea corteziensis*	Cortez oyster
	Crassostrea virginica	American cupped oyster
	Magallana [syn. *Crassostrea*] *ariakensis*	Ariake cupped oyster
	Saccostrea palmula	palmate oyster

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

~~All stages after settlement are susceptible.~~

Species for which there is incomplete evidence to fulfil the criteria for listing as susceptible to infection with *P. marinus* according to Chapter 1.5. of the *Aquatic Code* are: ~~Gasar cupped oyster (*Crassostrea tulipa*), mangrove cupped oyster (*Crassostrea rhizophorae*), and Pacific cupped oyster (*Magallana* [Syn. *Crassostrea*] *gigas*).~~

Family	Scientific name	Common name
Ostreidae	*Crassostrea gasar*	Gasar cupped oyster
	Crassostrea rhizophorae	mangrove cupped oyster
	Magallana [syn. *Crassostrea*] *gigas*	Pacific cupped oyster

In addition, pathogen-specific positive polymerase chain reaction (PCR) results have been reported in the following species, but no active infection has been demonstrated: ~~Columbia black oyster (*Crassostrea columbiensis*), soft shell clam (*Mya arenaria*), and stone oyster (*Striostrea prismatica*).~~

Family	Scientific name	Common name
Myidae	*Mya arenaria*	soft shell clam
Ostreidae	*Crassostrea columbiensis*	Columbia black oyster
	Striostrea prismatica	stone oyster

[…]

Annex 3. Item 4. – Work plan and priorities

EU comment

The EU supports the work plan and priorities

WORK PLAN FOR THE AQUATIC ANIMALS COMMISSION

(including provisional timelines for commenting and adoption)

Aquatic Code			
Chapter/Subject	**Status**		
	February 2024	**May GS 2024**	**September 2024**
Monitor emerging diseases and consider any required actions	On-going		
Glossary definitions: 'Competent Authority', 'Veterinary Authority' and 'Aquatic Animal Health Services'	Review comments (2nd round)	Propose for adoption	–
Glossary definitions: 'aquatic animal products'	Review comments (1st round)	Propose for adoption	–
Chapter 1.3. 'Diseases listed by WOAH' – Listing of infection with infectious spleen and kidney necrosis virus species	Review comments (3rd round)	Propose for adoption	–
Article 1.1.5. of Chapter 1.1. 'Notification of diseases and provision of epidemiological information'	Review comments (2nd round)	Propose for adoption	–
Chapter 4.3. 'Application of Compartmentalisation'	Review responses to discussion paper, revise and provide for information	–	Draft revised Chapter 4.3. and present for comment
Draft new Chapter 4.X. 'Emergency disease preparedness'	Review comments (1st round)	–	Review comments (2nd round)
Draft new Chapter 4.Y. 'Disease outbreak management'	Review comments (1st round)	–	Review comments (2nd round)
Draft new Chapter 4.Z. 'Control of pathogenic agents in traded milt and fertilised eggs of fish'	Review comments (1st round)	–	Review comments (2nd round)
Draft new Chapter 5.X. 'Movement of ornamental aquatic animals'	Review comments (1st round)	–	Review comments (2nd round)
Susceptible Species Assessment of new evidence for previously assessed diseases (as necessary)	On-going		
Safe commodities **Articles 8.X.3. – Amphibian**	Review comments (3rd round)	Propose for adoption	–

Aquatic Code			
Chapter/Subject	**Status**		
	February 2024	**May GS 2024**	**September 2024**
Safe commodities **Articles 9.X.3. – Crustacean**	Review comments (2nd round)	Propose for adoption	–
Safe commodities **Articles 10.X.3. – Fish**	Review comments (2nd round)	Propose for adoption	–
Safe commodities **Articles 11.X.3. – Mollusc**	Review comments (3rd round)	Propose for adoption	–
Assessment of default periods in Articles X.X.4.-X.X.8. for disease-specific chapters	Present assessment of default periods with proposed changes	–	–
Model Articles X.X.5. and X.X.6. for disease-specific chapters	Review comments (1st round)	Propose for adoption	–
Susceptible Species – Crustacean diseases – Articles 9.X.1. and 9.X.2. for: **– Infection with decapod iridescent virus** **– Infection with white spot syndrome virus** **– Infection with *Aphanomyces astaci* (Crayfish plague)**	DIV1: Review comments (1st round)	DIV1: Propose for adoption	–
	WSSV: Review *ad hoc* Group report and present amended articles for comment	–	WSSV: Review comments (1st round)
	–	–	Crayfish plague: Review interim *ad hoc* Group report
Article 10.6.2. of Chapter 10.6. Infection with infectious haematopoietic necrosis virus	Review comments (1st round)	Propose for adoption	–
Susceptible Species – Fish diseases – Articles 10.X.1. and 10.X.2. for: **– Infection with Tilapia lake virus** **– Infection with *Aphanomyces invadans* (Epizootic ulcerative syndrome)**	TiLV: Review comments (1st round)	TiLV: Propose for adoption	–
	EUS: Review interim *ad hoc* Group report	–	EUS: Review *ad hoc* Group report and present amended articles for comment
Susceptible species – Mollusc diseases – Articles 11.X.1. and 11.X.2. for: **– Infection with *Perkinsus marinus*** **– Infection with *Perkinsus olseni*** **– Infection with *Xenohaliotis californiensis***	*Perkinsus marinus*: Review comments (2nd round)	*Perkinsus marinus*: Propose for adoption	–
	Perkinsus olseni: Review *ad hoc* Group report and present amended articles for comment	–	*Perkinsus olseni*: Review comments (1st round)
	–	–	*Xenohaliotis californiensis*: Review *ad hoc* Group report and present amended articles for comment

Aquatic Manual			
Chapter/Subject	**Status**		
	February 2024	**May GS 2024**	**September 2024**
Chapter 1.1.1. 'Quality management in veterinary testing laboratories'	Provide comments to BSC	Propose for adoption	–
Chapter 1.1.2. 'Validation of diagnostic assays for infectious diseases of aquatic animals'	Review first draft	–	Review second draft presented by two AAC members with input from RLs
Chapter 2.2.0. 'General information: diseases of crustaceans'	Review comments (3rd round)	Propose for adoption	–
Chapter 2.2.2. 'Infection with *Aphanomyces astaci* (Crayfish plague)'	Review comments (3rd round)	Propose for adoption	–
Chapter 2.2.4. 'Infection with infectious hypodermal and haematopoietic necrosis virus'	–	–	Review updated draft and present for Member comments
Chapter 2.2.6. 'Infection with Macrobrachium rosenbergii nodavirus (white tail disease)'	Review comments (2nd round)	Propose for adoption	–
Chapter 2.2.9. 'Infection with yellow head virus genotype 1'	Review comments (2nd round)	Propose for adoption	–
Chapter 2.2.X. 'Infection with decapod iridescent virus 1'	Review comments (1st round)	Propose for adoption	–
Chapter 2.3.4. 'Infection with HPR-deleted or HPR0 infectious salmon anaemia virus'	–	–	Review updated draft and present for Member comments
Chapter 2.3.9. 'Infection with spring viraemia of carp virus'	Review validation or publication of real-time PCR	–	Review updated draft and present for Member comments
Chapter 2.3.X. 'Infection with tilapia lake virus'	–	–	Review first draft and present for Member comments
Chapter 2.4.0. 'General information: diseases of molluscs'	Review comments (1st round)	Propose for adoption	–
Chapter 2.4.1. 'Infection with abalone herpes virus'	Review comments (1st round)	Propose for adoption	–
Chapter 2.4.4. 'Infection with *Marteilia refringens*'	Review comments (1st round)	Propose for adoption	–
Chapter 2.4.2. 'Infection with *Bonamia exitiosa*'	Review updated draft	–	Review updated draft and present for comments
Chapter 2.4.3. 'Infection with *Bonamia ostreae*'	Review updated draft	–	Review updated draft and present for comments
Section 2.2.1. and 2.2.2. of Chapter 2.2.8. 'Infection with white spot syndrome virus'	Review *ad hoc* Group report and present amended sections for comment	–	Review comments (1st round)

Aquatic Manual			
Chapter/Subject	**Status**		
	February 2024	**May GS 2024**	**September 2024**
Sections 2.2.1. and 2.2.2. of Chapter 2.4.5. 'Infection with *Perkinsus marinus*'	Review comments (2nd round)	Propose for adoption	–
Section 2.2.1. and 2.2.2. of Chapter 2.4.6. 'Infection with *Perkinsus olseni*'	Review *ad hoc* Group report and present amended sections for comment	–	Review comments (1st round)

Annex 40. Item 7.1. – Draft new Chapter 4.X. 'Emergency disease preparedness'

SECTION 4

DISEASE PREVENTION AND CONTROL

CHAPTER 4.X.

EMERGENCY DISEASE PREPAREDNESS

EU comment

The EU thanks the Aquatic Animals Commission and in general supports this new chapter. However, the EU would like to reiterate comments that were not accepted before.

Article 4.X.1.

Purpose

To describe the essential elements of an emergency disease preparedness framework which a *Competent Authority* should develop in accordance with country priorities and resources to ensure that *outbreaks* of important and *emerging aquatic animal diseases* can be rapidly identified and efficiently managed, and which will guide a country, *zone* or *compartment*, towards a suitable path to recovery.

An important *aquatic animal disease* is one which has been identified by the *Competent Authority* in accordance with Article 4.X.6. Such *diseases* may be listed in Chapter 1.3., or they may be *emerging diseases* or other *aquatic animal diseases*.

Article 4.X.2.

Scope

This chapter describes recommendations for the development of an emergency disease preparedness framework. This framework encompasses all the elements that will enable the *Competent Authority* to activate an efficient response to a *disease outbreak*, thereby minimising the impact on *aquatic animal* populations, trade, the economy, and the financial resources that are required to manage *disease outbreaks*. The specific actions which are necessary to operationalise the framework in the event of a *disease outbreak* are described in Chapter 4.Y.

Article 4.X.3.

Introduction

*Aquatic animal disease*s have the potential to spread quickly, often with serious consequences. In many parts of the world, these *disease* events appear to be increasing in frequency and severity, due to increased *aquaculture* production and *international trade*. This chapter provides recommendations for a *Competent Authority* to identify and coordinate the elements of a framework, which will achieve a suitable level of preparedness for those emergencies.

When developing the framework, it is of fundamental importance to ensure that the *aquatic animal disease*s which are important to a country, *zone* or *compartment*, are identified in advance (i.e. in peacetime) by the *Competent Authority*, and that their future control is supported by adequate legislative and funding measures. The statutory list of important *disease*s that is developed after conducting a *risk analysis* as described in 4.X.6., may include *aquatic animal disease*s which are listed in Chapter 1.3., as well as other *disease*s which have been identified as being of importance to the country, *zone* or *compartment*.

EU comment

The EU reiterates the following suggestions concerning the final sentence of the above paragraph of Article 4.X.3., to account for situations where a disease may become important to a country, not only as a result of risk analysis, which could be a lengthy procedure but as a result of other considerations. For example, a disease might be listed in the national statutory list because it is listed by WOAH based on listing criteria, without conducting a risk analysis. Limiting the scope of the list of important diseases subject to disease preparedness to those where a risk analysis has already been conducted may hamper proper disease control.

"The statutory list of important *disease*s ~~that is developed after conducting a *risk analysis* as described in 4.X.6.,~~ may include *aquatic animal disease*s which are listed in Chapter 1.3., as well as other *disease*s which have been identified as being of importance to the country, *zone* or *compartment*."

Also in peacetime, the *Competent Authority* should take a systematic approach to planning every element of the framework that will be applied from the point at which an important *disease* is suspected during the alert phase, through the activation of the *contingency plan* in the emergency phase, to the point at which the recovery phase begins and the emergency officially ends.

The *Competent Authority* should consider whether the *contingency plan* and recovery plan elements of the emergency disease preparedness framework apply either to a specific *aquatic animal disease* or to a group of such *diseases*. The *Competent Authority* should decide in peacetime, which of these approaches best meets their needs, taking into account *aquatic animal diseases* that are listed in their country, the relevant *susceptible species,* and types of production.

Article 4.X.4.

General principles

Emergency *disease* preparedness is a core function of the *Competent Authority.* The various elements that are necessary to ensure that the *Competent Authority* is prepared to deal with an *outbreak* of an important *disease*, are elaborated in a framework. The framework is constructed in peacetime before the occurrence of a *disease outbreak*.

The ultimate success of the framework will be influenced by the quality of the preparations which have been made by the *Competent Authority*, and the commitment and coordination of the *Aquatic Animal Health Services*, and relevant industry stakeholders.

The general principles to be considered when developing an emergency disease preparedness framework are as follows:

1) legal provisions and funding should be available to allow a *Competent Authority* to execute all elements of the framework and to manage disease outbreaks in compliance with the *contingency plan*, and with the detailed operational measures which are referred to in Chapter 4.Y.;

2) risk analysis should be used in advance of, during and after a *disease outbreak* as described in Article 4.X.6. The *risk analysis* that is carried out in advance will identify the important *aquatic animal diseases* which will be subject to emergency measures. The *risk analysis* that is carried out during and after the *disease outbreak* will inform the response and recovery actions which will be taken by the *Competent Authority*, ~~and~~ the *Aquatic Animal Health Services*, and industry stakeholders;

3) a *contingency plan should be developed* for a specific *aquatic animal disease* or group of related *aquatic animal diseases*, following appropriate consultation with the *Aquatic Animal Health Services*, which contains at least the components outlined in points (a) to (f) of Article 4.X.7. The *contingency plan* is:

   a) partially activated in compliance with Article 4.Y.4.~~Chapter 4.Y.~~ when the presence of an important *disease* is suspected during the 'alert phase';

   b) fully activated in compliance with Article 4.Y.5.~~Chapter 4.Y.~~ once the *disease* emergency has commenced during the 'emergency phase'.

4) simulation exercises should be planned and executed to test, and ultimately to improve, relevant elements of the *disease* preparedness framework. Simulation exercises support ~~ensure that~~ *Competent Authorities* and *Aquatic Animal Health Services* to be ~~are~~ trained and properly equipped and resourced ~~to manage suspicion and confirmation of an important *disease* in their *territory*,~~ in accordance with Article 4.X.8.;

5) all elements of the framework should be regularly reviewed and revised as described in Article 4.X.9.;

6) a 'recovery plan' should be prepared as described in Article 4.X.11., which will be based on *risk analysis* and on the recovery options which are described in Article 4.X.10.

Article 4.X.5.

Legal provisions and funding

There are certain pre-requisites for an emergency disease preparedness framework including ~~. Such pre-requisites include~~ that the *Competent Authority* has:

1) ~~recourse to~~ *aquatic animal* health legislation which underpins the execution of all the elements and actions that are necessary to manage suspicion and confirmation of an *outbreak* of an important *aquatic animal disease* as described in Article 4.X.6.*;*

2) access to emergency resources including funds which are sufficient to allow the execution of the relevant elements of the *disease* preparedness framework as well as the operational measures which are set out in Chapter 4.Y.

Any delay in the ability of the *Competent Authority* to rely on legal provisions, or to access finance, can hamper the effective management of a *disease* emergency. Delays should be avoided, or at least minimised, by ensuring that all the administrative steps that must be followed to transmit the necessary funds from the central funding authority to the *Competent Authority* are identified.

Article 4.X.6.

Risk analysis

Risk analysis plays an important role before, during and after a *disease outbreak*. It is therefore, of critical importance that this expertise is available to the *Competent Authority* to ensure that the emergency disease preparedness framework can be efficiently executed. This article elaborates the principles described in Chapter 2.1. and applies them in the context of emergency *disease* preparedness.

Identification of *aquatic animal diseases* which will be subject to emergency measures

Risk analysis should be used by the *Competent Authority* to determine which important *diseases* of *aquatic animal*s present a threat and should, therefore, be subject to emergency measures in the event of a *disease outbreak*.

The *risk analysis* should take account of a country's circumstances. In particular, the knowledge of relevant wild and farmed *aquatic animal* species in the *territory*, as well as their geographic distribution, *disease* status and economic and trade importance, are critical to the completion of an effective *risk analysis*. Such *risk analysis* should also include information on the most important routes of introduction, transmission pathways, life cycle stages, persistence in the environment, likelihood of eradication, which will inform *disease* control strategies and response options which are referred to in Article 4.X.10.

The list of important *aquatic animal* dise*ases* that may be subject to emergency measures should be under regular ~~continual~~ review by the *Competent Authority*. The *risk analysis* should utilise ~~take into account~~ the latest relevant scientific findings and should be repeated regularly to assess the threat of *emerging diseases.* Changes in the species farmed, and in the distribution or virulence of known *pathogenic agents* should inform changes in national *disease* listings. *Competent Authorities* should ensure they collate the data required for completing and updating *risk analysis.*

Surveillance activities

Suspicion of an *outbreak* of an important *aquatic animal disease*, which is subject to statutory control, often results from *surveillance* activities. Therefore, emergency *disease* preparedness systems are heavily reliant on the *surveillance* and reporting activities carried out by the *Aquatic Animal Health Services*, and relevant industry stakeholders in accordance with Chapter 1.4. The outcomes from an emergency disease preparedness framework are fundamentally reliant on the quality of *surveillance* and reporting activities.

In addition, when the presence of an important *aquatic animal disease* is suspected or has been confirmed, *risk analysis* has a crucial role to play in prioritising *surveillance* activities as part of forward and backward epidemiological tracing, and establishing *protection zones* and *infected zones*.

Response actions during the *disease* emergency

As part of preparedness planning, *risk analysis~~assessment~~* protocols should be developed to support decision making by the *Competent Authority* during an *outbreak*. The *risk analysis* should be able to identify the *risk* mitigation measures and protocols that~~Protocols~~ are required to cover a range of *disease* control options e.g. the possibility to on-grow stock on an infected *aquaculture establishment* to slaughter weight (which will include an assessment of the *risk* of spread within a particular water body), and the possibility to move live *aquatic animals* within *infected zones*.

A *risk analysis~~assessment~~* of depopulation activities should be undertaken to ensure that they are carried out with the minimum risk of *disease* spread. In addition, prior to repopulation, a *risk analysis~~assessment~~* should be completed to determine if further *risk* mitigation measures are required to prevent reinfection of the new stock of *aquatic animals*.

Article 4.X.7.

Contingency plan

The *Competent Authority* should decide whether the *contingency plan* applies either to a specific *aquatic animal disease* or to a group of such *diseases* which, because of their similarity to each other, may be managed effectively using the same principles e.g. certain finfish *diseases* that occur in freshwater, certain mollusc *diseases* that occur in seawater.

EU comment
The EU suggest to delete the examples given in the above paragraph of Article 4.X.7., because we believe the examples given are too narrow :
"The *Competent Authority* should decide whether the *contingency plan* applies either to a specific *aquatic animal disease* or to a group of such *diseases* which, because of their similarity to each other, may be managed effectively using the same principles ~~e.g. certain finfish *diseases* that occur in freshwater, certain mollusc *diseases* that occur in seawater~~."

The *Competent Authority* should also consider that because of the nature of *emerging diseases*, the *contingency plan* and the recovery plan, which are devised for such *aquatic animal diseases*, should be generic. Such generic plans will, however, require rapid and effective fine-tuning, once the details of the *emerging disease* have become known, and the *Competent Authority* has assessed that the *disease* in question should be subject to emergency *disease* preparedness measures.

The *contingency plan* should include at least the following components:

1) the establishment of a clear chain of command within the country, from the central level to the regional and local levels, with the *Competent Authority* in overall command. This chain of command should include decision makers from the *Aquatic Animal Health Services* who may not deal directly with *aquatic animal* health, but who play a role in the emergency disease preparedness framework;

2) a framework for cooperation between the *Competent Authority*, ~~and the~~ *Aquatic Animal Health Services* and industry stakeholders. This cooperation should:

   a) ensure that all actions, and roles and responsibilities which form part of the plan are well understood and discussed in advance of and during, any *disease outbreaks*, thereby ensuring that rapid and effective decisions can be made when necessary;

   b) result in the establishment of at least the following groups which meet at frequencies which may vary depending on the phase of the emergency:

      i) a formally recognised emergency management group which is chaired by the *Competent Authority*;

ii) specialist sub-groups which will provide specific advice to the emergency management group~~Emergency Task Force~~ for consideration e.g. epidemiology group, laboratory group, logistics group, communications group, environmental group, producers' group, mental health and psychological support group.

3) identification of, and arrangements for access to, appropriate:

a) central and local *disease* control centres;

b) laboratories;

c) equipment;

d) trained personnel;

e) communications and media liaison;

f) data management or information systems;

g~~f~~) additional materials and resources that may be required, including for instance, telecommunications, transport, vaccines, experts (e.g. in the areas of logistics, fisheries management, environmental protection);

h~~g~~) service providers (e.g. waste disposal contractors, Personal Protective Equipment (PPE) suppliers, chemical suppliers, standby generators).

4) the general *biosecurity* and *disease* control measures which will be taken in the event of suspicion or confirmation of the presence of an important *aquatic animal disease* to which the *contingency plan* applies. The general *biosecurity* measures which will apply to *aquaculture establishments* should follow the guidance on~~comply with~~ the measures which are described in Chapter 4.1. Coordination of control measures with neighbouring countries with shared waterbodies should be taken into account;

5) concerning specific *disease* control measures, the duration of the *fallowing* period that may apply following de-population, cleaning and *disinfection*, should be considered~~, using *risk assessment*~~. The duration of the fallowing period~~Such an assessment~~ should take into account relevant factors such as the nature of the relevant *pathogenic agent*, the type and extent of the production system, hydrographical factors and the nature of local wild *aquatic animal* populations. ~~The *risk assessment* should also inform the need for synchronised~~Synchronised *fallowing* of a number of *aquaculture establishments*, should be considered in certain circumstances;

6) possible response options that can be applied to manage a *disease outbreak*, based on *risk assessment*. Such response options would depend on the progression of the *disease outbreak* and could include measures such as eradication, containment through *biosecurity* measures, mitigation of *disease* consequences, or no *disease* response;

7) *risk communication* strategy which will apply during each stage of the process, both within and between the various authorities and services and with relevant stakeholders. For example, the *contingency plan* should set out the nature and timing of communications with the personnel who are described in points 2(b)(i) and (ii) above, as well as taking community engagement into account, where appropriate. The *risk communication* strategy should be based on the principles of *risk communication* described in Chapter 2.1.

The actions necessary to operationalise points 1 to 7 above are described in Chapter 4.Y.

Article 4.X.8.

Simulation exercises

Simulation exercises are a crucial component of emergency *disease* preparedness. The objectives of such exercises are to validate and test the functionality and suitability of the *contingency plan* and the operational measures which are described in Chapter 4.Y. Simulation exercises will also validate and test the capacity of *Competent Authorities*, ~~and~~ *Aquatic Animal Health Services*, and industry stakeholders to respond to an important *aquatic animal disease*. The emergency disease preparedness framework should include a requirement for the regular completion of simulation exercises to test that all personnel are adequately trained and prepared for the tasks which have been allocated to them. An outcome report should be produced following each simulation exercise.

describing the actions necessary to close any gaps which have been identified in the *contingency plan*, or other amendments which are required to the operational measures which are described in Chapter 4.Y.

The *Competent Authority* should set a minimum frequency for the completion of such exercises, to ensure readiness to efficiently execute the various elements of the *contingency plan,* should it be activated. Simulation exercises may be organised within a country or among the *Competent Authorities* and *Aquatic Animal Health Services* of countries or *zones* with shared waterbodies where relevant.

A simulation exercise should have clearly defined objectives with respect to the elements of the emergency disease preparedness framework or *outbreak* response capability that is being evaluated. The objectives will inform the type of exercise, participation and the exercise design.

The planning, organisation, and completion of simulation exercises should take account of the following points:

1) different types of exercises may be used e.g. tabletop, limited field exercises or more extensive field exercises;

2) the scale, frequency and scope of the exercises should be based on *risk* prioritisation, which has been completed by the *Competent Authority,* taking account of any new *risk* factors which have been identified;

3) exercises should include the *Competent Authority* at different administrative levels, as well as the *Aquatic Animal Health Services,* and relevant industry stakeholders that will be involved in the application of the *contingency plan* in the event of a *disease* emergency;

4) exercises should test the capacity of the *Competent Authority* to manage every element of the emergency disease preparedness framework, from the initial *disease* alert to the end of the recovery phase;

5) once completed, each simulation exercise should be thoroughly evaluated by the organiser, and an outcome report should be prepared, with the objective of identifying:

    a) the elements of the emergency disease preparedness framework that are fit-for-purpose, and those that are not;

    b) the readiness and capacity of the *Competent Authority*, ~~and~~ the *Aquatic Animal Health Services*, and industry stakeholders to respond to the elements of the emergency disease preparedness framework, that were tested during the exercise.

    c) any gaps/issues raised and any actions to be taken forward, including a timeframe within which these should be addressed.

Article 4.X.9.

Revision and review

The *Competent Authority* should establish a mechanism to improve its emergency disease preparedness framework through regular review, and where necessary, revision of its various elements.

The list of *aquatic animal diseases* which are subject to the emergency disease preparedness framework should be under regular~~continual~~ review, as described in Article 4.X.6.

Review and revision of the *contingency plan* and the operational measures which are set out in Chapter 4.Y. should take into account, the outcomes from the evaluation of the simulation exercises described in Article 4.X.8., and the implementation of an emergency *disease* response, where this is relevant.

The review process consequently may necessitate a revision of the *contingency plan* or other elements of the emergency disease preparedness framework. Such exercises and responses should also be used to highlight the training needs of personnel from the *Competent Authority* and the *Aquatic Animal Health Services,* and to inform the possible revision of the legislation which underpins the framework.

The regular review and revision of the emergency disease preparedness framework should also take into account measures to strengthen the *contingency plan* or to prevent another *disease* emergency event~~,~~ (e.g. updated scientific information including

diagnostic tests, improvements in technology or relevant industry practices, as well as any other new elements which will improve the overall suitability and effectiveness of the framework).

All revisions which are made as a result of the review process described above should be communicated to the *Aquatic Animal Health Services* and industry stakeholders within an agreed timeframe.

Article 4.X.10.

Response Options

The *Competent Authority* should take into account that the initial objective of successfully completing an eradication programme and re-gaining *disease* freedom in a country, *zone* or *compartment* following a *disease outbreak*, may change as *the outbreak* develops.

While the purpose of the recovery plan, may be to re-establish the *disease-free* situation which existed before the *disease outbreak* occurred, it should be considered that in certain cases, the *aquatic animal health status* which is achieved after the emergency has ended, may not be the same as the one which existed before the *outbreak* occurred. Various response options should, therefore, be set out in the emergency disease preparedness framework, upon which the recovery plan can be based, depending on the epidemiological situation which exists at the end of the emergency.

Concerning the *aquatic animal diseases* which are listed in Chapter 1.3., and taking into account Chapter 1.4., the possible options the *Competent Authority* could consider as part of their recovery plan are as follows:

1) demonstrate the re-establishment of disease freedom at country, *zone* or *compartment* level;

2) establish a *disease free zone* in a previously *disease free country*;

3) establish a redefined (reduced) *disease free zone*;

4) establish one or more *disease-free compartments*;

5) relinquish *disease* free status and take measures to contain the *disease*;

6) take measures which are designed to mitigate the impacts of the *disease*;

7) accept that none of the options outlined above are feasible and no official disease control measures will be applied.

If *disease* control operations are halted before regaining the pre-*outbreak disease* free status at country or *zone* level, the recovery plan should set out how the *Competent Authority* could explore the potential to establish redefined *disease free zones* or *compartments.*

Where the options described in points 1 to 6 above are not possible for epidemiological, logistical or economic reasons, the *Competent Authority* may accept an evolution from the original *disease* free status, to one where the *disease* has become endemic, but where the epidemiological situation is stable.

Concerning important *aquatic animal diseases* which are not listed in Chapter 1.3., but which are listed in the national legislation of a country, the *Competent Authority* may decide to apply a similar range of options to those described in points 1 to 4 above. However, these would not fall within the scope of the official *disease free* statuses that may be established for a country, *zone* or *compartment*, as described in Chapter 1.4.

Article 4.X.11.

Recovery plan

The *Competent Authority* should decide whether the recovery plan applies either to a specific *aquatic animal disease* or to a group of such diseases which, because of their similarity to each other, may be managed effectively using the same principles e.g. certain finfish *diseases* that occur in freshwater, certain mollusc *diseases* that occur in seawater.

The recovery plan should be activated when the end of the emergency has been declared by the *Competent Authority*. The point at which the emergency ends, and the nature of the recovery plan, will be determined by *risk* *analysis*~~*assessment*~~, which will take account of the following factors as well as the options described in Article 4.X.10.:

1) the current geographic distribution of the *pathogenic agent;*

2) whether or not, the *disease* has become established in wild *aquatic animal* populations;

3) the costs and feasibility of establishing and maintaining *disease*-freedom at the level of country, *zone* or *compartment,* taking into account hydrological and epidemiological connections;

4) the socio-economic impact of the possible recovery option(s);

5) any *risk* the *disease* may pose to vulnerable wild *aquatic animal* populations in the infected or adjacent areas.

Concerning the response options described in points 1 to 6 of Article 4.X.10., the recovery plan should include details of the actions which the *Competent Authority* and the operators of *aquaculture establishments* should take to:

6) prepare a self-declaration of freedom from *disease*, as referred to in points 1 to 4 of Article 4.X.10.; or

7) put in place appropriate *biosecurity* measures in compliance with Chapter 4.1., to ensure the disease is contained, as referred to in point 5 of Article 4.X.10.; or

8) put in place the mitigation measures which are referred to in point 6 of Article 4.X.10.~~,~~ (e.g. vaccination, change of production species, or change in husbandry practices);

9) consider research requirements to support the actions referred to in points 6 to 8.

Annex 41. Item 7.1. – Draft new Chapter 4.Y. 'Disease outbreak management'

SECTION 4

DISEASE PREVENTION AND CONTROL

CHAPTER 4.Y.

DISEASE OUTBREAK MANAGEMENT

EU comment

The EU thanks the Aquatic Animals Commission for taking into account most of our previous comments on the draft chapter.

However, the EU provides additional comment to Articles 4.Y.4 and 4.Y.6, due to the changes made in February 2024 to the draft chapter below.

Article 4.Y.1.

Purpose

To provide recommendations concerning the actions which should be taken by the *Competent Authority* and the *Aquatic Animal Health Services* to manage the emergency response to suspicion or confirmation of the presence of an important *aquatic animal disease*, and activate its contingency plans as described in Chapter 4.X.

Article 4.Y.2.

Scope

To provide recommendations concerning the actions to be taken by the *Competent Authority* and the *Aquatic Animal Health Services,* from the point at which an important *disease*, as described in Article 4.X.6., is suspected in a *free country*, *free zone* or *free compartment*, or has been suspected or confirmed in an epidemiologically linked population, to the point at which the recovery phase begins. These actions operationalise the elements described in Chapter 4.X., which are required to manage the *disease outbreak*.

Article 4.Y.3.

General Principles

The successful management of an emergency response should take the following principles into account:

1) the actions to be taken by the *Competent Authority* and the *Aquatic Animal Health Services*, should be based on the emergency *disease* preparedness framework which has been developed in accordance with Chapter 4.X.;

2) the operational elements of the emergency *disease* preparedness framework should be described in an Operations Manual. The Operations Manual may be a single document or a series of documents which together, ~~The *Competent Authority* can rely on the Operations Manual to~~ provide guidance on all aspects of the emergency response, including actions to be taken during the alert, emergency, and recovery phases;

3) the initial response objective following a *disease outbreak* is to eradicate the *disease*, thereby allowing a country, *zone* or *compartment* to return to *disease* freedom. However, should the progression of the *outbreak* prevent this objective from being

achieved, other actions should be described, which will assist the *Competent Authority* to pursue an alternative pathway to recovery;

4) the actions described in the Operations Manual should be executed in a timely and co-ordinated fashion, by competent personnel, who have access to all the resources which are necessary to manage the *disease outbreak*.

Article 4.Y.4.

Alert phase

The alert phase begins when there is suspicion of the presence of an important *disease* of *aquatic animals*, generally as a consequence of active or *passive surveillance* in the country, or in another country, which is a neighbour or a trading partner.

The main actions to be taken into account during the alert phase of an emergency should take the following factors into account:

~~1) the alert phase begins when there is suspicion of the presence of an important *disease* of *aquatic animals*, generally as a consequence of active or *passive surveillance* in the country, or in another country, which is a neighbour or a trading partner. During this phase, the *Competent Authority* will take steps to detect the presence of the *disease* and to prevent possible *disease* spread;~~

1~~2~~) following the commencement of this phase, an epidemiological investigation should be initiated in order to:

a) confirm or rule out the presence of the *disease*, in the shortest possible time frame;

b) establish a working case definition for outbreak investigation where this is necessary (e.g. in the case of a disease which is not listed in Chapter 1.3., or of an emerging disease);

c~~b~~) determine if the *disease* has spread from or to *aquaculture establishments* or waterbodies other than the one in which the original suspicion was raised.

2~~3~~) during the epidemiological investigation:

a) *risk*-based *surveillance* is used to prioritise which *aquatic animal* populations, identified through tracing, should be prioritised for sampling. For example, *aquaculture establishments* which are highly connected to the *aquaculture establishment* or waterbody in which the suspicion arose, through movements of live *aquatic animals* and other transmission pathways, as described in Article 4.1.7., should be considered~~prioritised~~ for clinical inspection and sampling;

b) the samples should be submitted to laboratories identified in the *Contingency Plan,* as described in Chapter 4.X., as being suitably equipped and staffed to produce reliable results in the shortest possible timeframe.

3~~4~~) during the alert phase, taking into account Chapter 4.1., the *Competent Authority* should take steps to prevent *disease* spread by implementing *biosecurity* measures in the *aquaculture establishment* or waterbody in question. Additional specific *disease* control measures should also be considered, such as:

a) prohibiting the movement of *aquatic animals* and *aquatic animal products* as well as equipment, *vehicles*, *feed*, contaminated water and *aquatic animal waste* to or from the *aquaculture establishment* or waterbody, unless authorised by the *Competent Authority* based on a *risk assessment*;

EU comment

The EU disagree to add "contaminated water" in the sentence. Movement of contaminated water should not be prohibited in the alert phase (i.e. the suspicion phase). The water in an aquaculture establishment in the alert phase is not known to be contaminated, and can only be assumed to be contaminated. In addition, it is impossible to stop the movement of water to or from most of all the commonly used types of aquaculture establishment – e.g. how should movement of water to and from a fish farm in net cages at sea be stopped?

The newly added ", contaminated water" should therefore be deleted.

b) extending the measures described above to other *aquaculture establishments* or waterbodies that have an epidemiological link with the *aquaculture establishment* or waterbody in which the suspicion arose.

45) whilst awaiting the outcome of the epidemiological investigation referred to in point 1 a)~~described~~ above, in the case of suspicion of a *disease* outbreak in a previously *free country* or *free zone*, the *Competent Authority* should inform ~~communicate with~~ the emergency management group, as described in Chapter 4.X., and where necessary, convene a meeting to advise them of developments and review the *Contingency Plan*. The objectives of this review are to:

a) reinforce the structure of the chain of command and the framework for cooperation which are described in Article 4.X.6.;

b) ensure the *Contingency Plan*, as described in Chapter 4.X., is ready to be fully activated should the presence of the *disease* in question be confirmed in the country, *zone*, *compartment*; and

c) make any updates which are necessary to ensure the *Contingency Plan* is ready for immediate activation.

56) whilst confirmation of the presence of the *disease* in question is ongoing, the *Competent Authority* should communicate with relevant personnel, industry stakeholders, diagnostic laboratories, and contractors, putting them on alert to ensure they review their readiness to act quickly in compliance with the *Contingency Plan*, should the *disease* be confirmed. Such communications are made using the contact details which are kept in accordance with Chapter 4.X.;

67) the *Competent Authority* should endeavour to ensure that the alert phase is short enough to minimise *disease* spread, and long enough to ensure the suspicion has been accurately confirmed or ruled out;

78) should the suspicion not be confirmed, the alert phase is terminated, and any outcomes which warrant review of the *Contingency Plan*, are made;

89) the alert phase ends when the presence of an important *disease* is either confirmed or ruled out by the *Competent Authority.* Relevant actors in the *Aquatic Animal Health Services* should be communicated with to advise them that the alert phase is being terminated, and that the situation is either moving back to peacetime or forward to the emergency phase as described in Article 4.Y.5.

Article 4.Y.5.

Emergency Phase

The emergency phase of *disease outbreak* management commences when the presence of an important *disease* has been confirmed. The steps which should be taken during the emergency phase are set out in the *Contingency Plan*, and the associated detailed actions are set out in the Operations Manual, taking the following factors into account:

1) the chain of command as described in Article 4.Y.6.;

2) the appropriate facilities, ~~skills,~~ resources, personnel and skills as described in Article 4.Y.7.;

3) the *Biosecurity* and other *disease* control measures as described in Article 4.Y.8.

Article 4.Y.6

Chain of command

As soon as the *disease outbreak* has been confirmed, the *Competent Authority* convenes a meeting of the emergency management group as described in Chapter 4.X., and the activation of all elements of the *contingency plan* commences.

The ~~first meeting of the emergency management group considers at least the~~ following issues should be considered, with the assistance of relevant specialist sub-groups:

1) the most up-to-date epidemiological information available concerning the *disease* emergency, including:

a) location of confirmed case(s) including grid references and maps;

b) inventory of species kept in the infected *aquaculture establishment(s)* and the numbers ~~and weights of the *aquatic animals*~~;

EU comment

According to page 25 of this report, the *Aquatic Commission* agreed with a comment that the details on numbers and weights of animals should be removed from point 1 b). However, only the weight part has been removed in the text above.

"inventory of species kept in the infected aquaculture establishment(s) ~~and the numbers and weights of the aquatic animals~~;"

c) clinical situation including description of clinical signs and estimates of morbidity and mortality;

d) identification of the index *case*;

e) details of *susceptible species* in the vicinity of the confirmed case(s);

f) outcomes from preliminary tracing and *surveillance*;

g) outcome from preliminary *risk assessment*.

2) immediate response objectives and options, taking into account the available epidemiological information referred to above, including:

a) official confirmation of the *disease outbreak* to the operators concerned;

b) international notification in accordance with Chapter 1.1.;

c) the reinforcement of the preliminary *biosecurity* measures described in point 4 of Article 4.Y.4. which were put in place during the 'alert phase', the imposition of new biosecurity and other *disease* control measures described in Article 4.Y.8., or both.

3) trade issues which are likely to arise, both within the country and with trading partners elsewhere;

4) review of appropriate facilities, skills and resources, as well as the legal, administrative and financial arrangements which are in place to ~~ensure all relevant enablers are in place~~ enable the Competent Authority to immediately manage the *disease* emergency. This review should include:

a) details of the infrastructure, skill sets and other necessary resources which are available to support the effective management of the *disease* emergency;

b~~a~~) details of the legal instrument which supports the provision of funding for the management of disease emergencies concerning *aquatic animals*;

c~~b~~) contact details for the relevant department which will process the request for funds, and which ensure that payments are executed smoothly once the *contingency plan* has been activated;

~~c) details concerning the mechanisms by which the funds will be transferred, in addition to the frequency of transfer and the personnel who are authorised to draw down the funding~~.

5) agreed messages, format ~~for~~, and timing of, communications with the *Aquatic Animal Health Services* who are responding to the emergency, relevant trading partners, and the public. Communications may be based on generic templates which have been prepared in peacetime and are adapted as appropriate to the circumstances~~Those communications are based on generic~~

~~draft press releases and letters to the *Aquatic Animal Health Services* which have been prepared in peacetime, and which are appropriately fine-tuned to meet the current circumstances~~;

6) a schedule for future meetings throughout the emergency phase of the response, as well as a distribution list for the minutes of those meetings. Flexibility should be introduced to allow~~allowing for flexibility to schedule~~ meetings to be scheduled at short notice, should this be required.

Article 4.Y.7.

Appropriate facilities, skills, resources

1) Disease control centres

a) The *Competent Authority* establishes a central *disease* control centre and where necessary, an appropriate number of local *disease* control centres. Those centres, identified in the *Contingency Plan,* should be capable of providing at least the following:

i) appropriate information technology and telecommunication infrastructure;

ii) information systems to manage data collection concerning *aquaculture establishments*, details of sample collection and associated laboratory results, as well as the imposition of *disease* control measures on affected *aquaculture establishments* and other relevant stakeholders~~transporters~~;

iii) space for preparing and storing sampling kits for dispatch to the field;

iv) *disinfection* points for staff who are involved in sampling and inspection of *aquaculture establishments*, *vehicles* and other premises;

v) storage area for fields kits, personal protective equipment, cleaning and *disinfection* materials;

vi) *biosecurity* measures which are appropriate for the specific facilities and the purpose for which they are used.

b) The personnel from the *Aquatic Animal Health Services* who staff the central and local *disease* control centres have been identified in the *Contingency Plan.* Operationally, this group includes technical, administrative and legal personnel, as necessary, who are fully trained to complete the following tasks in accordance with detailed standard procedures which are set out in the Operations Manual:

i) clinical inspections of *aquaculture establishments*, other establishments and wild aquatic animals ~~and wild aquatic habitats,~~ as relevant;

ii) sample collection and transportation;

iii) preparation and issuance of legal notices;

iv) management of general biosecurity measures and other specific disease control measures;

v) communications with relevant personnel and stakeholders;

vi) data and record management;

vii) human resources management including workplace health and safety.

2) Laboratories

a) During the emergency, the *Aquatic Animal Health Services* should submit samples to the laboratories which have been identified in the *Contingency Plan.* Those laboratories provide rapid and accurate testing and reporting, which is dependent on the following resources:

i) appropriately trained and competent staff;

ii) appropriate equipment, which has been suitably serviced and is fit-for-purpose;

iii) a sufficient range and quantity of consumables;

iv) appropriate information systems to ensure sample traceability and reporting of laboratory results;

v) *biosecurity* measures which are suitable to contain the *pathogenic agent* in question.

Contact details of the staff which are referred to in point (i) and the companies which provide the services and goods, which are referred to in points (ii), (iii) and (iv), are detailed in the Operations Manual.

b) For *listed diseases*, laboratory methods should follow the relevant chapter of the ~~WOAH~~ *Aquatic Manual.* For diseases other than *listed diseases*, a procedure identified in the Operations Manual should be utilised, or another method which has been validated for the purpose of use.

3) Service Providers

The availability of relevant service providers during the emergency phase is of crucial importance, in particular, considering that a *disease outbreak* may extend to multiple *aquaculture establishments* in dispersed locations, and potentially to wild *aquatic animals*. Action should, therefore, be taken to ensure the availability of:

a) mortality management providers involved in retrieval and/or transport, who have capacity for the required daily tonnage;

b) sanitary slaughter facilities, which can cater for the required daily tonnage;

c) predatory animal and bird control specialists;

d) telecommunications providers;

e) communication specials or journalist for media liaison;

f~~c~~) telecommunications providers;

g~~d~~) providers of laboratory equipment and consumables who have an acceptable lead-in time for delivery of new and replacement items;

h~~e~~) companies which service relevant laboratory equipment and which have an acceptable response time for critical pieces of equipment;

i~~f~~) providers of vaccines/ veterinary medicines, who can supply an appropriate number of doses and have a suitable lead-in time for delivery;

j~~g~~) experts in areas which are relevant to the successful management of the emergency, and who have appropriate skills (e.g. in the areas of logistics, fisheries management, environmental protection, vaccination or treatment of *aquatic animals*), and who are available to deal with emergency situations;

k~~h~~) back-up providers for each type of service, should they be required for an extensive *disease outbreak*.

Subject to the relevant regulatory requirements which apply in a country, contact~~Contact~~ details of the providers referred to in points a) to k~~h~~) above are detailed in the Operations Manual.

Article 4.Y.8.

Biosecurity and other disease control measures

The actions which the *Competent Authority* should take~~s~~ concerning *biosecurity* and other *disease* control measures during the emergency phase, are described in the Operations Manual and may include:

1) defining the *infected zone* and *protection zones* which apply in freshwater or marine environments, as relevant, following confirmation of a *disease outbreak*, and taking into account the recommendations of Chapter 4.2.;

2) appropriate classification of the health status of *aquaculture establishments* to define their *disease* status or *risk* of infection;

32) providing maps which will demonstrate the *infected zone* and the surrounding *protection zone*, as well as the *aquaculture establishments* which are located within those *zones*;

43) coordinating actions concerning *biosecurity* and other *disease* control measures with other *Competent Authorities*, when the establishment of such *infected zone* or *protection zones* impacts neighbouring countries;

54) specifying relevant *biosecurity* and other specific *disease* control measures including:

a) controlling the movement of *aquatic animals*, *aquatic animal products*, *feed*, and equipment, *vehicles*, waste, fomites and *vectors* to or from the infected establishment(s) or *infected zone*, unless authorised by the *Competent Authority* following *risk assessment*;

b) extending the movement controls referred to above, to other *aquaculture establishments* or waterbodies which have an epidemiological link with the *aquaculture establishment* in which the suspicion arose;

c) exemptions from the movement prohibitions described above, should *risk assessment* have indicated that these represent an acceptable *risk* (e.g. emergency harvesting, on-site processing, cooking for human consumption), or alternatively that more stringent movement measures are required due to the developing *disease* situation;

d) specifying the procedures to be used when *aquatic animals* are slaughtered or killed, depending on their species, size and the number of *aquatic animals* involved, including:

i) details of the equipment and where relevant, veterinary products to be used, and their suppliers;

ii) the appointment of a named Welfare Officer to ensure that procedures are carried out to the highest possible standards, and in the case of fish, to ensure that slaughtering or killing is carried out in accordance with Chapter 7.4.;

iii) details of the *biosecurity* measures required to ensure the slaughter or killing process does not cause *disease* spread. This includes measures for the containment and safe disposal of dead or destroyed stock. Also measures which apply to *vehicles* which are authorised to move animals or products from the infected establishments (or from additional establishments, as directed by the *Competent Authority*), to processing factories or animal by product establishments;

e~~iv~~) the vaccination options that may be employed, depending on the circumstances of the *disease outbreak*, including:

i) no vaccination;

ii) vaccination which is implemented in aquaculture establishments within the infected zone i.e. suppressive vaccination, the aim of which is to reduce the spread of disease from the infected zone;

iii) vaccination which is implemented outside the infected zone where the disease has not been suspected or confirmed i.e. protective vaccination, the aim of which is to prevent the spread of the disease in populations of aquatic animals which are at risk of infection;

iv) a combination of suppressive and protective vaccination.

f~~e~~) the decontamination options which are available, taking into account the recommendations of Chapter 4.4.. A list of the cleaning agents, *disinfectants* and equipment that are appropriate to use, are commercially available, authorised for use by the relevant *Competent Authority*, and which meet the decontamination requirements concerning the *pathogenic agent* in question~~,~~ should also be specified;

g~~f~~) procedures for the containment of wastewaters which are produced following equipment, facility and *vehicle* *disinfection* activities, which have been drawn up in accordance with the instructions of the *Competent Authorities* with responsibility for discharges to the environment;

h) where relevant, specifying the procedures to be used for the containment, *disinfection* and disposal of *disease* contaminated water used for *aquatic animal* production.

Article 4.Y.9.

Recovery phase

The recovery phase of *disease outbreak* management is activated when the end of the emergency has been declared by the *Competent Authority*. This phase takes into consideration the recovery plan described in Chapter 4.X., and the associated detailed actions which are set out in the Operations Manual.

1. Return to freedom.

   In cases where the recovery phase includes the intention~~ambition~~ to return to *disease* freedom in accordance with ~~Pathway 4 as referred to in~~ Chapter 1.4. (Pathway 4), either for:

   a) the entity (country, *zone* or *compartment*), which was previously *disease* free; or ~~to make a *self-declaration of freedom from disease* for~~

   b) a smaller entity or entities (*zone*(s) or *compartment*(s));

   this phase should begin with a review of the *basic biosecurity conditions* which applied before the *disease outbreak* occurred. This review will determine if additional *sanitary measures* are required to strengthen the *basic biosecurity conditions* which will apply in the entity for which the new declaration of freedom will be made.

   This step will be followed in due course, by the re-population of *aquatic animals*, the required surveillance (as per Chapter 1.4.) and the re-commencement of trade. The ultimate aims of the recovery phase are to successfully return to peacetime operations.

2. In cases where the recovery phase does not include the ambition to return to disease-freedom, the actions which are necessary to either contain the *disease*, or to mitigate the impacts of the *disease*, should be identified and set out in the Operations Manual.

   a) Containment. Where the aim of the recovery plan is to contain the *disease*, the following measures may be described:

      i) zoning and movement controls;

      ii) *biosecurity* measures, as described in Chapter 4.1.;

      iii) *disinfection* of *aquaculture establishments* and equipment, as described in Chapter 4.4.;

      iv) periodic *fallowing*, as described in Chapter 4.7.;

      v) handling, disposal and treatment of *aquatic animal waste*, as described in Chapter 4.8.

   b) Mitigation. Where the aim of the recovery plan is to mitigate the impact of the *disease*, the following measures may be described:

      i) vaccination, using one or more of the strategies, which are referred to in Article 4.Y.5.;

      ii) the possibility to change to the production of a species of *aquatic animals*, which are not susceptible to the *disease* which caused the emergency;

      iii) the possibility to change production and husbandry practices, so that *risk* factors which are known to result in morbidity or mortality of *susceptible species* are minimised as far as possible;

iv) training which may be provided to operators to create improved awareness of the *disease* in question, as well as the steps that can be taken at establishment level to mitigate its impact.

3. In addition, the recovery plan may include details of:

a) the steps that are necessary to:

i) allow relevant movement controls to be partially or completely lifted (including permitting arrangements), so that affected trade may recommence within the country;

ii) start communications with producers and international partners, with a view to supporting an early recommencement of *international trade*, or to seek alternative trading partners.

b) any increased *surveillance* or *biosecurity* measures which may apply to facilitate resumption of trade, and that is undertaken once~~as~~ trade recommences within the country and with international partners;

c) any resources that the *Competent Authority* intends to provide including research, monetary, technical, or other relevant supports;

d) any review of national legislation and *disease outbreak* management procedures that may be required to underpin the recovery plan that has been developed concerning the *disease outbreak* in question;

e) ongoing communication with *Aquatic Animal Health Services* to explain relevant details of the recovery plan and to reinforce the role the *Aquatic Animal Health Services* play in future *disease* prevention and control.

Annex 42. Item 7.2. – Draft new Chapter 4.Z. 'Control of pathogenic agents in traded gametes and fertilised eggs of fish'

SECTION 4

DISEASE PREVENTION AND CONTROL

CHAPTER 4.Z.

CONTROL OF PATHOGENIC AGENTS IN TRADED GAMETES~~MILT~~ AND FERTILISED EGGS OF FISH

EU comment

The EU thanks the Aquatic Commission for this interesting initiative and for taking into account most of our preliminary comments on the draft chapter below.

However, the EU provides additional comments to Article 4.Z.3., to Article 4.5.Z and to Article 4.6.Z

In parallel with this process, we intend to work with the European Food Safety Authority (EFSA), to obtain a Scientific Opinion which will describe the various elements which are necessary to underpin safe trade in genetic material of aquaculture animals.

Ultimately, our final views on Chapter 4.Z. will be guided by that Scientific Opinion.

Article 4.Z.1.

Purpose

To provide recommendations for trade of *gametes*~~milt~~ and *fertilised eggs* of fish intended for *aquaculture* purposes and to define *risk management*~~mitigation~~ for trade~~import~~ to a *free country*, *free zone* or *free compartment* when:

1) the intention is to grow out and harvest the traded fish~~imported *aquatic animals*~~; or

2) the intention is to establish a new stock for *aquaculture*.

For disease-specific recommendations, refer to Article 10.X.15. (and Article 10.4.20. for infection with ISAV)~~Section 10~~.

Article 4.Z.2.

Scope

This chapter describes general recommendations for safe trade in *gametes*~~milt~~ and *fertilised eggs* of fish from an area other than a *free country*, *free zone* or *free compartment*. These recommendations include the measures outlined in Article 4.Z.3. which cumulatively reduce the *risk* of transfer of *infection* to *aquatic animal* populations in a *free country, free zone* or *free compartment*.

Trade of *gametes*~~milt~~ and *fertilised eggs* of fish from a *free country*, *free zone* or *free compartment* should meet the requirements in Articles 10.X.9. (and Article 10.4.14. for infection with ISAV) of the fish disease-specific chapters, and is not addressed in this chapter.

Article 4.Z.3.

Specific measures required for trade of gametes~~milt~~ and fertilised eggs of fish

Trade of *gametes*~~milt~~ and *fertilised eggs* of fish from a country, *zone* or *compartment* not declared free from infection with the *listed diseases* of concern should meet the following requirements:

1) the health status of the broodstock at the *aquaculture establishment* of origin must~~should~~ be determined. Only populations of broodstock which test free from the *pathogenic agents* of concern are suitable for movement~~supply~~ to *collection and incubation centres*, as described in Article 4.Z.4.;

2) *gametes*~~milt~~ and *fertilised eggs* should originate~~come~~ from a *collection and incubation centre* which has been approved for that purpose by the *Competent Authority* of the place of origin, and which operates in compliance with the conditions described in Articles 4.Z.5., 4.Z.6. and 4.Z.7.;

EU comment

The EU has the following comment to paragraph 2 of Article 4.Z.3., as regards the origin of gametes and fertilised eggs:

"2) *gametes* and *fertilised eggs* should finally originate from a *collection and incubation centre* which has been approved for that purpose by the *Competent Authority* of the place of origin, and which operates in compliance with the conditions described in Articles 4.Z.5., 4.Z.6. and 4.Z.7.;"

The rationale is to make it clear that gametes and fertilised eggs can only be subject to trade when they are originated from a collection and incubation centre and not directly from the aquatic establishment where the broodstock is kept.

3) in the event of a positive detection in a *collection and incubation centre*, the *Competent Authority* of the *importing country* should assess the *risks* associated with importation of *gametes* and *fertilised eggs* from that establishment, taking all relevant factors into account, including the *biosecurity plan* which is applied to prevent cross contamination of gametes and fertilised eggs from individual parents which have tested negative;

4~~3~~) ~~the~~ *fertilised eggs* should be~~have been~~ surface disinfected prior to the export using a method proven to inactivate *pathogenic agents*, for salmonid eggs as described in Chapter 4.5.~~ and in accordance with the recommendations in the fish disease specific chapters (Articles 10.X.15. for infection with SAV, infection with IHNV, and infection with VHSV; Article 10.4.20. for infection with ISAV)~~;

5~~4~~) when intended for *international trade*, the consignment should be accompanied by an *international aquatic animal health certificate* issued by the *Competent Authority* of the *exporting country* stating~~which should state~~ that the *gametes*~~milt~~ and the *fertilised eggs* originate~~come~~ from parents which have tested free from the relevant *disease*, and which meet the requirements in points 1, ~~and~~ 2 and 4.

Application of the measures recommended in this chapter should comply with the requirements of Chapters 5.1., 5.2. and 5.3.

Article 4.Z.4.

Health status of broodstock at the aquaculture establishment~~place~~ of origin

Aquaculture establishments keeping broodstock for movement to a *collection and incubation centre* for the production of~~and~~ *gametes*~~milt~~ and *fertilised eggs* of fish ~~from a country, *zone* or *compartment* not declared free from infection with a *listed disease*,~~ should meet the following requirements:

1) it should be approved for that purpose by the *Competent Authority* and be under its official control;

2) it should implement~~have in place~~ a *biosecurity plan* which has been drawn up in accordance with Chapter 4.1.;

3) the broodstock should be tested for the *pathogenic agents* of concern as close as possible to the date on which they enterprior to entry to the *collection and incubation centre* using a sample size that is sufficiently large to demonstrate with 95% confidence that the *pathogenic agent* would be detected if present above a prevalence of 2%, using the diagnostic methods provided in the *Aquatic Manual*. If the results of this testing produce a positive result, the broodstock should not be moved to the *collection and incubation centre*;

4) broodstock intended for movement to a *collection and incubation centre* should be clinically healthy at the time of movement, should not originatebe from a population experiencing recent or ongoing mortality, and should not be exposed to animals or other sources of *disease* that canof a lower their health status following the testing referred to inat point 3.

Article 4.Z.5.

Collection and incubation centres

Collection and incubation centres should be approved by the *Competent Authority* for that purpose on the basis that the *collection and incubation centre* should:

1) isbe under the supervision of an *Aquatic Animal Health Professional* or *veterinarian*, who takes overall responsibility for its operation;

2) implementhave a *biosecurity plan* which has been drawn up in accordance with Chapter 4.1.;

3) isbe structured to contain epidemiologically separate individual broodstock or groups of broodstock;

4) hashave in place a valid traceability system in place to ensure that milteach batch of *gametes* or *fertilised eggs* can be traced back to an epidemiologically separate individual or group, and which includesinclude documentation and auditing of testing results, *disease* history and movements of *aquatic animals*;

5) isbe separated into dedicated areas for:

   a) holding broodstock prior to *gamete* collection;

   ba) a collection ofroom for eggs and milt;

   c) milt testing and storage;

   d) disinfection of *fertilised eggs;*

   eb) an incubation ofcentre for *fertilised eggs*;

   c) a milt laboratory and milt storage area;

   fd) administration offices.

6) isbe subject to inspections carried outand pass audits by the *Competent Authority* or aan approved third party approved by the *Competent Authority* at a frequency sufficient to ensure that the *collection and incubation centre* is in compliance withleast once per year against the requirements of this chapter.

EU comment:

The EU proposes a new point 7) in Article 4.Z.5., as regards the requirements for the water source of the collection and incubation centres:

"7) uses water from a source without contact to wild or farmed susceptible species known or suspected of being infected with the pathogenic agent."

Article 4.Z.6.

Testing of broodstock at the collection and incubation centre

Broodstock for the production of~~and~~ *gametes*~~milt~~ and *fertilised eggs* of fish, should meet the following requirements at the *collection and incubation centre*:

1) stripping and sampling should be carried out under the supervision of the *Aquatic Animal Health Professional* or *veterinarian* who has responsibility for the *collection and incubation centre*;

2) at stripping the broodstock should be individually sampled, and tested for the *listed diseases* of concern, in accordance with the methods for diagnosis provided in the *Aquatic Manual,* in a laboratory that has been approved by the *Competent Authority*;

3~~2~~) fish that test positive, and any *gametes* or *fertilised eggs*~~milt or eggs~~ derived from them should not be traded;

4) details of the results from testing relevant cohorts of broodstock as described in paragraph 1 should be provided to the *Competent Authority* of an *importing country* on request;

5) in accordance with the *biosecurity plan* for the *collection and incubation centre*,~~and~~ all gametes and fish from the~~that~~ epidemiological group that tested positive should be disposed of in a biosecure manner. Affected facilities should be disinfected to ensure that cross-contamination of other batches of *gametes* or *fertilised eggs*~~milt or eggs~~ does not occur;

6) *fertilised eggs* should be surface disinfected using a method proven to inactive *pathogenic agents*, for salmonid eggs as described in Chapter 4.5.

EU comment:

The EU has the following comment to point 6 of Article 4.Z.6., as regards the disinfection of the fertilised eggs:

"6) *fertilised eggs* should be surface disinfected using a method proven to inactive *pathogenic agents*, for salmonid eggs as described in Chapter 4.5. and kept in water known to be free of the pathogenic agent."

Article 4.Z.7.

Conditions applicable to the collection and storage of milt and preparation of milt samples~~ in the laboratory~~

The following conditions should be in place ~~at the laboratory~~ for milt collection and storage:

1) the integrity of the traceability system as described in Article 4.Z.5. should be maintained at all times;

2) receptacles used to freeze milt should be sterilized before use;

3) diluents should be produced in a way to protect against contamination with *pathogenic agents*;

4) frozen milt should be stored in hermetically sealed containers in a separate room.

Annex 43. Item 7.2. – Model Article 10.X.10. for Chapter 10.5. 'Infection with SAV', Chapter 10.6. 'Infection with IHNV' and Chapter 10.10. 'Infection with VHSV', and Article 10.4.15. for Chapter 10.4. 'Infection with ISAV'

Model Article 10.X.10. for Chapter 10.5. 'Infection with SAV', Chapter 10.6. 'Infection with IHNV', and Chapter 10.10. Infection with VHSV'

EU comment

The EU thanks the Aquatic Commission for this proposed model Article. Ultimately, our final views on Model Article 10.X.10. will be guided by the future Scientific Opinion of EFSA, as per our earlier comments on Annex 42.

CHAPTER 10.X.

INFECTION WITH [PATHOGEN X]

[…]

Article 10.X.10.

Importation of aquatic animals, excluding gametes and fertilised eggs, for aquaculture from a country, zone or compartment not declared free from infection with [pathogen X]

When importing, for *aquaculture, aquatic animals*, excluding *gametes* and *fertilised eggs,* of a species referred to in Article 10.X.2. from a country, *zone* or *compartment* not declared free from infection with [pathogen X], the *Competent Authority* of the *importing country* should assess the *risk* in accordance with Chapter 2.1. and consider ~~applying~~ the *risk* mitigation measures in ~~either~~ points~~s~~ 1 and~~or~~and 2 below.~~:~~

1) If the intention is to grow out and harvest the imported *aquatic animals*, consider applying the following:

~~Either~~

a) the direct delivery to and lifelong holding of the imported *aquatic animals* in a *quarantine* facility; and

b) before leaving *quarantine* (either in the original facility or following biosecure transport to another *quarantine* facility) the *aquatic animals* are killed and processed into one or more of the *aquatic animal products* referred to in Article 10.X.3. or other products authorised by the *Competent Authority*; and

c) the treatment of all transport water, equipment, effluent and waste materials to inactivate [pathogen X] in accordance with Chapters 4.4., 4.8. and 5.5.

~~Or~~

~~d) apply the requirements of Chapter 4.Z.~~

OR

2) If the intention is to establish a new stock for *aquaculture*, consider applying the following:

~~Either~~

a) In the *exporting country*:

i) identify potential source populations and evaluate their *aquatic animal* health records;

ii) test source populations in accordance with Chapter 1.4. and select a founder population (F-0) of *aquatic animals* with a high health status for infection with [pathogen X].

b) in the *importing country*:

i) import the F-0 population into a *quarantine* facility;

ii) test the F-0 population for [pathogen X] in accordance with Chapter 1.4. to determine their suitability as broodstock;

iii) produce a first generation (F-1) population in *quarantine*;

iv) culture the F-1 population in *quarantine* for a duration sufficient for, and under conditions that are conducive to, the clinical expression of infection with [pathogen X], and sample and test for [pathogen X] in accordance with Chapter 1.4. of the *Aquatic Code* and Chapter 2.3.6. of the *Aquatic Manual;*

v) if [pathogen X] is not detected in the F-1 population, it may be defined as free from infection with [pathogen X] and may be released from *quarantine*;

vi) if [pathogen X] is detected in the F-1 population, those animals should not be released from *quarantine* and should be killed and disposed of in a biosecure manner in accordance with Chapter 4.8.

~~Or~~

~~c) apply the requirements of Chapter 4.Z.~~

[…]

CHAPTER 10.4.

INFECTION WITH INFECTIOUS SALMON ANAEMIA VIRUS

[…]

Article 10.4.15.

Importation of aquatic animals, excluding gametes and fertilised eggs, for aquaculture from a country, zone or compartment not declared free from infection with ISAV

In this article, all statements referring to infection with ISAV are for any detectable ISAV, including HPR0 ISAV.

When importing, for *aquaculture, aquatic animals*, excluding *gametes* and *fertilised eggs,* of a species referred to in Article 10.4.2. from a country, *zone* or *compartment* not declared free from infection with ISAV, the *Competent Authority* of the *importing country* should assess the *risk* in accordance with Chapter 2.1. and consider ~~applying~~ the *risk* mitigation measures in ~~either~~ points~~s~~ 1 and~~or~~ ~~and~~ 2 below.~~:~~

1) If the intention is to grow out and harvest the imported *aquatic animals*, consider applying the following:

~~Either~~

a) the direct delivery to and lifelong holding of the imported *aquatic animals* in a *quarantine* facility; and

b) before leaving *quarantine* (either in the original facility or following biosecure transport to another *quarantine* facility) the *aquatic animals* are killed and processed into one or more of the *aquatic animal products* referred to in Article 10.4.3. or other products authorised by the *Competent Authority*; and

c) the treatment of all transport water, equipment, effluent and waste materials to inactivate ISAV in accordance with Chapters 4.4., 4.8. and 5.5.

~~Or~~

~~d) apply the requirements of Chapter 4.7.~~

OR

2) If the intention is to establish a new stock for *aquaculture*, consider applying the following:

~~Either~~

a) In the *exporting country*:

i) identify potential source populations and evaluate their *aquatic animal* health records;

ii) test source populations in accordance with Chapter 1.4. and select a founder population (F-0) of *aquatic animals* with a high health status for infection with ISAV.

b) in the *importing country*:

i) import the F-0 population into a *quarantine* facility;

ii) test the F-0 population for ISAV in accordance with Chapter 1.4. to determine their suitability as broodstock;

iii) produce a first generation (F-1) population in *quarantine*;

iv) culture the F-1 population in *quarantine* for a duration sufficient for, and under conditions that are conducive to, the clinical expression of infection with ISAV, and sample and test for ISAV in accordance with Chapter 1.4. of the *Aquatic Code* and Chapter 2.3.6. of the *Aquatic Manual;*

v) if ISAV is not detected in the F-1 population, it may be defined as free from infection with ISAV and may be released from *quarantine*;

vi) if ISAV is detected in the F-1 population, those animals should not be released from quarantine and should be killed and disposed of in a biosecure manner in accordance with Chapter 4.8.

~~Or~~

~~c) apply the requirements of Chapter 4.Z.~~

[…]

Annex 44. Item 7.2. – Model Article 10.X.15. for Chapter 10.5. 'Infection with SAV', Chapter 10.6. 'Infection with IHNV' and Chapter 10.10. 'Infection with VHSV', and Article 10.4.20. for Chapter 10.4. 'Infection with ISAV'

Model Article 10.X.15. for Chapter 10.5. 'Infection with SAV', Chapter 10.6. 'Infection with IHNV', and Chapter 10.10. 'Infection with VHSV'

EU comment

Our final views on Model Articles 10.X.15. and 10.4.20. will be guided by the future Scientific Opinion of EFSA, as per our earlier comments on Annex 42.

CHAPTER 10.X.

INFECTION WITH [PATHOGEN X]

[…]

Article 10.X.15

Importation of gametes~~milt~~ and fertilised eggs of fish ~~disinfected eggs~~ for aquaculture from a country, zone or compartment not declared free from infection with [pathogen X]

When importing *gametes~~milt~~* or *fertilised eggs* of a species referred to in Articles 10.X.2., for *aquaculture* from a country, *zone* or *compartment* not declared free from infection with [pathogen X], the *Competent Authority* of the *importing country* should ensure that:

1) the consignment meets the requirements in Chapter 4.Z.; and

2) *fertilised eggs* have been disinfected using a method proven to inactivate *pathogenic agents*, for salmonid eggs in accordance with recommendations in Chapter 4.5.; and

3) all water (including ice), equipment, *containers* and packaging material used in transport are treated to ensure inactivation of [pathogen X] or disposed of in a biosecure manner in accordance with Chapters 4.4., 4.8. and 5.5.; and

4) all effluent and waste materials are treated to ensure inactivation of [pathogen X] or disposed of in a biosecure manner in accordance with Chapters 4.4. and 4.8.

The *Competent Authority* should consider internal measures, such as additional *disinfection* of the *fertilised eggs* upon arrival in the *importing country*.

The consignment should be accompanied by an *international aquatic animal health certificate* issued by the *Competent Authority* of the *exporting country* certifying that the *gametes~~milt~~* and *fertilised eggs* fulfil the recommendations in Articles 4.Z.3. to 4.Z.7.

~~1) When importing disinfected eggs of the species referred to in Article 10.X.2. for *aquaculture*, from a country, *zone* or *compartment* not declared free from infection with [pathogen X], the *Competent Authority* of the *importing country* should assess at least the following:~~

~~a) the likelihood that water used during the *disinfection* of the eggs is contaminated with [pathogen X];~~

~~b) the prevalence of infection with [pathogen X] in broodstock (including results from testing of ovarian fluid and milt).~~

2) If the *Competent Authority* of the *importing country* concludes that the importation is acceptable, it should request that *risk* mitigation measures are applied, including:

a) *disinfection* of the eggs prior to importing, in accordance with recommendations in Chapter 4.5.; and

b) that between *disinfection* and importation, eggs should not come into contact with anything which may affect their health status.

The *Competent Authority* should consider internal measures, such as additional *disinfection* of the eggs upon arrival in the *importing country*.

3) When importing *disinfected* eggs of the species referred to in Article 10.X.2. for *aquaculture*, from a country, *zone* or *compartment* not declared free from infection with [pathogen X], the *Competent Authority* of the *importing country* should require that the consignment be accompanied by an *international aquatic animal health certificate* issued by the *Competent Authority* of the *exporting country* certifying that the procedures described in point 2(a) and (b) of this article have been fulfilled.

[…]

CHAPTER 10.4.

INFECTION WITH INFECTIOUS SALMON ANAEMIA VIRUS

[…]

Article 10.4.20.

Importation of gametes~~milt~~ and fertilised eggs of fish ~~disinfected eggs~~ for aquaculture from a country, zone or compartment not declared free from infection with ISAV

When importing *gametes~~milt~~* or *fertilised eggs* of a species referred to in Articles 10.4.2., for *aquaculture* from a country, *zone* or *compartment* not declared free from infection with ISAV, the *Competent Authority* of the *importing country* should ensure that:

1) the consignment meets the requirements in Chapter 4.Z.; and

4) *fertilised eggs* have been disinfected in accordance with recommendations in Chapter 4.5.; and

5) all water (including ice), equipment, *containers* and packaging material used in transport are treated to ensure inactivation of ISAV or disposed of in a biosecure manner in accordance with Chapters 4.4., 4.8. and 5.5.; and

6) all effluent and waste materials are treated to ensure inactivation of ISAV or disposed of in a biosecure manner in accordance with Chapters 4.4. and 4.8.

The *Competent Authority* should consider internal measures, such as additional *disinfection* of the *fertilised eggs* upon arrival in the *importing country*.

The consignment should be accompanied by an *international aquatic animal health certificate* issued by the *Competent Authority* of the *exporting country* certifying that the *gametes~~milt~~* and *fertilised eggs* fulfil the recommendations in Articles 4.Z.3. to 4.Z.7.

~~1) When importing disinfected eggs of the species referred to in Article 10.4.2. for *aquaculture*, from a country, *zone* or *compartment* not declared free from infection with ISAV, the *Competent Authority* of the *importing country* should assess at least the following:~~

~~a) the likelihood that water used during the *disinfection* of the eggs is contaminated with ISAV;~~

~~b) the prevalence of infection with ISAV in broodstock (including results from testing of ovarian fluid and milt).~~

~~2) If the *Competent Authority* of the *importing country* concludes that the importation is acceptable, it should request that *risk* mitigation measures are applied, including:~~

~~a) *disinfection* of the eggs prior to importing, in accordance with recommendations in Chapter 4.5.; and~~

~~b) that between *disinfection* and importation, eggs should not come into contact with anything which may affect their health status.~~

~~The *Competent Authority* should consider internal measures, such as additional *disinfection* of the eggs upon arrival in the *importing country*.~~

~~3) When importing *disinfected* eggs of the species referred to in Article 10.4.2. for *aquaculture*, from a country, *zone* or *compartment* not declared free from infection with ISAV, the *Competent Authority* of the *importing country* should require that the consignment be accompanied by an *international aquatic animal health certificate* issued by the *Competent Authority* of the *exporting country* certifying that the procedures described in point 2(a) and (b) of this article have been fulfilled.~~

[…]

———————

Annex 45. Items 7.2. and 7.3. – Glossary

GLOSSARY

EU comment

The EU supports these new glossary terms.

[...]

COLLECTION AND INCUBATION CENTRE

means a facility approved by the *Competent Authority* in conformity with the provisions of Chapter 4.Z. for holding broodstock, the collection of eggs, fertilisation and incubation, and the collection, processing, and storage of milt.

[...]

FERTILISED EGG

means a viable fertilised *ovum* of an *aquatic animal*. 'Green eggs' means newly fertilised ova of fish. 'Eyed eggs' means *fertilised eggs* of fish where the eyes of the embryo are visible and that the *fertilised eggs* may be transported.

[...]

GAMETES

means the sperm (contained within seminal fluid or milt) or unfertilised eggs of aquatic animals that are held or transported separately prior to fertilisation.

[...]

ORNAMENTAL AQUATIC ANIMAL

means an *aquatic animal* that is intended for display, exhibition, competition, or to be kept as a pet.

[...]

Annex 46. Item 7.3. – Draft new Chapter 5.X. 'Movement of ornamental aquatic animals'

SECTION 5

TRADE MEASURES, IMPORTATION/EXPORTATION PROCEDURES AND HEALTH CERTIFICATION

CHAPTER 5.X.

MOVEMENT OF ORNAMENTAL AQUATIC ANIMALS

EU comment

The EU thanks the AAC and supports this new initiative.

Article 5.X.1.

Introduction

This chapter provides recommendations to address the *risk* of pathogen~~*disease*~~ transmission via the movement of *ornamental aquatic animals* to prevent entry into a country, *zone* or *compartment* that is free from the *pathogenic agents* of concern.

Ornamental aquatic animals may originate from the wild or from *aquaculture establishments*. Once they have entered the supply chain they may be epidemiologically separated from farmed or wild populations but can be diverted to other end uses for which they were not intended. This may provide a pathway for *disease* transmission and place other populations of *susceptible species* at *risk*.

International movement of *ornamental aquatic animals* is characterised by translocation of numerous individual animals comprised of many species of fish, crustaceans, molluscs and amphibians originating from diverse environments. Supply chains may involve the aggregation of animals from multiple sources and their dissemination through retail trade as pets, providing opportunities for *disease* transmission. These characteristics of the movement of *ornamental aquatic animals* may present challenges for managing *aquatic animal disease risks*.

Article 5.X.2.

Scope

This chapter provides recommendations for managing the pathogen~~*disease*~~ *risks* associated with movement of *ornamental aquatic animals*. The standards concerning trade in species that are susceptible to the *diseases* listed in Chapter 1.3., are set out in the disease-specific chapters. This Chapter provides additional guidance for managing *risk* associated with the movement of *ornamental aquatic animals* which are susceptible to *listed diseases* or other *diseases* identified as *hazards*. ~~that complement other provisions of the *Aquatic Code*, including the measures specified in the disease-specific chapters.~~

Article 5.X.3.

General principles

The general principles for the movement of *ornamental aquatic animals* that should be considered when developing *risk* mitigation measures are:

1) the legality~~eligibility~~ for the movement of a species (or a taxonomic group of species) should be determined considering existing regulatory measures in the importing country regarding ~~its conservation status (e.g. species listed in the Convention on International Trade in Endangered Species of Wild Fauna and Flora), and~~ potential biodiversity and ecosystem impacts ~~in the *importing country* (e.g. potential to become an invasive alien species)~~, as described in Article 5.X.4.;

2) *ornamental aquatic animals* intended for international movement should be clinically healthy at the time of movement, not exposed to animals of a lower health status, and should not be from an establishment experiencing recent or ongoing *disease* or unexplained mortality, as described in Article 5.X.5.;

3) *risk management* measures for *listed diseases* should be in accordance with the provisions of the disease-specific chapters, as described in Article 5.X.6.;

4) *risk management* measures for non-listed *diseases*, or any measures for *listed diseases* exceeding those described in the disease-specific chapters, should be justified by *risk analysis*, as described in Article 5.X.7.;

5) any *risk management* measures should be the least restrictive measures required to mitigate the *disease* risks identified by a *risk assessment,* as described in Articles 5.X.8. to 5.X.11.;

6) measures should be taken to maintain the welfare of *ornamental aquatic animals* during transit, including as described in Article 5.X.12.

Article 5.X.4.

Eligibility for the international movement of ornamental aquatic animals

Prior to considering the *aquatic animal* health *risks* associated with the import of a species of *ornamental aquatic animal*, the *Competent Authority* of an *importing country* should determine that import of the species would be compliant with ~~consult~~ relevant national regulations and international obligations ~~to determine that the species is eligible for import~~. ~~Species~~ For example, species of *ornamental aquatic animal* may be subject to controls on international movement or trade due to their conservation status (e.g. listed in the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) or listed as an endangered species or preserved species by a *Competent Authority* or other authorities of an *importing country*). These controls may prohibit international movement or may necessitate additional import documentation.

Species of *ornamental aquatic animals* (or taxonomic groups of species) may also be identified as invasive by a *Competent Authority* or other authority of an *importing country*. Such species may be prohibited to be traded, owned or farmed due to the risks they present to biodiversity, ecosystems, industry, ~~or~~ public amenity or public health in the *importing country*.

Article 5.X.5.

General health status of ornamental aquatic animals

Aquaculture establishments holding or packaging *ornamental aquatic animals* for international movement should have suitable facilities and husbandry practices for maintaining the health status of all species held within the facility.

The *Competent Authority* of an *exporting country* should ensure that *aquaculture establishments* are under sufficient supervision to ensure that requirements of the *Competent Authority* of the *importing country* for *ornamental aquatic animals* can be met. The *Aquatic Animal Health Services* relevant to meeting *importing country* requirements should comply with the principles of Chapter 3.1.

If *aquaculture establishments* are required by the *Competent Authority* to maintain a *biosecurity plan,* or if this is required to meet *importing country* requirements, the *biosecurity plan* should be developed as described in Chapter 4.1.

Ornamental aquatic animals should not be moved or traded from an *aquaculture establishment* if they are exhibiting clinical signs of *disease* or experiencing unexplained mortalities.

Article 5.X.6.

Application of measures for listed diseases

Sanitary measures applied to manage the *risk* of transmission of *listed diseases* associated with movement of *ornamental aquatic animals* should be in accordance with the relevant disease-specific chapters. The *Competent Authority* of an *importing country* can only require disease-specific measures if it is free from the *disease* of concern, or if the *disease* of concern is under an official control programme, as described in Chapter 5.1.

When importing *ornamental aquatic animals* of *susceptible species* (as listed in Article X.X.2. of each disease-specific chapter), from a *free country*, *free zone* or *free compartment*, the *Competent Authority* of the *importing country* should require, in accordance with Article X.X.9. of the relevant disease-specific chapter, that the consignment be accompanied by an *international aquatic animal health certificate* issued by the *Competent Authority* of the *exporting country* attesting that the consignment originates from a *free country*, *free zone* or *free compartment*.

The *Competent Authority* of an *importing country* can only require *sanitary measures* for a *listed disease* more stringent than the standards of the *Aquatic Code* if those measures are supported by a *risk analysis* in accordance with Chapter 2.1.

Article 5.X.7.

Risk analysis

The *Competent Authority* of an *importing country* should use *risk analysis* to justify any *sanitary measures* for non-listed *diseases* associated with imported *ornamental aquatic animals*. *Risk analysis* should also be used to justify any *sanitary measures* for *listed diseases* if the measures are more stringent than the standards of the *Aquatic Code*. The *Competent Authority* of an *importing country* can only require pathogen-specific *sanitary measures* if the country is free from the *disease* of concern, or if the *disease* of concern is under an official control programme, as described in Chapter 5.1.

Risk analysis for the import of *ornamental aquatic animals* should be conducted as described in Chapter 2.1. In addition to the factors provided in Chapter 2.1, the *risk analysis* should take into account the following factors relevant to the assessment of likelihood of entry and exposure of *hazards* associated with *ornamental aquatic animals*.

Entry

1) The *disease* status of identified *hazards* within the country, *zone* or *compartment* of origin, including information on the prevalence of identified *hazards* within populations of *ornamental aquatic animals* or within their source populations (e.g. wild animals).

2) The *disease* prevention and control practices within the supply chain for *ornamental aquatic animal*s in the *exporting country*, and the quality of the *aquatic animal health services* supporting disease prevention and control.

3) The range of species that are susceptible to the specific *pathogenic agents* identified as *hazards* and the evidence to substantiate susceptibility in accordance with Chapter 1.5.

4) The suitability of environmental conditions (e.g. temperature, salinity) for the *hazard* at the place of origin of the *ornamental aquatic animals*.

5) The nature of supply chains and the degree of mixing or epidemiological separation of populations originating from sources with different health status.

Exposure

6) The presence of populations of *susceptible species* in the *importing country*.

7) The suitability of environmental conditions (e.g. temperature, salinity) for the *susceptible species* of imported *ornamental aquatic animals* in the *importing country*.

8) The suitability of environmental conditions (e.g. temperature, salinity) for the *hazard* in the *importing country.*

9) Intended end uses of the *ornamental aquatic animals* and the implications for exposure. For example:

    a) display in zoos or public aquariums – *ornamental aquatic animals* may be displayed in professionally managed facilities which may have veterinary oversight and *biosecurity* measures in place;

    b) exhibition or competition – *ornamental aquatic animals* may be moved internationally for short periods for participation in exhibitions or competitions, may be kept epidemiologically isolated, and then returned to the country of origin;

c) pets – *ornamental aquatic animals* may be moved internationally in large numbers and widely distributed through retail trade for sale as pets.

10) Cultural practices that may influence exposure, including diversion from intended end-uses (e.g. deliberate release into waterways, use as bait).

11) Internal measures for disease prevention and control and to limit diversion to non-intended end uses.

Article 5.X.8.

Risk management

The standards of the *Aquatic Code* are the preferred choice of *sanitary measures* for *risk management* of *listed diseases* associated with *ornamental aquatic animals*.

To develop *sanitary measures* for non-listed *diseases*, or to justify measures for *listed diseases* that are more stringent than the standards of the *Aquatic Code*, the *Competent Authority* of an *importing country* should follow the recommendations for *risk management* as described in Chapter 2.1. The *sanitary measures* should also comply with the requirements of Section 5 of the *Aquatic Code*.

Sanitary measures for imported *ornamental aquatic animals* can be applied along the import pathway. The *Competent Authority* of the *importing country* should select the least restrictive measures required to mitigate the *disease risks* identified by a *risk assessment.* Options for *risk management* are provided in articles 5.X.9. to 5.X.11. and include those applied:

1) within the *exporting country,* as described in Article 5.X.9.;

2) at the *frontier post,* as described in Article 5.X.10.;

3) within the *importing country*, as described in Article 5.X.11.

Article 5.X.9.

Risk management measures in the exporting country

Where required by the *Competent Authority* of the *importing country* based on *risk analysis*, *risk management* measures can be applied within the *exporting country* to mitigate the *disease risks* associated with international movement of *ornamental aquatic animals* from a country, *zone* or *compartment* not declared free from *diseases* of concern. ~~The *Competent Authority* of the *importing country* should select the least restrictive measures required to mitigate the *disease risks* identified by a *risk assessment.*~~ *Risk management* measures may include:

1) registration or approval by a *Competent Authority* of *aquaculture establishments* that produce, hold or package *ornamental aquatic animals* for export. Registration or approval is a means for ensuring that any *aquaculture establishments* meet any necessary requirements for export of *ornamental aquatic animals* (e.g. general health requirements, *biosecurity*, record keeping);

2) confirmation that the exported *ornamental aquatic animals* are free from signs of *disease* or unexplained mortality at the place of origin (as described in point 2 of Article 5.X.7.) and meet general health requirements in accordance with Article X.X.5.;

3) pre-export *quarantine* in an *aquaculture establishment* (e.g. packaging facility) to ascertain the health status of the animals to be exported. The length of *quarantine* would be based on the *risk assessment* and may vary depending on the species and specific *diseases* of concern;

4) pre-export testing of consignments of *ornamental aquatic animals* to confirm they are free from *pathogenic agents* of concern;

5) systems for traceability and record keeping to ensure transparency of the health status of specific populations or consignments of *ornamental aquatic animals*;

6) appropriate packaging of *ornamental aquatic animals* to maintain their health status for the expected duration and conditions of the transport;

7) certification or provision of other documentation to verify that the *risk management* measures required by the *Competent Authority* of the *importing country* have been met.

Article 5.X.10.

Risk management measures at the border

Where required by the *Competent Authority* of the *importing country* based on *risk assessment*, *risk management* measures can be applied at the border to mitigate the *disease risks* associated with international movement of *ornamental aquatic animals* from a country, *zone* or *compartment* not declared free from *diseases* of concern. ~~The *Competent Authority* of the *importing country* should select the least restrictive measures required to mitigate the *disease risks* identified by a *risk assessment*.~~ *Risk management* measures may include:

1) upon arrival at the *frontier post,* the *Competent Authority* of the *importing country* may perform an inspection of the containers, checking that the consignment matches information included on the accompanying certificate or other documentation. The inspection may include checking for damage to the containers, and observing the animals for abnormal behaviour and suspected clinical signs;

2) at border *quarantine* under the supervision of the *Competent Authority*. The length of *quarantine* would be based on the *risk assessment* and may vary depending on the species and specific *diseases* of concern. Effluent and waste materials from the *quarantine* facilities should~~may~~ be treated or disposed of in a biosecure manner in accordance with Chapters 4.4. and 4.8.;

3) at border testing under the supervision of the *Competent Authority*. Any testing requirements would be based on the *risk assessment*;

4) destruction (as described in Chapter 7.4.) and biosecure disposal of clinically affected animals. All water (including ice), equipment, containers and packaging material used in transport should~~may~~ be treated or disposed of in a biosecure manner in accordance with Chapters 4.4., 4.8. and 5.5.

Article 5.X.11.

Risk management measures in the importing country

The *Competent Authority* of the *importing country* may apply internal *risk management* measures, including to address the *risks* associated with *ornamental aquatic animals* being used for non-intended purposes or being released into the wild. *Risk management* measures may include:

1) prohibiting the diversion of *ornamental aquatic animals* for an alternative end use (e.g. for *aquaculture*, *feed*, bait, research) or from being released into the wild;

2) notifying the *Competent Authority* of the *exporting country* of the detection of a *pathogenic agent* of concern in a consignment, in accordance with Chapter 5.3.;

3) traceability of imported *ornamental aquatic animals* to commercial establishments~~through the commercial supply chain~~.

Article 5.X.12.

Animal welfare during transport

Welfare of *ornamental aquatic animals* during international movement relies on the maintenance of environmental conditions appropriate to the biological characteristics of the species. The minimum requirements to maintain welfare will vary among different species.

Transport of *ornamental aquatic animals* in conditions that are not suited to their biological characteristics may increase vulnerability to infection and the development of clinical *disease*, leading to an increased likelihood of *disease* transmission and morbidity or mortality of animals not related to *disease*.

Transport of *ornamental aquatic animals* should follow protocols that are appropriate for maintaining the welfare of the species and life stage being transported (e.g. for packaging, water quality, temperature, stocking density, duration). Where existing protocols are

not available, they may be developed by considering the factors provided in Chapter 7.2. *Welfare of farmed fish during transport* and should accommodate other requirements during transport, (e.g. the need for inspection and external container repackaging). The International Air Transport Association (IATA) regulations for the transport of live animals should also be taken into account.

Contingency plans should be developed that identify possible adverse welfare events that may occur during transport, the procedures for managing each event, the actions to be taken and the responsibilities of the parties involved.

Annex 47. Item 7.4. – Recommendations for periods of basic biosecurity conditions and targeted surveillance for the disease-specific chapters of the *Aquatic Code*

EU comment

The EU supports the recommendations

Recommendations for periods of basic biosecurity conditions and targeted surveillance for the disease-specific chapters of the WOAH Aquatic Animal Health Code

February 2024

Executive summary and recommendations

- Chapter 1.4. 'Aquatic animal disease surveillance' of the *Aquatic Code* sets out the principles for declaration of disease freedom via four different pathways: 1. Absence of susceptible species, 2. Historical freedom, 3. Targeted surveillance and 4. Returning to freedom.
- The disease-specific chapters of the *Aquatic Code* provide recommendations for periods of basic biosecurity conditions (BBC) for all four pathways and targeted surveillance (TS) for pathways 3 and 4. Following the adoption of the revised Chapter 1.4. in May 2022, the periods of BBC and TS remained under study pending analysis.
- This report details how recommended periods for BBC and TS have been developed by applying the relevant criteria included in Chapter 1.4. 'Aquatic Animal Disease Surveillance' of the *Aquatic Code*.
- If a pathogen is present, it may be detected via the early detection system or passive surveillance throughout the periods of the BBC and TS.
- Pathogen-specific information relevant to the likelihood of pathogen detection by either the early detection system/passive surveillance and by TS (i.e. seasonality of transmission, persistence in the environment, the rapidity of onset of clinical signs or mortality, and rate of spread) was extracted from the disease-specific chapters of the *Aquatic Manual*, and are summarised in the attachments.
- For each pathway, the relevant information was used to rank pathogens and the rankings used to recommend periods for BBC for each pathway, and for TS for pathways 3 and 4. For countries and zones, pathways 1 to 4 apply. For compartments, only pathways 3 and 4 apply.

BBC periods

- For pathway 1, the default minimum period for BBC is 6 months (defined in Chapter 1.4.). Only information on the persistence of the pathogen in the external environment was used for ranking. It is recommended that the period of BBC for pathogens ranked 1 or 2 is 6 months. For pathogens ranked 3, a period of one year is recommended. This pathway is not considered suitable for three pathogens because, as a result of their broad host range, demonstrating absence of susceptible species is not considered possible.
- For pathway 2, the default minimum period for BBC prior to declaring freedom is 10 years (defined in Chapter 1.4.). Only information on the likelihood that infection results in observable clinical signs and a noticeable increase in mortality was used to rank pathogens. For pathogens ranked 1 and 2, the period for BBC prior to declaring freedom is recommended to be ten years. For pathogens ranked 3, a 15 year period for BBC prior to declaring freedom is recommended. For all declarations of freedom

utilising pathway 2, the requirements of passive surveillance in article 1.4.8 must be met (e.g. conditions must be conducive for clinical expression of infection).

- For pathway 3, the default minimum period of BBC preceding TS for countries and zones is one year (defined in Chapter 1.4.). The duration of BBC preceding TS should be long enough for the design prevalence used in TS design to be reached, assuming the pathogen became established immediately prior to commencement of BBC. Hence, the rate of spread between populations is critical.
- Pathogens whose transmission only occurs during limited periods (determined primarily by water temperature) require a longer period of BBC to ensure high confidence that the design prevalence has been reached before TS begins.
- During the period of BBC, the pathogen, if present, may be detected through passive surveillance, which is more likely for pathogens that cause observable signs or mortality. As passive surveillance is a secondary form of evidence for pathway 3 (refer to Article 1.4.3. of the *Aquatic Code*), this factor was also used to make recommendations for the period of BBC for pathway 3 (see Table 3).

TS periods

- The default minimum period for TS for countries and zones is two years. For pathogens whose transmission rate is significantly determined by environmental conditions the prevalence may fall below the design prevalence at periods when environmental or biological conditions are not conducive to transmission.
- For pathogens whose transmission is significantly influenced by environmental factors and where infection does not consistently result in observable clinical signs or mortality, it is recommended that the period of TS is extended to three years (see Table 3).
- For compartments seeking freedom in accordance with pathway 3, a period of one year for BBC and TS is considered sufficient for all pathogens, as the conditions required to maintain a compartment will generate a high confidence that the pathogen will be detected irrespective of its characteristics.
- Chapter 1.4. of the *Aquatic Code* requires that countries, zone or compartments attempting to return to freedom via pathway 4 following an outbreak, review measures to prevent the introduction of the pathogenic agent and implement changes for as long as necessary to evaluate success. As the circumstances of each disease outbreak leading to a breakdown in disease freedom are unique, setting the period of BBC (preceding TS to regain freedom) on a pathogen basis is not considered appropriate.
- In principle the minimum period of TS under pathway 4, should be consistent with the requirements for pathway 3. However, guidance in *Aquatic Code* Chapter 1.4., allows for flexibility in applying periods of TS to regain a disease free status if justified by the circumstances of the outbreak.

Table 1. Recommendations for periods of BBC using Pathway 1. 'Absence of susceptible' species.

Period	Diseases of fish	Diseases of crustaceans	Diseases of molluscs	Diseases of amphibians
6 months	EHNV *G. salaris* IHNV ISAV KHV RSIV SVCV TiLV	AHPND *H. penai* IHHNV IMNV MrNV WSSV YHV1	AbHV *B. exitiosa* *B. ostreae* *P. marinus* *M. refringens* *X. californiensis*	*B. dendrobatidis* *B. salamondrivorans* *Ranavirus*

Period	Diseases of fish	Diseases of crustaceans	Diseases of molluscs	Diseases of amphibians
12 months	SAV	Crayfish plague		
Pathway not suitable	EUS VHSV		*P. olseni*	

Table 2. Recommendations for periods of BBC using Pathway 2. 'Historical freedom'.

Period	Diseases of fish	Diseases of crustaceans	Diseases of molluscs	Diseases of amphibians
10 years	EHNV EUS IHNV ISAV RSIV SAV SVCV TiLV VHSV	AHPND Crayfish plague *H. penai* IHHNV IMNV MrNV WSSV YHV1	AbHV *B. exitiosa* *B. ostreae* *P. marinus* *M. refringens* *P. olseni* *X. californiensis*	*B. dendrobatidis* *B. salamondrivorans* *Ranavirus*
15 years	*G. salaris* KHV			

Table 3. Recommendations for periods of BBC and TS for claims of freedom for countries and zones using Pathway 3. 'Targeted surveillance'.

Period	Diseases of fish	Diseases of crustaceans	Diseases of molluscs	Diseases of amphibians
BBC				
1 year	EHNV EUS IHNV ISAV RSIV SAV SVCV VHSV TiLV	AHPND Crayfish plague *H. penai* IHHNV IMNV MrNV WSSV YHV1	AbHV	*B. dendrobatidis* *B. salamondrivorans* *Ranavirus*

Period	Diseases of fish	Diseases of crustaceans	Diseases of molluscs	Diseases of amphibians
2 years	KHV *G. salaris*		*B. exitiosa* *B. ostreae* *P. marinus* *M. refringens* *P. olseni* *X. californiensis*	
TS				
2 years	*A. astacii* EHNV EUS IHNV ISAV RSIV SAV SVCV VHSV	AHPND Crayfish plague *H. penai* IHHNV IMNV MrNV WSSV YHV1	AbHV	*B. dendrobatidis* *B. salamondrivorans* *Ranavirus*
3 years	*G. salaris* KHV		*B. exitiosa* *B. ostreae* *P. marinus* *M. refringens* *P. olseni* *X. californiensis*	

Contents

List of tables

Abbreviations

BBC	basic biosecurity conditions
TS	targeted surveillance

Abbreviations for 'listed diseases' of fish

EHNV	Infection with epizootic haematopoietic necrosis virus
EUS	Infection with *Aphanomyces invadans* (epizootic ulcerative syndrome)
G. salaris	Infection with *Gyrodactylus salaris*
IHNV	Infection with infectious haematopoietic necrosis virus
ISAV	Infection with HPR-deleted or HPR0 infectious salmon anaemia virus
KHV	Infection with koi herpesvirus
RSIV	Infection with red sea bream iridovirus
SAV	Infection with salmon alphavirus
SVCV	Infection with spring viraemia of carp virus
TiLV	Infection with tilapia lake virus
VHSV	Infection with viral haemorrhagic septicaemia virus

Abbreviations for 'listed diseases' of molluscs

AbHV	Infection with abalone herpesvirus
B. ostreae	Infection with *Bonamia ostreae*
B. exitiosa	Infection with *Bonamia exitiosa*
M. refringens	Infection with *Marteilia refringens*
P. marinus	Infection with *Perkinsus marinus*
P. olseni	Infection with *Perkinsus olseni*
X. californiensis	Infection with *Xenohaliotis californiensis*

Abbreviations for 'listed diseases' of crustaceans

AHPND	Acute hepatopancreatic necrosis disease
crayfish plague	Infection with *Aphanomyces astaci* (crayfish plague)
DIV1	Infection with decapod iridescent virus 1
H. penaei	Infection with *Hepatobacter penaei* (necrotising hepatopancreatitis)
IHHNV	Infection with infectious hypodermal and haematopoietic necrosis virus
IMNV	Infection with infectious myonecrosis virus
MrNV	Infection with Macrobrachium rosenbergii nodavirus (white tail disease)
TSV	Infection with Taura syndrome virus
WSSV	Infection with white spot syndrome virus
YHV1	Infection with yellow head virus genotype 1

Abbreviations for 'listed diseases' of amphibians

B. dendrobatidis	Infection with *Batrachochytrium dendrobatidis*
B. salamandrivorans	Infection with *Batrachochytrium salamandrivorans*
Ranavirus	Infection with *Ranavirus* species

Introduction

The World Organisation for Animal Health (WOAH) provides standards for Members to allow them to demonstrate freedom from specified pathogens at the country, zone or compartment level. The disease-specific chapters of the Aquatic Animal Health Code[1] (*Aquatic Code*) set default minimum periods for the duration of basic biosecurity conditions (BBC) before a declaration of freedom can be made by pathways 1, 2 and 3, and the period of targeted surveillance (TS) for pathway 3. Attachment 1 details the minimum periods for each listed pathogen and pathway stipulated in the disease-specific chapters before the adoption of the revised Chapter 1.4. 'Aquatic animal disease surveillance' in 2022. Since 2022, the default minimum periods have been under study.

This paper presents a rationale for determining, for each aquatic animal disease, the minimum periods of BBC for pathways 1, 2 and 3, and the duration of targeted surveillance for pathway 3, for declarations of freedom for a country, zone or compartment (only pathway 3 applies for compartments). In addition, the guidance for the BBC for a country, zone or compartment to return to freedom under pathway 4 is reviewed.

The duration of the minimum period of BBC required before declaration of freedom using pathway 1 (absence of susceptible species) should be long enough for any pathogen introduced by a fomite (e.g. via trade) before measures were implemented, to lose viability.

The duration of BBC before declaring freedom via pathway 2 should allow the early detection system (EDS) and passive surveillance to generate a high level of confidence that if present the pathogen would be detected (EDS and passive surveillance are components of basic biosecurity).

The design of the TS to demonstrate freedom (via pathway 3) will be largely based on the selected design prevalence (i.e. the minimum prevalence that will be detected with 95% confidence). Guidance on setting the design prevalence is provided in Chapter 1.4. of the *Aquatic Code*. At a zone and country level, the BBC needs to be in place long enough to generate a high level of certainty that the design prevalence would have been reached prior to the start of TS (assuming the pathogen is present before BBC were implemented). The duration of BBC (preceding TS) may need to be longer than the default minimum period (one year) if the pathogen: i) has a long lifecycle; ii) spreads only slowly within and between populations (e.g. requires a high infectious dose); iii) transmission only takes place during limited periods of the year (i.e. when water temperatures are permissive for replication); or iv) only remains viable for only short periods (<14 days) outside the host (survival outside the host correlates with likelihood of transmission).

For pathways 3 and 4, information from passive surveillance can be used as secondary evidence in demonstration of disease freedom. Therefore, in addition to the pathogen transmission (i.e. the rate at which the design prevalence is reached), the likelihood of detection during the period of BBC may also be used to determine the period of BBC. Infections which result in rapid onset of clinical disease or mortality following introduction to a naïve population, are more likely to be detected during the period of BBC compared with pathogens which cause low levels of clinical disease or mortality.

The default minimum period of TS specified in chapter 1.4. is two years for a country or zone and one year for compartments. The rationale for setting the minimum period of TS used in this paper, assumes that the design prevalence has been reached before TS starts. However, for many pathogens transmission, and therefore prevalence, is influenced by environmental factors. Unseasonably low water temperatures in the first year of sampling may result in the prevalence falling below the design prevalence. In addition, the likelihood that a sampled infected fish will test positive may be reduced if levels of infection are lower (e.g. due to a reduced exposure level). A longer sampling period increases the time before freedom is declared, which allows for further pathogen spread (i.e. a higher prevalence and geographic distribution), and thus making detection more likely. Secondly, if sites are sampled on multiple occasions, then the lifecycle of the pathogen becomes

[1] https://www.woah.org/en/what-we-do/standards/codes-and-manuals/aquatic-code-online-access/

relevant, as in the second year of sampling, the likelihood that the prevalence has increased above the design prevalence increases. Seasonality is the key factor driving variation in prevalence from year to year (i.e. the likelihood detecting the pathogen is strongly influenced by water temperature). As passive surveillance can be combined with active surveillance to demonstrate freedom, the likelihood that infection results in clinical signs or mortality detectable through passive surveillance is also considered in determining the minimum period of TS.

Terms of reference

1. Develop an approach to determine for each listed pathogen the minimum period of basic biosecurity conditions for demonstration of freedom at country or zone level via pathway 1 (absence of susceptible species) and pathway 2 (historical freedom) and preceding targeted surveillance for pathway 3 (targeted surveillance[2]).
2. Apply the method to WOAH listed aquatic animal diseases and recommend periods of BBC for pathway 1 and 2, and to precede targeted surveillance to demonstrate freedom at country and zone level (via pathway 3) for the disease-specific chapters of Aquatic Animal Health Code.
3. Review guidance for the minimum period of BBC for compartments seeking disease freedom under pathway 3 (TS)
4. Review the guidance for the BBC for countries, zones or compartments to regain freedom under pathway 4.

Method

Information on pathogen specific characteristics that influence i) the speed at which the design prevalence will be reached and ii) likelihood of early detection through passive surveillance, was extracted from the *Aquatic Manual* disease-specific chapters (summarised in Attachments 2-5). The characteristics are:

1. lifecycle;
2. rate of spread within and between populations (e.g. infectious dose);
3. period of the year during which transmission takes place (i.e. when water temperatures are permissive for replication);
4. persistence outside the host (in the environment);
5. likelihood of early detection (i.e. rapid onset of clinical disease/ mortality following introduction).

For pathway 1 (absence of susceptible species), only information on persistence outside the host in the environment was considered relevant to determining the BBC. This factor was used to rank (from 1-3) pathogens at host group level (i.e. fish, molluscs, crustaceans, amphibians). Recommendations for the duration of BBC for each pathogen are made.

For pathway 2 (historical freedom), only information on the likelihood of detection was considered relevant to determining the BBC. This factor was used to rank (from 1-3) pathogens at host group level (i.e. fish, molluscs, crustaceans, amphibians). Recommendations for the duration of BBC for each pathogen groups were made.

For the BBC of pathway 3, pathogens are ranked (from 1-3) at host group level based on all the characteristics assessed (see Table 4 for details). The rankings indicate the relative rate at which design prevalence will be reached and/or a higher likelihood of detection by passive surveillance.

[2] Described in Article 1.4.3. of the *Aquatic Code*

Table 4. Rankings used to assess the period of basic biosecurity conditions for pathway 3. 'Targeted surveillance'.

Rank 1.
• little or no seasonal variation in transmission • evidence of rapid onset of clinical signs/mortality following pathogen introduction • evidence of rapid spread between populations • persistence outside of host in the environment for > 14 days
Rank 2.
• seasonal variation in transmission, at least some evidence of low to negligible level of transmission during some period of the year • evidence of rapid onset of clinical signs/mortality following pathogen introduction • evidence of at least moderate rate of spread between populations • persistence outside of host in the environment for > 7 days
Rank 3.
• strong seasonal variation in transmission, good evidence of low to negligible level of transmission during some period of the year • slow onset of clinical signs/mortality following pathogen introduction AND / OR • slow spread between populations

For the duration of TS (pathway 3), the factors listed in Table 5 are compared between pathogens for each host group (i.e. fish, molluscs, crustaceans, amphibians) considering:

1. limited period of the year during which transmission occurs, that may vary between years due to environmental factors (e.g. water temperatures);
2. likelihood of early detection (i.e. rapid onset of clinical disease/ mortality following introduction).

For each category of host (i.e. fish, molluscs, crustaceans, amphibians), pathogens are ranked on the basis of the characteristics assessed (see Table 5 for details).

Table 5. Definitions of rankings used to determine the minimum period of targeted surveillance for pathway 3

Rank 1.
• little or no seasonal variation in transmission, • evidence of rapid onset of clinical signs/mortality following pathogen introduction
Rank 2.
• seasonal variation in transmission, at least some evidence of low to negligible level of transmission during some period of the year • evidence of rapid onset of clinical signs/mortality following pathogen introduction
Rank 3.
• strong seasonal variation in transmission, good evidence of low to negligible level of transmission during some period of the year • slow onset of clinical signs/ mortality following pathogen introduction

Results and Recommendations

Pathway 1: Assessment of duration of basic biosecurity conditions (absence of susceptible species)

The rankings of pathogens within host group are set out in Table 6.

Table 6. Summary rankings of pathogens to determine the minimum period of BBC for pathway 1. 'Absence of susceptible species'. Pathogens marked * are considered unsuitable for application of this pathway.

Ranking	Diseases of fish	Diseases of crustaceans	Diseases of molluscs	Diseases of amphibians
1	KHV *G. salaris*	AHPND WSSV YHV1		
2	VHSV* IHNV SVCV RSIV ISAV EHNV TiLV	*H. penaei* IHHNV IMNV MrNV TSV	AbHV *B. exitiosa* *B. ostreae* *P. marinus* *M. refringens* *X. californiensis*	*B. dendrobatidis* *B. salamandrivorans* Ranavirus
3	EUS* SAV	crayfish plague	*P. olseni**	

Based on the analysis, it is recommended that for cases demonstrating freedom at a country or zone level, pathogens ranked 1 and 2 should retain the default minimum six month period of BBC. For pathogens ranked 3, it is recommended that the BBC is extended to 12 months.

This pathway is not considered suitable for pathogens with a broad host range and for which new susceptible species are expected to be determined with further research or spread of the pathogens to new geographic areas. For these species, demonstrating absence of susceptible species in a country or zone is not considered possible. Pathway 1 is thus unsuitable for three species - EUS, VHSV, and *P. olseni*. This recommendation is consistent with the provisions of the 2021 Aquatic Code (i.e. prior to the adoption of revised articles for declaration of freedom in the disease specific chapters in 2022). See Attachment 1.

Pathway 1 is not appropriate to demonstrate freedom at the compartment level as the Aquatic Code does not currently include provisions for compartment freedom via pathway 1.

Pathway 2: Assessment of duration of basic biosecurity conditions (historic freedom)

The rankings of pathogens by host group are set out in Table 7. All fish pathogens with the exception of KHV and *G. salaris* have a high likelihood of detection by the early detection systems or passive surveillance, and hence the default minimum period of ten years will generate a high likelihood of detection (for populations that meet the requirements of Article 1.4.8. and assuming an annual surveillance systems sensitivity of at least 30%). For *G. salaris* and KHV annual surveillance systems sensitivity may be less than 30% and therefore an extended period of 15 years is recommended.

All crustacean pathogens have a high or moderate likelihood of detection and the default minimum period of ten years can be recommended. It should be noted that for all pathogens the passive surveillance requirements of Article 1.4.8. must be met. For example, this pathway may be suitable for declarations of freedom from crayfish plague (*A. astaci*) in populations of susceptible species in which infection results in clinical signs and

observable levels of mortality (e.g. native European species). However, it may not be appropriate to declare freedom for species in which *A. astaci* causes subclinical infection (e.g. North American species of crayfish).

Many mollusc species only cause mortality in older animals and thus may not be detected for some years after introduction. If the pathogen is introduced shortly before the period of BBC starts, mortality will become apparent within the default minimum ten year time period. Hence a period of ten years for BBC can be recommended.

The ranking and recommendation for ISAV applied only to applications of disease freedom for the HPR deleted strain (not the HPR0 strain) where there are populations of Atlantic salmon in which infection will lead to clinical signs and an observable level of mortality. Pathway 2 is not considered appropriate to claim freedom from HPR0 ISAV for which clinical disease is not expected. Similarly claims of freedom from *B. dendrobatidis* and *B. salamondrivorans* need to provide evidence of the presence of susceptible species in which infection will cause mortality and clinical signs.

Table 7. Summary rankings of pathogens to determine the minimum period of BBC for pathway 2. 'Historic freedom'.

Ranking	Diseases of fish	Diseases of crustaceans	Diseases of molluscs	Diseases of amphibians
1	SAV	AHPND Crayfish plague *H. penai* IHHNV IMNV MrNV WSSV YHV1	AbHV	*B. dendrobatidis* *B. salamondrivorans* *Ranavirus*
2	EHNV EUS IHNV ISAV HPR-deleted RSIV TiLV SVCV VHSV		*B. exitiosa* *B. ostreae* *M. refringens* *P. marinus* *P. olseni* *X. californiensis*	
3	KHV *G. salaris*			

It is recommended that pathogens ranked 1 and 2 retain the default minimum ten year period for BBC. For pathogens ranked 3, the minimum BBC period is extended to 15 years.

Pathway 2 should not be used to demonstrate freedom at compartment level.

Pathway 3: Assessment of duration of basic biosecurity conditions preceding targeted surveillance to demonstrate freedom

The current default minimum BBC period of one year is considered the minimum period. The results of the assessments for each pathogen (Attachments 2-5) are summarised in the following sections. The

requirements for passive surveillance described in Article 1.4.8. are a pre-requisite for application of this pathway.

Fish pathogens

Details summarised below can be found in Attachment 2.

- All the fish pathogens had direct lifecycles and therefore lifecycle information was uninformative and not used for ranking pathogens.
- Information in the *Aquatic Manual* chapters did not allow for levels of 'infectiousness' to be compared between pathogens; this criterion could not be used for ranking.
- Based on seasonality and persistence in the environment, only SAV achieved a ranking of 1.
- All pathogens with exception of KHV and *G. salaris*, had a high likelihood of rapid detection post-introduction by passive surveillance.
- The ranking and recommendation for ISAV applied only to applications of disease freedom for the HPR deleted strain. The HPR0 strain is not known to cause clinical disease and exists at very low prevalences in wild Atlantic salmon populations. Historical freedom is not considered a suitable pathway for HPR0 ISAV.

Crustacean pathogens

Details summarised below can be found in Attachment 3.

- All crustacean pathogens have simple direct lifecycles.
- Information on survival outside the host and on environmental factors affecting replication/transmission was not available for most pathogens.
- No basis was found to recommend different durations of BBC on pathogen characteristics.
- All pathogens have high rates of spread and high likelihood of detection by passive surveillance so the minimum period of one year can be applied to all crustacean pathogens.
- The ranking for *Aphanomyces astaci* (crayfish plague) applies to infection in populations of susceptible species in which infections leads to signs and mortality. Demonstration of freedom in populations of crayfish species which do not display clinical signs and experience mortality, cannot be used as evidence from passive surveillance to demonstrate disease freedom.

Molluscan pathogens

Details summarised below can be found in Attachment 4.

- Little information is available on environmental persistence of molluscan pathogens.
- All molluscan pathogens showed seasonality in prevalence/mortality indicating transmission was restricted or reduced for a period of the year (usually during winter months).
- Likelihood of early detection is low for all molluscan pathogens (except abalone herpesvirus) as onset of clinical signs/mortality occurs months to years after exposure.
- *Marteillia refringens* is an outlier, having an indirect lifecycle and the best evidence for restricted periods of transmission.

Amphibian pathogens

Details summarised below can be found in Attachment 5

- Little evidence of strong seasonal impact on the rate of transmission of *B. salamondrivorans* or *B. dendrobatidis.*
- Evidence of limited spread between infected populations leads *B. salamondrivorans* to be ranked lower than *B. dendrobatidis.*
- Ranavirus is listed as a genus. Rate of spread and transmission varies considerably between hosts and viral species (multiple), making ranking at genus level invalid.

The rankings are summarised in 8.

Table 8. Summary rankings of pathogens to determine minimum periods of basic biosecurity conditions for pathway 3. 'Targeted surveillance'.

Ranking	Diseases of fish	Diseases of crustaceans	Diseases of molluscs	Diseases of amphibians
1	SAV	All	AbHV	*B. dendrobatidis*
2	EHNV EUS IHNV ISAV RSIV SVCV TILV VHSV			*B. salamondrivorans* (*Ranavirus**)
3	KHV *G. salaris*		*B. exitiosa* *B. ostreae* *P. marinus* *P. olseni* *M. refringens* *X. californiensis*	

*not assessed, given same ranking as EHNV which is a ranavirus

It is recommended that for pathogens ranked 1 and 2, the default minimum BBC period of one year is retained. For pathogens ranked 3, the period is extended to two years.

Compartments

The default minimum period of BBC is one year for compartments, zones and countries demonstrating freedom using pathway 3 (targeted surveillance). At a compartment level, a case can be made to apply a one year minimum period for all pathogens. Compartments are epidemiologically isolated and factors associated with spread between populations (assessed in this paper) are not relevant. In addition, the high level of management required by Competent Authorities authorising a compartment, should generate a very high likelihood of detection via passive surveillance (e.g. through monitoring of feed consumption and growth rates) even for infections with pathogens that result in few clinical signs or only low mortality. On this basis, the period of BBC (preceding TS) of one year can be adopted for all pathogens.

Pathway 3. Assessment of duration of targeted surveillance to demonstrate freedom

The results of the assessments can be found in Attachments 2-5, and summarised in the following sections.

Fish pathogens

Details summarised below can be found in Attachment 2.

- Based on seasonality and persistence in the environment, SAV is the only pathogen to rank 1.
- All pathogens, with exception of KHV and *G. salaris*, have a high likelihood of rapid detection following introduction into a naïve population by passive surveillance.

Crustacean pathogens

Details summarised below can be found in Attachment 3.

- Little evidence for seasonality of transmission of any pathogens.
- All pathogens have a high likelihood of rapid detection following introduction into a naïve population by passive surveillance.

Molluscan pathogens (Attachment 3)

Details summarised below can be found in Annex 4.

- All pathogens showed seasonality in prevalence/mortality indicating transmission was restricted or reduced for a period of year (usually during winter months).
- Likelihood of early detection is low for all molluscan pathogens (except abalone herpesvirus) as onset of clinical signs /mortality occurs months to years after exposure.
- *Marteillia refringens* is an outlier, having an indirect lifecycle, and the best evidence for seasonally restricted periods of transmission.

Amphibian pathogens

Details summarised below can be found in Attachment 5.

- Little evidence of strong seasonal impact on the rate of transmission of *B. salamondrivorans* or *B. dendrobatidis*
- Good evidence of rapid onset of mortality and morbidity in many (but not all) host species for *B. salamondrivorans* and *B. dendrobatidis*

Rankings for TS are summarised in Table 9.

Table 9. Summary rankings of pathogens to determine the minimum period of targeted surveillance for pathway 3. Targeted surveillance

Ranking	Fish	Crustacean	Molluscs	Amphibian
1	SAV	ALL	AbHV	*B. dendrobatidis* *B. salamondrivorans*
2	VHSV IHNV SVCV RSIV ISAV TiLV EUS EHNV			(*Ranavirus**)
3	KHV *G. salaris*		*B. exitiosa* *B. ostreae* *P. marinus* *P. olseni* *M. refringens* *X. californiensis*	

*not assessed, given same ranking as EHNV which is a ranavirus

It is recommended that for pathogens ranked 1 and 2, the minimum period for TS is two years and for pathogens ranked 3 it is three years.

Compartments

The current default minimum period for TS is one year for compartments for pathway 3. A case can be made to keep a one year period for TS for all pathogens. The high level of management required by Competent Authorities authorising a compartment, should generate a very high likelihood of detection via passive surveillance if the pathogen was present. On this basis, TS for a minimum period of one year is sufficient for all pathogens.

Pathway 4: returning to disease freedom

In Chapter 1.4. of the *Aquatic Code* a default minimum period for BBC before TS to regain freedom is not specified. Instead the guidance requires that 'the pathway of disease introduction should be investigated and basic biosecurity conditions should be reviewed and modified' and that 'mitigation measures should be implemented following eradication of the disease, and prior to commencement of any targeted surveillance'. As the circumstances of each disease outbreak leading to a breakdown in disease freedom are unique setting periods for BBC (preceding TS to regain freedom) on a pathogen basis is not required.

Chapter 1.4. of the *Aquatic Code* suggests that for 'a country or a zone, the default minimum period of surveillance to regain freedom is consistent with the requirements for pathway 3', and thus the periods of TS recommended in this paper can be used for pathway 4. However, it should be noted that guidance in Chapter 1.4. allows for earlier self-declarations of freedom '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'. As outbreaks leading to a breakdown in disease freedom will vary considerably in size and circumstance, flexibility in applying periods of TS to regain a disease free status is justified.

Discussion

Pathway 1. 'Absence of susceptible species'.

Based on the analysis in this paper, it is recommended a minimum period of 6 months for BBC before claiming freedom based on the absence of susceptible species is sufficient for most pathogens. However, for pathogens for which there is evidence of persistence in the environment for months, a minimum period of 12 months is recommended. The viability of pathogens in the environment (outside the host) will be influenced by environmental factors, which following guidance in Chapter 1.4. of the *Aquatic Code*, should be considered in any claim for disease freedom using pathway 1.

Pathway 2. 'Historical freedom'.

In editions of the *Aquatic Code* before revision of Chapter 1.4., a minimum period of ten years over which the pathogen had not been observed was required for all but a few diseases (see Attachment 1). Evidence that the pathogen has not been observed is only reliable if BBC (i.e. passive surveillance) have been implemented. A ten year period of BBC will generate a high likelihood of confidence that the pathogen is present for all but two fish diseases (KHV and *G. salaris*). Guidance in Chapter 1.4. is clear that pathway 2 can only be used if infection results in observable clinical signs. As well, in addition to meeting standards for duration of BBC set in the *Aquatic Manual* disease-specific chapters, evidence of the effectiveness of the passive surveillance component of BBC is required in any application for recognition of disease freedom.

Pathway 3. 'Targeted surveillance' (period of BBC).

The BBC period will only formally start once a Competent Authority is confident that the disease is absent (as a result of stamping-out or a long period of no detections). For pathogens with high rates of spread and high likelihood of detection (i.e. ranked 1 and 2), it is reasonable to assume that one year is a sufficient minimum period for the design prevalence to be reached (assuming introduction just preceding implementation of BBC) or detection through passive surveillance.

For pathogens ranked 3, a longer BBC may be required to allow either a second window for spread, or for clinical signs or mortality to occur. For example, infection with a number of molluscan diseases may only become apparent in older animals and thus a longer period is needed for detection during the period of BBC via passive surveillance. For pathogens ranked 3 with limited periods of transmission and low likelihood of detection by passive surveillance, the period of BBC should be extended to two years. All fish disease were ranked 1 or 2, except KHV and *G. salaris* (ranked 3), both of which had limited periods of transmission during some periods of the year and low likelihood of detection by passive surveillance. It is recommended that BBC be extended to 2 years for these pathogens.

Compared with fish diseases, less evidence is available to rank crustacean diseases. On the basis that they are all i) highly infectious and cause rapid onset of morbidity and mortality after introduction to a naïve population, and ii) observational evidence of rapid spread between population, all crustacean diseases met the criteria for a rank of 1. By contrast, for all the molluscan parasites seasonal variation in prevalence indicates water temperature dependent rates of transmission. Only abalone herpesvirus has a high likelihood of detection by passive surveillance within one year of introduction into a naïve population. It proposed that the BBC (preceding TS) is one years for abalone herpesvirus and 2 years for all the other pathogens.

It did not prove possible to assess ranavirus genus (due to the large variation in characteristics between the multiple host-pathogen combinations). Ranavirus was given the same ranking as EHNV (which is a ranavirus). Based primarily on observations on a low level of spread between populations, it is suggested that the BBC for *Batrachochytrium salamandrivorans* is at least 2 years. The largely observational evidence for *B. dendrobatidis* indicates higher rate of spread and rapid onset of clinical signs and a one year BBC is appropriate.

Pathways 3. 'Targeted surveillance' (duration of targeted surveillance).

It is suggested that for pathogens ranked 1 and 2 in this analysis, the minimum period of TS is two consecutive years (the default minimum period stipulated in Chapter 1.4. of the *Aquatic Code*). The design of the surveillance should follow guidance in Chapter 1.4. that requires surveillance to take place in consecutive years. Sampling should take place when conditions for pathogen detection is optimal, which may occur during a period of weeks or months during each year of the surveillance period. Whilst transmission for pathogens ranked 1 and 2 are not strongly seasonal, stochastic inter-annual variation in transmission (and therefore prevalence) justifies the default minimum period of two years for TS.

For pathogens ranked 3, three consecutive years of TS can be justified. This means that sampling is done at the time of year when likelihood of detection is highest in at least three consecutive years, on the basis that environmental conditions in the years one and two may result in a low likelihood of detection by either TS (sampling) or passive surveillance. It is therefore recommended that the minimum period of TS is three years for pathogens ranked 3.

Conditions making detection of the pathogen suboptimal may persist for more than two or three years. Therefore, it is important that Members follow guidance in Chapter 1.4. when making a case for disease freedom and provide evidence that sampling took place when conditions were optimal for pathogen detection.

Conclusion

The aim of this assessments is to provide a justification for the durations of the BBC and TS for the disease-specific chapters of the *Aquatic Code*. Therefore, the analysis was focused on pathogen characteristics and has not attempted to provide recommendations based on host and environment. Arguably, it may be problematic to assess the importance of pathogen characteristics without considering the host (for pathogens with multiple hosts) and environment (for pathogens with a wide geographic distribution). To some extent the rankings are based on the pathogen characteristics in the major hosts and on environmental conditions in the main areas where these hosts are found. Nevertheless, it is possible to cite specific examples where pathogen/host/environmental combinations for which the ranking is not appropriate. Therefore, it is important that the provisions of Chapter 1.4. requiring that passive surveillance is effective (as infection will cause observable clinical signs), and sampling is undertaken when conditions are optimal for detection and populations with higher likelihoods of infection are preferentially sampled.

It is important to recognise the lack of data, especially for environmental persistence for many of the pathogens, and especially those of molluscs and crustaceans. Ideally, quantitative assessments from observational epidemiological studies would be available to assess the rate of spread between populations. However, in general these data are not available and are not necessarily thoroughly reviewed in the disease-specific chapters of the *Aquatic Manual*.

Despite these possible criticisms and weaknesses in the available data, the analysis presented provides a sound evidence base to justify recommendations for duration of the BBC and TS that should be used when developing surveillance programmes to claim freedom from WOAH listed diseases as described in Chapter 1.4. 'Aquatic animal disease surveillance' of the *Aquatic Code*.

Attachments

Attachment 1. Summary of the previously recommended minimum periods of BBC and TS for all listed diseases and all pathways in the 2021 Aquatic Code (i.e. preceding the adoption of Chapter 1.4. in 2022). Periods for country freedom are shown. NA = not applicable (pathway not available).

	Epizootic haematopoietic necrosis disease	A. invadans (EUS)	Infection with *Gyrodactylus salaris*	ISA virus HPR0 and HPR deleted	ISA virus HPR deleted	Infection with salmonid alphavirus	Infectious haematopoietic necrosis	Koi herpesvirus disease	Red sea bream iridoviral disease	Spring viraemia of carp	Viral haemorrhagic septicaemia	Infection with abalone herpesvirus	Infection with *Bonamia ostreae*	Infection with *Bonamia exitiosa*	Infection with *Martelilia refringens*	Infection with *Perkinsus marinus*	Infection with *Perkinsus olseni*	Infection with *Xenohaliotis californiensis*	Acute hepatopancreatic necrosis disease	Crayfish plague (*Aphanomyces astaci*)	Infection with yellow head virus	Infectious hypodermal and haematopoietic necrosis	Infectious myonecrosis	Necrotising hepatopancreatitis	Taura syndrome	White spot disease	White tail disease	Infection with *Batrachochytrium dendrobatidis*	Infection with ranavirus	*Infection with Batrachochytrium salamandrivorans*
1. Absence of susc species	2	NA	2	2	NA	2	2	2	2	2	NA	2	2	2	3	3	NA	3	2	2	2	2	2	2	2	2	2	2	2
2. Historical freedom																													
-Not observed	10	10	10	NA	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	25	10	10	10	10	10	10	10	10	10
-Basic biosec conds	10	10	10	NA	10	10	10	10	10	10	10	2	2	2	3	3	3	3	2	10	2	2	2	2	2	2	10	10	10
3. Targeted surv																													
-Basic biosec conds	2	2	5	2	2	2	2	2	2	2	2	2	2	2	3	3	3	3	2	5	2	2	2	2	2	2	2	2	2
-Targeted surv	2	2	5	2	2	2	2	2	2	2	2	2	2	2	3	3	3	2	2	5	2	2	2	2	2	2	2	2	2
4. Return to freedom	2	2	5	2	2	2	2	2	2	2	2	2	2	2	3	3	3	2	2	5	2	2	2	2	2	2	2	2	2

Attachment 2. Fish pathogens: Assessment of duration of BBC preceding TS to demonstrate freedom (pathway 3).

Pathogen	Life-cycle	Rate of spread	Early detection (LH)	Transmission period	Environmental persistence	Ranking
VHSV	Simple-direct	High – very infectious, low minimum infectious dose	High: Rapid onset clinical signs	Restricted (when water temp <14 C)	Moderate- Days to weeks	2
IHNV	Simple-direct	High – very infectious, low minimum infectious dose	High: Rapid onset clinical signs	Restricted (when water temp <14 C)	Moderate- Days to weeks	2
SVCV	Simple-direct	High – very infectious, low minimum infectious dose	High: Rapid onset clinical signs	Restricted (when water temp 11-17 C)	Moderate- Days to weeks	2
KHV	Simple-direct	High – very infectious, low minimum infectious dose Slow spread between populations when water temp <16 C	Low: Subclinical infection at low water temp	Restricted (when water temp <16 C)	Low - days	3
SAV	Simple-direct	High – very infectious, low minimum infectious dose	High: Rapid onset clinical signs	Unrestricted (seasonal variation observed but outbreaks occur throughout the year)	High – weeks to months	1
EHNV	Simple-direct	High – very infectious, low minimum infectious dose	High: Rapid onset clinical signs	Restricted (outbreaks occur at water temperatures between and 11-20 C)	Very high – months to years	2
RSIV	Simple-direct	High – very infectious, low minimum infectious dose	High: Rapid onset clinical signs	Restricted to summer months (water temp >25 C)	unknown	2
ISAV (HPR deleted strain)	Simple-direct	High – very infectious, low minimum infectious dose	High: Rapid onset clinical signs	Unrestricted with mortality peaks in early summer and winter	Low persistence – hours to days	2
TiLV	Simple - direct	High – very infectious, low minimum infectious dose	High: Rapid onset clinical signs	Outbreaks generally when water temp >22 C	unknown	2
A invadans (EUS)	Simple-direct	High (single spore sufficient for pathogen to establish)	High: Rapid onset clinical signs	Restricted 18-22 C.	Month-years (encysted form)	2

Pathogen	Life-cycle	Rate of spread	Early detection (LH)	Transmission period	Environmental persistence	Ranking
G. salaris	Simple-direct	High (single parasite sufficient for infestation to establish) Evidence of slow spread between wild populations	Low: Months to years to detect populations declines in wild *Salmo salar;* Clinical signs not apparent in rainbow trout	Rate of replication and spread low below 6.5 C (and on rainbow trout)	Hours to days on dead host; temperature dependent	3

LH = likelihood

Attachment 3. Crustacean pathogens: Assessment of duration of BBC preceding TS to demonstrate freedom (pathway 3).

Pathogen	Life-cycle	Rate of spread	Early detection (LH)	Transmission period	Environmental persistence	Ranking
AHPND	Simple-direct	100% prevalence achieved indicating high rate of spread	High: Rapid onset mortality	Unrestricted	9-18 d	1
A. astaci	Simple-direct	Very rapid spread in susceptible species crayfish, reaching 100% prevalence	High: Rapid onset mortality (in susc. spp.)	Unrestricted – Infection over wide temp range	Several weeks, spores 2 months	1
H. penaei	Simple-direct	Little some information but evidence of rapid spread in farmed P. vannaemi	High: Rapid onset mortality	Unrestricted – High rate of spread at high temp and salinity	No information available	1
IHHNV	Simple-direct	Very rapid spread in P. stylirostris; low in P. vannamei (may go undetected for months)	High; P stylirostric Low: P vannamei	Unrestricted – reduced replication at high temp	No information available	1
IMNV	Simple-direct	Little information	Medium : mortality following stress events in endemic areas	No information available	No information available	1
MrNV	Simple-direct	Rapid spread on introduction to naïve populations	High: Rapid onset mortality in juveniles	No information available	No information available	1
TSV	Simple-direct	Dependent of strain/spp susceptibility	High Rapid onset mortality Rapid onset mortality	No information available - (outbreaks more frequent when salinities are below 30 ppt	No information available	1
WSSV	Simple-direct	High rates of spread and mortality	High Rapid onset mortality	Outbreaks generally at water temp between 18-30 C.	3-4 d in pond water, 3-5 wks in sediment	1
YHV1	Simple-direct	Very rapid – 100% mortality with 3-5 d of clinical signs	High Rapid onset mortality	Little information – probably unrestricted	viable in aerated seawater for 3 d	1

LH = likelihood

Attachment 4. Molluscan pathogens : Assessment of duration of BBC preceding TS to demonstrate freedom (pathway 3).

Pathogen	Life-cycle	Rate of spread	Early detection (LH)	Transmission period	Environmental persistence	Ranking
abalone herpesvirus	Simple-direct	High – rapid rise in prevalence and onset of mortality in all age classes	High	Evidence of seasonal variation in transmission: Outbreaks at 16-19 C but impact of temp not established.	No information available	1
B. exitiosa	Simple-direct	Slow - spread in O chilensis, causing mortality of 80% over 2-3 years; lower prevalence /mortality in O. edulis	Low	Evidence of seasonal variation in transmission: Peak infection in O chilensis in autumn & winter; seasonality not established for infection in O. edulis	No information available	3
B. ostreae	Simple-direct	Slow – infection observed >3 mon after introduction – highest prevalence 2 yr old animals	Low	Evidence of seasonal variation in transmission: Peak infection in late winter/early spring	>7d in seawater	3
M. refringens	Indirect via intermediate host	Slow – prevalence peaks 1 yr post-introduction.	Low	Evidence of seasonal variation in transmission: When water temp > 17 C; higher transmission at high salinity	Up to 21 d	3
P. marinus	Simple-direct	Slow - prevalence highest in animals 1 yr post introduction; mortality observed 1-2 yr post introduction	Low	Evidence of seasonal variation in transmission: Peak transmission when water temp high	No information available	3
P. olseni	Simple-direct	Slow – mortality 1-2 yrs post introduction; low mortality	Low	Evidence of seasonal variation in transmission: Transmission low/ negligible when temp < 15 C.	Several months (spores)	3

Pathogen	Life-cycle	Rate of spread	Early detection (LH)	Transmission period	Environmental persistence	Ranking
X. californiensis	Simple-direct	Slow – prevalence increases with age (size); infection may persist months without signs (3-7 month pre-patent period) esp. at lower water temp	Medium	Evidence of seasonal variation in transmission: Transmission higher at elevated when water temp >15	Demonstrated but not quantified	3

LH = likelihood

Attachment 5. Amphibian pathogens: Assessment of duration of BBC preceding TS to demonstrate freedom (pathway 3).

Pathogen	Life-cycle	Rate of spread	Early detection (LH)	Transmission period	Environmental persistence	Ranking
B. dendrobatidis	Simple - direct	Very high: in susceptible species	High: Rapid onset mortality in susceptible populations (host species dependent)	Unrestricted: Transmission probably higher in cooler months	Suspected but not confirmed	1
B. salamondrivorans	Simple - direct	High within susceptible species in the invasive range; spread between populations is limited	High: Rapid onset mortality in susceptible populations (host species dependent)	Unrestricted:	Encysted spores viable for up to 31 d	2
Ranavirus	Simple - direct	Host species / viral species dependent	Host species / viral species dependent	Not known: Outbreaks area seasonal	Months	?

LH = likelihood

Annex 48. Item 7.5. – Article 9.9.2. of Chapter 9.9. 'Infection with white spot syndrome virus'

CHAPTER 9.9.

INFECTION WITH WHITE SPOT SYNDROME VIRUS

EU comment

The EU supports the proposed changes to this chapter.

[…]

Article 9.9.2.

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.~~:to all decapod (Order Decapoda) crustaceans from marine, brackish and freshwater sources. These recommendations also apply to any other *susceptible species* referred to in the *Aquatic Manual* when traded internationally.~~

Family	Scientific name	Common name
Astacidae	*Austropotamobius pallipes*	white-clawed crayfish
	Pacifastacus leniusculus	signal crayfish
	Pontastacus leptodactylus	Danube crayfish
Calanidae	*Calanus pacificus californicus*	no common name
Cambaridae	*Faxonius limosus*	spinycheek crayfish
	Procambarus spp. (all species)	N/A
Cancridae	*Cancer pagurus*	edible crab
Nephropidae	*Homarus gammarus*	European lobster
	Nephrops norvegicus	Norway lobster
Nereididae	*Dendronereis sp.*	N/A
Paguridae	*Pagurus benedicti*	no common name
Palaemonidae	*Palaemon spp.* (all species)	N/A
Palinuridae	*Panulirus spp.* (all species)	N/A
Parastacidae	*Cherax quadricarinatus*	red claw crayfish
Penaeidae	all species	N/A
Polybiidae	*Liocarcinus depurator*	blue-leg swimcrab
	Necora puber	velvet swimcrab
Portunidae	all species	N/A
Varunidae	*Eriocheir sinensis*	Chinese mitten crab

[…]

Annex 49. Item 7.6. – Articles 11.6.1. and 11.6.2. of Chapter 11.6. 'Infection with *P. olseni*'

CHAPTER 11.6.

INFECTION WITH *PERKINSUS OLSENI*

EU comment

The EU supports the proposed changes to this chapter.

Article 11.6.1.

For the purposes of the *Aquatic Code*, infection with *Perkinsus olseni* means *infection* with the pathogenic agent *P. olseni* of the Family Perkinsidae.

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

Article 11.6.2.

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.:

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

~~primarily venerid clams (*Austrovenus stutchburyi*, *Venerupis pullastra*, *Venerupis aurea*, *Ruditapes decussatus* and *Ruditapes philippinarum*), abalone (*Haliotis rubra*, *Haliotis laevigata*, *Haliotis Cyclobates* and *Haliotis scalaris*) and other species (*Anadara trapezia*, *Barbatianovaezelandiae*, *Macomonaliliana*, *Paphies australis* and *Crassostrea ariakensis*). These recommendations also apply to any other *susceptible species* referred to in the *Aquatic Manual* when traded internationally.~~

[…]

_____

Annex 60. Item 10.1.1. – Sections 2.2.1. and 2.2.2. of Chapter 2.2.8. 'Infection with white spot syndrome virus'

CHAPTER 2.2.8.

INFECTION WITH WHITE SPOT SYNDROME VIRUS

[…]

EU comments

The EU supports the proposed changes to this chapter.

2.2. Host factors

2.2.1. Susceptible host species

Species that fulfil the criteria for listing as susceptible to infection with WSSV according to Chapter 1.5. of the *Aquatic Animal Health Code* (*Aquatic Code*) are:

Family	Scientific name	Common name
Astacidae	*Austropotamobius pallipes*	white-clawed crayfish
	Pacifastacus leniusculus	signal crayfish
	Pontastacus leptodactylus	Danube crayfish
Calanidae	*Calanus pacificus californicus*	no common name
Cambaridae	*Faxonius limosus*	spinycheek crayfish
	Procambarus spp. (all species)	N/A
Cancridae	*Cancer pagurus*	edible crab
Nephropidae	*Homarus gammarus*	European lobster
	Nephrops norvegicus	Norway lobster
Nereididae	*Dendronereis sp.*	N/A
Paguridae	*Pagurus benedicti*	no common name
Palaemonidae	*Palaemon spp.* (all species)	N/A
Palinuridae	*Panulirus spp.* (all species)	N/A
Parastacidae	*Cherax quadricarinatus*	red claw crayfish
Penaeidae	all species	N/A
Polybiidae	*Liocarcinus depurator*	blue-leg swimcrab
	Necora puber	velvet swimcrab
Portunidae	all species	N/A
Varunidae	*Eriocheir sinensis*	Chinese mitten crab

~~Of all the species that have been tested to date, no decapod (order Decapoda) crustacean from marine, brackish or freshwater sources has been reported to be refractory to infection with WSSV (Flegel, 1997; Lightner, 1996; Lo & Kou, 1998; Maeda *et al.*, 2000; Stentiford *et al.*, 2009).~~

~~[**Note:** an assessment of species that meet the criteria for listing as susceptible to infection with WSSV in accordance with Chapter 1.5. has not yet been completed]~~

2.2.2. Species with incomplete evidence for susceptibility

Species for which there is incomplete evidence to fulfil the criteria for listing as susceptible to infection with WSSV according to Chapter 1.5. of the *Aquatic Code* are:

Family	Scientific name	Common name
Carcinidae	*Carcinus maenas*	green crab
Ergasilidae	*Ergasilus manicatus*	no common name
Gecarcinucidae	*Spiralothelphusa hydrodroma*	no common name
	Vela pulvinata	no common name
Grapsidae	*Metopograpsus sp.*	N/A
Macrophthalmidae	*Macrophthalmus (Mareotis) japonicus*	no common name
Ocypodidae	*Leptuca pugilator*	Atlantic sand fiddler
Palaemonidae	*Macrobrachium idella*	slender river prawn
	Macrobrachium lamarrei	Kuncho river prawn
	Macrobrachium nipponense	Oriental river prawn
	Macrobrachium rosenbergii	giant river prawn
Scyllaridae	*Scyllarus arctus*	lesser slipper lobster
Sergestidae	*Acetes sp.*	N/A
Sesarmidae	*Sesarma sp.*	N/A
Varunidae	*Helice tientsinensis*	N/A
Veneridae	*Meretrix lusoria*	Japanese hard clam

In addition, pathogen-specific positive polymerase chain reaction (PCR) results have been reported in the following species, but no active infection has been demonstrated:

Family	Scientific name	Common name
Alpheidae	*Alpheus brevicristatus*	teppo snapping shrimp
	Alpheus digitalis	forceps snapping shrimp
	Alpheus japonicus	Japanese snapping shrimp
	Alpheus lobidens	brownbar snapping shrimp
Artemiidae	*Artemia salina*	brine shrimp
	Artemia sp.	N/A
	Nitokra sp.	N/A
Astacidae	*Astacus astacus*	noble crayfish
Balanidae	*Belanus sp.*	N/A
Brachionidae	*Brachionus plicatilis*	no common name
	Brachionus urceolaris	no common name
Calappidae	*Calappa lophos*	common box crab

	Calappa philargius	spectacled box crab
Cambaridae	*Faxonius punctimanus*	spothand crayfish
Crangonidae	*Crangon affinis*	Japanese sand shrimp
Cyclopidae	*Apocyclops royi*	no common name
Diogenidae	*Diogenes nitidimanus*	no common name
Dorippidae	*Paradorippe granulata*	granulated mask crab
Epialtidae	*Doclea muricata*	no common name
Eunicidae	*Marphysa gravelyi*	polychaete worm
Euphausiidae	*Euphausia pacifica*	Isada krill
Galenidae	*Halimede ochtodes*	no common name
Grapsidae	*Grapsus albolineatus*	no common name
	Metopograpsus messor	no common name
Hippolytidae	*Latreutes anoplonyx*	medusa shrimp
	Latreutes planirostris	flatnose shrimp
Leucosiidae	*Philyra syndactyla*	no common name
Lithodidae	*Lithodes maja*	stone king crab
Macrophthalmidae	*Macrophthalmus (Macrophthalmus) sulcatus*	no common name
Matutidae	*Ashtoret miersii*	no common name
	Matuta planipes	flower moon crab
Menippidae	*Menippe rumphii*	maroon stone crab
Ocypodidae	*Gelasimus vocans*	orange fiddler crab
	Leptuca panacea	gulf sand fiddler
	Leptuca spinicarpa	spined fiddler
	Minuca longisignalis	gulf marsh fiddler
	Minuca minax	redjointed fiddler
	Minuca rapax	mudflat fiddler
Ostreidae	*Magallana gigas*	Pacific oyster
Paguridae	*Pagurus angustus*	no common name
Parthenopidae	*Parthenope prensor*	no common name
Pasiphaeidae	*Leptochela gracilis*	lesser glass shrimp
Sergestidae	*Acetes chinensis*	northern mauxia shrimp
Sesarmidae	*Armases cinereum*	squareback marsh crab
	Circulium rotundatum	no common name
Solenoceridae	*Solenocera crassicornis*	coastal mud shrimp
Squillidae	*Squilla mantis*	spottail mantis squillid
Thiaridae	*Melanoides tuberculata*	red-rim melania
Upogebiidae	*Austinogebia edulis*	no common name

Varunidae	*Chhapgarus intermedius*	no common name
	Cyrtograpsus angulatus	no common name
	Helice tridens	no common name
	Neohelice granulata	no common name
Xanthidae	*Atergatis integerrimus*	red egg crab
	Demania splendida	no common name
	Liagore rubronaculata	no common name

~~All life stages are potentially susceptible, from eggs to broodstock (Lightner, 1996; Venegas *et al.*, 1999). WSSV genetic material has been detected in reproductive organs (Lo *et al.*, 1997), but susceptibility of the gametes to WSSV infection has not been determined definitively.~~

[…]

Annex 61. Item 10.2.1. – Sections 2.2.1. and 2.2.2. of Chapter 2.4.6. 'Infection with *P. olseni*'

CHAPTER 2.4.6.

INFECTION WITH *PERKINSUS OLSENI*

[…]

EU position

The EU supports the proposed changes to this chapter.

2.2. Host factors

2.2.1. Susceptible host species

Species that fulfil the criteria for listing as susceptible to infection with *Perkinsus olseni* according to Chapter 1.5. of the *Aquatic Animal Health Code* (*Aquatic Code*) are:

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

~~*Perkinsus olseni* has an extremely wide host range. Known hosts include the clams *Anadara trapezia*, *Austrovenus stutchburyi*, *Ruditapes decussatus*, *R. philippinarum*, *Tridacna maxima*, *T. crocea*, *Protothaca jedoensis* and *Pitar rostrata* (Cremonte *et al.*, 2005; Goggin & Lester, 1995; Park *et al.*, 2006; Sheppard & Phillips, 2008; Villalba *et al.*, 2004;); oysters *Crassostrea gigas*, *C. ariakensis*, and *C. sikamea* (Villalba *et al.*, 2004); pearl oysters *Pinctada margaritifera*, *P. martensii*, and *P. fucata* (Goggin & Lester, 1995; Sanil *et al.*, 2010); abalone *Haliotis rubra, H. laevigata, H. scalaris,* and *H. cyclobates* (Goggin & Lester, 1995). Other bivalve and gastropod species might be susceptible to this parasite, especially in the known geographical range. Members of the families Arcidae, Malleidae, Isognomonidae, Chamidae and Veneridae are~~

~~particularly susceptible, and their selective sampling may reveal the presence of *P. olseni* when only light infections occur in other families in the same habitat.~~

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

~~All stages after settlement are susceptible.~~

Species for which there is incomplete evidence to fulfil the criteria for listing as susceptible to infection with *P. olseni* according to Chapter 1.5. of the *Aquatic Code* are:

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

In addition, pathogen-specific positive polymerase chain reaction (PCR) results have been reported in the following species, but no active infection has been demonstrated:

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

[…]