

Report of the Meeting of WOAH Aquatic Animal Health Standards Commission

Original: English (EN)

13 to 20 September 2023

Introduction and Member contribution

This report presents the work of the Aquatic Animal Health Standards Commission (hereinafter “the Aquatic Animals Commission”) who met in Paris, France from 13 to 20 September, 2023.

The WOAHA Aquatic Animals Commission wished to thank the following Members for providing written comments on draft texts for the WOAHA *Aquatic Animal Health Code* (Hereinafter “the *Aquatic Code*”) and WOAHA *Manual of Diagnostic Tests for Aquatic Animals* (hereinafter “the *Aquatic Manual*”) circulated in the Commission’s February 2023 report: Australia, Canada, Chile, China (People’s Republic of), Chinese Taipei, Japan, Korea (Republic of), Mexico, New Caledonia, New Zealand, Norway, Switzerland, Thailand, the United Kingdom (UK), the United States of America (USA), the African Union Interafrican Bureau for Animal Resources (AU-IBAR), 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 WOAHA scientific network.

The Aquatic Animals Commission reviewed all comments that were submitted on time 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 significant issues. Where amendments were of an editorial nature, no explanatory text has been provided. The Commission wished to note that not all texts proposed by Members to improve clarity were accepted; in these cases, it considered the text clear as currently written. The Commission made amendments to draft texts 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 1 to 6](#) and [8 to 34](#) are presented for comment. [Annex 7](#) is presented for information.

How to submit comments

The Aquatic Animals Commission strongly encourages Members and International Organisations with a Cooperation Agreement with WOAHA to participate in the development of WOAHA International Standards by submitting comments on relevant annexes of this report. All comments should be submitted to WOAHA through the WOAHA Delegates or from Organisations with which WOAHA has a Cooperative Agreement.

The Commission wished to draw the attention of Members to those instances where an *ad hoc* Group has addressed a specific topic at the request of the Aquatic Animals Commission. In such cases, Members are encouraged to review these reports together with the report of the Commission. *Ad hoc* Group reports are no longer annexed to the Commission’s report. Instead, they are available on the dedicated webpages on the WOAHA website, e.g., *ad hoc* Group reports:

<https://www.woah.org/en/what-we-do/standards/standards-setting-process/ad-hoc-groups/>

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

Comments should be presented within the relevant annex, and include any amendments to the proposed text, supported by a structured rationale or by published scientific references. Proposed deletions should be indicated in ‘~~strikethrough~~’ and proposed additions with ‘double underline’. Members should not use the



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automatic 'track-changes' function provided by word processing software, as such changes may be lost in the process of collating submissions into working documents.

Deadline for comments

Comments on relevant texts in this report must reach the Secretariat by **5 January 2024** to be considered at the February 2024 meeting of the Aquatic Animals Commission.

Where to send comments

All comments should be sent to the Standards Department at: AAC.Secretariat@woah.org

Date of the next meeting

The Aquatic Animals Commission noted the dates for its next meeting: **14 to 21 February 2024**.

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1. Welcome

1.1. Deputy Director General, International Standards and Science

Dr Montserrat Arroyo, WOAHA Deputy Director General, International Standards and Science, met with the Aquatic Animals Commission on 6 September 2023, welcomed members and thanked them for their ongoing contributions to the work of WOAHA. Dr Arroyo commended the Commission for its ambitious agenda and extended her appreciation to the members' employing institutions and national governments.

Dr Arroyo informed the Commission that, with the objective of improving the transparency, documentation, and traceability of the Standard-Setting Process, the Director General has agreed to a step-wise approach for the publication of comments considered by the relevant Specialist Commission (see item 1.3.1. of this report).

Dr Arroyo informed the Commission that the Organisation is currently dedicating efforts to various IT projects with the aim of creating tools that will facilitate access to WOAHA information and input mechanisms, including: i) the evolution of the system for collecting annual reports from Reference Centres; ii) a digitised system for navigating WOAHA International Standards, including a mechanism for the visualisation of sanitary measures recommended for the international trade of commodities for terrestrial animals; iii) an improved system for self-declaration of disease status and; iv) a repository of PVS reports. The goal of all of these IT projects is to improve and simplify access to the relevant information, ensure transparency, and enhance the traceability of WOAHA's work, and interconnecting all the tools.

Dr Arroyo informed the Commission that the Call for experts for seeking nomination for election to the next term of the WOAHA Specialist Commissions (2024-2027) has closed and the next step is the assessment of eligible applicants by the Nomination Evaluation Committee. She noted that more information will be provided to the Delegates in due course. Dr Arroyo acknowledged the strengthening of collaboration with the other Specialist Commissions, emphasising the importance of harmonising and adopting a consistent approach to common work themes. Dr Arroyo highlighted the outcomes of 2022 and 2023 meetings of the Presidents of the Specialist Commissions and the agreed approach for the procedure for the elaboration of WOAHA Standards.

The members of the Commission thanked Dr Arroyo for these updates.

1.2. WOAHA Director General

Dr Monique Eloit, the WOAHA Director General, met the Aquatic Animals Commission on 19 September and thanked its members for their support and commitment to achieving WOAHA objectives.

Dr Eloit informed the Commission that WOAHA is currently undergoing a consultancy to evaluate the Organisation's Basic Texts from both a technical and legal viewpoint. The importance of this consultancy is to introduce a more robust and transparent approach to the Organisation's procedures, supported by a solid legal basis. The revision of the Basic Texts is essential to maintaining WOAHA's credibility among stakeholders, and Members. She noted that more information will be provided to the Delegates in due course.

The Commission thanked Dr Eloit for these updates and agreed on the importance of evaluating the Organisation's Basic Texts. The Commission highlighted the importance of building awareness at national level on the WOAHA Standards Setting process to support the Delegates in achieving a sustainable and effective engagement in the process. The Commission considered that strengthening this connection will help build a more robust system which will also improve knowledge at national level on WOAHA Standards and the associated processes.

1.3. Updates from WOAAH Headquarters

1.3.1. Transparency of the WOAAH process for the elaboration of Standards

The Secretariat informed the Aquatic Animals Commission that the WOAAH Director General had agreed to implement a step-wise approach to improve the transparency of the WOAAH process for the elaboration of Standards that will include the publication of comments considered, responses, and an evolution of the reports of the Aquatic Animals Commission, the Terrestrial Animal Health Standards Commission (Terrestrial Animals Commission) and the Biological Standards Commission. This is also to align with the 7th Strategic Plan. The Secretariat also noted that this proposal had been discussed with the Presidents of the three Commissions at a meeting after the 90th General Session in May 2023 and that they supported this approach.

The Secretariat explained that this process also aims to ensure that Members can gain a better understanding of the complexity and range of opinions, as well as of Commission decisions, and that this will result in a better understanding of Members concerns. This process should also improve the quality of the comments received.

The Secretariat explained that this would be a progressive process that will start in March/April 2024. The comments received on new and revised standards that were considered during the February 2024 meetings of the respective Commissions will be published on the Delegates portal at the same time as the respective February 2024 Commission reports. This process will include an evolution of the Commission reports towards full transparency of comments considered and Commissions' responses, which will result in better documentation and traceability of the WOAAH process for the elaboration of Standards. The Secretariat noted that Delegates will be kept informed throughout this process, and a detailed communication will be sent after the publication of this report.

1.3.2. WOAAH standards online navigation tool

The WOAAH Standards Department informed the Aquatic Animals Commission of the project to develop a new WOAAH Standards Online Navigation Tool. This project is aimed at improving how WOAAH Standards are displayed and made available to Members and other users. The project will enhance the display of the *Aquatic Code*, *Terrestrial Code*, *Aquatic Manual*, and *Terrestrial Manual* on the WOAAH website. The tool is also expected to simplify the annual process of updating the content of the Standards.

The project is aligned with the goals of the 7th Strategic Plan and will provide significant benefits for WOAAH Members, including enhanced accessibility to WOAAH Standards, efficiency in information retrieval, supporting the implementation of WOAAH Standards. The project will also bring benefits to the Organisation itself, by improving the efficiency of internal processes and the interoperability across various datasets related to WOAAH Standards.

The Commission expressed interest and support for the project and recognised the importance of facilitating Members' access to achieve better understanding and use of WOAAH Standards.

1.3.3. Observatory

The WOAAH Observatory provided an update on the state of play of its programme and presented a summary of the main developments in the last 12 months. The Aquatic Animals Commission was informed that the deliverables of the WOAAH Observatory will now comprise:

- Dashboards: The Observatory indicators will be monitored on an annual basis, and the dashboards will be updated annually as well. The publication of the annual report will be discontinued.

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- Comprehensive monitoring report: This report will be issued every five years to provide insights into specific aspects of WOA's strategic plans.
 - Observatory report for the Specialist Commissions: A short report will be produced every three years to support the newly elected Specialist Commissions in developing their work plans.
 - Thematic studies: One to two thematic studies will be conducted each year, depending on the workload and the needs. The outcomes of these studies will be published in reports or dashboards or other types of deliverables depending on the topic.

The Observatory informed the Commission on the progress of the thematic studies on the implementation of WOA Standards on animal welfare during transport and on zoning and compartmentalisation.

The Commission thanked the Observatory for the update and specifically noted the valuable information that these thematic studies and reports will provide on the level of implementation of standards. Additionally, the Commission recognised the significance of understanding the challenges faced by Members to implement these standards. This information will greatly assist its work to review the relevant standards.

1.3.4. Editorial board of the scientific and technical review

The Head of the Publications Unit explained why a new Editorial Board was being established for WOA's peer reviewed journal, the *Scientific and Technical Review*. Although the content is of high quality, and robust editorial and reviewing processes are in place, the publication lacks governance to ensure its scientific credibility.

The Editorial Board will monitor and foster the quality and impact of the *Scientific and Technical Review* and will also advise on WOA's overall publications strategy on request. The role of the Board will be mainly advisory, but they will also participate in reviewing content occasionally and will attend two meetings per year.

The Aquatic Animals Commission was asked to nominate a candidate for the Editorial Board who could commit to the role. Given that the mandate of the current Commission will end in May 2024, the term of the first nominated candidate will run until May 2024.

The Commission agreed that the creation of a new Editorial Board would be a positive step forward for WOA's publications and agreed to inform the Head of the Publications Unit of a possible candidate.

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 Terrestrial Animal Health Standards Commission

The Bureaus (i.e. the President and the two Vice-Presidents) of the Terrestrial Animals Commission and the Aquatic Animals Commission held a meeting on 14 September 2023, chaired by WOA Deputy Director General, International Standards and Science. The purpose of the meeting was to share information and ensure a harmonised approach for the revision of horizontal chapters. Both Commissions committed to convene Bureau meetings at least annually to ensure enhanced coordination. The Bureaus discussed issues of mutual interest in the *Aquatic Code* and the *Terrestrial Code* notably:

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- The approach taken by both Commissions in the development of their respective work plans and criteria for prioritisation of items, as well as a stepwise procedure for the elaboration of WOAHS Standards;
 - Update on the usage of 'Competent Authority', 'Veterinary Authority', 'Veterinary Services' and 'Aquatic Animal Health Services' to ensure a harmonised approach;
 - Work to develop a framework for disease-specific chapters of the *Terrestrial Code* to provide a standardised structure for disease-specific chapters aimed at consistent drafting and understanding across these chapters;
 - Proposed plan to revise the User's guide in the *Terrestrial Code* to improve the usefulness of this section for Members;
 - New draft Chapter 4.X. Biosecurity, in the *Terrestrial Code* that is going out for Member comment. The Terrestrial Animals Commission Bureau agreed to share working documents as comments are received;
 - New draft Chapter 4.X. Emergency disease preparedness, and new draft Chapter 4.Y. Disease outbreak management, in the *Aquatic Code* that will go out for Member comment. The Aquatic Animals Commission Bureau agreed to share working documents as comments are received;
 - Plan to progress work on Chapter 4.2. Zoning and Compartmentalisation, and Chapter 4.3. Application of Compartmentalisation, in the *Aquatic Code*. The Aquatic Animals Commission is developing a Discussion Paper describing the proposed amendments to share with Members for comment. The Aquatic Animals Commission Bureau agreed to share working documents as developed;
 - Update on the work to revise Chapters 5.4. to 5.7. in the *Terrestrial Code*. Draft Chapter 5.4. Measures and procedures applicable in the exportation of commodities, and draft Chapter 5.6. Measures and procedures applicable in the importation of commodities, are going out for Member comment. The Terrestrial Animals Commission Bureau agreed to share draft chapters and to continue to share working documents as developed and comments are received;
 - Revision of Chapter 6.10. Responsible and Prudent Use of Antimicrobial Agents in Veterinary Medicine, in the *Terrestrial Code*. The Terrestrial Animals Commission Bureau agreed to continue to share working documents as comments are received.

4. Work plan and priorities

Comments were received from Australia, New Caledonia, New Zealand, Norway, the EU.

The Aquatic Animals Commission reviewed comments received and noted the support for the development of Chapter 4.X. Emergency disease preparedness, Chapter 4.Y. Disease outbreak management, Chapter 4.Z. Control of pathogenic agents in traded milt and fertilised eggs of fish, and Chapter 5.X. Ornamental aquatic animals, and informed the Members that it will circulate the four draft new chapters for comment as part of this report.

The Commission reviewed the status of ongoing items on its work plan and agreed on the anticipated milestones for their completion. The Commission reviewed the prioritisation of new work items, taking into account a number of criteria, including the expected improvement to the standards and its impact, the benefit to Members, Member comments, relevance to activities of the WOAHS Aquatic Animal Health Strategy, WOAHS Headquarters' comments, and progress of ongoing work plan items.

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 2023. The list of current and planned *ad hoc* Groups for 2023 are available on the WOAHS website.

The updated work plan is presented as [Annex 3](#) for comments.

5. Aquatic Animal Health Strategy

The coordinator of the Aquatic Animal Health Strategy, provided an update on implementation of the Strategy. The Commission was informed of the key milestones and achievements since the last update in February 2023, new activities underway, communication initiatives and priorities for 2024. The Commission also agreed to provide input on a number of proposed activities of relevance to the development of standards and Reference Centres.

The WOAHA Aquatic Animal Health Code

6. Items for Member comments

6.1. Glossary definitions: 'Aquatic Animal Health Services', 'Competent Authority', and 'Veterinary Authority'

Comments were received from Australia, China (People's Rep. of), the UK, the 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 in coordination with the Terrestrial Animals Commission.

At its September 2022 meeting, the Aquatic Animal Commission and the Terrestrial Animals Commission 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* and circulated the proposed changes for comment.

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 meeting

The Aquatic Animals Commission reviewed comments received and noted that Members were generally supportive of the proposed changes. The Commission agreed that 'Aquatic Animal Health Services' should be reinstated into point 6 of Article 4.2.3. as the 'Aquatic Animal Health Services' have responsibilities for the biosecurity plan of a compartment.

The proposed amendments to 'Aquatic Animal Health Services', 'Competent Authority' and 'Veterinary Authority' are presented as [Annex 4](#) for comments.

6.2. Glossary definitions: 'aquatic animal products'

September 2023 meeting

While ensuring alignment of glossary terms throughout the *Aquatic Code* following the safe commodities assessments, it was noted that there were some occurrences of 'products of aquatic animal origin' that should be replaced by the glossary term 'aquatic animal products'. The Aquatic Animals Commission agreed to revise the relevant text to ensure that the glossary term 'aquatic animal product' is used throughout the *Aquatic Code*.

The proposed amendments to 'aquatic animal products' are presented as [Annex 5](#) for comments.

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

Comments were received from Australia, Norway, the UK, 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 duplication within the *Aquatic Code* and to ensure alignment with Chapter 1.1. Notification of diseases and provision of epidemiological information, of the *Terrestrial Code*.

September 2023 meeting

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

The revised Article 1.1.5. of Chapter 1.1. Notification of diseases and provision of epidemiological information, is presented as [Annex 6](#) for comments.

6.4. Article 1.3.1. of Chapter 1.3. Diseases listed by WOAHA

Comments were received from Australia, Canada, Chile, China (People's Rep. of), Chinese Taipei, Japan, Norway, Thailand, the UK, the USA, the AU-IBAR, 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 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 Chapter 1.2. Criteria for listing aquatic animal diseases. 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 Chapter 1.2. Criteria for listing aquatic animal diseases. The Commission agreed that the species ISKNV, including the RSIV genogroup (currently listed in 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) 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 is 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. This proposal would maintain the RSIV genogroup as a listed disease and would also include the infectious spleen and kidney necrosis virus genogroup and the TRBIV genogroup.

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 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 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 considered comments that the International Committee on Taxonomy of Viruses (ICTV) is currently reviewing the classification and nomenclature of the genus *Megalocyticvirus* and that the listing of the disease should use a new proposed name to avoid confusion between ISKNV species and ISKNV genogroup. The Commission confirmed that new nomenclature is under consideration by the ICTV but noted a decision and publication is pending. The Commission will continue to monitor this issue and adopt new nomenclature once published by the ICTV.

The Commission agreed that there are some limitations on the validation of diagnostic tests for detection of the TRBIV genogroup due to the availability of TRBIV-infected tissues. However, the Commission reiterated that there are a variety of methods that are inclusive of all three genogroups (Kawato *et al.*, 2021a, Koda *et al.*, 2023 and Kim *et al.*, 2022). Further diagnostic accuracy studies are warranted, in particular using TRBIV-infected tissues, however, this is not an impediment to this criterion being met.

Regarding the request to revise the wording of Article 1.2.2. Criterion 2 on the demonstration of country freedom, and change 'may' to 'has', the Commission noted that similar comments were considered when Chapter 1.2. was revised in 2015-2016. The Commission reiterated that 'may' is more appropriate to avoid a Member having to demonstrate freedom (in the absence of disease-specific standards) before the listing criteria could be applied. Furthermore, a Member indicated in a comment that they have a surveillance program in place for ISKNV species and are free of infection with ISKNV species in one of the susceptible species farmed.

In the assessment for listing document, the Commission agreed to add that each genogroup is further subdivided into two clades, including the relevant references.

The revised assessment of infection with all genogroups of the virus species infectious spleen and kidney necrosis virus against the criteria in Chapter 1.2. Criteria for listing aquatic animal diseases, is presented as [Annex 7](#) for information.

The revised Article 1.3.1. of Chapter 1.3. Diseases listed by WOA, is presented as [Annex 8](#) for comments.

6.5. Chapter 4.3. Application of compartmentalisation – discussion paper

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. While compartmentalisation has particular relevance for aquatic animal diseases - because eradication is often not possible - it has not been implemented or recognised widely among Members. The Commission emphasised that the revision of Chapter 4.3. aims to provide clarity on the requirements of compartments, improve acceptance and make private sector investment in it more attractive.

The discussion paper proposes 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.

This discussion paper has been 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 Commission explained that questions are included throughout the discussion paper to prompt responses from Members on issues of particular importance to the direction of the chapter's revision. Members are invited to submit comments on the discussion paper, including responses to the questions posed and other matters relevant to the revision of Chapter 4.3.

The discussion paper on Chapter 4.3. Application of compartmentalisation, is presented as [Annex 9](#) for comments.

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

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.

These chapters will support the implementation of other standards in the *Aquatic Code*, in particular the path to returning to disease freedom.

September 2023 meeting

The Commission finalised work on the draft new Chapter 4.X. Emergency disease preparedness and the draft new Chapter 4.Y. Disease outbreak management.

The Commission noted that these two draft new 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 Chapter 4.X. Emergency disease preparedness and draft new Chapter 4.Y. Disease outbreak management, are presented as [Annex 10](#) and [Annex 11](#), respectively, for comments.

6.7. Draft new Chapter 4.Z. Control of pathogenic agents in traded milt and fertilised eggs of fish

September 2023 meeting

The Aquatic Animals Commission reviewed the draft new Chapter 4.Z. Control of pathogenic agents in traded milt and fertilised eggs of fish, which had been developed in collaboration with industry. The Commission reminded Members that the purpose of this chapter is 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 agreed to revise model Article 10.X.10. for infection with SAV, infection with IHNV and infection with VHSV and Article 10.4.15. for infection with ISAV, and to add an option in both points 1 and 2 to follow the requirements described in Chapter 4.Z.

The Commission revised model Articles 10.X.15. for infection with SAV, infection with IHNV and infection with VHSV, and Article 10.4.20. for infection with ISAV to align with the recommendations of the new Chapter 4.Z.

The Commission agreed to add 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 Commission agreed that it would further discuss any suitable article changes for fish disease chapters other than infection with SAV, infection with IHNV, infection with VHSV and infection with ISAV at its February 2024 meeting.

The new Chapter 4.Z. Control of pathogenic agents in traded milt and fertilised eggs of fish, is presented as [Annex 12](#) for comments.

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, is presented as [Annex 13](#) 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, is presented as [Annex 14](#) for comments.

The new glossary term is presented as [Annex 15](#) for comments.

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

September 2023 meeting

The Aquatic Animals Commission reviewed the draft new Chapter 5.X. Movement of ornamental aquatic animals, which had been developed in the Commission and also considered feedback from Focal Point seminars where the proposed need, purpose and scope had been discussed.

The Commission reminded Members of the importance of the development of this new draft chapter given that the international movement of ornamental aquatic animals is characterised by the 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.

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 agreed to add 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 new Chapter 5.X. Movement of ornamental aquatic animals, is presented as [Annex 16](#) for comments.

The new glossary term is presented as [Annex 15](#) for comments.

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

Background

At its September 2020 meeting, the Aquatic Animals Commission reviewed Article X.X.3. of all disease-specific chapters to address comments that the recommended time/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 to assess the safety of aquatic animal products imported (or transited) for any purpose regardless of the disease X status of the exporting country, zone or compartment (as described in Article 1.4.1.) needed to be reviewed based on any new evidence on thermal stability, and requested that a consultant be contracted to undertake this review.

At its February 2023 meeting, the Commission reviewed the Safe commodity assessments that had been 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.

September 2023 meeting

The Commission considered a comment to include more than one time/temperature for inactivation for aquatic animal products listed in Articles X.X.3., when supported by the assessment. The Commission agreed that this was a good proposal and that it would consider it at a future meeting.

The Commission reminded Members that all the information on the Safe Commodities assessments is available on the WOAHP website: <https://www.woah.org/en/document/safe-commodity-assessments-for-woah-listed-aquatic-animal-diseases-2023/>

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

Comments were received from Australia, Korea (Rep. of), New Caledonia, Norway, Thailand, the UK, and the EU.

Background

At its February 2022 meeting, the 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 and circulated these for comment.

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, and circulated them for comment.

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 meeting

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

The revised Articles 8.1.3., 8.2.3. and 8.3.3., are presented in [Annex 17](#) in track changes and clean versions, for comments.

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

Comments were received from Australia, China (People's Rep. of), Korea (Rep. of), New Caledonia, Norway, Thailand, the UK, the USA, and the EU.

Background

At its February 2023 meeting, the 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., 9.8.3. and 9.10.3. accordingly, and circulated them for comment.

Previous Commission reports where this item was discussed

February 2023 report (Item 8.4.2., page 23).

September 2023 meeting

The Commission agreed to add 'that' which was missing in the English version of point 1 of Article 9.4.3.

The Commission agreed to amend an error in the English version of Article 9.7.3. to read five minutes and not 50 minutes.

The revised Articles 9.3.3., 9.4.3., 9.6.3., 9.7.3. and 9.8.3., are presented in [Annex 18](#), for comments.

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

Comments were received from Australia, China (People's Rep. of), Korea (Rep. of), New Caledonia, Norway, Thailand, the UK, the USA, and the EU.

Background

At its February 2023 meeting, the Commission considered the revised Safe commodity assessments for products listed in Articles 10.X.3. and amended these articles accordingly, and circulated them for comment.

Previous Commission reports where this item was discussed

February 2023 report (Item 8.4.3., page 23).

September 2023 meeting

The Commission did not agree to add 'using a process that mitigates potential contamination of the chilled fish products' to the end of point 7 of Article 10.3.3., as it considered that the removal of skin, fins and gills from chilled fish products is sufficient.

The Commission did not agree to add a comma after i.e. as this is not aligned with the style guide for the *Aquatic Code*.

In Article 10.9.3., the Commission did not agree to change the time/temperature for inactivation for SVCV from 60°C for 60 minutes to 60°C for 15 minutes based on a reference by Dixon, 2019 (DIXON, P. (2019). Spring viraemia of carp. CABI Compendium. doi:10.1079/cabicompendium.96466). The Commission noted that this was a review paper and that neither the Commission nor the consultant that performed the safe commodity assessments was able to find scientific evidence to support the statement made in the publication.

The Commission reminded Members that at the 2023 General Session, points 1 and 2 of Article 10.11.3. of Chapter 10.11. Infection with tilapia lake virus were placed under study following an intervention regarding the time/temperature for inactivation. The Commission considered the concerns raised and agreed to add Article 10.11.3. to the texts out for comment with this report using the time/temperature for inactivation identified in the Safe Commodities assessment.

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 19**, for comments.

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

Comments were received from Australia, Korea (Rep. of), New Caledonia, Norway, Thailand, the UK, the USA, and the EU.

Background

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

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, and circulated them for comment.

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 2.1.1.2., page 5), February 2023 report (Item 8.4.4., page 24).

September 2023 meeting

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

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 20**, in track changes and clean versions, respectively, for comments.

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

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 2023 General Session, 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, which were

inconsistent with point 1 of Article 1.4.14. As a result, these proposed changes were placed ‘under study’ and the Commission agreed to review model Articles X.X.5. and X.X.6. for disease-specific chapters at its September 2023 meeting.

September 2023 meeting

The Commission reviewed the final paragraph of point 4 of model Article X.X.5., and agreed to use the wording in the final paragraph of Article 10.11.5. of Chapter 10.11. Infection with tilapia lake virus, 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 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 21](#) for comments.

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

Background

The *ad hoc* Group on Susceptibility of crustacean species to infection with WOAHA listed diseases met in March 2023 to assess the susceptibility of crustacean species to infection with decapod iridescent virus 1 (DIV1) using the criteria in Chapter 1.5. Criteria for listing species as susceptible to infection with a specific pathogen.

September 2023 meeting

The Aquatic Animals Commission considered the *ad hoc* Group report on Susceptibility of crustacean species to infection with WOAHA listed diseases and commended its members for their comprehensive work.

The Commission agreed to remove the ‘under study’ and amend the list of susceptible species in Article 9.3.2. in line with recommendations of the *ad hoc* Group, i.e.

- Six species currently listed in Article 9.3.2. ‘under study’, giant river prawn (*Macrobrachium rosenbergii*), Oriental river prawn (*Macrobrachium nipponense*), red claw crayfish (*Cherax quadricarinatus*), red swamp crawfish (*Procambarus clarkii*), ridgetail prawn (*Palaemon carinicauda*), and whiteleg shrimp (*Penaeus vannamei*), were assessed to meet the criteria for listing as susceptible to infection with DIV1 and are therefore proposed to remain in Article 9.3.2.
- Three new susceptible species, fleshy prawn (*Penaeus chinensis*), gazami crab (*Portunus trituberculatus*) and kuruma prawn (*Penaeus japonicus*) were assessed to meet the criteria for listing as susceptible to infection with DIV1 and are therefore proposed to be added to Article 9.3.2.
- One species currently listed in Article 11.5.2. ‘under study’, giant tiger prawn (*Penaeus monodon*), was assessed and did not meet the criteria for listing as a susceptible species to infection with DIV1 and is therefore proposed to be deleted from Article 9.3.2.

The Commission noted that *P. monodon* had been reported as an affected species in outbreaks of infection with DIV1 and sought further information to inform the assessment for this species.

The Commission encouraged Members to refer to the *ad hoc* Group’s March 2023 report available on the WOAHA Website for details of the assessments conducted by the *ad hoc* Group.

The revised Article 9.3.2. of Chapter 9.3. Infection with decapod iridescent virus 1, is presented as [Annex 22](#) for comments.

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

September 2023 meeting

In Article 10.6.2., the Commission agreed to amend the list of susceptible species 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 10.6.2. of Chapter 10.6. Infection with infectious haematopoietic necrosis virus, is presented as [Annex 23](#) for comments.

6.13. Article 10.11.2. of Chapter 10.11. Infection with tilapia lake virus

Background

The *ad hoc* Group on Susceptibility of fish species to infection with WOAHA listed diseases met in April 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 fish species to tilapia lake virus (TiLV).

September 2023 meeting

The Aquatic Animals Commission considered the *ad hoc* Group report on Susceptibility of fish species to infection with WOAHA listed diseases and commended its members for their comprehensive work.

The Commission agreed to remove the 'under study' and amend the list of susceptible species in Article 10.11.2. in line with recommendations of the *ad hoc* Group, i.e.

- Five species currently listed in Article 10.11.2. 'under study', blue-Nile tilapia hybrid (*Oreochromis aureus* x *O. niloticus*), mango tilapia (*Sarotherodon galilaeus*), Mozambique tilapia (*Oreochromis mossambicus*), Nile tilapia (*Oreochromis niloticus*), and red hybrid tilapia (*Oreochromis niloticus* x *O. mossambicus*), were assessed to meet the criteria for listing as susceptible to infection with TiLV and are therefore proposed to remain in Article 10.11.2.
- Three species currently listed in Article 10.11.2. 'under study', blue tilapia (*Oreochromis aureus*), redbelly tilapia (*Tilapia zillii*) and Tvarnun simon (*Tristramella simonis*), were assessed and did not meet the criteria for listing as a susceptible species to infection with TiLV and are therefore proposed to be deleted from Article 10.11.2.

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

The revised Article 10.11.2. of Chapter 10.11. Infection with tilapia lake virus, is presented as [Annex 24](#) for comments.

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

Comments were received from Australia, China (People's Rep. of), 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 WOAHA listed diseases who undertook assessments

of susceptible species for infection with *Perkinsus marinus* against the criteria presented in Chapter 1.5., Criteria for listing species as susceptible to infection with a specific pathogen.

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. Relevant sections of Chapter 2.4.4. Infection with *Perkinsus marinus*, in the *Aquatic Manual* were also amended in line with the recommendations of the *ad hoc* Group (see item 11.2.1.).

Previous Commission reports where this item was discussed

February 2023 report (Item 8.5., page 24).

September 2023 meeting

The Commission agreed to add 'to the following species that meet the criteria for listing as susceptible in accordance with Chapter 1.5.' after 'The recommendations in this chapter apply' to align with other disease-specific chapters.

The Commission did not agree to add multiple common names for a species in the list of susceptible species in Article 11.5.2. as this was not in line with the convention used in Article X.X.2. of the *Aquatic Code*, and noted that common names are in accordance with FAOTerm <http://www.fao.org/faoterm/collection/faoterm/en/>.

The revised Articles 11.5.1. and 11.5.2. of Chapter 11.5. Infection with *Perkinsus marinus*, are presented as [Annex 25](#) for comments.

7. Items for Member Information

7.1. Emerging diseases

Background

A standing agenda item for each meeting of the Aquatic Animals Commission is to review scientific information on emerging diseases to determine whether a disease should be considered as an emerging disease by WOAHP Members or whether any other actions are warranted. The Commission also considered information from other sources such as WOAHP Members, experts and Reference Centres.

7.1.1. Covert mortality nodavirus (CMNV)

Comments were received from the EU.

Background

At its September 2022 meeting, the Aquatic Animals Commission considered scientific information available on covert mortality nodavirus (CMNV) and agreed that infection with CMNV meets the definition of an emerging disease and should be reported to WOAHP in accordance with Article 1.1.4. of the *Aquatic Code*.

At its February 2023 meeting, the Commission reviewed scientific information and agreed that infection with CMNV continued to meet the definition of an 'emerging disease' and encouraged Members to investigate mortality and morbidity events in the range of aquatic animal species affected.

Previous Commission reports where this item was discussed

September 2022 report (Item 6.2.2., page 13); February 2023 report (Item 9.1.2., page 26).

September 2023 meeting

The Commission reviewed new scientific evidence for CMNV and will update the technical disease card accordingly.

The Commission agreed that CMNV still met the WOAHA definition of an 'emerging disease'. Once again, the Commission requested Members to provide any relevant information on CMNV to the Commission to support its next review of this disease, and encouraged Members to investigate mortality and morbidity events in the range of aquatic animal species affected.

The Commission wished to inform Members that a technical disease card for CMNV is available on the WOAHA website at: <https://www.woah.org/en/document/infection-with-covert-mortality-nodavirus-cmnv-technical-disease-card/>.

7.1.2. Infection with *Enterocytozoon hepatopenaei*

Background

At its September 2021 meeting, the Aquatic Animals Commission considered scientific information available on infection with *Enterocytozoon hepatopenaei* (EHP) and agreed that infection with EHP meets the definition of an emerging disease and should be reported to WOAHA in accordance with Article 1.1.4. of the *Aquatic Code*.

At its February 2022 meeting, the Commission reviewed scientific information and agreed that infection with EHP continues to meet the definition of an 'emerging disease' and encouraged Members to investigate mortality and morbidity events in the range of aquatic animal species affected.

Previous Commission reports where this item was discussed

September 2021 (Item 5.2.1.2., page 28); February 2022 Part B (Item 2.2.1.2., page 8).

September 2023 meeting

The Commission reviewed new scientific evidence for infection with EHP and will update the technical disease card accordingly.

The Commission agreed that infection with EHP still met the WOAHA definition of an 'emerging disease'. Once again, the Commission requested Members to provide any relevant information on infection with EHP to the Commission to support its next review of this disease, and encouraged Members to investigate mortality and morbidity events in the range of aquatic animal species affected.

The Commission wished to inform Members that a technical disease card for EHP is available on the WOAHA website at: <https://www.woah.org/en/document/infection-with-enterocytozoon-hepatopenaei/>.

The WOAHA 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 WOAHS Standards Department (AAC.Secretariat@WOAH.org).

8. Items for Member comments

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 |
|--|---|--|
| The three introductory chapters on diseases of crustaceans, fish, or molluscs (2.2.0; 2.3.0 or 2.4.0), Section on Use of molecular techniques for confirmatory testing and diagnosis | No comment, this resulted from discussions within the Commission | Add guidance text on nested PCR: “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.” |
| 3.6. Pooling of samples | No comment, this resulted from the Commission’s review of the standard paragraph. | Amend the first sentence of standard text: Pooling of samples from more than one individual animal for a given purpose should only be <u>is only</u> recommended where robust supporting data on diagnostic sensitivity and diagnostic specificity have been evaluated and found to be suitable. |
| 4.4. Nucleic acid amplification | In the tables of PCR primers and probe sequences and cycling conditions, clarify that an initial denaturation step and a final elongation step are not included | Agree, added a footnote to these tables in all chapters |
| 6.3. Diagnostic sensitivity and specificity for diagnostic test | In situations where no data are provided in Tables 6.3.1 and 6.3.2, remove the text on how the data can be used in surveys. | Agree |

8.1. Section 2.2. Diseases of crustaceans

8.1.1. Chapter 2.2.0. General information: diseases of crustaceans

Comments were received from Australia, Chinese Taipei, Mexico, Norway, Thailand, the USA, and the AU-IBAR.

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.

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 meeting

| Section/ paragraph | Comment | Decision |
|--|--|--|
| 1.2. Specifications according to crustacean populations | Indicate how to determine that the sample is proportionally represented. | Disagree, no need to include further explanation as the proposed wording is already self-explanatory. |
| 2.2. Virological examination | Clarify and correct the text. | Agree, modified the first sentence and deleted the second sentence as it is incorrect: nodavirus is isolated and not the shrimp <i>Macrobrachium rosenbergii</i> . |
| 2.3. Bacteriological examination | Clarify how the test method should be used. | Disagree, according to Chapter 2.2.3. Infection with <i>Hepatobacter penaei</i> (necrotising hepatopancreatitis) <i>H. penaei</i> has not been cultured and the text states that bacteriological examination is not routine used. |
| 5. Techniques | Request to keep the numeric examples as they provide clearer guidance on sampling/sample sizes. | Disagree, the purpose of the text on pooling is to emphasise that a sufficient sample volume is necessary for the test in question. Specific numerical guidance is provided in the disease-specific chapters: the numbers here might not be applicable to all susceptible species and thus are proposed for deletion. The sample volume does not influence the number of tests that are required for the purpose of use. |
| 5.5.1. Sample preparation and types, point vi) <i>Fixed tissues for in-situ hybridisation:</i> | Request to specify the concentration of ethanol. | Agree, amended the text accordingly and also clarified the fixation times in Davidson's fixative. |
| 5.5.2. Preservation of RNA and DNA in tissues | Clarify the text regarding the length of time preserved samples can be stored and the acceptability of other products commercially available for the same purpose. | Agree |

| Section/ paragraph | Comment | Decision |
|---|--|--|
| 5.5.4. Preparation of slides for <i>in-situ</i> hybridisation | Request to specify the concentration of ethanol. | Agree |
| 6. Additional information to be collected | Delete the Section. | Disagree, the title of Section 6 is “Additional information to be collected”, and the current text fits with that title. The comment strays from the central focus of the Section. |

The revised Chapter 2.2.0. General information (diseases of crustaceans), is presented as [Annex 26](#) for comments.

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

Comments were received from Australia, China (People’s Rep. of), Mexico, Norway, the USA, the 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 WOAHP 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.

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 meeting

| Section/ paragraph | Comment | Decision |
|--|---|--|
| 2.2.3. Likelihood of infection by species, host life stage, population or sub-population | Delete the sentence stating that the only non-crayfish crustacean species known to be susceptible is the Chinese mitten crab (<i>Eriocheir sinensis</i>) as there is no scientific evidence to support this claim | Disagree, the relevant reference has been added. |
| 2.2.5. Aquatic animal reservoirs of infection | Provide information on the nature and health conditions of species that are reservoirs of infection with <i>Aphanomyces astaci</i> . | Such information cannot currently be added to the chapter as not all species of crayfish have been tested. |
| 2.3.2. Clinical signs, including behavioural changes and 2.3.3. Gross pathology | Change the subheading from “Susceptible species” to “Species prone to clinical disease” | Agree, as different to susceptible species as defined in Aquatic Code Chapter 1.5. Criteria for listing species as susceptible to infection with a specific pathogen |

| Section/ paragraph | Comment | Decision |
|---|--|--|
| | Change the subheading from “North American crayfish species” to “Species that do not normally develop clinical disease” | Agree |
| 2.3.6. Geographical distribution | Add a reference and additional text on distribution in North America | Agree to add the reference, but disagree to add the text as the information is already stated in the sentence. |
| 4.4.1. Real-time PCR | Correct the cycling conditions for the real-time PCR in line with the reference. | Agree |
| | Delete method 2 because it has not yet been published and modify the accompanying text accordingly. | Agree |
| | Amend text in the paragraph under the table to provide clarity on the science and the steps necessary to accurately identify the listed pathogen | Agree |
| 6.1.1. Definition of suspect case in apparently healthy animals | Replace “at least” by “one of”, otherwise the criteria are not accurate. | Agree |

The revised Chapter 2.2.2. Infection with *Aphanomyces astaci* (crayfish plague), is presented as [Annex 27](#) for comments.

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

Comments were received from Australia, Canada, China (People’s Rep. of), Mexico, Norway, Thailand, and the USA.

Background

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

Previous Commission reports where this item was discussed

February 2023 report (Item 11.1.3., page 46).

September 2023 meeting

| Section/ paragraph | Comment | Decision |
|---|---|---|
| General comment | Review the chapter to better clarify which pathogens (<i>Macrobrachium rosenbergii</i> nodavirus [MrNV] and extra small virus [XSV]), and when, should be considered for activities such as surveillance, case definition, reporting, freedom declaration claims, etc. | The disease is defined in the scope as infection with MrNV. Included text in the scope that XSV is associated with the disease but its role has not been determined. Maintained information on XSV in the chapter apart from Table 4.1 on recommended test methods for MrNV and Section 6 on the case definitions. As its role is unknown, information on XSV adds value to the chapter and many of the literature references refer to both MrNV and XSV. |
| 2.1.1. Aetiological agent | Delete mention of XSV. | Disagree, see general comment. |
| 2.1.2 Survival and stability in processed or stored samples | Delete mention of XSV. | Disagree, see general comment. |
| 2.2.2 Species with incomplete evidence for susceptibility | Delete the aquatic insect species from the Table in this Section as they have already been defined as vectors in Section 2.2.6. | Agree. |
| | Delete the Table in this Section because it is not appropriate to require testing for a disease in species for which there is incomplete evidence of susceptibility. | Disagree, rationale provided in previous reports and approach agreed by WOAHA Members. The scope of trade standards in the <i>Aquatic Code</i> applies only to susceptible species; any measures applied to species with incomplete evidence of susceptibility (which are included in the <i>Aquatic Manual</i> only) would need to be supported by a risk assessment. |
| | Add text and a reference to the finding of MrNV genomes in <i>Cyprinus carpio carpio</i> | To be considered by the <i>ad hoc</i> Group on susceptibility of crustacean species to WOAHA listed diseases will review the reference. |
| 2.2.3. Likelihood of infection by species, host life stage, population or sub-populations | Add a reference on MrNV causing mortality in freshwater prawns in experimental studies. | Agree. |
| 2.2.5. Aquatic animal reservoirs of infection | Add text and a reference on recent reports of MrNV and XSV infections in Pacific white shrimp. | Agree to add reference, but not to add the new text as susceptibility and transmission were not likely to have been demonstrated in the study (PCR positive only): amended the current sentence to accurately reflect the situation. |

| Section/ paragraph | Comment | Decision |
|----------------------------------|--|--|
| 2.3.6. Geographical distribution | Add two references and include the expansion of the geographical range to named Members and potentially to a new region. | Disagree, one of the two references already in the text, and the second is not a peer-reviewed paper. This Section reports disease occurrence at the regional and not country level. |
| Table 4.1. | Request to review the completeness of data in Table 4.1. | Agree, first clarified in the title of the Table that the recommended diagnostic methods are <u>for MrNV</u> only. Added that the level of validation of histopathology for presumptive diagnosis of clinically affected animals is NA (Not available) |
| 4.4.1. Real-time PCR | Consider maintaining XSV in the real-time RT-PCR table | Agreed to maintain: see general comment. |
| 4.4.2. Conventional RT-PCR | Review and complete or correct the information in the table, including deletion of the reference to a test method that not given in the table. | Agree except to add the initial denaturation and final extension steps (see decision on horizontal changes to the <i>Aquatic Manual</i> above) |

The revised Chapter 2.2.6. Infection with *Macrobrachium rosenbergii* nodavirus (white tail disease), is presented as [Annex 28](#) for comments.

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

Comments were received from Australia, China (People's Rep. of), Chinese Taipei, Japan, Mexico, Norway, the USA, the AU-IBAR, 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 (YHD), which had been updated by the WOAHP Reference Laboratory expert and reformatted using the new disease chapter template.

Previous Commission reports where this item was discussed

February 2023 report (Item 11.1.4., page 46).

September 2023 meeting

| Section/ paragraph | Comment | Decision |
|---|--|--|
| 2.2.2. Species with incomplete evidence for susceptibility | Delete the Table in this Section because it is not appropriate to require testing for a disease in species for which there is incomplete evidence of susceptibility. | Disagree, rationale provided in previous reports and approach agreed by WOAHA Members. The scope of trade standards in the <i>Aquatic Code</i> applies only to susceptible species; any measures applied to species with incomplete evidence of susceptibility (which are included in the <i>Aquatic Manual</i> only) would need to be supported by a risk assessment. |
| 2.2.5. Aquatic animal reservoirs of infection | Delete the last sentence as it does not use the appropriate exposure pathways for demonstrating infection due to the use of experimental infection via injection. | Agree. |
| 2.3.6. Geographical distribution | Include statement regarding genotype 7 (YHV7). | Disagree, the subject of this chapter is YHV-1. For completeness and for the purposes of distinguishing genotypes YHV7 is mentioned in Section 2.1.1 Aetiological agent. |
| 3.1. Selection of populations and individual specimens | Delete the text in this Section unless references can be provided. | Disagree, the text is clear and useful, and is aligned with the content of other chapters. |
| 3.2. Selection of organs or tissues | Delete the text in this Section unless references can be provided. | Disagree, the text is clear and there is no need for a reference: the choice of tissue depends on many factors including method to be used, tissue predilection, reliability/ease of sampling, method validation, likelihood of contamination, storage, inhibitors etc. |
| 3.3. Samples or tissues not suitable for pathogen detection | The current text contradicts Section 3.1: add an explanation regarding tissue type and early postlarvae life stages. | Disagree: samples and specimens are not the same – sample type suitability is not necessarily binary. |
| 4.4.1. Real-time PCR | Add the real-time PCR method being developed by the WOAHA Reference Laboratory. In particular it should replace the conventional nested RT-PCR in Section 4.4.2. as the recommended test for surveillance. | Agree: the test will be added once it has been published or the expert can provide a completed validation report template (see item 9.1.). |
| 4.4.2. Conventional RT-PCR | Specify the name of the detected genotype. | Agree: YHV8 genotype. |

| Section/ paragraph | Comment | Decision |
|---|---|---|
| | There are three RT-PCR methods in the Tables in this Section but they are not explicitly designated in Table 4.1 or Section 6. To avoid confusion, those test protocols should be specified in the relevant sections. | Disagree, the selection of the RT-PCR depends on the user's purpose. The three RT-PCR tests are all recommended for two purposes with the same level of validation. |
| 4.4.3. Other nucleic acid amplification methods | Add text and a reference to a real-time RT-PCR with higher sensitivity and specificity than the methods already in the chapter. | Disagree, reviewing the reference it was noted that no direct comparison of methods was done on the same sample set/matrix. |
| 4.8. Bioassay | Add a new paragraph on how to interpret bioassay results in line with standard text in other chapters. | Agree |
| 6.1. Apparently healthy animals or animals of unknown health status | In the second sentence of the introductory paragraph, insert "suspect or" before "known infected population" because follow up would be warranted if there was an epidemiological link to either a known or suspect population. | Disagree, the text discusses populations becoming suspect based on their hydrogeographical proximity or epidemiological link to an infected population. That implies that all linked populations are therefore suspect and do not become suspect by proximity to another suspect population that is linked to an infected population. |
| 6.1.1. Definition of suspect case in apparently healthy animals | Replace "recommended" with "conventional" RT-PCR to reflect the recommendation in Table 1.4. | Agree |
| 6.1.2. Definition of confirmed case in apparently healthy animals | Amend to align with Section 6.2.2. | Agree |
| 6.2.2. Definition of confirmed case in clinically affected animals | Delete "targeting non-overlapping parts of the genome" following "RT-PCR". | Agree, PCR and sequence analysis of the amplicons are sufficient for confirmation. |

The revised Chapter 2.2.9. Infection with yellow head virus genotype 1, is presented as [Annex 29](#) for comments.

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

September 2023 meeting

The Aquatic Animals Commission reviewed Chapter 2.2.X. Infection with decapod iridescent virus 1, which had been developed by the WOAHP Reference Laboratory expert and formatted using the new disease chapter template.

The new Chapter 2.2.X. Infection with decapod iridescent virus 1, is presented as [Annex 30](#) for comments.

8.2. Section 2.4. Diseases of molluscs

8.2.1. Chapter 2.4.0. General information: diseases of molluscs

September 2023 meeting

The Aquatic Animals Commission reviewed Chapter 2.4.0. General information: diseases of molluscs, which had been updated by the WOAHP 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.

The main amendments include:

| Section/ paragraph | Change |
|--|--|
| A.1.2. Specifications according to mollusc populations | Amended to be consistent with chapters 2.2.0 and 2.3.0, the general information chapters on diseases of crustaceans and fish. |
| A.1.3. Specifications according to clinical status | Clarified the text and deleted unnecessary details. |
| A.1.4. Specifications according to mollusc size | Deleted disease-specific information as available in the disease-specific chapters. |
| A.2. General processing of samples | Moved the final paragraph to Section B.6. Additional information to be collected. |
| B.5. Techniques | Deleted information that is repeated elsewhere in the chapter and simplified text on transport and shipping. |
| B.5.5. Molecular methods | Aligned the title with chapters 2.2.0 and 2.3.0 to "Use of molecular techniques for surveillance, confirmatory testing and diagnosis". |
| B.5.5.2. Preservation of DNA in tissues | Edited the text to align with chapters 2.2.0 and 2.3.0. |

The revised Chapter 2.4.0. General information: diseases of molluscs, is presented as [Annex 31](#) for comments.

8.2.2. Chapter 2.4.1. Infection with abalone herpesvirus

September 2023 meeting

The Aquatic Animals Commission reviewed Chapter 2.4.1. Infection with abalone herpesvirus, which had been updated by the WOAHP Reference Laboratory expert and reformatted using the new disease chapter template.

The main amendments include:

| Section/ paragraph | Change |
|--------------------|---|
| 1. Scope | Updated the taxonomy of the pathogenic agent. |

| Section/ paragraph | Change |
|--|---|
| 2.2.1 Susceptible host species and 2.2.2 Species with incomplete evidence for susceptibility | Completed these two sections in accordance with the findings of the <i>ad hoc</i> Group on susceptibility of mollusc species to WOAH listed diseases. |
| Table 4.1. | Completed Table 4.1. and aligned with the case definitions in Section 6. |
| 4.6. <i>In-situ</i> hybridisation | Deleted the details of the test method as it is in the reference. |
| 6. Corroborative diagnostic criteria | Revised definitions of suspect and confirmed case in apparently healthy and clinically affected animals. |
| 7. References | Updated the references. |

The revised Chapter 2.4.1. Infection with abalone herpesvirus, is presented as [Annex 32](#) for comments.

8.2.3. Chapter 2.4.4. Infection with *Marteilia refringens*

September 2023 meeting

The Aquatic Animals Commission reviewed 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.

The main amendments include:

| Section/ paragraph | Change |
|--|---|
| 1. Scope | Amended the scope to align with the <i>Aquatic Code</i> . Moved most of the text in the second paragraph to Section 2.1.1 Aetiological agent. |
| 2.2.5 Aquatic animal reservoirs of infection and 2.2.6 Vectors | Amended to align with the disease chapter template. |
| Table 4.1. | Completed Table 4.1. and aligned with the case definitions in Section 6. |
| 4.5. Nucleic acid amplification | Completed the tables of PCR primer and probe sequences and cycling parameters and removed the details of the PCR methods. |
| 6. Corroborative diagnostic criteria | Revised definitions of suspect and confirmed case in apparently healthy and clinically affected animals. |
| 7. References | Updated the references. |

The revised Chapter 2.4.4. Infection with *Marteilia refringens*, is presented as [Annex 33](#) for comments.

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

Comments were received from Australia, Chinese Taipei, 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 WOAHA listed diseases.

Previous Commission reports where this item was discussed

February 2023 report (Item 11.2.1., page 47).

September 2023 meeting

The Commission did not agree 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. The Commission also did not agree to change the spelling of 'fulfil' to 'fulfill' as WOAHA uses UK spelling.

The revised Section 2.2.1. and 2.2.2. of Chapter 2.4.5. Infection with *Perkinsus marinus*, is presented as [Annex 34](#) for comments.

9. Items for Member information

9.1. Develop a mechanism to speed up the process of making updates to diagnostic methods in the *Aquatic Manual* available to Members quicker

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 Biological Standards Commission had decided that the template should be used as a 'validation report' form for tests recommended in the *Terrestrial Manual*.

The Aquatic Animals Commission stressed the importance of validating diagnostic tests for aquatic animal diseases. It 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.

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 meeting

The Commission made the final amendments to the template for the validation report form for tests recommended in the WOAHA 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. The template was provided to a WOAHA Reference Laboratory expert who is in the final stages of developing a new PCR method. At the next meeting, the Commission will review the data submitted along with the expert's feedback on the template's suitability and usability.

10. Ad hoc Groups

10.1. 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 June 2023 to conduct assessments for susceptibility of mollusc species to infection with *Perkinsus olseni*.

The Aquatic Animals Commission was informed that the *ad hoc* Group did not complete their assessments of *P. olseni* due to the volume of research associated with the pathogenic agent. The Commission reviewed the *ad hoc* Group's interim report which outlined the work completed to date and provided feedback on. The *ad hoc* Group is planning to meet in November/December 2023 to finalise the assessments of species susceptible to infection with *P. olseni*.

10.2. 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 April 2023 to complete the assessments for susceptibility of fish species to infection with tilapia lake virus (see Item 6.13.).

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

The report of the *ad hoc* Group on susceptibility of fish species to infection with WOAH listed diseases is available on the WOAH Website at: <https://www.woah.org/app/uploads/2023/10/a-fish-ahg-tilv-april-2023.pdf>

10.3. 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 April 2023 to complete the assessments for susceptibility of fish species to infection with Decapod iridescent virus 1 (see Item 6.11.).

The Commission was informed that the *ad hoc* Group is planning to meet in November 2023 to progress its work assessing species susceptible to infection with white spot syndrome virus.

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: <https://www.woah.org/app/uploads/2023/10/a-crustacean-ahg-div1-mars-2023.pdf>

11. Reference centres or change of experts

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

The WOAH Collaborating Centre for Emerging Aquatic Animal Diseases hosted by the Centre for Environment, Fisheries and Aquaculture Sciences in the UK had proposed that the Centre be led by two experts with complementary skills. The Commission agreed with this approach.

The Delegate of the Member concerned had submitted to WOAH the following nomination for a change of expert at a WOAH Reference Laboratory. The Aquatic Animals Commission reviewed the application and recommended its acceptance:

Infection with *Hepatobacter penaei* (necrotising hepatopancreatitis)

Dr Arun Dhar to replace Dr Luis Fernando Aranguren at the Aquaculture Pathology Laboratory, The University of Arizona, Tucson, Arizona, UNITED STATES OF AMERICA

11.2. Review of the annual reports of activities in 2022 of the WOAHA Reference Centres

Annual reports had been received from 35 of 37 WOAHA Reference Laboratories for diseases of aquatic animals and all four Collaborating Centres for aquatic animal issues.

In accordance with the adopted Procedures for designation of WOAHA Reference Laboratories (the SOPs) ([Procedure for Designation of Reference Laboratories](#)) and the Procedures for designation of WOAHA Collaborating Centres ([Procedures for designation of Collaborating Centres](#)), the Aquatic Animals Commission reviewed all the reports received, noting in particular the performance of each Reference Centre with regard to fulfilling the Terms of Reference (ToR) to the benefit of WOAHA Members.

The Commission noted the significant contributions that had been made by Reference Centres and wished to thank designated experts for leading these valuable contributions to the WOAHA mission. The Commission expressed its on-going appreciation for the enthusiastic support and expert advice given to the WOAHA by the Reference Centres. In particular, the Commission is grateful for the ongoing support and essential contributions of Reference Laboratory experts for revision of the disease-specific chapters of the *Aquatic Manual*.

The Commission proposed some amendments to the annual report template such as indicating that experts can inform WOAHA of the need to update an *Aquatic Manual* chapter due to new scientific findings.

11.3. Improving the engagement of the Reference Laboratories for aquatic animal diseases with the Aquatic Animals Commission

The Aquatic Animals Commission would like to build closer relationships with the Reference Laboratories for aquatic animal diseases to improve their engagement with the Commission. Some ways to achieve this goal include inviting designated Reference Laboratory experts to the Commission's meetings, inviting the Reference Laboratories to the Commission's post-meeting webinars, promoting research projects such as projects to validate some of assays in the *Aquatic Manual*. A member of the Commission was identified to draft a summary of possible ways to progress this undertaking, which will be reviewed at the next meeting in February 2024.

11.4. Review of the main focus areas and specialties for Collaborating Centres

The Aquatic Animals Commission reviewed the changes proposed by the Biological Standards Commission to the list of Main Focus Area and Specialities for WOAHA Collaborating Centres. The Aquatic Animals Commission proposed some further amendments to the list. The updated document with the changes shown is available at [Annex 20](#) of the report of the September 2023 meeting of the Biological Standards Commission.

11.5. Call for applications for candidates for WOAHA Reference Laboratory status

The Aquatic Animals Commission noted the need to designate WOAHA Reference Laboratories for the following listed diseases:

- Infection with *Aphanomyces invadans* (epizootic ulcerative syndrome)
- Infection with *Batrachochytrium dendrobatidis*
- Infection with *Batrachochytrium salamandrivorans*
- Infection with infectious myonecrosis virus
- Infection with *Perkinsus marinus*
- Infection with *Perkinsus olseni*

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- Infection with tilapia lake virus
 - Infection with *Xenohaliotis californiensis*.

The Commission invites applications from Members with appropriate expertise in these diseases.

12. Any other business

12.1. Registration of Diagnostic Kits

The Aquatic Animals Commission was updated on the status of work to reshape the WOAHA procedure of registration of diagnostic kits. The Commission was informed that WOAHA held a meeting with two main stakeholders, Health for Animals and Diagnostic for Animals on 7 June 2023, exploring the possibility of establishing mechanisms that could facilitate regulatory harmonization of diagnostic kits. A joint meeting is planned with the above-mentioned stakeholders and a selection of key national Competent Authorities that represent all geographic regions. The Commission will be informed of future progress at their next meeting.

The Commission noted that the SRDK will need to consider processes for those aquatic diagnostic kits that are already registered and which may need to submit a new dossier and evaluation in order to be renewed.

.../Annexes

Annex 1. Item 2. – Adopted Agenda

MEETING OF THE WOA AQUATIC ANIMAL HEALTH STANDARDS COMMISSION

13 to 20 September 2023

1. Welcome from the Deputy Director General
2. Adoption of the agenda
3. Meeting with the Director General
4. Cooperation with Terrestrial Animal Health Standards Commission
 - 4.1. Alignment between the *Terrestrial Code* and *Aquatic Code*
 - 4.1.1. Consideration of new items for inclusion in AAHSC work plan & TAHSC work program, prioritisation (criteria and methods), documentation of the process
 - 4.1.2. Glossary definitions: 'Competent Authority', 'Veterinary Authority' and 'Aquatic Animal Health Services' – review usage (based on Member comments)
 - 4.1.3. Framework for the TC disease specific chapters
 - 4.2. Synergies and area of common interest
 - 4.2.1. TAHSC: Plan to revise User's guide
 - 4.2.2. TAHSC: Plan to progress work on Section 5: Revision of Chapters 5.4. to 5.7.
 - 4.2.3. AAHSC: Plan to progress work on Chapter 4.3. Application of Compartmentalisation
 - 4.2.4. AAHSC: New chapters 4.X. Emergency disease preparedness and 4.Y. Disease outbreak management
 - 4.2.5. TAHSC: Plan to progress work on Section 4: Emergency preparedness
 - 4.2.6. TAHSC: Plan to progress work on Section 4: Biosecurity
 - 4.2.7. TAHSC: Plan to progress work on the revised Chapter 6.10. Responsible and prudent use of antimicrobial agents in veterinary medicine
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. Focal points participation
 - 6.3. Plan to review the science of aquatic animal welfare
7. *Aquatic Code*
 - 7.1. Items for Member comment
 - 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 WOA
 - 7.1.4.1. Infection with infectious spleen and kidney necrosis virus
 - 7.1.5. Chapter 4.3. Application of compartmentalisation – discussion paper
 - 7.1.6. New Chapter 4.X. Emergency disease preparedness
 - 7.1.7. New Chapter 4.Y. Disease outbreak management

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- 7.1.8. New Chapter 4.Z. Control of pathogenic agents in traded milt and fertilised eggs of fish
 - 7.1.9. New Chapter 5.X. Movement of ornamental aquatic animals
 - 7.1.10. Safe commodities – Articles X.X.3. for disease-specific chapters
 - 7.1.10.1. Revised Articles 8.X.3. for amphibian disease-specific chapters
 - 7.1.10.2. Revised Articles 9.X.3. for crustacean disease-specific chapters
 - 7.1.10.3. Revised Articles 10.X.3. for fish disease-specific chapters
 - 7.1.10.4. Revised Articles 11.X.3. for mollusc disease-specific chapters
 - 7.1.11. Model Articles X.X.5.-X.X.6. for disease-specific chapters
 - 7.1.12. Assessment of default periods in Articles X.X.4.-X.X.8. for disease-specific chapters
 - 7.1.13. Article 9.10.2. of Chapter 9.10. Infection with decapod iridescent virus 1
 - 7.1.14. Article 10.6.2. of Chapter 10.6. Infection with infectious haematopoietic necrosis virus
 - 7.1.15. Article 10.11.2. of Chapter 10.11. Infection with tilapia lake virus
 - 7.1.16. Articles 11.5.1. and 11.5.2. of Chapter 11.5. Infection with *Perkinsus marinus*
 - 7.2. Items for consideration
 - 7.2.1. Consideration of emerging diseases
 - 7.2.1.1. Covert mortality nodavirus (CMNV) in zebrafish
 - 7.2.1.2. Infection with *Enterocytozoon hepatopenaei*
 - 7.2.1.3. Other diseases
 - 8. *Aquatic Manual*
 - 8.1. Items for Member comment
 - 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 2.3. Diseases of fish
 - 8.1.2.1. Chapter 2.3.9. Infection with spring viraemia of carp virus
 - 8.1.3. Section 2.4. Diseases of molluscs
 - 8.1.3.1. Chapter 2.4.0. General information: diseases of molluscs
 - 8.1.3.2. Chapter 2.4.1. Infection with abalone herpesvirus
 - 8.1.3.3. Chapter 2.4.4. Infection with *Marteilia refringens*
 - 8.1.3.4. Section 2.2.1. and 2.2.2. of Chapter 2.4.5. Infection with *Perkinsus marinus*
 - 8.2. Items for consideration
 - 8.2.1. 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
 - 8.2.2. Inclusion of videos on diagnostic techniques in the *Aquatic Manual*
 - 9. *Ad hoc* groups
 - 9.1. Report of the *ad hoc* Group on Susceptibility of mollusc species to infection with WOAHA listed diseases
 - 9.2. Report of the *ad hoc* Group on Susceptibility of fish species to infection with WOAHA listed diseases
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- 9.3. Report of the *ad hoc* Group on Susceptibility of crustacean species to infection with WOAHA 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. Review of the annual reports of activities in 2022 of the WOAHA Reference Centres; determine what outputs could be derived from the data collected
 - 10.3. Questionnaire to Reference Laboratories for aquatic animal diseases
 - 10.4. Review of the main focus areas and specialties for Collaborating Centres
 - 10.5. Develop a list of WOAHA-approved reference reagents for aquatic animal diagnostic diseases
 11. Other issues
 - 11.1. For discussion
 - 11.1.1. Registration of Diagnostic Kits
 - 11.1.1.1. Genics : Shrimp MultiPath™ (SMP)- Assessment Report and Review Panel Final Report (on the resubmitted dossier 2022)
 - 11.1.1.2. Update on the implementation of the New Concept of Registration of the Diagnostic Kits
 - 11.1.1.3. Decision on the renewals of the already registered aquatic diagnostic kits
 - 11.2. For information
 - 11.2.1. Transparency of the WOAHA process for the elaboration of Standards
 - 11.2.2. WOAHA standards online navigation tool
 - 11.2.3. Observatory
 - 11.2.4. Editorial Board of the Scientific and Technical Review
 12. Meeting review
 13. Next meeting: 14-21 February 2024

Annex 2. Item 2. – List of Participants

MEETING OF THE AQUATIC ANIMAL HEALTH STANDARDS COMMISSION

13 to 20 September 2023

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 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 Fiona Geoghegan
(Vice-President)
Legislative Officer,
European Commission,
DG SANTE
Brussels,
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Specialist Scientist,
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and the Environment,
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Annex 3. Item 4. – Work plan and priorities

WORK PLAN FOR THE AQUATIC ANIMALS COMMISSION
(including provisional timelines for commenting and adoption)

| <i>Aquatic Code</i> | | | |
|--|---|--|----------------------|
| Chapter/Subject | Status | | |
| | September 2023 | February 2024 | May GS 2024 |
| Monitor emerging diseases and consider any required actions | On-going | | |
| Glossary definitions: ‘Competent Authority’, ‘Veterinary Authority’ and ‘Aquatic Animal Health Services’ | Review comments (1st round) | Review comments (2nd round) | Propose for adoption |
| Glossary definitions: ‘aquatic animal products’ | Reviewed usage in the <i>Aquatic Code</i> and present amendments for comments | Review comments (1st round) | Propose for adoption |
| Chapter 1.3. Diseases listed by WOA – Listing of infection with infectious spleen and kidney necrosis virus species | Review comments (2nd round) | 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 (1st round) | Review comments (2nd round) | Propose for adoption |
| Chapter 4.3. Application of Compartmentalisation | Present discussion paper for comment | Review responses to discussion paper | |
| Draft new Chapter 4.X. Emergency disease preparedness | Present draft chapter for comment | Review comments (1 st round) | |
| Draft new Chapter 4.Y. Disease outbreak management | Present draft chapter for comment | Review comments (1 st round) | |
| Draft new Chapter 4.Z. Control of pathogenic agents in traded milt and fertilised eggs of fish | Present draft chapter for comment | Review comments (1 st round) | |
| Chapters 5.6. to 5.9. (Chapters 5.4. to 5.7. in <i>Terrestrial Code</i>) | – | Review TCC <i>ad hoc</i> Group report and review Chapters 5.4. and 5.6. of <i>Terrestrial Code</i> out for comment | |
| Draft new Chapter 5.X. Movement of ornamental aquatic animals | Present draft chapter for comment | Review comments (1st round) | |
| Chapter 6.2. Principals for responsible and prudent use of the antimicrobial agents in aquatic animal | Update from TCC on the on-going revision to <i>Terrestrial Code</i> Chapter 6.10. | Update from TCC on the on-going revision to <i>Terrestrial Code</i> Chapter 6.10. | |
| Susceptible Species Assessment of new evidence for previously assessed diseases (as necessary) | On-going | | |

| Aquatic Code | | | |
|---|---|---|--|
| Chapter/Subject | Status | | |
| | September 2023 | February 2024 | May GS 2024 |
| Safe commodities Articles 8.X.3. – Amphibian | Review comments (2nd round) | Review comments (3rd round) | Present for adoption |
| Safe commodities Articles 9.X.3. – Crustacean | Review comments (1st round) | Review comments (2nd round) | Present for adoption |
| Safe commodities Articles 10.X.3. – Fish | Review comments (1st round) | Review comments (2nd round) | Present for adoption |
| Safe commodities Articles 11.X.3. – Mollusc | Review comments (2nd round) | Review comments (3rd round) | Present for adoption |
| Assessment of default periods in Articles X.X.4.-X.X.8. for disease-specific chapters | – | Present assessment of default periods for comment | |
| Model Articles X.X.5. and X.X.6. for disease-specific chapters | Present amendments for comments | 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 <i>Aphanomyces astaci</i> (Crayfish plague) | DIV1: Review <i>ad hoc</i> Group report and present amended articles for comment | DIV1: Review comments (1st round) | DIV1: Present for adoption |
| | – | WSSV: Review <i>ad hoc</i> Group report and present amended articles for comment | |
| Article 10.6.2. of Chapter 10.6. Infection with infectious haematopoietic necrosis virus | Present amendments for comments | 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 <i>Aphanomyces invadans</i> (Epizootic ulcerative syndrome) | TiLV: Review <i>ad hoc</i> Group report and present amended articles for comment | TiLV: Review comments (1st round) | TiLV: Present for adoption |
| | – | EUS: Review interim <i>ad hoc</i> Group report | |
| Susceptible species – Mollusc diseases – Articles 11.X.1. and 11.X.2. for: – Infection with <i>Perkinsus marinus</i> – Infection with <i>Perkinsus olseni</i> – Infection with <i>Xenohaliotis californiensis</i> | <i>Perkinsus marinus</i> : Review comments (1st round) | <i>Perkinsus marinus</i> : Review comments (2nd round) | <i>Perkinsus marinus</i> : Present for adoption |
| | <i>Perkinsus olseni</i> : Review interim <i>ad hoc</i> Group report | <i>Perkinsus olseni</i> : Review <i>ad hoc</i> Group report and present amended articles for comment | |

| Aquatic Manual | | | |
|--|--|--|----------------------|
| Chapter/Subject | Status | | |
| | September 2023 | February 2024 | May GS 2024 |
| Chapter 1.1.1. Quality management in veterinary testing laboratories | Receive update from BSC | Provide comments to BSC | |
| Chapter 1.1.2. Validation of diagnostic assays for infectious diseases of aquatic animals | – | Review first draft | |
| Chapter 2.2.0. General provisions – Crustaceans | Review comments (2nd round) | Review comments (3rd round) | Propose for adoption |
| Chapter 2.2.2. Infection with <i>Aphanomyces astaci</i> (Crayfish plague) | Review comments (2nd round) | Review comments (3rd round) | Propose for adoption |
| Chapter 2.2.6. Infection with <i>Macrobrachium rosenbergii nodavirus</i> (white tail disease) | Review comments (1st round) | Review comments (2nd round) | Propose for adoption |
| Chapter 2.2.9. Infection with yellow head virus genotype 1 | Review comments (1st round) | Review comments (2nd round) | Propose for adoption |
| Chapter 2.2.X. Infection with decapod iridescent virus 1 | Review updated draft and present for Member comments | Review comments (1st round) | |
| Chapter 2.3.9. Infection with spring viraemia of carp virus | – | Review validation or publication of real-time PCR | |
| Chapter 2.3.X. Infection with tilapia lake virus | – | Review updated draft and present for Member comments | |
| Chapter 2.4.0. General Information | Review updated draft and present for comments | Review comments (1st round) | |
| Chapter 2.4.1. Infection with abalone herpes virus | Review updated draft and present for comments | Review comments (1st round) | |
| Chapter 2.4.4. Infection with <i>Marteilia refringens</i> | Review updated draft and present for comments | Review comments (1st round) | |
| Chapter 2.4.3. Infection with <i>Bonamia ostreae</i> | – | Review updated draft and present for comments | |
| Sections 2.2.1. and 2.2.2. of Chapter 2.4.5. Infection with <i>Perkinsus marinus</i> | Review comments (1st round) | Review comments (2nd round) | Propose for adoption |

Annex 4. Item 6.1. – Glossary definitions: ‘Aquatic Animal Health Services’, ‘Competent Authority’ and ‘Veterinary Authority’

| 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 <u>Aquatic Animal Health Services and the Competent Authorities</u> 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 <u>relevant Aquatic Animal Health Services’ Competent Authority’s</u> ethical approach to the provision of international health certificates and <u>the Veterinary Authority’s</u> history in meeting their notification obligations. |
| Glossary | NOTIFICATION means the procedure by which: a) the Competent Authority <u>Veterinary Authority</u> informs the <i>Headquarters</i> , b) the <i>Headquarters</i> inform Competent Authority <u>the Veterinary Authority</u> of Member Countries of the occurrence of a <i>disease</i> in accordance with the provisions of Chapter 1.1. |
| Article 1.1.1. | For the purposes of the <i>Aquatic Code</i> 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 <i>Headquarters</i> to communicate directly with the Competent Authority <u>Veterinary Authority</u> of its <i>territory or territories</i> . All <i>notifications</i> and all information sent by WOAHP to the Competent Authority <u>Veterinary Authority</u> shall be regarded as having been sent to the country concerned and all <i>notifications</i> and all information sent to WOAHP by the Competent Authority <u>Veterinary Authority</u> shall be regarded as having been sent by the country concerned. |
| Article 1.1.3. paragraph 1 | The Competent Authority <u>Veterinary Authority</u> shall, under the responsibility of the Delegate, send to the <i>Headquarters</i> : |
| Article 1.1.4. paragraph 1 | Competent Authority <u>Veterinary Authority</u> shall, under the responsibility of the Delegate, send to the <i>Headquarters</i> : |
| Article 1.1.5. point 1 | The Competent Authority <u>Veterinary Authority</u> of a country in which an <i>infected zone</i> or <i>compartment</i> is located shall inform the <i>Headquarters</i> when this country, <i>zone</i> or <i>compartment</i> becomes free from the <i>disease</i> . |
| Article 1.1.5. point 3 | The Competent Authority <u>Veterinary Authority</u> of a Member Country which establishes one or several <i>free zones</i> or <i>free compartments</i> shall inform the <i>Headquarters</i> , 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 <i>zones</i> or <i>compartments</i> 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 <i>international aquatic animal health certificates</i> . |
| Article 3.1.2. point 10 | <u>Information, complaints and appeals</u> The relevant Competent Authority Aquatic Animal Health Services should undertake to reply to requests from Aquatic Animal Health Services <u>the Competent Authority</u> 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 <u>the Competent Authority</u> Aquatic Animal Health Services . |
| Article 3.1.5. paragraph 4 | The expert(s) facilitate(s) the evaluation of the <i>Aquatic Animal Health Services</i> of the Member Country using the WOAHP 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 <u>Aquatic Animal Health Services</u> of the Member Country. |
| Article 3.2.1. paragraph 2 | The recognition of communication as a discipline of the <i>Aquatic Animal Health Services</i> 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 <i>zone</i> should be established by the Aquatic Animal Health Service <u>Competent Authority</u> on the basis of the definition of zone and made public through official channels. |
| Article 4.2.3. point 3 | The factors defining a <i>compartment</i> should be established by the Aquatic Animal Health Service <u>Competent Authority</u> on the basis of relevant criteria such as management and husbandry practices related to <i>biosecurity</i> , and made public through official channels. |
| Article 4.2.3. point 6 | For a <i>compartment</i> , the <i>biosecurity plan</i> should describe the partnership between <u>among</u> the relevant enterprise/industry, <u>and the Aquatic Animal Health Service Competent Authority and the Aquatic Animal Health Services</u> , and their respective responsibilities, including the procedures for oversight of the operation of the <i>compartment</i> by the Aquatic Animal Health Service <u>Competent Authority</u> . |
| Article 5.3.4. point 2(a) | infrastructure including the legislative base (e.g. <i>aquatic animal health law</i>) and administrative systems (e.g. organisation of Veterinary Services or Aquatic Animal Health Services <u>Competent Authority</u>); |
| Article 5.3.7. point 1(d)(i) | an evaluation of the <i>exporting country's</i> Veterinary Services or Aquatic Animal Health Services ; |
| Article 5.3.7. point 2(e)(i) | an evaluation of the <i>exporting country's</i> Veterinary Services or Aquatic Animal Health Services ; |

Annex 5. Item 6.2. – Glossary definitions: ‘Aquatic Animal Products’

| 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 <u>aquatic animal products</u> and as a tool for disease management. |
| 5.9.2. point 2 | An <i>importing country</i> may require sufficient advance notification regarding the proposed date of entry into its <i>territory</i> of a consignment of products of aquatic animal origin <u>aquatic animal products</u> destined for human consumption, together with information on the nature, quantity and packaging of the products, as well as the name of the <i>frontier</i> 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 <u>aquatic animal products</u> |
| 5.11.1. Box I.9. | For products of aquatic animal origin <u>aquatic animal products</u> : 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 <u>aquatic animal products</u> that have to be further processed before being suitable for end use. |
| 5.11.1. Box I.24. | For products of aquatic animal origin <u>aquatic animal products</u> : 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 <u>aquatic animal products</u> |

CHAPTER 1.1.

NOTIFICATION OF DISEASES, AND PROVISION OF
EPIDEMIOLOGICAL INFORMATION

[...]

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 WOAHA 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 7. Item 6.4. – Assessment of infection with all genogroup of the virus species infectious spleen and kidney necrosis virus for listing in the *Aquatic Code*

ASSESSMENT OF INFECTION WITH ALL GENOGROUPS OF THE VIRUS SPECIES INFECTIOUS SPLEEN AND KIDNEY NECROSIS VIRUS (ISKNV) FOR LISTING IN THE WOAHP AQUATIC ANIMAL HEALTH CODE

Assessment summary

1. The Aquatic Animal Health Standards Commission assessed the virus species *Infectious spleen and kidney necrosis virus*, including its three genogroups red sea bream iridovirus (RSIV), infectious spleen and kidney necrosis virus (ISKNV), and turbot reddish body iridovirus (TRBIV) against the criteria for listing aquatic animal diseases in Article 1.2.2. of the *Aquatic Code*.
2. The Aquatic Animals Commission agreed that the RSIV genogroup (currently listed in the *Aquatic Code*), as well as the two genogroups ISKNV and TRBIV meet the listing criteria 1, 2, 3, and 4b (see Table 1 below).
3. The Aquatic Animals Commission noted that the three genogroups have overlapping susceptible species, similar epidemiology, and similar diagnostic methods. The Commission agreed that the proposed listed disease should be named “infection with all genogroups of the virus species ISKNV”. Infection with all genogroups of the virus species ISKNV would be defined to include the genogroups ISKNV, RSIV and TRBIV but would exclude the other recognized species of *Megalocytivirus*, *Scale drop disease virus*.

| | Listing criteria | | | | | | Conclusion |
|--|------------------|---|---|----|----|----|---|
| | 1 | 2 | 3 | 4a | 4b | 4c | |
| Infection with all genogroups of the viral species ISKNV | + | + | + | NA | + | - | The disease meets the criteria for listing. |

NA = not applicable.

Listing Criteria (Chapter 1.2. of the *Aquatic Code*)

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

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

AND

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

AND

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

AND

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

OR

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

OR

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

Background

Megalocytivirus is one of seven genera of the family *Iridoviridae* and is classified within the subfamily *Alphairidovirinae* together with the genera *Ranavirus* and *Lymphocystivirus* (Chinchar *et al.*, 2017; Chinchar *et al.*, 2020). Megalocytiviruses are distinguished from ranaviruses and lymphocystiviruses by their ability to trigger marked cell enlargement in infected tissues and by sequence analysis of key viral genes (Chinchar *et al.*, 2017). Megalocytiviruses are the aetiological agents of severe disease associated with high mortality in a range of marine and freshwater finfish species (Kurita & Nakajima, 2012; Hick *et al.*, 2016).

The ICTV recognises two species of *Megalocytivirus*: *Infectious spleen and kidney necrosis virus* (ISKNV) and *Scale drop disease virus* (SDDV) (Chinchar *et al.*, 2017). SDDV is genetically and epidemiologically distinct from the species ISKNV and is not considered further in this assessment.

Within the species ISKNV, three genogroups have been recognised: ISKNV, RSIV and TRBIV (Song *et al.*, 2008). However, it remains to be resolved whether these genogroups represent distinct species, or strains of a single species (Chinchar *et al.*, 2017). Megalocytiviruses have been given numerous unique names based on the species that they were detected in; however, all variants of the species ISKNV that have had their genomes analysed are placed within the three genogroups: ISKNV, RSIV and TRBIV (Chinchar *et al.*, 2017). Each genogroup is further subdivided into two clades (Koda *et al.* 2018, 2019, 2023; Fusianto *et al.* 2023).

The name ISKNV is used for one of two recognised species of *Megalocytivirus* and also for one of the three genogroups within the ISKNV species. When used within this document, “ISKNV genogroup” refers to the genogroup ISKNV and “the species ISKNV” is used whenever referring to the species ISKNV.

Infection with red sea bream iridovirus (RSIV) was first listed by WOAHA in the 2003 *Aquatic Animal Health Code*¹ and remains listed in the 2023 *Aquatic Code*. Disease caused by RSIV was first detected in cultured red sea bream (*Pagrus major*) in Japan in 1990 (Inouye *et al.*, 1992). RSIV has been detected principally from marine fish. Species currently listed as susceptible to infection with RSIV in the WOAHA *Aquatic Code* include:² red sea bream (*Pagrus major*), yellowtail (*Seriola quinqueradiata*), amberjack (*Seriola dumerilii*), sea bass (*Lateolabrax* sp.), Asian sea bass (*Lates calcarifer*), albacore (*Thunnus thynnus*), Japanese parrotfish (*Oplegnathus fasciatus*), striped jack (*Caranx delicatissimus*), mandarin fish (*Siniperca chuatsi*), red drum (*Sciaenops ocellatus*), mullet (*Mugil cephalus*) and groupers (*Epinephelus* spp.).

The ISKNV genogroup is not currently listed in the WOAHA *Aquatic Code*. Virions morphologically consistent with iridoviruses and presenting enlarged cells with inclusion bodies consistent with megalocytiviruses have been reported in species of freshwater fish since the late 1980s and 1990s (e.g. Armstrong & Ferguson, 1989; Anderson *et al.*, 1993). ISKNV genogroup has been detected in archival ornamental fish samples from as early as 1996 (Go *et al.*, 2006; Go *et al.*, 2016; Becker *et al.*, 2022). Infectious spleen and kidney necrosis disease was described from mandarin fish (*Siniperca chuatsi*; He *et al.*, 2000; He *et al.*, 2002) and in 2001 the genome of ISKNV genogroup was analysed and found to be genetically similar to RSIV (He *et al.*, 2001). ISKNV genogroup has been detected from numerous freshwater fish species, including many associated with ornamental fish trade (see review by Johan & Zainathan, 2020; Becker *et al.*, 2022). This genogroup has been reported from numerous species of ornamental fish that have been traded internationally (see Rimmer *et al.*, 2015). ISKNV genogroup has also been reported as a cause of mass mortality in species important for human consumption (e.g. Subramaniam *et al.*, 2016; Ramírez-Paredes *et al.*, 2020; Fusianto *et al.*, 2021).

The TRBIV genogroup is not currently listed in the WOAHA *Aquatic Code*. TRBIV was first described as causing disease in turbot, *Scophthalmus maximus* (Shi *et al.*, 2004). TRBIV has been known to principally cause disease in flatfishes in China and Korea (e.g. Shi *et al.*, 2004; Do *et al.*, 2005) but it has also been detected in other species including in the ornamental fish trade (Go *et al.*, 2016; Koda *et al.*, 2018). TRBIV has also caused disease in other economically important farmed fish species such as Asian sea perch (*Lates calcarifer*) (Tsai *et al.*, 2020) and barred knifejaw (*Oplegnathus fasciatus*) (Huang *et al.*, 2011).

The Aquatic Animals Commission previously proposed an approach to differentiating pathogen strains (refer to the Commission’s [February](#) and [October 2011](#) meeting reports). Three main criteria were considered for the applicability of pathogen strain differentiation in the standards of the *Aquatic Code* and *Aquatic Manual*: 1) variants of the pathogen are clearly recognized in the scientific literature and have different disease characteristics; 2) there are robust methods for consistently differentiating the variants; and 3) there is, or there is potential for, different management of variants within or between countries. In the case of the species ISKNV, RSIV was listed prior to research that defined the 3 genogroups

¹ RSIV was included in the *Aquatic Code* prior to 2003 as an “other disease of significance”.

² Note that the species listed as susceptible to infection with RSIV in accordance with Chapter 1.5. of the *Aquatic Code* has not been revised based on the recommendations of the *ad hoc* Group.

within the species ISKNV, and the genetic and epidemiological relationships among them. Given the precedent of infection with RSIV having been listed, but not the ISKNV and TRBIV genogroups, this assessment presents information for each of these three genogroups, despite the three genogroups, being proposed for listing collectively as the species ISKNV.

Assessment against listing criteria

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

Assessment

The species ISKNV can be transmitted horizontally via water and is known to remain viable in frozen host tissues. The likelihood of transmission is expected to be greater for trade in live fish but is also possible in aquatic animal products, particularly if not eviscerated.

Numerous marine and freshwater species are susceptible to the species ISKNV and are traded internationally, either as live aquatic animals (for human consumption, aquaculture or for ornamental purposes) or as aquatic animal products.

RSIV has been detected in several countries in Asia where it has been associated with disease in species of farmed marine fish (Kurita & Nakajima, 2012). Some susceptible species are traded live for human consumption (e.g. red sea bream, groupers), others are traded as aquatic animal products.

ISKNV genogroup has been detected in numerous species traded as ornamental fish and the ornamental fish trade has been implicated in disease spread and outbreaks (e.g. Jeong *et al.*, 2008; Johan & Zainathan, 2020). Infected ornamental fish may not present clinical signs (e.g. Subramaniam *et al.*, 2014; Rimmer *et al.*, 2015) and as such may act as carriers of the virus. ISKNV genogroup has also been detected in important farmed species for human consumption that are traded internationally, such as tilapia (Ramírez-Paredes *et al.*, 2020). ISKNV genogroup has also been detected in unprocessed fish used for aquaculture feed (Lajimin *et al.*, 2015) suggesting that fish traded for aquaculture feed or bait may present a pathway. Transmission from freshwater finfish species to marine finfish species has been demonstrated by direct inoculation and cohabitation (Jeong *et al.*, 2008b; Go & Whittington, 2019).

TRBIV is known to occur in several species that are important for international trade (e.g. turbot, flounder, Asian sea bass), including live trade or as aquatic animal products. Phylogenetic analysis indicates that there has been recent international spread of TRBIV (Tsai *et al.*, 2020).

Variants of the species ISKNV have been detected in numerous species of marine and freshwater species that are traded internationally. Each of the three genogroups has been detected in traded commodities and there is evidence of international spread associated with trade.

Conclusion

The criterion is met.

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

Assessment

Infection with RSIV has been notifiable to the WOAHA since 2003. Several countries continue to report that RSIV has never been reported from their territory (refer to WOAHA World Animal Health Information System) and it is likely that some of these countries could demonstrate country freedom.

ISKNV genogroup has been reported from numerous fish species traded through the ornamental fish trade and it is likely that this genogroup is widespread through ornamental fish supply chains. However, some countries maintain *basic*

*biosecurity measures*³ for ISKNV genogroup and may be able to demonstrate freedom. Further, PCR assays used in surveillance for RSIV would also detect ISKNV genogroup, providing evidence of freedom from ISKNV genogroup.

TRBIV has been primarily detected in farmed flatfish from China and Korea but has also been detected in ornamental fish and in farmed Asian sea bass. PCR assays recommended in the WOA *Aquatic Manual* chapter for RSIV may not be inclusive of TRBIV resulting in a lower confidence in the distribution of TRBIV. However, given TRBIV has demonstrated pathogenicity in farmed populations of several species, it is likely that TRBIV would be detected in those species if it had occurred. Although there is less certainty regarding the distribution of TRBIV, it seems likely that at least one country could claim freedom at the level of country or zone.

Conclusion

The criterion is met.

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

Assessment

Case definitions for suspicion and confirmation of infection with RSIV are available in the WOA *Aquatic Manual*. As some PCR assays for RSIV (and some other methods, e.g. histopathology), are inclusive of ISKNV genogroup, the case definitions could be easily adapted to include ISKNV genogroup. Kawato *et al.* (2021) compared the analytical performance of four real-time PCR methods for the detection of megalocytiviruses (excluding SDDV) and found that three of the four assays detected ISKNV, RSIV, and TRBIV genogroups. Kim *et al.* (2022) reported on the performance of a real-time PCR assay with inclusivity for RSIV, ISKNV and TRBIV genogroups. There are sufficient diagnostic tools available to detect the species ISKNV and to construct case definitions inclusive of the three genogroups.

Conclusion

Criterion is met.

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

Assessment

There is no evidence of transmission to humans.

Conclusion

Criterion not applicable.

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

Assessment

RSIV has caused mass mortalities in cultured fish populations. The disease was first detected in red sea bream in Japan with affected fish becoming lethargic, exhibiting severe anaemia, petechiae of the gills, and enlargement of the spleen (Inouye *et al.*, 1992; Jung *et al.*, 1997; Nakajima & Maeno, 1998). RSIV has been reported to cause production losses, morbidity and mortality in many other species (e.g. Chao *et al.*, 2004; Chen *et al.*, 2003; Girisha *et al.*, 2020; Ni *et al.*, 2021; Sumithra *et al.*, 2022).

ISKNV genogroup has been associated with numerous cases of disease in ornamental fish (see review by Johan & Zainathan, 2020; Becker *et al.*, 2022). ISKNV genogroup has also been associated with high mortalities in important species farmed for human consumption; for example, in Asian sea bass (Dong *et al.*, 2017; Kerddee *et al.*, 2021), Tilapia

³ Basic Biosecurity conditions are defined in article 1.4.6. of the *Aquatic Code* and include requirements for an early detection system (as described in Article 1.4.7.) and measures to prevent the introduction of the pathogenic agent.

(e.g. Figueiredo *et al.*, 2021; Ramírez-Paredes *et al.*, 2021) and groupers (e.g. Chao *et al.*, 2004; Huang *et al.*, 2020; Fusianto *et al.*, 2021).

TRBIV has caused disease and high mortality in turbot aquaculture in China (e.g. Shi *et al.*, 2010). Mortalities of up to 90% have occurred in Asian sea bass farms in Taiwan (Tsai *et al.*, 2020).

Conclusion

Criterion is met.

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

Assessment

There is limited information on the occurrence of RSIV, ISKNV or TRBIV genogroups in wild fish populations and their consequences such as morbidity, mortality or ecological impacts. ISKNV genogroup has been reported as the cause of a mass mortality event in a population of wild cichlids in India (Swaminathan *et al.*, 2022), but has also been detected in many apparently healthy wild fish from a diverse range of fish species (Wang *et al.*, 2007).

Conclusion

Criterion is not met.

References

ARMSTRONG, R. & FERGUSON, H. (1989). Systemic viral disease of the chromide cichlid *Etilopis maculatus*. *Diseases of Aquatic Organisms*, **7**, 155-157.

ANDERSON, I.G., PRIOR, H.C., RODWELL, B.J. & HARRIS, G.O. (1993). Iridovirus-like virions in imported dwarf gourami (*Colisa lalia*) with systemic amoebiasis. *Australian Veterinary Journal*, **70**(2), 66-67.

BECKER, J.A., FUSIANTO, C., HICK, P.M. (2022). Infection with Megalocytivirus in Ornamental Fish. In: *Aquaculture Pathophysiology, Pharmacology and Toxicology* (F. Kibenge, R.S. Chong, B. Baldisserotto, eds), Elsevier. <https://doi.org/10.1016/B978-0-12-812211-2.00016-0>.

CHAO, C.B., CHEN, C.Y., LAI, Y.Y., LIN, C.S. & HUANG, H.T. (2004). Histological, ultrastructural, and in situ hybridization study on enlarged cells in the grouper *Epinephelus* hybrids infected with grouper iridovirus in Taiwan (TGIV). *Diseases of Aquatic Organisms*, **58**, 127–142.

CHEN, X.H., LIN, K.B. & WANG, X.W. (2003). Outbreaks of an iridovirus disease in maricultured large yellow croaker, *Larimichthys crocea* (Richardson), in China. *Journal of Fish Diseases*, **26**, 615-619.

CHINCHAR, V.R., HICK, P., INCE, I.A., JANCOVICH, J.K., MARSCHANG, R., QIN, Q., SUBRAMANIAM, K., WALTZEK, T.B., WHITTINGTON, R., WILLIAMS, T. & ZHANG, Q. (2017). ICTV Report Consortium ICTV Virus Taxonomy Profile: *Iridoviridae*. *Journal of General Virology*, **98**, 890–891.

CHINCHAR, V.G., HICK, P.H., HUANG, J., INCE, I.A., JANCOVICH, J.K., MARSCHANG, R., QIN, Q., SUBRAMANIAM, K., WALTZEK, T.B., WHITTINGTON, R., WILLIAMS, T. & ZHANG, Q. (2020) ICTV Virus Taxonomy Profile: *Iridoviridae*, *Journal of General Virology*, **98**, 890-891.

DO, J.W., CHA, S.J., KIM, J.S., AN, E.J., LEE, N.S., CHOI, H.J., LEE, C.H., PARK, M.S., KIM, J.W., KIM, Y.C. & PARK, J.W. (2005). Phylogenetic analysis of the major capsid protein gene of iridovirus isolates from cultured flounders *Paralichthys olivaceus* in Korea. *Diseases of Aquatic Organisms*, **64**, 193–200.

DONG, H.T., JITRAKORN, S., KAYANSAMRUJ, P., PIRARATE, N., RODKHUM, C., RATTANAROJPONG, T., SENAPIN, S., SAKSMERPROME, V. (2017). Infectious spleen and kidney necrosis disease (ISKND) outbreaks in farmed barramundi (*Lates calcarifer*) in Vietnam. *Fish & Shellfish Immunology*, **68**, 65-73.

-
- FIGUEIREDO, H.C.P., TAVARES, G.C., DORELLA, F.A., ROSA, J.C.C., MARCELINO, S.A.C., PIERZAN, F. & PEREIRA, F.L. (2022). First report of infectious spleen and kidney necrosis virus in Nile tilapia in Brazil. *Transboundary and Emerging Diseases*, 69(5), 3008-3015.
- FUSIANTO, C.K., BECKER, J.A., SUBRAMANIAM, K., WHITTINGTON, R.J., KODA, S.A., WALTZEK, T.B., MURWANTOKO & HICK, P.M. (2023). Genotypic characterization of Infectious spleen and kidney necrosis virus (ISKNV) in Southeast Asian aquaculture. *Transboundary and Emerging Diseases*, 3, 1-16.
- FUSIANTO, C., HICK, P.M., HERLAMBANG, A., WHITTINGTON, R.J. & BECKER, J.A. (2021). Outbreak investigation attributes Infectious spleen and kidney necrosis virus as a necessary cause of a mortality epidemic in farmed grouper (*Epinephelus* spp.) in Bali, Indonesia. *Aquaculture Reports* 20, 100723.
- GIRISHA, S.K., PUNEETH, T.G., NITHIN, M.S., NAVEEN KUMAR, B.T., AJAY, S.K., VINAY, T.N. & RAMESH, K.S. (2020). Red sea bream iridovirus disease (RSIVD) outbreak in Asian seabass (*Lates calcarifer*) cultured in open estuarine cages along the west coast of India: first report. *Aquaculture*, 520, 734712.
- GO, J., WALTZEK, T.B., SUBRAMANIAM, K., YUN, S.C., GROFF, J.M., ANDERSON, I.G., CHONG, R., SHIRLEY, I., SCHUH, J.C.L., HANDLINGER, J.H., TWEEDIE, A. & WHITTINGTON, R.J. (2016). Complete genome analysis of the mandarin fish infectious spleen and kidney necrosis iridovirus. *Virology*, 291, 126–139.
- GO, J. & WHITTINGTON, R. (2006). Experimental transmission and virulence of a megalocytivirus (Family Iridoviridae) of dwarf gourami (*Colisa lalia*) from Asia in Murray cod (*Maccullochella peelii peelii*) in Australia. *Aquaculture*, 258, 140-149.
- GO, J. & WHITTINGTON, R.J. (2019). Experimental transmission of Infectious Spleen and Kidney Necrosis Virus (ISKNV) from freshwater ornamental fish to silver sweep *Scorpiis lineolata*, an Australian marine fish. *Diseases of Aquatic Organisms*, 137(1), 1-21.
- HE, J.G., DENG, M., WENG, S.P., LI, Z., ZHOU, S.Y., LONG, Q.X., WANG, X.Z. & CHAN, S.M. (2001). Complete genome analysis of the mandarin fish infectious spleen and kidney necrosis iridovirus. *Virology*, 291, 126–139, doi: 10.1006/viro.2001.1208.
- HE, J.G., ZENG, K., WENG, S.P. & CHAN, S.M. (2000). Systemic disease caused by an iridovirus-like agent in cultured mandarin fish *Siniperca chuatsi* (Basillewsky), in China, *Journal of Fish Diseases*, 23, 219–222.
- HE, J.G., ZENG, K., WENG, S.P. & CHAN, S.M. (2002), Experimental transmission, pathogenicity and physical-chemical properties of infectious spleen and kidney necrosis virus (ISKNV), *Aquaculture*, 204, 11–24. doi: 10.1016/S0044-8486(01)00639-1.
- HICK, P.M., BECKER, J.A. & WHITTINGTON, R.J. (2016). Iridoviruses of fish In: *Aquaculture Virology* (F. Kibenge and M. Godoy, eds), Elsevier, London, UK, 127-152.
- HUANG, S.M., TU, C., TSENG, C.H., HUANG, C.C., CHOU, C.C., KUO, H.C. & CHANG, S.K. (2011). Genetic analysis of fish iridoviruses isolated in Taiwan during 2001-2009. *Archives of Virology*, 156, 1505-1515.
- HUANG, Y., CAI, S., JIAN, J., LUI, G., & XU, L. (2020). Co-infection of infectious spleen and kidney necrosis virus and *Francisella* sp. in farmed pearl gentian grouper (♀*Epinephelus fuscoguttatus* × ♂*E. lanceolatus*) in China — A case report. *Aquaculture*, 526, 735409.
- INOUE, K., YAMANO, K., MAENO, Y., NAKAJIMA, K., MATSUOKA, M., WADA, Y. & SORIMACHI, M. (1992). Iridovirus infection of cultured red sea bream, *Pagrus major*, *Fish Pathology*, 27, 19–27.
- JEONG, J.B., KIM, H.Y., JUN, L.J., LYU, J.H., PARK, N.G., KIM, J.K. & JEONG, H.D. (2008a). Outbreaks and risks of infectious spleen and kidney necrosis virus diseases in freshwater ornamental fishes, *Diseases of Aquatic Organisms*, 78, 209–215. doi: 10.3354/dao01879.
- JEONG, J., CHO, H., JUN, L., HONG, S., CHUNG, J. & JEONG, H. (2008b). Transmission of Iridovirus from freshwater ornamental fish (pearl gourami) to marine (rock bream). *Diseases of Aquatic Organisms*, 82(1), 27-36.
- JOHAN, C.A.C. & ZAINATHAN, S.C. (2020). Megalocytiviruses in ornamental fish: A review. *Veterinary World*, 13, 2565–2577.
-

-
- JUNG, S.J., OH, M.J. (2000). Iridovirus-like infection associated with high mortalities of striped beakperch, *Oplegnathus fasciatus* (Temminck et Schlegel), in southern coastal areas of the Korean peninsula, *Journal of Fish Diseases*, **23**, 223–226. doi: 10.1046/j.1365-2761.2000.00212.x.
- KERDDEE, P., DINH-HUNG, N., THANH DONG, H., HIRONO, I., SOONTARA, C., AREECHON, N., SRISAPOOME, P. & KAYANSAMRUJ, P. (2021). Molecular evidence for homologous strains of infectious spleen and kidney necrosis virus (ISKNV) genotype I infecting inland freshwater cultured Asian sea bass (*Lates calcarifer*) in Thailand. *Archives of Virology*, **166**, 3061–3074.
- KIM, K.H., CHOI, K.M., KANG, G., WOO, W.S., SOHN, M.Y., SON, H.J., YUN, D., KIM, D.H. & PARK, C.I. (2022). Development and Validation of a Quantitative Polymerase Chain Reaction Assay for the Detection of Red Sea Bream Iridovirus. *Fishes*, **7**, 236. <https://doi.org/10.3390/fishes7050236>
- KODA, S.A., SUBRAMANIAM, K., HICK, P.M., HALL, E., WALTZEK, T.B. & BECKER, J.A. (2023). Partial validation of a TaqMan quantitative polymerase chain reaction for the detection of the three genotypes of *Infectious spleen and kidney necrosis virus*. *PLoS ONE*, **18**(2):e0281292.
- KODA, S.A., SUBRAMANIAM, K., POUDEY, D.B., YANONG, R.P. & WALTZEK, T.B. (2019). Phylogenomic characterization of red seabream iridovirus from Florida pompano *Trachinotus carolinus* maricultured in the Caribbean Sea. *Archives of Virology*, **164**, 1209-1212.
- KODA, S.A., SUBRAMANIAM, K., FLOYD-FRANCIS, R., YANONG, R.P., FRASCA, S., GROFF, J.M., POPOV, V.L., FRASER, W.A., YAN, A., MOHAN, S. & WALTZEK, T.B. (2018). Phylogenomic characterization of two novel members of the genus Megalocytivirus from archived ornamental fish samples. *Diseases of Aquatic Organisms*, **130**(1), 11-24.
- KURITA, J., NAKAJIMA, K., (2012), Megalocytiviruses, *Viruses*, **4**(4), 521-538.
- KAWATO, Y., CUMMINS, D.M., VALDETER, S., MOHR, P., ITO, T., MIZUNO, K., KAWAKAMI, H., WILLIAMS, L.M., CRANE, M.ST.J. & MOODY, N.J.G. (2021). Development of New Real-time PCR Assays for Detecting *Megalocytivirus* Across Multiple Genotypes. *Fish Pathology*, **56** (4), 177-186. doi.org/10.3147/jfsfp.56.177
- LAJIMIN, S., RAZAK, A.A., DENIL, D. J., RANSANGAN, J., ABDUL WAHID, M.E. & SADE, A. (2015). First detection of Megalocytivirus (*Iridoviridae*) in trash fish used for aquaculture feed in Sabah, Malaysia. *Int. J. of Aquatic Science*, **6**(1): 54-66.
- NAKAJIMA, K., MAENO, Y., HONDA, A., YOKOYAMA, K., TOORIYAMA, T. & MANABE, S. (1999). Effectiveness of a vaccine against red sea bream iridoviral disease in a field trial test, *Diseases of Aquatic Organisms*, **36**(1), 73-5.
- NI, S.Z., WANG, Y.J., HU, J. B., SHI, J., XU, Y., ZHOU, S.M., LI, J.J., HONG, B.H. & QIAN, D. (2021). Identification, histopathology, and phylogenetic analysis of an iridovirus from cultivated silver pomfret in Zhejiang Province, East China. *Aquaculture*, **530**, 735619.
- RAMÍREZ-PAREDES, J.G., PALEY, R.K., HUNT, W., FEIST, S.W., STONE, D.M., FIELD, T.R., HAYDON, D.J., ZIDDAH, P.A., NKANSA, M., GUILDER, J., GRAY, J., DUODU, S., PECKU, E.K., AWUNI, J.A., WALLIS, T.S. & VERNER-JEFFREYS, D.W. (2021). First detection of infectious spleen and kidney necrosis virus (ISKNV) associated with massive mortalities in farmed tilapia in Africa. *Transboundary Emerging Diseases*, **68**, 1550–1563. <https://doi.org/10.1111/tbed.13825>
- RIMMER A.E., BECKER J.A., TWEEDIE A., LINTERMANS M., LANDOS M. & WHITTINGTON R.J. (2015). Detection of dwarf gourami iridovirus (Infectious spleen and kidney necrosis virus) in populations of ornamental fish prior to and after importation into Australia, with the first evidence of infection in domestically farmed Platy (*Xiphophorus maculatus*). *Preventive Veterinary Medicine*, **122**, 181-194.
- SHI, C.Y., WANG, Y.G., YANG, S.L., HUANG, J. & WANG, Q.Y. (2004). The first report of an iridovirus-like agent infection in farmed turbot, *Scophthalmus maximus*, in China. *Aquaculture*, **236**, 11-15. <https://doi.org/10.1016/j.aquaculture.2003.11.007>
- SONG, J.-Y., KITAMURA, S.-I., JUNG, S.-J., MIYADAI, T., TANAKA, S., FUKUDA, Y., KIM, S.-R. & OH, M.-J. (2008). Genetic variation and geographic distribution of megalocytiviruses. *Journal of Microbiology*, **46**, 29-33.
-

-
- SUBRAMANIAM, K., SHARIFF, M., OMAR, A.R., HAIR-BEJO, M. & ONG, B.L. (2014). Detection and molecular characterisation of infectious spleen and kidney necrosis virus from major ornamental fish breeding states in peninsular Malaysia, *Journal of Fish Diseases*, **37**, 609–618, <https://doi.org/10.1111/jfd.12152>
- SUBRAMANIAM, K., GOTESMAN, M., SMITH, C.E., STECKLER, N.K., KELLEY, K.L., GROFF, J.M. & WALTZEK, T.B. (2016). *Megalocytyivirus* infection in cultured Nile tilapia *Oreochromis niloticus*. *Diseases of Aquatic Organisms*, **119**, 253–258. <https://doi.org/10.3354/dao02985>
- SUMITHRA, T.G., KRUPESHA SHARMA, S.R., NEELIMA, L., DHANUTHA, N.R., JOSHY, A., ANUSREE, V.N., GAYATHRI, S., RAGHU, R.K., PRAVEEN, N.D., THOMAS, S. & RAJESH, K.M. (2022). Red sea bream iridovirus infection in cage farmed Asian sea bass (*Lates calcarifer*): Insights into the pathology, epizootiology, and genetic diversity. *Aquaculture*, **548**, 737571. <https://doi.org/10.1016/j.aquaculture.2021.737571>
- SWAMINATHAN, T.R., JOHNY, T.K., NITHIANANTHAM, S.R., SUDHAGAR, A., PRADHAN, P.K., SULUMANE RAMACHANDRA, K.S., NAIR, R.R., & SOOD, N. (2022). A natural outbreak of infectious spleen and kidney necrosis virus (ISKNV) threatens wild pearlspot, *Etroplus suratensis* in Peechi Dam in the Western Ghats biodiversity hotspot, India. *Transboundary and Emerging Diseases*, **69(5)**, 1595-1605. <https://doi.org/10.1111/tbed.14494>
- TSAI, J.M., HUANG, S.L. & YANG, C.D. (2020). PCR Detection and Phylogenetic Analysis of *Megalocytyivirus* Isolates in Farmed Giant Sea Perch *Lates calcarifer* in Southern Taiwan. *Viruses*, **12(6)**, 681. <https://doi.org/10.3390/v12060681>
- WANG, Y.Q., LÜ, L., WENG, S.P., HUANG, J.N., CHAN, S.M. & HE, J.G. (2007). Molecular epidemiology and phylogenetic analysis of a marine fish infectious spleen and kidney necrosis viruslike (ISKNV-like) virus. *Archives of Virology*, **152**, 763–773.

CHAPTER 1.3.
DISEASES LISTED BY WOAH

[...]

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 HPRO infectious salmon anaemia virus
- Infection with infectious haematopoietic necrosis virus
- Infection with 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.

[...]

Revised standards for compartmentalisation in the *WOAH Aquatic Animal Health Code*

A discussion paper for Members' comment developed by the
WOAH Aquatic Animal Health Standards Commission
September 2023

Summary

This discussion paper provides a means to engage WOA members on issues relevant to the revision of *Aquatic Code* Chapter 4.3. Application of Compartmentalisation. 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. While compartmentalisation has particular relevance for aquatic animal diseases—because eradication is often not possible—it has not been adopted and recognised widely among member countries. Ultimately, revision of Chapter 4.3. aims to provide clarity on the requirements of compartments, improve acceptance and make private investment in it more attractive.

The discussion paper proposes a range of purposes for applying compartments (section 4), high-level principles to guide their application (section 6) and the concept of dependent and independent compartments (section 5). 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.

Section 7 provides an analysis of the existing articles of Chapter 4.3., and provides recommendations for the revision of existing articles and the development of new articles following the framework of principles proposed in section 6. A proposed article structure for the revised Chapter 4.3 is included at [Attachment 2](#).

Questions are included throughout the document to prompt responses from members on issues of particular importance to the direction of the chapter's revision. Member comments are invited in response to these questions and other matters relevant to the revision of Chapter 4.3. The questions are collated in [Attachment 3](#).

Following consideration of member comments, the commission will recirculate the discussion paper to members with a summary of member responses and consensus views. Together the discussion paper and member responses will set the direction for the revision of Chapter 4.3.

1. Introduction

Compartmentalisation provides an opportunity to trade aquatic animal commodities with a specific disease-free status from zones or countries not declared free from those diseases. The application of compartmentalisation for aquatic animal diseases is considered an important mechanism to enhance safe trade—this is because eradication of aquatic animal diseases is often not possible, limiting alternative approaches to trade disease-free commodities from areas where listed diseases occur.

Chapter 4.3. of the *Aquatic Code* sets out recommendations concerning the application of compartmentalisation. Despite the passage of time since the chapter was first adopted in 2010 (and most recently updated in 2016) the concept of compartmentalisation for aquatic animal diseases has failed to be widely adopted. There are likely to be a range of reasons for this; however, it is clear that one key factor is the differing conceptual understanding of compartmentalisation among users of Chapter 4.3.

This paper aims to engage WOA members on issues relevant to the revision of Chapter 4.3. such that the revised chapter will provide consistent and clear guidance on compartmentalisation. This discussion paper has been informed by member responses to a short questionnaire provided in the Commission's September 2022 meeting report. A summary of member responses to the questionnaire is included at [Attachment 1](#).

This paper also aims to explore and seek consensus on key conceptual issues relevant to compartmentalisation. For example, some member countries recognise two types of compartments: those that are dependent on the health status of the surrounding waters and those which are not. The potential for each type of compartment to engage in different types of trade (e.g. trade for human consumption versus trade for aquaculture) will be explored.

As the implementation of compartments may involve investment risk (i.e. a compartment must be established without certainty that desired market access will be granted), it is imperative that Competent Authorities, Aquatic Animal Health Services and the operators of aquaculture establishments have a common understanding of the requirements for establishing a free compartment guided by the standards of the *Aquatic Code*.

2. Objectives of the paper

The primary objective of this paper is to engage WOAHA members in issues relevant to the revision of Chapter 4.3. such that the revised chapter will provide consistent and clear guidance on compartmentalisation to facilitate trade from compartments declared free from WOAHA-listed diseases. Ultimately, revision of Chapter 4.3 aims to improve acceptance of compartmentalisation and make private investment in it more attractive.

In exploring issues relevant to revision of Chapter 4.3. this discussion paper aims to:

- Explore the conceptual understanding of what a compartment is and what its purpose is
- Draw on member's experiences with compartmentalisation to inform revision of the standards to provide maximum common benefit while supporting safe trade
- Develop consensus on key conceptual issues prior to the commencement of drafting of the revised chapter.

In addressing the objectives described above, several principles are proposed to achieve these objectives, including that the provisions of the revised chapter should:

- A. provide confidence among Members in the strength of self-declarations of compartment freedom in accordance with any proposed approaches in the *Aquatic Code*;
- B. articulate the variety of purposes for which compartmentalisation might be applied;
- C. provide risk management that is appropriate for different production system/product/pathway combinations;
- D. provide standards that are as clear as possible to develop common understanding of requirements;
- E. integrate with existing standards in other chapters of the *Aquatic Code*.

Q1. Are the above principles (points A-E) to guide revision of Chapter 4.3. Compartmentalisation appropriate? If not, please suggest alternatives.

Response:

3. Member responses to 2022 questionnaire

This discussion paper has been informed by member responses to a short questionnaire provided in the Commission's September 2022 meeting report. The questionnaire invited responses from Members on their experiences in the application of compartments, including the purpose of compartments, positive experiences, acceptance by trading partners and constraints. A summary of member responses to the questionnaire is included at [Attachment 1](#).

4. Purposes of compartmentalisation

The current definition of a compartment in the glossary of the *Aquatic Code* limits the purpose of a compartment to international trade (see section 8 of this discussion paper, definitions). However, free compartments are established to provide an assurance of disease freedom for a range of commodity types, trade pathways and intended end uses. These factors have implications for management of disease risk.

The commodities traded from a free compartment may include live aquatic animals (gametes, fertilised eggs, juveniles or adults) or aquatic animal products (ranging from whole slaughtered animals to any number of processed products consisting of parts of animals).

There are numerous potential end-uses for commodities traded from a compartment. Some of the principal end-uses that might be anticipated include:

- Human consumption – directly as live aquatic animals or products; or indirectly following grow out of juveniles in another aquaculture establishment.
- Breeding – use as brood stock in hatcheries or breeding centres to produce animals for grow-out; or for establishment of a new aquaculture species, or genetically selected lines of species, in a territory.
- Stock enhancement – release into open systems to enhance or recover wild populations.
- Ornamental purposes – for sale within the pet trade; or for display at zoos or aquariums.
- Research – provision of aquatic animals for scientific purposes.

Trade pathways from a compartment may include domestic or international trade (note that the current *Aquatic Code* definition is limited to international trade). Trade from a free compartment could, in most circumstances, be expected to occur from a zone or country not declared free to a country, zone or compartment declared free. Compartmentalisation might also be applied to provide epidemiological separation from populations of susceptible aquatic animals within a free country or zone to protect valuable aquatic animals (e.g. selected lines) in the event of a disease outbreak within the previously free zone/country.

Q2. Do these purposes encompass the principal reasons for establishing a compartment, defined by product type, pathway and end use? If not, please provide alternative suggestions.

Response:

5. Independent versus dependent compartments

Members have noted that there are two major types of compartments that have been recognised for international trade and which are categorised by the degree of epidemiological separation from the surrounding environment: independent and dependent compartments (see [Attachment 1](#)). Chapter 4.3. of the *Aquatic Code* does not currently differentiate types of compartments based on the degree of epidemiological separation.

Independent compartments have complete epidemiological separation from surrounding environments. These compartments have high levels of physical and management measures to maintain effective biosecurity. Independent compartments are closed-systems that have control over all transmission pathways into the compartment. An independent compartment may use disease free water sources (e.g. bore water) or have disinfection procedures in place to prevent the entry of pathogens of concern. Independent compartments may be used for high value aquatic animals (e.g. genetically improved lines, brood stock) and may be suited to end uses such as aquaculture and restocking programmes.

Dependent compartments do not have complete epidemiological separation from the surrounding environment and maintenance of their health status is dependent on freedom from diseases of concern in the surrounding natural waters. Dependent compartments are semi-closed systems which may have control over all transmission pathways but may not utilise sterile water sources (e.g. pump ashore tank or pond aquaculture). A dependent compartment would need to be established considering epidemiological factors to maintain epidemiological independence of the compartment (e.g. geographical location; environmental conditions;

proximity to populations of susceptible species; presence, abundance and behaviour of populations of susceptible species; disease status of any nearby populations of susceptible species; hydrological conditions in the adjacent water bodies). Dependent compartments can be considered to provide a lower degree of assurance of disease freedom relative to independent compartments; however, additional assurance may be provided through increased targeted surveillance and other epidemiological circumstances. Dependent compartments may be best suited for certain product types and end uses, e.g. processed product intended for human consumption.

Q3. Do you support including the concepts of independent and dependent compartments in the revised Chapter 4.3? What are your reasons?

Response:

Q4. Should a dependent compartment be able to supply live aquatic animals for aquaculture or restocking? If yes, under what conditions should this trade be allowed (e.g. epidemiological separation, targeted surveillance)?

Response:

6. General principles of compartmentalisation

The following principles are proposed as high-level guidance for the development of compartments and to frame the article structure of a revised Chapter 4.3.

1. A disease-free compartment represents a functional epidemiological separation of a population of aquatic animals within it from other sources of infection.
2. The purpose of the compartment must be clearly defined (e.g. species and commodities produced, disease(s) for which freedom will be claimed, end uses of commodities) as this will have implications for the design of risk management measures.
3. Compartments may include two principal categories: those that are dependent on the disease status of the surrounding environment and those which are independent from it.
4. A compartment must have an effective biosecurity plan in accordance with Chapter 4.1. that is applied consistently across all elements of the compartment.
5. Surveillance measures to establish the compartment as free, and the measures to maintain compartment freedom, must be clearly described in accordance with Chapter 1.4., including elements of internal and external surveillance as appropriate.
6. Reliable laboratory testing services are required to underpin surveillance testing. The laboratory services must have independence from the compartment operator and have quality management accreditation.
7. Traceability systems must provide assurance of provenance of commodities from the free compartment.
8. Record keeping must support the transparent and ongoing application of all measures on which the compartment has been granted disease free status.
9. Official oversight responsibilities must be clearly documented, including registration or approval by the Competent Authority, an auditing schedule and underpinning regulatory instruments.
10. Notification and response measures must be in place in the event of detection of the disease for which the compartment has been declared free, or for other diseases relevant to trade from the compartment.

Q5. Do the general principles of compartmentalisation described above provide an appropriate high-level framework for the establishment and recognition of a compartment? Please suggest any amendments or additional principles that should be considered?

Response:

7. Analysis of the current adopted text of Chapter 4.3.

7.1. Article 4.3.1. Introduction and objectives

Current situation and analysis

Article 4.3.1. provides a broad description of compartments and comparison to declaration of freedom at a country or zone level. The current text describes compartments through comparison, for example to zones, rather than describing what a compartment is more directly. The current text lacks clarity on some basic concepts related to compartments; for example, their purposes, benefits, and roles for establishment and maintenance. The article is titled introduction and objectives; however, the article does not clearly state any objectives for the chapter.

Recommended approach

It is important that article 4.3.1. clearly define what a compartment is. This is important to convey a common understanding and to avoid varying conceptual interpretations, which has been indicated as a constraint (see [Attachment 1](#)).

Text could be added to this article to articulate a clear objective of the chapter, for example, to describe the requirements for establishing a free compartment and for meeting the requirements for a self-declaration of compartment freedom to be made.

It is proposed that article 4.3.1. be revised to more directly describe the concept of a compartment, rather than by comparison to zones. The text should also introduce the purposes of establishing compartments, the benefits for facilitating trade and disease management, and the roles of the private sector and competent authorities broadly.

It is also proposed that a new article 4.3.X. be included in the revised chapter to clearly describe the varying purposes of establishing compartments, as indicated by member responses to the survey (see [Attachment 1](#)). These would include facilitating trade of disease-free animals and animal products (not limited to international trade), to contribute to disease management, and to protect and preserve valuable aquatic animals (e.g. selected lines) in the event of a disease outbreak in an otherwise free country or zone.

7.2. Article 4.3.2. Principles for defining a compartment

Current situation and analysis

Article 4.3.2. indicates that a compartment should have its components and interrelationships described and that epidemiological factors should be defined. This text does not adequately articulate a set of principles for defining a compartment.

Recommended approach

It is proposed that this article be revised to clearly state the high-level principles that must be met for a compartment to be established and for a self-declaration of compartment freedom made. These principles would then align with the article structure for the chapter which would provide further details on how to meet the requirements of each principle. This approach has been used in Chapter 4.1. Biosecurity for

aquaculture establishments (see article 4.1.2.) and Chapter 4.4. Disinfection of aquaculture establishments and equipment (see article 4.4.2.).

Possible principles for inclusion in this article could reflect those of section 6 above.

Q6. Do you support the revision of article 4.3.2. to include the principles in section 6 above (as modified based on member comments)? Are there any additional key issues or requirements that should be addressed within a set of principles?

Response:

7.3. Article 4.3.3. Separation of a compartment from potential sources of infection

Current situation and analysis

Article 4.3.3. is a large article that covers four main topics as subpoints:

1. Physical or spatial factors that affect the status of biosecurity in a compartment
2. Infrastructural factors
3. Biosecurity plan
4. Traceability system

A significant portion of this article covers biosecurity planning and measures that are addressed more comprehensively in Chapter 4.1. Biosecurity in aquaculture establishments.

Recommended approach

It is proposed that article 4.3.3. be revised to focus on the description of a compartment and the nature of its epidemiological independence. This would include describing the concepts of dependent and independent compartments (see section 5 above).

It is proposed that biosecurity plan and traceability requirements be addressed in separate articles as appropriate to align with the principles proposed for article 4.3.2.

7.4. Article 4.3.4. Documentation

Current situation and analysis

Article 4.3.4. provides guidance on the records that should be kept to provide evidence that the requirements of a compartment are being met. Much of this article focuses on record keeping relevant to matters addressed in a biosecurity plan or for surveillance requirements. The article indicates that the time periods for maintaining records may vary.

Recommended approach

For the elements of this article relevant to documentation of a biosecurity plan, it is proposed that a cross reference to the relevant articles in Chapter 4.1. be included.

For the elements of this article relevant to surveillance, it is proposed that this text be revised and replaced with more specific requirements of the evidence to meet surveillance requirements to claim self-declaration of compartment freedom, and to maintain freedom. This would include reference to Article 4.3.5. (as revised, see below) and any relevant articles in Chapter 1.4.

It is proposed that guidance be provided on factors for determining the time periods for keeping records. These should be linked to production cycles, surveillance, biosecurity plan requirements, auditing, and traceability requirements.

It is proposed that this article be moved lower such that it would follow all relevant articles for which there is a record keeping requirement.

7.5. Article 4.3.5 Surveillance for the pathogenic agent or disease

Current situation and analysis

This article advises that the surveillance system should comply with Chapter 1.4. on surveillance and the specific recommendations for surveillance for the disease(s) for which the compartment was defined. The article notes that the sensitivity of the surveillance system should be reviewed if there is an increased risk of exposure to the agent for which the compartment has been defined.

The article also describes internal and external surveillance requirements. Internal surveillance is described as allowing the Competent Authority to certify that animals within the compartment comply with its defined status and to enable early detection of disease. External surveillance is intended to identify a significant change in the level of exposure for the identified pathways of disease introduction into the compartment.

Recommended approach

It is proposed that this article be revised to align more closely with the requirements for making a self-declaration of compartment freedom and the requirements for maintaining freedom. These requirements are included in Chapter 1.4. and the relevant disease specific chapters of the *Aquatic Code*.

The concepts of internal and external surveillance are likely to be useful however they are not terms that are used in Chapter 1.4. or in disease specific chapters. It is proposed that these concepts be considered and perhaps applied in the context of dependent and independent compartments. See section 5 above.

7.6. Article 4.3.6. Diagnostic capabilities and procedures

Current situation and analysis

This Article advises that testing laboratories should be officially designated and that testing procedures should comply with recommendations of the *Aquatic Manual*. It also advises that testing laboratories should have procedures in place for reporting results to the Competent Authority.

Article 4.3.6. provides guidance on diagnostic procedures that underpin surveillance within a compartment and confidence in the compartment's disease-free status. Several factors that influence the quality of diagnostic testing are not referenced in the article.

Recommended approach

It is suggested that article 4.3.6. be revised to address additional factors that contribute to reliable diagnostic testing. These include independence of the testing laboratory from management and ownership structures of the compartment and a requirement for officially approved testing laboratories to be accredited to ISO Standard 17025 or equivalent.

There should be a mandatory requirement for testing laboratories to report positive test results to the competent authority for compartments declared disease free for the purposes of international trade. This is necessary to meet the requirements of basic biosecurity conditions of a compartment as specified in Article 1.4.6. of Chapter 1.4 of the *Aquatic Code*.

Q7. Do you support the recommended approach to revision of article 4.3.6., including requirements for independence, accreditation and mandatory laboratory reporting? Please provide rationale or further comments.

Response:

7.7. Article 4.3.7. Emergency response and notification

Current situation and analysis

This article provides guidance on the actions to be taken if there is suspicion of occurrence of the disease from which the compartment has been declared free. Paragraph 1 advises that if there is suspicion of occurrence of the disease, free status should be suspended, and importing countries notified in accordance with Chapter 1.1. The language in this paragraph differs from Chapter 1.1 which requires notification of occurrence or recurrence, not suspicion.

Paragraph 2 advises that a review of biosecurity measures should be initiated to determine if there has been a breach of biosecurity measures and free status should only be reinstated after the compartment has adopted the necessary measures to re-establish the original biosecurity level and the Competent Authority has re-approved the status of the compartment. The requirements of this paragraph differ subtly from those of Chapter 1.4. and disease specific chapters which require that basic biosecurity measures be reviewed and amended as appropriate. Further, for the purposes of international trade, free status can only be reclaimed once the requirements of Chapter 1.4 and the relevant disease specific chapters have been met.

Paragraph 3 advises that any changes in disease risk in the surrounding area should be considered, the status of the compartment re-evaluated and the need for additional biosecurity measures implemented. This paragraph appears to be most relevant for dependent compartments; however, could be considered as part of the review of basic biosecurity conditions. Specific mention of factors to be reviewed for either dependent or independent compartments may be warranted.

Recommended approach

Article 4.3.7. requires revision to ensure guidance is consistent with other provisions of the *Aquatic Code*, for example, notification requirements of Chapter 1.1. and the requirements for returning to compartment freedom specified in Chapter 1.4. and the relevant disease-specific chapter(s). The article may also require cross-referencing to new chapters under development for Section 4 of the *Aquatic Code* on emergency preparedness and outbreak management.

7.8. Article 4.3.8. Supervision and control of a compartment

Current situation and analysis

Article 4.3.8. requires that the authority, organisation, and infrastructure of the Aquatic Animal Health Services be clearly documented to provide confidence in the integrity of the compartment. The article cross references to Chapter 3.1. Quality of Aquatic Animal Health Services but does not limit documentation of the Aquatic Animal Health Services to those aspects relevant to the self-declaration of compartment freedom. The article specifies that authority, organisation, and infrastructure of the Aquatic Animal Health Services should be documented; however, Chapter 3.1. includes 14 fundamental principles of quality. The article might be improved by clarifying that the Aquatic Animal Health Services relevant to the self-declaration of freedom should be documented, including how these relevant Aquatic Animal Health Services meet the requirements of Chapter 3.1.

The article also advises that “the” Competent Authority has final authority on approving or suspending status and that the Competent Authority should continuously supervise compliance with all requirements critical to maintaining compartment status. This is a principal concept of Competent Authority oversight of

a disease-free compartment. It may be beneficial to more clearly articulate the role of competent authorities and the Veterinary Authority in establishing and approving a disease-free compartment, providing ongoing oversight (including of relevant Aquatic Animal Health Services) and for communication with WOH and trading partners as specified in relevant chapters of the *Aquatic Code*.

Recommended approach

It is suggested that Article 4.3.8. be separated into two articles: one on quality of Aquatic Animal Health Services and one on supervision and authority. The article on quality of Aquatic Animal Health Services should clarify that the Aquatic Animal Health Services relevant to the self-declaration of freedom should be documented, including how they meet the requirements of Chapter 3.1. The second article should clearly articulate the role of competent authorities and the veterinary authority in establishing and approving a disease-free compartment, and providing ongoing oversight.

Q8. Do you support the proposed revision of article 4.3.8., including division into two articles: one on quality of Aquatic Animal Health Services and one on Competent Authority oversight? Please provide rationale or further comments.

Response:

8. Definitions

Current status

Two terms specific to compartments are included within the glossary of the *Aquatic Code* and will require consideration during the revision of Chapter 4.3. Compartmentalisation. These include the definitions for “compartment” and “free compartment”. The current definitions for these terms as included in the 2023 edition of the *Aquatic Code* are:

COMPARTMENT means one or more *aquaculture establishments* under a common *biosecurity* management system containing an *aquatic animal* population with a distinct health status with respect to a specific *disease* or *diseases* for which required *surveillance* and control measures are applied and *basic biosecurity conditions* are met for the purpose of *international trade*. Such must be clearly documented by the *Competent Authority(ies)*.

FREE COMPARTMENT means a *compartment* that fulfils the requirements for *self-declaration of freedom from disease* with respect to the *disease(s)* under consideration in accordance with the relevant chapter(s) in the *Aquatic Code*.

Many additional defined terms are relevant to the revision of Chapter 4.3., for example those related to surveillance and biosecurity. Many of these terms, have been recently revised during the development and adoption of the new Chapter 4.1. Biosecurity for Aquaculture Establishments (adopted 2021) and revision of Chapter 1.4. Aquatic Animal Health Surveillance (adopted 2022).

Analysis

The specific terms related to compartments are likely to require revision to ensure they are fit for purpose for the agreed scope, purposes and concepts included in the revised chapter. For example, some issues that may need to be addressed are:

- the current definition limits the purpose of a compartment to international trade. This may be too narrow based on Member comments (see [Attachment 1](#)) and consideration of the concepts in sections 5 and 6 above.
- there may be a need to define “types” of compartments that offer different levels of risk management based on their purpose (e.g. dependent and independent compartments). Members raised different

types and purposes of compartments in their survey responses (see [Attachment 1](#)) that may need to be reflected in revised definitions, or be the subject of new definitions.

9. Other interacting standards

There are several chapters within the WOA *Aquatic Code* that are relevant to a revision of Chapter 4.3. It is important that these standards are considered so that appropriate cross references are provided, and that duplication or conflicting guidance avoided. This section of the discussion paper identifies key standards of the *Aquatic Code* that should be considered in the revision of Chapter 4.3.

Disease specific chapters.

Each disease specific chapter of the *Aquatic Code* provides guidance on the requirements for declaring a free compartment for that disease. The requirements in these articles are consistent with and cross reference Chapter 1.4. Surveillance.

In addition, the disease-specific chapters provide recommendations on risk management for aquatic animal commodities (of susceptible species for that disease) for different end uses; in particular, where the source of the commodities is a country, zone or compartment not declared free.

Chapter 1.4 Surveillance.

Chapter 1.4. provides guidance on the surveillance required to demonstrate freedom at the level of a compartment. The provisions in Chapter 1.4. for surveillance to declare a compartment free complement the provisions of disease-specific chapters.

Chapter 3.1. Quality of Aquatic Animal Health Services

Chapter 3.1. sets out the fundamental principles of an ethical, organisational, legislative, regulatory and technical nature which define the quality of aquatic animal health services. The provisions of Chapter 3.1 are important to characterise the transparent and independent oversight and services that underpin confidence in the ongoing disease free status of a compartment.

Chapter 4.1. Biosecurity of aquaculture establishments.

Chapter 4.1. provides detailed guidance on the requirements for developing and implementing a biosecurity plan. The provisions in Chapter 4.1. are fundamental to establishing and maintaining compartment freedom.

Chapter 5.3. WOA procedures relevant to the Agreement on the Application of Sanitary and Phytosanitary Measures of the World Trade Organization.

Article 5.3.7 describes the sequence of steps to be taken to establish a zone or compartment and have it recognised for international trade purposes.

10. Discussion

This paper has aimed to engage WOA members on issues relevant to the revision of Chapter 4.3. so that the revised chapter will provide consistent and clear guidance on compartmentalisation. Principles are presented in section 3 to guide the intended outcome of the revision. The discussion paper has explored conceptual issues relevant to the revision of the paper, analysed the current structure of the existing Chapter 4.3 and sought responses from members on key issues of importance to its revision.

A proposed article structure for the revised Chapter 4.3. has been proposed at [Attachment 2](#), based on the analysis and discussion presented in this paper.

Following consideration of member comments, the commission will recirculate the discussion paper to members with a summary of member responses and consensus views. Together the discussion paper and member responses will set the direction for the revision of Chapter 4.3.

Attachment 1. Summary of member responses to 2022 questionnaire

Comments were received from Australia, Brazil, Canada, China, Germany, Ireland, Japan, New Zealand, Slovenia, Spain, Sweden, Switzerland, UK, USA and the EU.

At its September 2022 meeting the Commission agreed to circulate a questionnaire for Members to inform the revision of Chapter 4.3. Application of compartmentalisation. For those Members who responded that they have established or are in the process of establishing compartments, the purpose of the compartments was primarily for:

- Domestic or international trade (aquaculture species and ornamental aquatic animals)
- Support and protect hatcheries from introduction of disease or disease response activities in the event of incursion of disease within the zone.
- Enhancement of wild aquatic animal populations
- Human consumption

Members indicated positive experiences related to establishment of compartments mainly related to benefits for trade and disease control such as:

- Increased market access and ease or facilitation of trade;
- overall increased health status of the defined aquatic animal populations;
- protection of health status in the event of disease incursion within the surrounding zone;
- shorter time duration for return to disease -free status

For those Members with established compartments, the acceptance of these compartments by trade partners varied. When compartments were not accepted or had delayed acceptance by trade partners, it was related to constraints/impediments that must be overcome such as:

- Members may have a different understanding or application of compartmentalisation which can impact acceptance of recognised compartments by their trade partners;
- The use of dependent compartments may limit potential market access;
- Audit of the established compartments by trade partners were required prior to acceptance and initiation of trade.

In addition to trade related constraints and impediments there were other constraints or threats that either had to be overcome or prevented the establishment of compartments. These threats were mainly relating to the industry and competent authority:

Industry

- may be constrained or prevented from establishing compartments by the type of aquaculture production system used (open/ semi-open/semi-closed systems). The requirements for establishing a compartment may not be achievable
- the Aquaculture establishment must make the business decision to invest the money and effort to establish a compartment based on the potential market access. The real return on investment won't be known until the compartment has been established.
- Once a free status has been established, the introduction of new genetics/live animals may be limited due to a potential resulting change in health status.

Competent Authority

-
- Development of parameters to ensure separation of the compartment from the surrounding zone and implementation of compartments based on the zone health status requires Competent Authority oversight and corresponding resources (human and financial)
 - Potential lack of understanding by the Competent Authority

Specifically in regard to the revision of the Chapter 4.3. Application of Compartmentalisation, Members were supportive and identified several gaps in the current chapter where additional detail could be incorporated:

- Introducing when compartmentalisation is appropriate for use;
- Incorporate cross references to Chapter 4.1. Biosecurity in aquaculture establishments and the different types of aquaculture production systems where compartmentalisation is possible (e.g. dependent and independent compartments)
- Indicate the difference between standards for the establishment of a compartment health status, standards for maintenance of the health status and recovery after a disease incursion to regain freedom.

Attachment 2. Proposed article structure for the revised Chapter 4.3.

| Article number | Content |
|-----------------------|---|
| 4.3.1. | Objective and introduction |
| 4.3.2. | Purposes of compartments |
| 4.3.3. | Principles for establishing a compartment |
| 4.3.4. | Dependent and independent compartments |
| 4.3.5. | Biosecurity |
| 4.3.6. | Surveillance requirements to claim and maintain freedom |
| 4.3.7. | Laboratory testing |
| 4.3.8. | Traceability |
| 4.3.9. | Record keeping |
| 4.3.10. | Official oversight |
| 4.3.11. | Quality of aquatic animal health services |
| 4.3.12. | Notification and response measures |

Attachment 3. Questions for response by Members

The questions below are included in the body of the discussion paper text and are collated here for ease of reference.

| Question | Section reference |
|---|--|
| Q1. Are the above principles (points A-E) to guide revision of Chapter 4.3. Compartmentalisation appropriate? If not, please suggest alternatives. | 2. Objectives of the paper |
| Q2. Do these purposes encompass the principal reasons for establishing a compartment, defined by product type, pathway and end use? If not, please provide alternative suggestions. | 4. Purposes of compartmentalisation |
| Q3. Do you support including the concepts of independent and dependent compartments in the revised chapter 4.3.? What are your reasons? | 5. Independent versus dependent compartments |
| Q4. Should a dependent compartment be able to supply live aquatic animals for aquaculture or restocking? If yes, under what conditions should this trade be allowed (e.g. epidemiological separation, targeted surveillance)? | 5. Independent versus dependent compartments |
| Q5. Do the general principles of compartmentalisation described above provide an appropriate high-level framework for the establishment and recognition of a compartment? Please suggest any amendments or additional principles that should be considered? | 6. General principles of compartmentalisation |
| Q6. Do you support the revision of article 4.3.2. to include the principles in section 6 above (as modified based on member comments)? Are there any additional key issues or requirements that should be addressed within a set of principles? | 7.2. Article 4.3.2. Principles for defining a compartment |
| Q7. Do you support the recommended approach to revision of article 4.3.6., including requirements for independence, accreditation and mandatory laboratory reporting? Please provide rationale or further comments. | 7.6. Article 4.3.6. Diagnostic capabilities and procedures |
| Q8. Do you support the proposed revision of article 4.3.8., including division into two articles: one on quality of Aquatic Animal Health Services and one on Competent Authority oversight? Please provide rationale or further comments. | 7.8. Article 4.3.8. Supervision and control of a compartment |

SECTION 4

DISEASE PREVENTION AND CONTROL

CHAPTER 4.X.

EMERGENCY DISEASE PREPAREDNESS

Article 4.X.1.

Purpose

To describe the essential elements of an emergency disease preparedness framework which a *Competent Authority* should develop to ensure that *outbreaks* of important *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.

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 diseases 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 diseases* 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 *diseases* that is developed after conducting a *risk analysis* as described in 4.X.6., may include *aquatic animal diseases* which are listed in Chapter 1.3., as well as other *diseases* 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*.

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*;
- 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 Chapter 4.Y. when the presence of an important *disease* is suspected during the 'alert phase';
 - b) fully activated in compliance with Chapter 4.Y. once the *disease* emergency has commenced during the 'emergency phase'.
- 4) simulation exercises should be planned and executed to test relevant elements of the *disease* preparedness framework. Simulation exercises ensure that *Competent Authorities* and *Aquatic Animal Health Services* are trained and properly equipped 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. 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 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.

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 animals* 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 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 diseases* that may be subject to emergency measures should be under continual review by the *Competent Authority*. The *risk analysis* should 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* activities carried out by the *Aquatic Animal Health Services*, in accordance with Chapter 1.4. The outcomes from an emergency *disease preparedness* framework are fundamentally reliant on the quality of *surveillance* 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.

Response actions during the disease emergency

As part of preparedness planning, *risk assessment* protocols should be developed to support decision making by the *Competent Authority* during an *outbreak*. 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 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 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.

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*. This cooperation should:
 - a) ensure that all actions 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 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) data management or information systems;
 - 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);
 - 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 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 depopulation, cleaning and *disinfection*, should be considered, using *risk assessment*. 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 *fallowing* of a number of *aquaculture establishments*, 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 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* 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.

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.

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* 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, with the objective of identifying:
 - a) the elements of the emergency disease preparedness framework that are fit-for-purpose, and those that are not;

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- b) the readiness and capacity of the *Competent Authority* and the *Aquatic Animal Health Services* to respond to the elements of the emergency disease preparedness framework, that were tested during the exercise.

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 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, improvements in technology or relevant 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* 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*;

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- 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 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.

SECTION 4

DISEASE PREVENTION AND CONTROL

CHAPTER 4.Y.

DISEASE OUTBREAK MANAGEMENT

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 *Competent Authority* can rely on the Operations Manual to provide guidance on all aspects of the 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 actions to be taken 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;
- 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) determine if the *disease* has spread from or to *aquaculture establishments* or waterbodies other than the one in which the original suspicion was raised.
- 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 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.
- 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* and *aquatic animal waste* to or from the *aquaculture establishment* or waterbody, unless authorised by the *Competent Authority* based on a risk assessment;
 - 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.
- 5) whilst awaiting the outcome of the epidemiological investigation described above, the *Competent Authority* should communicate with the emergency management group, as described in Chapter 4.X., and 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.
- 6) whilst confirmation of the presence of the *disease* in question is ongoing, the *Competent Authority* should communicate with relevant personnel, 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.;
- 7) 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;

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- 8) should the suspicion not be confirmed, the alert phase is terminated, and any outcomes which warrant review of the *Contingency Plan*, are made;
 - 9) 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 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, 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*;
 - 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.;

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- c) the reinforcement of the preliminary *biosecurity* measures which were put in place during the 'alert phase', the imposition of new *disease* control measures, or both.
 - 3) trade issues which are likely to arise, both within the country and with trading partners elsewhere;
 - 4) review of legal, administrative and financial arrangements to ensure all relevant enablers are in place to immediately manage the *disease* emergency. This should include:
 - a) details of the legal instrument which supports the provision of funding for the management of disease emergencies concerning *aquatic animals*;
 - b) contact details for the relevant department which will process the request for funds 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) format for, and timing of, communications with the *Aquatic Animal Health Services* who are responding to the emergency, relevant trading partners, and the public. 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, allowing for flexibility to schedule meetings 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 *aquaculture establishments* and 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*;
 - 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*, and wild aquatic habitats, as relevant;

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- ii) sample collection;
 - 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.

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

- a) 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:
 - i) mortality management providers involved in retrieval and/or transport, who have capacity for the required daily tonnage;
 - ii) sanitary slaughter facilities, which can cater for the required daily tonnage;
 - iii) telecommunications providers;
 - iv) providers of laboratory equipment and consumables who have an acceptable lead-in time for delivery of new and replacement items;
 - v) companies which service relevant laboratory equipment and which have an acceptable response time for critical pieces of equipment;
 - vi) providers of vaccines/ veterinary medicines, who can supply an appropriate number of doses and have a suitable lead-in time for delivery;
 - vii) 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;

viii) back-up providers for each type of service, should they be required for an extensive *disease outbreak*.

Contact details of the providers referred to in points (i) to (viii) above are detailed in the Operations Manual.

Article 4.Y.8.

Biosecurity and other disease control measures

The actions which the *Competent Authority* takes concerning *biosecurity* and other *disease control* measures during the emergency phase, are described in the Operations Manual and 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) 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;
- 3) 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;
- 4) specifying relevant *biosecurity* and other specific *disease control* measures including:
 - a) controlling the movement of *aquatic animals*, *aquatic animal products*, *feed* and equipment to or from the infected establishment(s), 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*, 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 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;
 - iv) the vaccination options that may be employed, depending on the circumstances of the *disease outbreak*, including:
 - no vaccination;
 - 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*;
 - 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;

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- a combination of suppressive and protective vaccination.
 - 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 and which meet the decontamination requirements concerning the *pathogenic agent* in question, should also be specified;
 - f) procedures for the containment of wastewaters which are produced following *disinfection*, which have been drawn up in accordance with the instructions of the *Competent Authorities* with responsibility for discharges to the environment.

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. In cases where the recovery phase includes the ambition to return to *disease freedom* in accordance with Pathway 4 as referred to in Chapter 1.4., either for the entity (country, *zone* or *compartment*), which was previously *disease free*, or to make a *self-declaration of freedom from disease* for 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* 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) Where the aim of the recovery plan is to contain the *disease*, the following measures may be described:
 - i) 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) 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 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.
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SECTION 4
DISEASE PREVENTION AND CONTROL

CHAPTER 4.Z.

CONTROL OF PATHOGENIC AGENTS IN TRADED MILT AND FERTILISED EGGS OF FISH

Article 4.Z.1.

Purpose

To provide recommendations for trade of milt and fertilised eggs of fish for *aquaculture* and to define *risk* mitigation for import to a *free country, free zone or free compartment* when:

- 1) the intention is to grow out and harvest the imported *aquatic animals*; or
- 2) the intention is to establish a new stock for *aquaculture*.

For disease-specific recommendations, refer to Section 10.

Article 4.Z.2.

Scope

This chapter describes general recommendations for safe trade in milt and fertilised eggs of fish from an area other than a *free country, free zone or free compartment*. These recommendations cumulatively reduce the *risk* of transfer of infection to *aquatic animal* populations in a *free country, free zone or free compartment*.

Trade of 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 milt and fertilised eggs of fish

Trade of 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 should be determined. Only populations of broodstock which test free from the *pathogenic agents* of concern are suitable for supply to *collection and incubation centres*, as described in article 4.Z.4.;
- 2) milt and fertilised eggs should come from a *collection and incubation centre* approved by the *Competent Authority* of the place of origin, which operates in compliance with the conditions described in Articles 4.Z.5., 4.Z.6. and 4.Z.7.;

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- 3) the fertilised eggs should 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);
 - 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* which should state that the milt and the fertilised eggs come from parents which have tested free from the relevant *disease*, and meet the requirements in points 1 and 2.

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 place of origin

Aquaculture establishments keeping broodstock for the production and 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) be approved by the *Competent Authority*;
- 2) have in place a *biosecurity plan* in accordance with Chapter 4.1.;
- 3) the broodstock should be tested for the *pathogenic agents* of concern prior to entry to the *collection and incubation centre* 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 be from a population experiencing recent or ongoing mortality, and should not be exposed to animals of a lower health status following the testing at point 3.

Article 4.Z.5.

Collection and incubation centres

Collection and incubation centres should be approved by the *Competent Authority* on the basis that the *collection and incubation centre* should:

- 1) be under the supervision of an *Aquatic Animal Health Professional* or *veterinarian*;
- 2) have a *biosecurity plan* in accordance with Chapter 4.1.;
- 3) be structured to contain epidemiologically separate groups of broodstock;
- 4) have in place a valid traceability system to ensure that each batch of gametes or fertilised eggs can be traced back to an epidemiologically separate group, and include documentation and auditing of testing results, *disease history* and movements of *aquatic animals*;
- 5) be separated into:
 - a) a collection room for eggs and milt;
 - b) an incubation centre for fertilised eggs;
 - c) a milt laboratory and milt storage area;

-
- d) administration offices.
 - 6) be subject to and pass audits by the *Competent Authority* or an approved third party at least once per year against the requirements of this chapter.

Article 4.Z.6.

Testing of broodstock at the collection and incubation centre

Broodstock for the production and milt and fertilised eggs of fish, should meet the following requirements at the *collection and incubation centre*:

- 1) 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*;
- 2) fish that test positive, and any milt or eggs derived from them should not be traded and all gametes and fish from that epidemiological group should be disposed of in a biosecure manner. Affected facilities should be disinfected to ensure that cross-contamination of other batches of milt or eggs does not occur.

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.
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Model Article 10.X.10. for Chapter 10.5. Infection with SAV, Chapter 10.6. Infection with VHSV, and Chapter 10.10. Infection with IHN

CHAPTER 10.X. INFECTION WITH [PATHOGEN X]

[...]

Article 10.X.10.

Importation of aquatic animals for aquaculture from a country, zone or compartment not declared free from infection with [pathogen X]

When importing, for *aquaculture*, *aquatic animals* 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 1 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 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* 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 1 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.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 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*;

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- 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.

[...]

Model Article 10.X.15. for Chapter 10.5. Infection with SAV, Chapter 10.6. Infection with VHSV, and Chapter 10.10. Infection with IHNV

CHAPTER 10.X. INFECTION WITH [PATHOGEN X]

[...]

Article 10.X.15

Importation of 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 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 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 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 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 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.

[...]

GLOSSARY

[...]

COLLECTION AND INCUBATION CENTRE

means a facility for the collection of eggs, fertilisation and incubation, and the collection, processing, and storage of milt approved by the Competent Authority in conformity with the provisions of Chapter 4.Z.

[...]

ORNAMENTAL AQUATIC ANIMAL

means an aquatic animal that is intended for display, exhibition, competition, or to be kept as a pet.

[...]

SECTION 5

**TRADE MEASURES, IMPORTATION/EXPORTATION
PROCEDURES AND HEALTH CERTIFICATION**

CHAPTER 5.X.

MOVEMENT OF ORNAMENTAL AQUATIC ANIMALS

Article 5.X.1.

Introduction

This chapter provides recommendations to address the *risk of 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 *disease risks* associated with movement of *ornamental aquatic animals* 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 eligibility for the movement of a species (or a taxonomic group of species) should be determined considering 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 mortality, as described in Article 5.X.5.;

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- 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 consult relevant national regulations and international obligations to determine that the species is eligible for import.

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)). 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 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 program, 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 animals* 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;

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- 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. 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 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*;

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- 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 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 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* 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.

Transport of *ornamental aquatic animals* should follow protocols that are appropriate for maintaining the welfare of the species 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 repackaging.

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.

(TRACK CHANGES 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

~~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*);~~
 - e) ~~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°C for at least five30 minutes, or any time/temperature equivalent that has been demonstrated to inactivate *B. dendrobatidis*);~~

e)3) ~~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.

[...]

(TRACK CHANGES 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

~~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 10060°C for at least five30 minutes, or any time/temperature equivalent that has been demonstrated to inactivate *B. salamandrivorans*;~~

~~e)3) 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.

[...]

(TRACK CHANGES 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 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 6560°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);
 - d2) mechanically dried amphibian products that have been subjected to (i.e. a heat treatment sufficient to attain a core temperature of at least 10065°C for at least 30 minutes, or any time/temperature equivalent that has been demonstrated to inactivates *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.

[...]

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~~68°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~~68°C for at least 30 minutes, or a time/temperature equivalent that inactivates DIV1;
- 3) ~~crayfish~~crustacean oil;
- 4) chemically extracted chitin. ~~(under study).~~

[...]

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~~69°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~~69°C for at least ~~30~~five minutes, or a time/temperature equivalent that inactivates *H. penaei*;
- 3) crustacean oil;
- 4) chemically extracted chitin.

[...]

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*.

[...]

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.

[...]

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*.

[...]

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) 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) fish oil;
- 5) fish skin leather.

[...]

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 minutes, 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) 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 minutes, or a time/temperature equivalent that inactivates *A. invadans*;
- 4) fish oil;
- 5) frozen eviscerated fish;
- 6) frozen fish fillets or steaks.

[...]

CHAPTER 10.3.

INFECTION WITH *GYRODACTYLUS SALARIS*

[...]

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) naturally dried eviscerated fish (i.e. sun-dried or wind-dried);
- 4) frozen eviscerated fish that have been subjected to minus 18°C or lower temperatures;
- 5) frozen fish fillets or steaks that have been subjected to minus 18°C or lower temperatures;
- 6) chilled eviscerated fish that have been harvested from seawater with a salinity of at least 25 parts per thousand (ppt);
- 7) chilled fish fillets or steaks derived from fish that have been harvested from seawater with a salinity of at least 25 ppt;
- 8) chilled fish products from which the skin, fins and gills have been removed;
- 9) non-viable fish roe;
- 10) fish oil;
- 11) fish meal;
- 12) fish skin leather.

[...]

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 HPRO 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) 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) fish oil;~~
- ~~5) fish skin leather.~~

[...]

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) 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) fish oil;
- 5) fish skin leather.

[...]

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 IHN 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 IHN, regardless of the IHN 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 IHN;
- 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 IHN;~~
- 3) 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 IHN;
- 4) fish oil;
- 5) fish skin leather.

[...]

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 minutes, 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) 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 minutes, or a time/temperature equivalent that inactivates KHV;
- 4) fish oil.

[...]

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) 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) fish oil;
- 5) fish skin leather.

[...]

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) 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) fish oil.

[...]

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) 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) naturally dried, eviscerated fish (i.e. sun-dried or wind-dried);~~
- ~~5) fish oil;~~
- ~~6) fish skin leather.~~

[...]

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.

[...]

(TRACK CHANGES VERSION)

CHAPTER 11.1.

INFECTION WITH ABALONE HERPESVIRUS

[...]

Article 11.1.3.

Measures for the importation 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 measure 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 121.5°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);
 - b) mechanically dried abalone products (i.e. that have been subjected to a heat treatment sufficient to attain a core temperature of at least 100/121°C for at least 3 minutes and 36 seconds, 30 minutes or any 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.

[...]

(TRACK CHANGES VERSION)

CHAPTER 11.2.

INFECTION WITH *BONAMIA EXITIOSA*

[...]

Article 11.2.3.

Measures for the importation 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 measure 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) 2) frozen oyster meat; and~~

~~b) 2) 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.

[...]

(TRACK CHANGES VERSION)

CHAPTER 11.3.

INFECTION WITH *BONAMIA OSTREAE*

[...]

Article 11.3.3.

Measures for the importation 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 measure 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.

[...]

(TRACK CHANGES VERSION)

CHAPTER 11.4.

INFECTION WITH *MARTEILIA REFRINGENS*

[...]

Article 11.4.3.

Measures for the importation 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 measure 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 three3 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*.

[...]

(TRACK CHANGES VERSION)

CHAPTER 11.5.

INFECTION WITH PERKINSUS MARINUS

[...]

Article 11.5.3.

Measures for the importation 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 measure 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°C for at least three60 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*.

[...]

(TRACK CHANGES VERSION)

CHAPTER 11.6.

INFECTION WITH *PERKINSUS OLSENI*

[...]

Article 11.6.3.

Measures for the importation or transit of aquatic animals and aquatic animal products for any purpose regardless of the infection with *P. olsenii* 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 measure conditions related to infection with *P. olsenii*, regardless of the infection with *P. olsenii* 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°C for at least three60 minutes and 36 seconds minutes, or a time/temperature equivalent that inactivates *P. olsenii*.~~
- ~~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. olsenii* 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. olsenii*, 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. olsenii* 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. olsenii*, regardless of the infection with *P. olsenii* 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. olsenii*.

[...]

(TRACK CHANGES VERSION)

CHAPTER 11.7.

INFECTION WITH *XENOHALIOTIS CALIFORNIENSIS*

[...]

Article 11.7.3.

Measures for the 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 these aquatic animal products listed below, Competent Authorities should not require any sanitary measure 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.~~

- ~~a1) 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, for 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 *XENOHALLOTIS 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*.

[...]

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 following 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 following 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.

[...]

CHAPTER 9.3.
INFECTION WITH DECAPOD IRIDESCENT VIRUS 1

[...]

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).

[...]

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 (*Oncorhynchus 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*).

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

[...]

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).

[...]

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.~~

[...]

SECTION 2.2.

DISEASES OF CRUSTACEANS

CHAPTER 2.2.0.

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 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 WOAHP *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.
- 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 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 WOAAH-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 WOAAH-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 (NHIP) 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 \[ictvonline.org\]](http://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 viruses, stocks, 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 WOAHA-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 or 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 WOA. H.

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 samples 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, amplifications 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 (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.

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NB: FIRST ADOPTED IN 2000; MOST RECENT UPDATES ADOPTED IN 2012.

CHAPTER 2.2.2.

INFECTION WITH *APHANOMYCES ASTACI* (CRAYFISH PLAGUE)

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-Urbeondo *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-Urbeondo *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 (Kozubiková *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 development of clinical disease and mortalities, and those that are infected without associated-but do not display any significant clinical disease or mortalities. All life stages 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 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 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

~~North American crayfish~~ **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 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. 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

~~North American crayfish~~ **Susceptible 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 intersternal 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 pereopods (walking legs), particularly the proximal joint, eyestalks and finally the gills.

~~North American crayfish~~ **Susceptible 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-Urbeondo *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-Urbeondo *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. CaCl₂ stimulates zoospore emergence from primary cysts, whereas MgCl₂ 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 WOAHS 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) (Oidtman *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 animals (crayfish, finfish) only from sources known to be free from infection with *A. astaci*).
 2. Movements of potentially infected live or dead crayfish, potentially contaminated water, equipment or any other item that might carry the pathogen from an infected to an uninfected site holding susceptible species should be prevented.
 3. If transfers of finfish or crayfish are being planned, these should not come 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 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.

The time between sampling of live animals and delivery to the investigating laboratory should not exceed 24 hours.

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 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, 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

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. 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 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 |
| <i>In-situ</i> hybridisation | | | | | | | | | | | | |
| 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); 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.

¹For confirmatory diagnoses, methods need to be carried out in combination (see Section 6). ²Susceptibility of early and juvenile life stages is described in Section 2.2.3.

³Specify 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. 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 Laboratory⁴.

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 H₂O) clean disposable swabs. The soft abdominal cuticle is then excised and 30–50 mg 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

⁴ <https://www.woah.org/en/what-we-offer/expertise-network/reference-laboratories/#ui-id-3>

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 <i>et al.</i> , 2009, Strand, 2013; GenBank Accession No.: AM947024 | | | |
| <i>Aphanomyces astaceus</i> / <i>A. fennicus</i> / 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 <u>500 nM</u> 200 nM | <u>50-40</u> cycles of: 95°C/15 sec and <u>60-58</u> °C/ <u>30</u> <u>60</u> sec |
| Alternative method 2: Strand <i>et al.</i> to be published; GenBank Accession No.: AM947024 | | | |
| <i>Aphanomyces astaceus</i> / <i>astaci</i> /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 <u>500 nM</u> 200 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.*, 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 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 <i>et al.</i> , 2006; GenBank Accession No.: AY310499; <u>Product amplicon</u> size: 569 bp | | | |
| <i>Aphanomyces astaceus</i> / <i>A. fennicus</i> / ITS | Fwd: GCT-TGT-GCT-GAG-GAT-GTT-CT Rev: CTA-TCC-GAC-TCC-GCA-TTC-TG- | 500 nM <u>500 nM</u> | 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

~~The size of the PCR amplicon is verified by agarose gel electrophoresis, 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, the modified assay by Strand *et al.* (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 WOAHP 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 WOAHA 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 status⁵

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:

- 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

⁵ For example transboundary commodities.

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 |
|-----------|--------------|--------------------|------------------------|---------|---------|---------|----------------|----------|
| | | | | | | | | |

DSe = diagnostic sensitivity, DSp = diagnostic specificity, n = number of samples used in the 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 |
|-----------|--------------|--------------------|------------------------|---------|---------|---------|----------------|----------|
| | | | | | | | | |

DSe = diagnostic sensitivity, DSp = diagnostic specificity, n = number of samples used in the 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: Zoospore 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., GIAN SANTE 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 über 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., 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., 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.

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.

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.

VRÅLSTAD 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.

VRÅLSTAD 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.

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* *

NB: There is a WOA Reference Laboratory for infection with *Aphanomyces astaci* (crayfish plague)
(please consult the WOA 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 WOAHA 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)

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 (PvNV) (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 *Sarothroviridae*.

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 | <i>Aeshna</i> sp. | dragonfly |
| Artemiidae | <i>Artemia</i> sp. | brine shrimps |
| Belostomatidae | <i>Belostoma</i> sp. | giant water bug |
| Dytiscidae | <i>Cybister</i> sp. | beetle |
| Notonectidae | <i>Notonecta</i> sp. | backswimmer |
| Palaemonidae | <i>Macrobrachium rude</i> | hairy river prawn |
| | <i>Macrobrachium malcolmsonii</i> | monsoon river prawn |
| Parastacidae | <i>Cherax quadricarinatus</i> | red claw crayfish |
| Penaeidae | <i>Penaeus japonicus</i> | kuruma prawn |
| | <i>Penaeus indicus</i> | Indian white prawn |
| | <i>Penaeus monodon</i> | 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 (moult) 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 WOA-H-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.

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 WOH 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 |
| <i>In-situ</i> 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.

¹For confirmatory diagnoses, methods need to be carried out in combination (see Section 6). ²Susceptibility of early and juvenile life stages is described in Section 2.2.3.

³Specify 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 <i>et al.</i> (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 <i>et al.</i> (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 <i>et al.</i> (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 <i>et al.</i> , 2003; Sahul Hameed <i>et al.</i> , 2004a, b; Sudhakaran <i>et al.</i> , 2007a) GenBank Accession No.: AY222840 (<i>MrNV</i>) and AY247793 (<i>XSV</i>); amplicon size: 425 bp (<i>MrNV</i>) and 546 bp (<i>XSV</i>) | | | |
| <i>MrNV</i> | Fwd: GCG-TTA-TAG-ATG-GCA-CAA-GG Rev: AGC-TGT-GAA-ACT-TCC-ACT-GG | 0.02 nM <u>400 nM</u> 0.02 nM <u>400 nM</u> | 30 cycles of: 94°C/40 sec, 55°C/40sec and 68°C/60 sec |
| <i>XSV</i> | 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 <u>400 nM</u> 0.02 nM <u>400 nM</u> | 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 <i>et al.</i> , 2007a); amplicon size: <u>205 bp (<i>MrNV</i>) and 236 bp (<i>XSV</i>)</u> | | | |
| <i>MrNV</i> | Internal primers: Fwd: GAT-GAC-CCC-AAC-GTT-ATC-CT Rev: GTG-TAG-TCA-CTT-GCA-AGA-GG | 0.02 nM <u>1000 nM</u> 0.02 nM <u>1000 nM</u> | 30 cycles of: 94°C/60 sec, 55°C/60 sec and 72°C/60 sec |
| <i>XSV</i> | Internal primers: Fwd: ACA-TTG-GCG-GTT-GGG-TCA-TA Rev: GTG-CCT-GTT-GCT-GAA-ATA-CC-3 | 0.02 nM <u>1000 nM</u> 0.02 nM <u>1000 nM</u> | 30 cycles of: 94°C/60 sec, 55°C/60 sec and 72°C/60 sec |
| Method 3: Multiplex RT-PCR (Yoganandhan <i>et al.</i> , 2005); GenBank Accession No.: AY222840 (<i>MrNV</i>) and AY247793 (<i>XSV</i>); amplicon size: 681 bp (<i>MrNV</i>) and 500 bp (<i>XSV</i>) | | | |
| <i>MrNV</i> | Fwd: GAT-ACA-GAT-CCA-CTA-GAT-GAC-C Rev: GAC-GAT-AGC-TCT-GAT-AAT-CC | 0.02 nM <u>400 nM</u> 0.02 nM <u>400 nM</u> | 30 cycles of: 94°C/40 sec, 55°C/40 sec and 68°C/60 sec |
| <i>XSV</i> | Fwd: GGA-GAA-CCA-TGA-GAT-CAC-G Rev: CTG-CTC-ATT-ACT-GTT-CGG-AGT-C | 0.02 nM <u>400 nM</u> 0.02 nM <u>400 nM</u> | 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 WOAHP 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 status⁶

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 *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

⁶ For example transboundary commodities.

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 | <i>Macrobrachium rosenbergii</i> | 100 (n=20) | 100 (n=20) | Western blot or ELISA | Sri Widada et al. (2003); Sahul Hameed et al. (2011) |
| Lateral flow immune-assay | Surveillance | PL from prawn hatcheries | Whole post-larvae | <i>Macrobrachium rosenbergii</i> | 100 (n=80) | 90 (n=80) | RT-PCR | Jamalpure et al. (2021) |

DSe = diagnostic sensitivity, DSp = diagnostic specificity, n = number of samples used in the 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 = number of samples used in the 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., BUNNONTAE M., PHIWSAIYAA K., SENAPINA S. & DHARA 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.

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- 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.2013.01.003><https://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., WENS 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., JAENGSAKONG C., PHIWISAIYA 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](https://doi.org/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. & HAMEED 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.

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NB: There is a WOA Reference Laboratory for infection with *Macrobrachium rosenbergii* nodavirus (white tail disease) (please consult the WOA 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

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*).

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 | <i>Palaemon serrifer</i> | carpenter prawn |
| | <i>Palaemon styliferus</i> | Pacific blue prawn |
| | <i>Macrobrachium sintangense</i> | Sunda river prawn |
| Parastacidae | <i>Cherax quadricarinatus</i> | red claw crayfish |
| Penaeidae | <i>Metapenaeus brevicornis</i> | yellow shrimp |
| | <i>Penaeus aztecus</i> | northern brown shrimp |
| | <i>Penaeus duorarum</i> | northern pink shrimp |
| | <i>Penaeus japonicus</i> | kuruma prawn |
| | <i>Penaeus merguensis</i> | banana prawn |
| | <i>Penaeus setiferus</i> | 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.).

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. merguensis*, *P. setiferus*, *Metapenaeus 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 WOA 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 | | | | | | | | | | | | |
| Conventional RT-PCR | ++ | ++ | ++ | 1 | ++ | ++ | ++ | 1 | | | | |
| Conventional PCR followed by amplicon sequencing | | | | | | | | | ++ | +++ | +++ | 1 |
| <i>In-situ</i> hybridisation | | | | | | ++ | ++ | 1 | | | | |
| Bioassay | | | | | + | + | + | 1 | | | | |
| 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 WOAHPathway (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.

¹For confirmatory diagnoses, methods need to be carried out in combination (see Section 6). ²Susceptibility of early and juvenile life stages is described in Section 2.2.3.

³Specify 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.

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 <i>et al.</i> , 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 <i>et al.</i> , 2004; GenBank Accession No.: FJ848675.1) | | | |
| YHV1 and GAV / ORF1b | <p>Primary (Amplicon size: 794 bp) GY1: 5GAC-ATC-ACT-CCA-GAC-AAC-ATC-TG GY4: GTG-AAG-TCC-ATG-TGT-GTG-AGA-CG</p> <p>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</p> <p>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</p> | 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 <i>et al.</i> , 2008b; GenBank Accession No.: FJ848675.1) | | | |
| YHV1 to YHV7 / ORF1b | <p>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</p> <p>YC-R1ab pool: TCT-TCR-CGT-GTG-AAC-ACY-TTC-TTR-GC TCT-GCG-TGG-GTG-AAC-ACC-TTC-TTG-GC</p> <p>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</p> <p>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).</p> | 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 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-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 WOAHA 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 status⁷

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

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) ~~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 conventional RT-PCR
- iv) Positive result by ISH

⁷ For example transboundary commodities.

- v) 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) 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 |
|-----------|--------------|--------------------|------------------------|---------|---------|---------|----------------|----------|
| | | | | | | | | |

DSe = diagnostic sensitivity, DSp = diagnostic specificity, n = number of samples used in the 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 used in the 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., SATTAMANS., CHAVISUTHANGKURA 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., CHAVISUTHANGKURA 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., 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>

WIJEGONAWARDANE P.K.M., COWLEY J.A., SITTIDILOKRATNA, N., PHETCHAMPAI, N., COWLEY, J.A., GUDKOV, N. & WALKER P.J. (2009). Homologous genetic recombination in the yellow head complex of nidoviruses infecting *Penaeus monodon* shrimp. *Virology* doi: 10.1016/j.virol.2009.04.015.

WIJEGONAWARDANE 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.

WIJEGONAWARDANE 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., SRIURAIATANA S., VICKERS J.E., AKRAJAMORN A., BOONSAENG V., PANYIM S., TASSANAKAJON A., WITHYACHUMNARNKUL B. & FLEGEL T.W. (1995). Yellow-head virus of *Penaeus monodon* is an RNA virus. *Dis. Aquat. Org.*, **22**, 45–50.

*
* *

NB: There is a WOA Reference Laboratory for infection with yellow head virus genotype 1
(please consult the WOA web site:
<https://www.woah.org/en/what-we-offer/expertise-network/reference-laboratories/#ui-id-3>).
Please contact the WOA 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.

CHAPTER 2.2.X.

INFECTION WITH DECAPOD IRIDESCENT VIRUS 1

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)* include: 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*).

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* include: giant tiger prawn (*Penaeus monodon*).

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*.

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, pereopods, 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 (*Helice 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 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 a 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 ISDL results, indicating DIV1 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, pereopods, 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, pereopods, 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 WOA 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 | Surveillance of apparently healthy animals | | | | Presumptive diagnosis of clinically affected animals | | | | 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 PCR | ++ | +++ | +++ | NA | +++ | +++ | +++ | 1 | +++ | +++ | +++ | 1 |
| Conventional PCR | ++ | ++ | ++ | NA | ++ | ++ | ++ | NA | | | | |
| Conventional nested PCR followed by amplicon sequencing | | | | | | | | | + | + | + | 1 |
| Conventional PCR followed by amplicon sequencing | | | | | | | | | +++ | +++ | +++ | 1 |
| <i>In-situ</i> 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 WOAHPathway (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

¹For confirmatory diagnoses, methods need to be carried out in combination (see Section 6). ²Susceptibility of early and juvenile life stages is described in Section 2.2.3.

³Specify 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 <i>et al.</i> , 2018a; GenBank Accession No.: MF599468.1 | | | |
| ATPase | SHIV-F: AGG-AGA-GGG-AAA-TAA-CGG-GAA-AAC | 500 nM | 40 cycles of 95°C/100 sec and 60°C/30 sec |
| | SHIV-R: CGT-CAG-CAT-TTG-GTT-CAT-CCA-TG Probe: FAM-CTG-CCC-ATC-TAA-CAC-CAT-CTC-CCG-CCC-TAMRA | 200 nM | |
| Method 2: Qiu <i>et al.</i> , 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 <i>et al.</i> , 2021; GenBank Accession No.: MF599468.1 | | | |
| ATPase | DIV1-F: AGG-AAA-GGA-AAC-GAA-AGA-AAT-TAT-ACC | 400 nM | 40 cycles of: 95°C/10 sec and 60°C/30 sec |
| | DIV1-R: GCT-TGA-TCG-GCA-TCC-TTG-A Probe: FAM-CAC-ATG-ATT-TGC-AAC-AAG-CTT-CCA-GCA-TAMRA | 200 nM | |

^(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 <i>et al.</i> , 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 <i>et al.</i> , 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 | 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 |
| | Nested PCR: SHIV-F2: CGG-GAA-ACG-ATT-CGT-ATT-GGG SHIV-R2: TTG-CTT-GAT-CGG-CAT-CCT-TGA | 400 nM | |

^(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 <i>et al.</i> , 2019; GenBank Accession No.: | | | |
| 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 <i>et al.</i> , 2020; GenBank Accession No.: | | | |
| 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 <i>et al.</i> , 2021; GenBank Accession No.: | | | |
| 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). 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 WOAHP 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 status⁸

⁸ For example transboundary commodities.

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,
- 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 used in the 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 used in the 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.

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.

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* *

NB: There is a WOAHP Reference Laboratory for infection with decapod iridescent virus 1
(please consult the WOAHP web site:
<https://www.woah.org/en/what-we-offer/expertise-network/reference-laboratories/#ui-id-3>).
Please contact the WOAHP Reference Laboratories for any further information on
infection with decapod iridescent virus 1

NB: FIRST ADOPTED IN 20XX.

SECTION 2.4.
DISEASES OF MOLLUSCS

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 WOAHA *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 WOA-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.

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 \[ictvonline.org\]](http://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 WOAHA-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 *Xenohalotis 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 WOAHA-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 ⁹ | 800 ml |
| Glycerol | 400 ml |

⁹ A saturated 37–39% aqueous solution of formaldehyde gas.

| | |
|---------------------|-----------------------------|
| Glacial acetic acid | 10% (add just prior to use) |
|---------------------|-----------------------------|

Carson's solution:

| | |
|---|--------|
| NaH ₂ PO ₄ ·2H ₂ O | 23.8 g |
| Sodium hydroxide (NaOH) | 5.2 g |
| Distilled water | 900 ml |
| 40% Formaldehyde ¹ | 100 ml |

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 OsO₄) 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 NCCOS5*, 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.

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NB: FIRST ADOPTED IN 1997. MOST RECENT UPDATES ADOPTED IN 2012.

CHAPTER 2.4.1.

INFECTION WITH ABALONE HERPESVIRUS

1. Scope

Infection with abalone herpesvirus means infection with the pathogenic agent *Aurivirus haliotidmalaco1* (commonly known as *Haliotid herpesvirus 1* [AbHV-1]) of the genus *Aurivirus* and the Family *Malacoherpesviridae*.

2. Disease information

2.1. Agent factors

2.1.1. Aetiological agent

AbHV-1 is the aetiological agent of abalone viral ganglioneuritis (AVG), 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 pāua (*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 laevis*), Blacklip abalone (*Haliotis rubra*) and hybrids of Greenlip × Blacklip abalone (*Haliotis laevis* × *Haliotis rubra*).

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: 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

2.2.3. Likelihood of infection by species, host life stage, population or sub-populations

All age classes of *H. diversicolor*, *H. rubra*, *H. laevis*, and hybrids of *H. rubra* × *H. laevis* 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 WOAHP 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 | D. Surveillance of apparently healthy animals | | | | E. Presumptive diagnosis of clinically affected animals | | | | F. 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 |
| <i>In-situ</i> 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 WOAHPathway (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.

¹For confirmatory diagnoses, methods need to be carried out in combination (see Section 6). ²Susceptibility 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.

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 <i>et al.</i> , 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 <i>et al.</i> , 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 <i>et al.</i> , 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 <i>et al.</i> , 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 digoxigenin (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 WOAHP 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 status¹⁰

¹⁰ For example transboundary commodities.

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) 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:

- 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

6.2 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 | <i>Haliotis rubra</i> | 100 (48) | 100 (48) | Histopathology | Corbeil et al., 2010 |
| Conventional PCR | | | | | | | | |
| Histopathology | | | | | | | | |

DSe = diagnostic sensitivity, DSp = diagnostic specificity, n = number of samples used in the 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 | <i>Haliotis laevigata</i> ; <i>H. rubra</i> ; <i>H. laevigata</i> x <i>H. rubra</i> hybrids | 90.1 (1452) | 97.7 (1452) | Histopathology | Caraguel et al., 2019 |
| Histopathology | | | | | | | | |

DSe = diagnostic sensitivity, DSp = diagnostic specificity, n = number of samples used in the 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 *Haliotis 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., MOHAMMADI., HYATT A.D., CRAMER S.G., FEGAN M. & CRANE M.ST.J. (2012a). Abalone viral ganglioneuritis: 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 pāua 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.ST.J. (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.ST.J., 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. *Virology*, **7**, 308. <http://www.virology.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.

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NB: There is a WOA Reference Laboratory for infection with abalone herpesvirus
(please consult the WOA web site:

<https://www.woah.org/en/what-we-offer/expertise-network/reference-laboratories/#ui-id-3>).

Please contact WOA Reference Laboratories for any further information on infection with abalone herpesvirus

NB: FIRST ADOPTED IN 2012.

CHAPTER 2.4.4.

INFECTION WITH *MARTEILIA REFRINGENS*

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 (Mérout 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*). 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*). 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*).

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 chlorophyll-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 (Mérout *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 | G. Surveillance of apparently healthy animals | | | | H. Presumptive diagnosis of clinically affected animals | | | | I. 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 |
| Real-time PCR | +++ | +++ | +++ | 3 | +++ | +++ | +++ | NA | +++ | +++ | +++ | NA |
| Conventional PCR | ++ | ++ | ++ | 2 | +++ | +++ | +++ | NA | | | | |
| Conventional PCR followed by amplicon sequencing | | | | | | | | | +++ | +++ | +++ | NA |
| <i>In-situ</i> 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.

¹For confirmatory diagnoses, methods need to be carried out in combination (see Section 6). ²Susceptibility of early and juvenile life stages is described in Section 2.2.3.

³Specify 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 oysters/mussels or freshly dead 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.

After 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.

Sections 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 <i>et al.</i> (2017); GenBank Accession No.: MH304865.1 | | | |
| <i>M. refringens</i> types O and M | Fwd Mare-F: YCA-GGC-GAG-TGC-TCT-CGT-T Rev Mare-R: TGA-TCT-GAT-ATT-ATT-CAG-CTG-TTC-GA | 400 nM 400 nM | 50 cycles of: 95°C/3 sec and 60°C/30 sec |
| | Probe Mare-O: CCT-TTC-CCC-GAC-GGC (VIC MGB-NFQ) Probe MareM: GCT-TGC-CCT-ACG-GCC (FAM MGB-NFQ) | 80 nM 80 nM | |
| Method 2: EURL (2023); GenBank Accession No.: MH304863.1 | | | |
| <i>M. refringens</i> types O and M | Fwd TaqMar-F: GTG-TTC-GGC-ACG-GGT-AGT Rev TaqMar-R: TGA-TCT-GAT-ATT-ATT-CAG-CTG-TTC-G | 100 nM 300 nM | 40 cycles of: 95°C/30 sec and 60°C/1 min |
| | TaqProb-O: GCC-CTT-TCC-CCG-ACG-GCC-G (FAM-BHQ-1) TaqProb-M: GCG-CTT-GCC-CTA-CCG-CCG-TGC (HEX-BHQ-1) | 250 nM 250 nM | |

| Method 3: Canier et al. (2020); GenBank Accession No.: MH342044.1 | | | |
|--|---|----------------------------|--|
| <i>M. refringens</i> Also amplifies <i>M. cochillia</i> and <i>M. sydneyi</i> | 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 |
| 18S | | | |

^(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 | | | |
| <i>M. refringens</i> types M and O Also amplifies <i>M. cochillia</i> 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] | | | |
| <i>M. refringens</i> types M and O Also amplifies <i>M. cochillia</i> 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 1 130 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] | | | |
| <i>Marteilia</i> spp. amplifies <i>M. refringens</i> types M and O, <i>M. cochillia</i> , and possibly other species | 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 |
| 18S | | | |
| Note: according to the alignment of available sequences Rev SAS1 primer sequence should be: TTC-GG-TGG-TCT-TGA-AAG-GC | | | |

^(a)A denaturation step prior to cycling has not been included.

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 WOA 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 <i>et al.</i> (1999) | <i>Marteilia</i> sp. 18S | Digoxigenin-labelled PCR product obtained with SS2/SAS1 primers | 266 bp |
| Le Roux <i>et al.</i> (2001) | <i>M. refringens</i> types M and O ITS1 | Digoxigenin-labelled PCR product obtained with Pr4/Pr5 primers | 412 bp |
| Lopez-Flores <i>et al.</i> (2004) | <i>M. refringens</i> 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 5 µl 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 WOA 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 WOAHP 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 status¹¹

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

¹¹ For example transboundary commodities.

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

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 = number of samples used in the 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 The Netherlands, representative of 3 different levels of prevalence (free, mild, high) | Section of tissues including visceral mass | Flat oysters | 70% (200) | 99% (200) | <i>In-situ</i> hybridisation (18S probe) Bayesian analyses | Thébault <i>et al.</i> , 2005 |

| Test type | Test purpose | Source populations | Tissue or sample types | Species | DSe (n) | DSp (n) | Reference test | Citation |
|---|--------------|---|--|--------------|-------------|-------------|---|-------------------------------|
| <i>In-situ</i> hybridisation (18S probe) | Surveillance | Field samples from France and The Netherlands, representative of 3 different levels of prevalence (free, mild, high) | Section of tissues including visceral mass | Flat oysters | 90% (200) | 99% (200) | Histology Bayesian analyses | Thébault <i>et al.</i> , 2005 |
| Real-time PCR (Canier <i>et al.</i> , 2020) | Surveillance | Field samples from the 3 main producing areas in France, representative of 3 different levels of prevalence (free, low, high) | Gills and digestive gland tissues | Flat oysters | 87,2% (386) | 98,4% (386) | Conventional PCR (Le Roux <i>et al.</i> , 2001) Bayesian analyses | Canier <i>et al.</i> , 2020 |
| Conventional PCR (Le Roux <i>et al.</i> , 2001) | Surveillance | Field samples from the 3 main producing areas in France, representative of 3 different levels of prevalence (free, low, high) | Gills and digestive gland tissues | Flat oysters | 60.7% (386) | 99.9% (386) | Real-time PCR (Canier <i>et al.</i> , 2020) Bayesian analyses | Canier <i>et al.</i> , 2020 |

DSe = diagnostic sensitivity, DSp = diagnostic specificity, n = number of samples used in the 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 Paramyxia 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). Martelliosis 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* (Paramyxia). *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.

THEBAULT A., BERGMANN S., POUILLON 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.

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NB: There is a WOA Reference Laboratory for infection with *Marteilia refringens*
(please consult the WOA web site:

<https://www.woah.org/en/what-we-offer/expertise-network/reference-laboratories/#ui-id-3>).

Please contact WOA Reference Laboratories for any further information on infection with *Marteilia refringens*

NB: FIRST ADOPTED IN 1995 AS MARTEILIOSIS. MOST RECENT UPDATES ADOPTED IN 2012.

CHAPTER 2.4.5.

INFECTION WITH *PERKINSUS MARINUS*

[...]

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*).

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*).

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*).

[...]
