



Organisation
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Original: English
September 2017

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

Paris, 13–20 September 2017

The OIE Aquatic Animal Health Standards Commission (Aquatic Animals Commission) met at OIE Headquarters in Paris from 13 to 20 September 2017. The list of participants is presented in [Annex 1](#).

The Aquatic Animals Commission thanked the following Member Countries for providing written comments: Australia, Brazil, Canada, China (People's Rep. of), Chinese Taipei, Cook Islands, Japan, New Caledonia, New Zealand, Singapore, Switzerland, United States of America (USA), Thailand, the Member States of the European Union (EU), and the African Union Interafrican Bureau for Animal Resources (AU-IBAR) on behalf of African Member Countries of the OIE.

The Aquatic Animals Commission reviewed Member Country comments and amended relevant chapters of the OIE *Aquatic Animal Health Code* (the *Aquatic Code*) and OIE *Manual of Diagnostic Tests for Aquatic Animals* (the *Aquatic Manual*) where appropriate. The amendments are shown in the usual manner by 'double underline' and '~~strike through~~', and are presented in the Annexes to this report. In Annexes, amendments proposed at this meeting are highlighted with a coloured background in order to distinguish them from those proposed previously.

The Aquatic Animals Commission considered all Member Country comments that were submitted on time and supported by a rationale. However, the Commission was not able to draft a detailed explanation of the reasons for accepting or not each of the proposals received and focused its explanations on the most significant issues.

The Aquatic Animals Commission encourages Member Countries to refer to previous reports when preparing comments on longstanding issues. The Commission also draws the attention of Member Countries to the reports of *ad hoc* Groups, which include important information, and encourages Member Countries to review these reports together with the report of the Commission, where relevant. These reports are readily available on the [OIE website](#).

The table below summarises the texts as presented in the Annexes. Member Countries should note that texts in [Annexes 3 to 27B](#) are presented for Member Countries' comments and [Annexes 28 to 31](#) are presented for Member Countries' information.

Comments on [Annexes 3 to 27B](#) of this report must reach OIE Headquarters by the **9th January 2018** to be considered at the February 2018 meeting of the Aquatic Animals Commission. Comments received after the due date will not be submitted to the Code Commission for its consideration.

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

The Aquatic Animals Commission again strongly encourages Member Countries to participate in the development of the OIE's international standards by submitting comments on this report, and prepare to participate in the process of adoption at the General Session. Comments should be submitted as Word files rather than pdf files because pdf files are difficult to incorporate into the Aquatic Animals Commission's working documents. Comments should be submitted as specific proposed text changes, supported by a structured rationale or by published scientific references. Proposed deletions should be indicated in 'strickethrough' and proposed additions with 'double underline'. Member Countries should not use the automatic 'track-changes' function provided by word processing software as such changes are lost in the process of collating Member Countries' submissions into the Aquatic Animals Commission's working documents. Member Countries are also requested **not** to reproduce the full text of a chapter as this makes it easy to miss comments while preparing the working documents.

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4.11	Infection with white spot syndrome virus (Chapter 2.2.8.)	Annex 22	149
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2.7.and 5.3.	Infection with infectious hypodermal and haematopoietic necrosis virus (Chapter 5.5.4., Sections 2.2.1. and 2.2.2.)	Annex 26	225
5.4.	Assessment of kuruma shrimp (<i>Penaeus japonicus</i>); Section 2.2.2. Acute hepatopancreatic necrosis disease Chapter (2.2.1.).	Annex 27A Annex 27B	227 229
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2.11.	Assessment of a novel orthomyxo-like virus, tilapia lake virus, for inclusion in the OIE list of diseases	Annex 28	231
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I.	Aquatic Animal Health Standards Commission Work Plan for 2017/2018	Annex 31	267

A. Meeting with the Director General

Dr Monique Eloit, OIE Director General, met with the Aquatic Animals Commission and informed them that the new process for selection of experts for Specialist Commissions had been launched and that she considered this to be a significant step towards implementation of goals of the Sixth Strategic Plan. She also highlighted that this was an opportunity to create a list of suitable experts for consideration when convening *ad hoc* Groups, noting that there currently is no formal process for gathering such information. Dr Eloit encouraged the Aquatic Animals Commission to work with the Biological Standards Commission on the issue of how to better engage the network of Collaborating Centres in the goals of the OIE.

Dr Ingo Ernst, President of the Aquatic Animals Commission, informed Dr Eloit that the Aquatic Animals Commission recognised the importance of identifying how to better engage Reference Laboratories and Collaborating Centres in support of the work in aquatic animal health and are working with the Biological Standards Commission in reviewing the process of selecting and approving Collaborating Centres.

Dr Ernst noted that the Aquatic Animals Commission had discussed three important issues – emerging diseases, antimicrobial resistance (AMR) and improving engagement with Reference Centres – with the objective of identifying actions the Commission could take to address these issues. He emphasised the ongoing poor record of reporting emerging diseases by Member Countries and highlighted the Commission's willingness to continue to explore ways to address this issue noting that they will continue to raise awareness of emerging diseases through several means, including the development of technical disease cards. The Commission also discussed how Reference Centres could better support Member Countries in the area of aquatic animal health noting that some Collaborating Centres' annual reports included aquatic animal health related activities, for example the Collaborating Centre for New and Emerging Diseases. On the topic of AMR, Dr Ernst commented that there is a need to identify risk factors and possible pathways for the emergence of AMR resulting from the administration of antimicrobial agents in aquatic animals and the required data needed for their assessment. He informed Dr Eloit that the Commission is considering whether the designation of a Collaborating Centre for AMR in aquatic animals may be useful to assist in managing risks associated with AMR in aquatic animals.

B. Adoption of the agenda

The provisional agenda circulated prior to the meeting was discussed, updated, and agreed. The adopted agenda of the meeting is presented in [Annex 2](#).

C. Meeting with the President of the OIE Terrestrial Animal Health Standards Commission

The President of the Aquatic Animals Commission met with the President of the Terrestrial Animal Health Standards Commission (Code Commission) during the week when both Commissions were meeting. The Presidents discussed issues of mutual interest in the *Aquatic* and *Terrestrial Codes*, to facilitate harmonisation of relevant chapters in the two *Codes* when under review by the respective Commissions. Issues discussed included:

- Harmonisation of the User’s Guides for the *Aquatic* and *Terrestrial Codes*, where appropriate.
- Development of a guidance document on the application of the criteria for listing an OIE disease.
- Proposed changes to the Glossary for definitions of ‘biosecurity’ and ‘biosecurity plan’ in the *Aquatic Code* which are necessary for the new draft chapter on Biosecurity in Aquaculture Establishments in Section 4. The Code Commission expressed an interest in this work and the new chapter, noting that it would look at this in the future.
- The President of the Code Commission noted it was continuing with the proposed deletion of the definition of disease from the Glossary but will keep the definitions for listed disease, emerging and notifiable disease.
- Revisions of Chapter 1.4. on Surveillance in the *Terrestrial Code* and future revisions of the corresponding chapter in the *Aquatic Code*.
- Chapters on zoning and compartmentalisation – the President of the Aquatic Animals Commission noted its plan to develop a new chapter on the application of zoning. The President of the Code Commission noted that the chapter in the *Terrestrial Code* was currently under revision and it was not planned to develop a new chapter on the application of zoning.
- With regard to the Code Commission’s proposed new chapter on management of outbreaks of listed diseases, the President of the Aquatic Animals Commission noted its plans for a different approach, which will include the development of two new chapters, one on emergency disease preparedness and one on management of disease outbreaks.

D. Meeting with the President of the OIE Biological Standards Commission

The President of the Aquatic Animals Commission met with the President of the Biological Standards Commission during the week when both Commissions were meeting. The Presidents discussed issues of mutual interest in the *Aquatic* and *Terrestrial Manuals*, notably how the newly adopted SOPs for Reference Laboratories would be implemented by both Commissions from January 2018. The President of the Biological Standards Commission also provided an update on the outcome of its brainstorming review of the procedures for approval and maintenance of OIE Collaborating Centre status and provided a list of six main focus areas and specialties it had identified. The President of the Aquatic Animals Commission noted that the Aquatic Animals Commission would review the document during its meeting and provide feedback on the list for consideration by the Biological Standards Commission.

E. OIE Aquatic Animal Health Code

1. Texts circulated for Member Country comments at the February 2017 meeting

1.1. General comments

The Aquatic Animals Commission noted that some Member Countries submitted comments related to revised texts that had been adopted at the 2017 General Session. The Commission did not consider these comments as they did not consider that any comments were critical to the understanding of the adopted text.

The Aquatic Animals Commission noted that, as stated in previous reports, they did not consider Member Country comments submitted without a rationale.

The Aquatic Animals Commission reminded Member Countries that with the adoption of the revised definition for aquatic animals the term ‘live aquatic animal’ would be amended to ‘aquatic animal’ throughout the *Aquatic Code* given that the revised definition explicitly refers to live aquatic animals.

1.2. Criteria for listing species as susceptible to infection with a specific pathogen (Chapter 1.5.)

Comments were received from Australia, Canada, Chinese Taipei, Japan, New Caledonia, New Zealand, Switzerland, Thailand, USA, EU and OIE experts.

The Aquatic Animals Commission noted that of the Member Country comments received all but one was in support of the intent of the new Article 1.5.9. The new Article 1.5.9. includes criteria to designate susceptibility to diseases that have a broad host range at a taxonomic ranking of genus or higher, rather than at the level of individual species.

It is intended that Article 1.5.9. will only apply to diseases that have a broad host range. This is defined in Article 1.5.9. as a disease that has at least one susceptible species from within each of three or more families. If this criterion is not met then Article 1.5.9. would not be applied and individual susceptible species would be listed in the relevant disease-specific chapter.

The Aquatic Animals Commission wished to emphasise that assessments would be made on the basis of information available for individual species; however, in accordance with Article 1.5.9, the outcome of the assessment may be listing of susceptibility at a taxonomic ranking of genus or higher.

The Aquatic Animals Commission wished to clarify that the criteria in Chapter 1.5. are used to determine which species (or taxonomic groups of species should Article 1.5.9. be adopted) are listed in the scope (Article X.X.2.) of each disease-specific chapter of the *Aquatic Code*. The criteria are applied by *ad hoc* Groups and the outcomes of those assessments are considered by the Commission and then provided to Member Countries for comment.

The Aquatic Animals Commission wished to remind Member Countries that the aim of the *Aquatic Code* is to prevent the spread of aquatic animal diseases and assure the sanitary safety of international trade in aquatic animals. Application of the current criteria in Chapter 1.5. to diseases with a proven broad host range (*e.g.*, infection with *Aphanomyces astaci* and infection with white spot syndrome virus) would result in a substantial reduction in the list of susceptible species for these diseases. As a consequence, the *Aquatic Code* measures for these diseases would not apply to many host species that are likely to be susceptible. The Commission noted that this circumstance would be contrary to the purposes of the *Aquatic Code* and could lead to the spread of listed diseases.

In response to requests for clarification of the criteria for listing susceptible species at a taxonomic ranking of genus or higher, the Aquatic Animals Commission proposed several amendments to the text in Article 1.5.9.

The Aquatic Animal Commission acknowledged that it is difficult to demonstrate that a species is refractory to infection, but did not agree to delete point 1 c) of Article 1.5.9. The Commission reminds Member Countries that the rationale for the new article is to list susceptible species at a taxonomic ranking higher than species only when the evidence supports a high likelihood that all the species at the taxonomic ranking are susceptible. Listing cannot be made at a taxonomic ranking if there is evidence of refractory susceptible species within that ranking.

For reasons of consistency and in response to comments, the Aquatic Animal Commission made editorial changes throughout the chapter. The word “pathogen” was replaced by “pathogenic agent” and “disease chapter” by “disease-specific chapter”.

The Aquatic Animals Commission did not agree to include “expression of clinical disease or pathological changes” to the considerations of environmental factors in Article 1.5.4., because clinical disease is not necessary for transmission.

The Aquatic Animal Commission agreed with a Member Country to amend the wording of Article 1.5.8.

The revised Chapter 1.5. Criteria for listing species as susceptible to infection with a specific pathogen is presented in [Annex 3](#) for Member Country comment.

1.3. Criteria to assess the safety of aquatic animal commodities (Chapter 5.4.)

Comments were received from Chinese Taipei, New Zealand, Switzerland and EU.

The Aquatic Animals Commission did not agree with a Member Country comment to revert to the word commodities in the title noting that the definition for commodities means ‘aquatic animals, aquatic animal products, biological products and pathological material’ but only aquatic animal products have been assessed to meet the criteria presented in this chapter.

The Aquatic Animals Commission did not agree with the rationale that cleaned and disinfected eggs could be regarded as safe commodities reminding Member Countries that disinfected eggs do not meet the criteria in Chapter 5.4. and are addressed in a specific article in relevant disease-specific chapters.

The Aquatic Animals Commission did not agree with a Member Country comment to include additional items such as equipment, in point 1 *b*) of Article 5.4.1. noting that this article refers to the criteria to assess the safety of aquatic animal products, noting that point 2) specifically addresses the management of cross contamination of the product and is not about managing biosecurity risks.

In response to a Member Country comment requesting clarification as to what constitutes a ‘small amount of raw waste tissues’ in criterion 2 in Article 5.4.2., the Aquatic Animals Commission referred to the February 2009 report of the *ad hoc* Group on Safety of Products Derived from Aquatic Animals where it is stated that:

“The *ad hoc* Group advised that the meaning of the terminology ‘small amount of raw waste tissues’ will depend on the commodity, and should be described as part of the assessment of each commodity. For example, a skinless fillet would be expected to generate a minimal amount of waste tissues when used by the consumer, whereas a whole shrimp would be expected to generate a larger amount of waste tissues (*e.g.*, shell, legs, head and tail fan), as there is a larger quantity of inedible tissues.”

The Aquatic Animals Commission reminded Member Countries that all the assessments by the *ad hoc* Group took into consideration what was considered to be ‘only a small amount of waste tissues’, *e.g.*, ‘wastes include head, backbone and skin.’ Assessments of aquatic animals and aquatic animal products made against the criteria in Chapter 5.4. are available on the [OIE website](#).

The Aquatic Animals Commission reminded Member Countries that all assessments are evidence based so there was no need to include the word ‘evidence’ in criterion 2 of Article 5.4.2.

In response to a Member Country comment the Aquatic Animals Commission reminded Member Countries that the meaning of the word ‘safety’ in this chapter is described in the first sentence of the chapter, *i.e.* ‘safety’ is applied only to animal health considerations for listed diseases.

The Aquatic Animals Commission did not agree with a Member Country comment to add a new point in Article 5.4.2. to address transmission risk of pathogenic agents noting that transmission risks are addressed within each disease-specific chapter *i.e.* Articles X.X.11. (crustacean, fish and mollusc chapters)/Article X.X.12. (amphibian chapters).

The revised Chapter 5.4. Criteria to assess the safety of aquatic animal commodities is presented in [Annex 4](#) for Member Country comment.

2. Other issues

2.1. User's Guide

The Aquatic Animals Commission reviewed amendments that had been adopted in the User's Guide of the *Terrestrial Code* in 2016 and made amendments to the User's Guide in the *Aquatic Code* to ensure alignment between the two Guides, where relevant.

The Aquatic Animals Commission also amended point 3) of Section C. regarding susceptibility of species to reflect recent work undertaken to review the list of susceptible species in disease-specific chapters. The Commission also amended point 5) of Section C. regarding safety of aquatic animal products for trade to improve readability.

The revised User's Guide is presented in [Annex 5](#) for Member Country comment.

2.2. Glossary

Aquatic animal health status

The Aquatic Animals Commission reviewed amendments adopted at the 2017 OIE General Session to the definition for 'animal health status' in the *Terrestrial Code*. The Commission agreed to make similar amendments to the *Aquatic Code* definition for 'aquatic animal health status' to improve readability.

Self-declaration of freedom from disease

The Aquatic Animals Commission proposed to delete the words 'from disease' from the definition of 'self-declaration of freedom from disease' noting that the reference to OIE-listed disease is included in the definition. They agreed that this amendment would result in a more extensive use of this defined term in relevant disease-specific chapters.

Biosecurity plan

The Aquatic Animals Commission agreed with the recommendation of the *ad hoc* Group on Aquatic Animal Biosecurity for Aquaculture Establishments to review and amend the definition for 'biosecurity plan' to ensure that it includes aquaculture establishments. The Commission also agreed to make some additional amendments to improve clarity and readability.

Biosecurity

In light of the proposed changes to the definition for 'biosecurity plan' the Aquatic Animals Commission proposed some amendments to the definition for 'biosecurity' to ensure it is aligned with the proposed changes to 'biosecurity plan'.

Susceptible species

The Aquatic Animals Commission amended the definition for 'susceptible species' to ensure it is aligned with Chapter 1.5. Criteria for listing species as susceptible to infection with a specific pathogen.

The revised Glossary definitions are presented in [Annex 6](#) for Member Country comment.

2.3. Diseases listed by the OIE (Chapter 1.3.)

Amended fish disease names

The Aquatic Animals Commission reviewed the names used for all listed fish diseases in Article 1.3.1. and made changes in line with the accepted convention: ‘infection with pathogenic agent X’. They noted that when this naming convention is applied for diseases commonly recognised by the name of the disease (and which differ significantly from the pathogen name) then the disease name would be retained in brackets in the relevant chapter title.

The Commission agreed that these amended names would be applied to the relevant fish disease-specific chapters in the *Aquatic Code* (see Item 2.8.).

The revised Chapter 1.3. is presented in Annex 7 for Member Country comment.

Assessment of a novel orthomyxo-like virus, tilapia lake virus, for inclusion in the OIE list of diseases

The Aquatic Animals Commission reviewed the assessment of tilapia lake virus (TiLV) against the new criteria in Chapter 1.2. Criteria for listing aquatic animal diseases noting that revised criteria had been adopted at the 2017 OIE General Session. The Commission also considered new scientific information published since their last meeting in February 2017.

The Aquatic Animals Commission re-evaluated evidence for the third criterion for listing a disease by the OIE: “a precise case definition is available and a reliable means of detection and diagnosis exists”. The Commission considered information in a recent publication describing a new diagnostic assay for TiLV (Dong *et al.*, Emergence of tilapia lake virus in Thailand and an alternative semi-nested RT-PCR for detection. *Aquaculture*, doi: 10.1016/j.aquaculture.2017.04.019). The Commission agreed that with this additional information the criterion is still not met because of insufficient information concerning analytical and diagnostic specificity and sensitivity of the assay. The Commission expressed their thanks to the People’s Republic of China, Vietnam and Thailand for providing information on the performance of available assays for TiLV.

The Aquatic Animals Commission noted that TiLV continues to be reported in new countries and poses a significant threat to many countries given the worldwide importance of tilapia farming and international trade in this species. It was also noted that some recent disease events associated with TiLV have not been reported to the OIE. An understanding of the geographic distribution of TiLV is essential for efforts to control its possible spread. Member Countries are, therefore, encouraged to investigate mortality and morbidity events in tilapines and submit gene sequences to the National Center for Biotechnology Information (NCBI) gene bank.

The Aquatic Animals Commission again reminded Member Countries that TiLV meets the definition of an “emerging disease” and, as such, should be reported to the OIE in accordance with Article 1.1.4. of the *Aquatic Code*.

In the absence of a Reference Laboratory for TiLV Member Countries investigating mortality and morbidity events in tilapines and requiring advice could contact the Collaborating Centre for New and Emerging Diseases for assistance (hosted by the Australian Animal Health Laboratory, CSIRO, refer to <http://www.oie.int/our-scientific-expertise/collaborating-centres/list-of-centres/>). The Aquatic Animals Commission encouraged Member Countries which do not have assays established for TiLV to take up an offer made by the Chilean OIE Reference Laboratory for ISA (Dr Sergio Hernán Marshall González; <http://www.oie.int/our-scientific-expertise/reference-laboratories/list-of-laboratories/>) to assist Member Countries in the diagnosis of TiLV.

The assessment of TiLV against the new criteria for listing in Chapter 1.2. is presented in Annex 28 for Member Country information.

2.4. OIE procedures relevant to the Agreement on the Application of Sanitary and Phytosanitary Measures of the World Trade Organization (Chapter 5.3.)

The Aquatic Animals Commission noted that a revised Chapter 5.3. of the *Terrestrial Code* had been adopted at the 2017 General Session. Given the importance of aligning these two chapters in both Codes, the Aquatic Animals Commission agreed to amend Chapter 5.3. of the *Aquatic Code* to ensure alignment with the equivalent chapter of the *Terrestrial Code*, where relevant.

The Aquatic Animals Commission proposed some additional amendments to those of Chapter 5.3. of the *Terrestrial Code* chapter including:

In paragraph 1 of Article 5.3.1., the Aquatic Animals Commission proposed to replace ‘more stringent’ with ‘that exceed’ regarding sanitary measures, noting that stringent refers to precision and exactness, whereas the article concerns implementing measures which achieve a higher level of protection.

Amendments were made in point 4) of Article 5.3.3. and point 2) of Article 5.3.4. for improved readability.

Amendments were made in paragraph 2 of Article 5.3.7. to reflect text in the *Aquatic Code* noting that there are differences between the two Codes on this point.

The revised Chapter 5.3. is presented in Annex 8 for Member Country comment.

2.5. Amphibian diseases

2.5.1. New draft chapter for Infection with *Batrachochytrium salamandrivorans* (Chapter 8.X.)

In light of the adoption of *Batrachochytrium salamandrivorans* in Chapter 1.3. Diseases listed by the OIE, at the 2017 OIE General Session, the Aquatic Animals Commission developed a new draft Chapter 8.X. Infection with *Batrachochytrium salamandrivorans* for inclusion in the *Aquatic Code*.

The Aquatic Animals Commission highlighted that the proposed list of susceptible species in Article 8.X.2. is based on a recent European Food Safety Authority* report but as these species have not been assessed against the criteria in Chapter 1.5., the Commission placed these species ‘under study’. The Commission requested that an *ad hoc* Group be convened to undertake these assessments. The Commission also requested that the *ad hoc* Group undertake assessments for the list of susceptible species for Chapter 8.1. Infection with *Batrachochytrium dendrobatidis* considering that the two species are closely related.

The Aquatic Animals Commission noted that the proposed lists of aquatic animal products in Articles 8.X.3. and 8.X.12. are the same as those listed in Articles 8.1.3. and 8.1.12. Infection with *Batrachochytrium dendrobatidis* (Chapter 8.1), a pathogen in the same genus. The Commission agreed that this was an appropriate approach given that there are insufficient data on the stability of the agent (effective inactivation methods). In addition, the Commission noted that the heat-treated products would be reviewed as part of the proposed new work on safe aquatic animal products (see Item 2.9.).

The Aquatic Animals Commission noted that this draft chapter includes horizontal changes being proposed in the fish disease-specific chapters (see Item 2.8.1.).

Reference:

*European Food Safety Authority, Baláž V, Gortázar Schmidt C, Murray K, Carnesecchi E, Garcia A, Gervelmeyer A, Martino L, Munoz Guajardo I, Verdonck F, Zancanaro G and Fabris C, 2017. Scientific report: scientific and technical assistance concerning the survival, establishment and spread of *Batrachochytrium salamandrivorans* (Bsal) in the EU. EFSA Journal 2017;15(2):4739, 73 pp. doi:10.2903/j.efsa.2017.4739.

The new draft Chapter 8.X. is presented in [Annex 9A](#) for Member Country comments.

2.5.2. Infection with *Batrachochytrium dendrobatidis* (Chapter 8.1.) and Infection with ranavirus (Chapter 8.2.)

In light of the development of a new draft chapter Infection with *Batrachochytrium salamandrivorans* (Chapter 8.X.) that also includes the horizontal amendments being proposed (see Item 2.8.1.), the Aquatic Animals Commission proposed to make the same horizontal changes to Chapters 8.1. and 8.2. to ensure that all three amphibian chapters are aligned, where relevant.

The revised chapter Infection with *Batrachochytrium dendrobatidis* (Chapter 8.1.) and Infection with ranavirus (Chapter 8.2.) are presented in [Annex 9B](#) and [Annex 9C](#), respectively, for Member Country comment.

2.6. Acute hepatopancreatic necrosis disease (Chapter 9.1.)

In light of recent publications of new non-*Vibrio* species that cause acute hepatopancreatic necrosis disease (AHPND) the Aquatic Animals Commission reviewed this information (see papers reviewed below) and determined that no amendments to the scope in Article 9.1.1. were required.

While recent publications demonstrated the presence of the plasmid carrying the PirA and PirB toxin genes, none of the studies re-isolated and identified the bacteria to demonstrate definitively that these bacterial species could reproduce the disease AHPND. The Aquatic Animals Commission noted that given the widespread presence of the PirA and PirB genes in nature it is important to ensure that before expanding the scope of the disease, there is definitive evidence to support any bacterial species as the pathogenic agent. Re-isolation and identification of the bacterial agent after determining the presence of the toxin genes as well as evidence to demonstrate that the bacterial species is the cause of AHPND (e.g. bioassay) is required to fulfil Koch's postulates.

References:

Kondo *et al.* 2015. Draft Genome Sequence of Non-*Vibrio* parahaemolyticus Acute Hepatopancreatic Necrosis Disease Strain KC13.17.5, Isolated from Diseased Shrimp in Vietnam. *Genome Ann* 3(5):1

Liu *et al.* (2015). Draft Genome Sequence of *Vibrio owensii* Strain SH-14, Which Causes Shrimp Acute Hepatopancreatic Necrosis Disease. *Genome Ann* 3(6):1

Han *et al.*, (2017) Four AHPND strains identified on Latin American shrimp farms. *Global Aquaculture Advocate*. Feb 3

Xiao *et al.* (2017). Shrimp AHPND-causing plasmids encoding the PirAB toxins as mediated by pirAB-Tn903 are prevalent in various *Vibrio* species. *Nature Scientific Reports*. 7.41277

Dong *et al.* (2017). Complete genome sequence of *Vibrio campbellii* strain 20130629003S01 isolated from shrimp with acute hepatopancreatic necrosis disease. *Gut Pathogens*. 9:31

Dong *et al.* (2017). An isolate of *Vibrio campbellii* carrying the pirVP gene causes acute hepatopancreatic necrosis disease. *Emerging Microbes and Infections*. 6.e2

Han *et al.* (2017). Characterization and pathogenicity of acute hepatopancreatic necrosis disease natural mutants, pirABvp (-) *V. parahaemolyticus*, and pirABvp (+) *V. campbellii* strains. *Aquaculture* 470:84

Liu *et al.* (2017). Rapid diagnosis of *Vibrio owensii* responsible for shrimp acute hepatopancreatic necrosis disease with isothermal recombinase polymerase amplification assay. *Molecular and Cellular Probes*. 33:4

2.7. Infection with infectious hypodermal and haematopoietic necrosis virus (Chapter 9.4.)

Since their February 2017 meeting, the Aquatic Animals Commission was made aware of new scientific information regarding susceptibility of *Macrobrachium rosenbergii* to infection with infectious hypodermal and haematopoietic necrosis virus (IHHNV) and had proposed that in Article 9.4.2. *M. rosenbergii* be placed 'under study' so that the Commission could undertake further assessment of its susceptibility. The Commission subsequently requested that the *ad hoc* Group on Susceptibility of Crustacean Species to Infection with OIE Listed Diseases re-assess the susceptibility of *M. rosenbergii* to infection with IHHNV against the criteria for listing in Chapter 1.5. taking into account the new scientific information that had been provided by a Member Country.

The *ad hoc* Group reviewed the reference Hsieh *et al.* (2006) and noted that both the location of lesions (hepatopancreas) and controls for *in situ* hybridization (ISH) were somewhat inconsistent for infection with IHHNV. The *ad hoc* Group agreed with the Member Country comment that other viruses infecting the hepatopancreas of *M. rosenbergii* can cause similar histopathology to that described in the figures presented in the Hsieh *et al.* (2006) paper. Thus it cannot be conclusively demonstrated that the lesions were the result of infection with IHHNV.

Taking this information into account the *ad hoc* Group agreed that *M. rosenbergii* does not fully fulfil categories C and D (pathology and location), leaving only A (replication) to be considered. Replication (A) can be determined by electron microscopy or by other means (*e.g.*, ISH). However, as there was mislabelling of the figures in the paper and potentially a lack of essential controls (*e.g.*, labelling of serial sections viewed in histology), the *ad hoc* Group concluded that the ISH reported in the Hsieh *et al.* (2006) paper should be considered inconclusive.

On the basis of this re-assessment, the *ad hoc* Group agreed that *M. rosenbergii* did not meet the criteria in Chapter 1.5. for listing in the *Aquatic Code* but agreed it should be included in Section 2.2.2. (Species with incomplete evidence for susceptibility) of Chapter 2.2.4. Infection with IHHNV of the *Aquatic Manual* (see Item 5.3).

The Aquatic Animals Commission agreed with the *ad hoc* Group recommendation and amended Article 9.4.2. accordingly.

The revised Article 9.4.2. is presented in [Annex 10](#) for Member Country comment.

2.8. Fish disease-specific chapters

2.8.1. Horizontal changes

The Aquatic Animals Commission reminded Member Countries that they had undertaken a thorough review of all disease-specific crustacean chapters in the *Aquatic Code* which had been circulated for Member Country comments and subsequently adopted at the 2017 General Session. The Commission had noted in their February 2017 report that these changes, of a horizontal nature, would also be made in other disease-specific chapters as the work related to susceptible species is applied. Given that this work has commenced in the fish disease-specific chapters, the Commission proposed to apply these horizontal amendments to all fish disease-specific chapters.

The Aquatic Animals Commission also reviewed and amended, where relevant, the title, Article 10.X.1. and made changes throughout the chapter in line with proposed amendments to the disease name, *i.e.* 'infection with pathogenic agent X' (see Item 2.3.).

The Aquatic Animals Commission noted that proposed amendments to Articles X.X.8., X.X.9., X.X.10. and X.X.11. (see Item 2.10.) have also been applied to all the amended fish disease-specific chapters.

Revised chapters Infection with *Aphanomyces invadans* (epizootic ulcerative syndrome) (Chapter 10.2.), Infection with salmonid alphavirus (Chapter 10.5.), Infectious haematopoietic necrosis (Chapter 10.6.), Koi herpesvirus disease (Chapter 10.7.), Red sea bream iridoviral disease (Chapter 10.8.), Spring viraemia of carp (Chapter 10.9.) and Viral haemorrhagic septicaemia (Chapter 10.10.) are presented in Annexes 14, 15, 16, 17, 18, 19 and 20, respectively, for Member Country comment.

2.8.2. List of susceptible species

In addition to the horizontal changes proposed above (see Item 2.8.1.) the Aquatic Animals Commission considered the report of the *ad hoc* Group on Susceptibility of fish species to infection with OIE listed diseases, which had applied the criteria for listing species as susceptible to infection with a specific pathogen according to Chapter 1.5. (see Item 3.1.). The Aquatic Animals Commission agreed to amend the list of susceptible species in Article X.X.2. for Chapters 10.1. Epizootic haematopoietic necrosis, Chapter 10.3. Infection with *Gyrodactylus salaris* and Chapter 10.4. Infection with infectious salmon anaemia virus in line with recommendations made by the *ad hoc* Group.

Epizootic haematopoietic necrosis (Chapter 10.1.)

The Aquatic Animals Commission noted that the two species currently listed in Article 10.1.2. were assessed to meet the criteria for listing as susceptible species i.e. European perch (*Perca fluviatilis*) and rainbow trout (*Oncorhynchus mykiss*) (see Item 3.1.).

The Aquatic Animals Commission noted that the common name for *Perca fluviatilis* was changed from redfin perch to European perch in line with FAOTERM (<http://www.fao.org/faoterm/collection/faoterm/en/>).

The Aquatic Animals Commission noted that nine new susceptible species were proposed for inclusion in Article 10.1.2.: black bullhead (*Ameiurus melas*), crimson spotted rainbow fish (*Melanotaenia fluviatilis*), eastern mosquito fish (*Gambusia holbrooki*), macquarie perch (*Macquaria australasica*), mosquito fish (*Gambusia affinis*), mountain galaxias (*Galaxias olidus*), northern pike (*Esox lucius*), pike-perch (*Sander lucioperca*), and silver perch (*Bidyanus bidyanus*) (see Item 3.1.).

The revised Chapter 10.1. is presented in Annex 11 for Member Country comment.

Infection with *Gyrodactylus salaris* (Chapter 10.3.)

Regarding the list of susceptible species listed in Article 10.3.2., the Aquatic Animals Commission noted that six of the seven species currently listed were assessed to meet the criteria for listing as susceptible species, i.e. Arctic char (*Salvelinus alpinus*), Atlantic salmon (*Salmo salar*), brown trout (*Salmo trutta*), grayling (*Thymallus thymallus*), North American brook trout (*Salvelinus fontinalis*) and rainbow trout (*Oncorhynchus mykiss*) (see Item 3.1.).

The Aquatic Animals Commission noted that North American lake trout (*Salvelinus namaycush*), currently listed in Article 10.3.2., was assessed and did not meet the criteria for listing as a susceptible species and was therefore proposed to be deleted from Article 10.3.2. (see Item 3.1.).

The Aquatic Animals Commission agreed to include the words ‘non-viable’ before fish roe in point 3 j) of Article 10.3.3. to clarify that only non-viable fish roe would be eligible for inclusion in this article as a safe aquatic animal product.

The Aquatic Animals Commission noted that point 1) of Article 10.3.8. of the 2016 edition of the *Aquatic Code* had been inadvertently deleted when the model Article X.X.8. was applied in the 2017 edition. The Commission therefore proposed to re-instate the text in point 1) of Article 10.3.7.

The revised Chapter 10.3. is presented in [Annex 12](#) for Member Country comment.

Infection with infectious salmon anaemia virus (Chapter 10.4.)

The Aquatic Animals Commission noted that the three species currently listed in Article 10.4.2. were assessed and met the criteria for listing as susceptible species, i.e. Atlantic salmon (*Salmo salar*), brown trout (*Salmo trutta*) and rainbow trout (*Onchorynchus mykiss*) (see Item 3.1.).

The revised Chapter 10.4. Infection with infectious salmon anaemia virus is presented in [Annex 13](#) for Member Country comment.

2.9. Articles X.X.3.

The Aquatic Animals Commission reminded Member Countries that the aquatic animal products included in Article X.X.3. of each disease-specific chapter are those that satisfied the criteria in Article 5.4.1. and were assessed against these criteria by a panel of experts. The assessments are available on the [OIE website](#).

In response to Member Country comments, the Aquatic Animals Commission reviewed the aquatic animal products in Article X.X.3. that referred to heat (time/temperature) inactivation treatments. The Commission agreed that it was not clear why non-equivalent heat (time/temperature) inactivation treatments were provided for different products and agreed that it would be more logical to provide a minimum heat (time/temperature) inactivation treatment for each OIE listed disease. This would allow a focussing on the minimum required heat treatment necessary to inactivate the pathogenic agent rather than on different and possibly variable commercial processing methods. The Commission noted that it would be the responsibility of the Competent Authority of the exporting country to provide evidence that the required minimum time/temperature had been met for a particular product.

The Aquatic Animals Commission requested that the *ad hoc* Group on Safety of Products Derived from Aquatic Animals be reconvened to review the heat treatments provided in Article X.X.3. of each disease-specific chapter and provide a minimum heat time/temperature treatment that has been demonstrated to be effective at inactivating the relevant pathogenic agent.

In addition, the Aquatic Animals Commission agreed that the wording “subjected to” a certain temperature/time combination was not appropriate because the product would need to have a core temperature for the required time for inactivation to occur. The heat treatment that a product would need to be “subjected to” to reach that core temperature would vary depending on several circumstances (*e.g.*, product size, initial temperature). The Commission therefore requested that the *ad hoc* Group also define a core temperature.

2.10. Model Articles X.X.8., X.X.9., X.X.10. and X.X.11.

2.10.1. Article X.X.8.

The Aquatic Animals Commission amended point 2 *b) iv)* of Article X.X.8. to ensure the correct cross referencing to the relevant sections of the *Aquatic Code* and *Manual*.

2.10.2. Articles X.X.9. and X.X.10.

In response to Member Country comments the Aquatic Animals Commission reviewed point 2) in Articles X.X.9. and X.X.10. and agreed to add ‘ice and waste material’ from transport to ensure all risk items are included.

The Aquatic Animals Commission also amended the first paragraph of Article X.X.10. to more accurately reflect the title of this article.

Other amendments reflect amendments adopted previously in the crustacean disease-specific chapters.

2.10.3. Article X.X.11.

In developing the new draft chapter for *B. salamandrivorans* (see Item 5.1.) the Aquatic Animals Commission noted that Article X.X.11. that addresses the ‘Importation of aquatic animals intended for use in laboratories or zoos from a country, zone or compartment not declared free from infection with *B. salamandrivorans*’ is only included in the other amphibian disease-specific chapters (Chapters 8.1. and 8.2.).

The Aquatic Animals Commission considered this article to be relevant for all other disease-specific chapters and proposed that it be included in all disease-specific chapters in Sections 9, 10 and 11, once adopted.

Model Articles X.X.8., X.X.9., X.X.10. and X.X.11. are presented in Annex 21 for Member Country comment.

The Aquatic Animals Commission agreed to apply these changes to all disease-specific chapters in Sections 8, 9 and 10 of the *Aquatic Code* once adopted. The Commission agreed to amend mollusc disease-specific chapters in Section 11 when work on susceptible species commences to ensure consistency in the alignment of relevant amendments.

2.11. Technical disease card for tilapia lake virus

The Aquatic Animals Commission reviewed the technical disease card for tilapia lake virus (TiLV) taking into consideration new scientific information and considered it did not need amending.

The Aquatic Animals Commission reminded Member Countries that the technical disease card for TiLV can be accessed on the [OIE website](#).

2.12. Technical disease card for *Batrachochytrium salamandrivorans*

In order to provide information for Member Countries on available detection methods and transmission risks for *Batrachochytrium salamandrivorans* while a disease-specific chapter for the *Aquatic Code* and *Manual* are being developed, the Aquatic Animals Commission developed a technical disease card for *B. salamandrivorans*.

The technical disease card for *B. salamandrivorans* has been uploaded onto the [OIE website](#).

The technical disease card for *B. salamandrivorans* is also presented in Annex 29 for Member Country information.

3. *Ad hoc* Groups

3.1. Report of the *ad hoc* Group on Susceptibility of Fish Species to Infection with OIE Listed Diseases

The Aquatic Animals Commission reviewed the report of the meeting of the *ad hoc* Group on Susceptibility of Fish Species to Infection with OIE Listed Diseases held from 25–27 April 2017. The Commission commended the *ad hoc* Group for their substantial work.

The OIE *ad hoc* Group had undertaken assessments of susceptible species using the ‘Criteria for listing species as susceptible to infection with a specific pathogen’ (Chapter 1.5. of the *Aquatic Code*) for inclusion in the relevant articles of fish disease-specific chapters in the *Aquatic Code* and *Aquatic Manual* for epizootic haematopoietic necrosis (Chapter 10.1. and Chapter 2.3.1., respectively), infection with *Gyrodactylus salaris* (Chapter 10.3. and Chapter 2.3.3., respectively), and infection with infectious salmon anaemia virus (Chapter 10.4. and Chapter 2.3.5., respectively).

Refer to Items 2.8.2. and 5.2. for details.

The Aquatic Animals Commission also requested that the *ad hoc* Group continue its work to review the list of susceptible species for the remaining fish disease-specific chapters.

The report of the OIE *ad hoc* Group on Susceptibility of fish species to infection with OIE listed diseases is presented in [Annex 30](#) for Member Country information.

3.2. Report of the *ad hoc* Group on Demonstration of Disease Freedom

The Aquatic Animals Commission reviewed the report of the meeting of the *ad hoc* Group on Demonstration of Disease Freedom held from 4–6 July 2017. The Commission acknowledged the excellent progress made by the *ad hoc* Group on this complex topic.

The *ad hoc* Group had, as requested by the Aquatic Animals Commission, developed and applied principles for demonstrating disease freedom to a model disease-specific chapter. Following consideration of the work of the *ad hoc* Group the Commission requested that the *ad hoc* Group further develop the principles they used for the model chapter so that these principles could be applied to the other disease-specific chapters. The Commission requested that the *ad hoc* Group work electronically and finalise a report for consideration by the Commission when they meet in February 2018.

3.3. Report of the *ad hoc* Group on Aquatic Animal Biosecurity for Aquaculture Establishments

The Aquatic Animals Commission reviewed the report of the meeting of the *ad hoc* Group on Aquatic Animal Biosecurity for Aquaculture Establishments, held from 20–22 June 2017, and commended them on their work.

The Aquatic Animals Commission reviewed the report and draft chapter on Aquatic animal biosecurity for aquaculture establishments and requested that the *ad hoc* Group meet again to finalise the draft chapter for the Commission’s consideration at their next meeting in February 2018.

F. MANUAL OF DIAGNOSTIC TESTS FOR AQUATIC ANIMALS

4. Texts circulated for Member Country comments at the February 2017 meeting

Comments were received from Australia, Brazil, Canada, China (People’s Rep. of), Chinese Taipei, Japan, New Zealand, Switzerland, Thailand, USA, AU-IBAR and EU.

The Aquatic Animals Commission reminded Member Countries that an *ad hoc* Group was developing a new template for disease-specific chapters for the *Aquatic Manual* (see Item 5.7.) and that issues such as definitions of suspect and confirmed cases, validation of diagnostic test methods, the structure and layout of the disease-specific chapters, which are frequently the subject of Member Country comments, would be addressed by the *ad hoc* Group and implemented in the new chapter template.

4.1. Infection with white spot syndrome virus (Chapter 2.2.8.)

The Aquatic Animals Commission reviewed all Member Country comments on Chapter 2.2.8. Infection with white spot syndrome virus (WSSV) and made relevant amendments.

In Section 2.2.1. Susceptible host species, one Member Country proposal for additional text was not accepted as it was not specific to the topic of species susceptibility to WSSV. The Aquatic Animals Commission reiterated that species susceptibility to WSSV had been thoroughly reviewed by the *ad hoc* Group on Susceptibility of Crustacean Species to Infection with OIE Listed Disease. Further revisions to this section would be considered following resolution of proposed changes to Chapter 1.5. (see Item 1.2. above).

In Section 3.3. Pooling of samples, one Member Country proposed reinstating deleted text on pooling juvenile and subadult samples to avoid creating too much work for laboratories. Another Member Country proposed reinstating the text despite the absence of scientific publications because pooling makes surveillance less expensive and the diagnosis process quicker and less complex. The Aquatic Animals Commission did not accept the rationale provided.

In Section 4.3.1.1.1., a Member Country requested that wet mounts and electron microscopy be deleted as test methods as results are difficult to define and the methods are impractical for users. The Aquatic Animals Commission reminded Member Countries that the chapters would be fully revised once the new disease chapter template developed by the *ad hoc* Group on the *Aquatic Manual* (see Item 5.7.) is approved and implemented. All test methods would be carefully evaluated for fitness for purpose and any irrelevant tests would not be included in the updated chapters.

A Member Country suggested that it was not necessary to include in the chapter published references to the PCR and real-time PCR protocols, but rather to include only the recommended primers, probe sequences and annealing temperatures. The Aquatic Animals Commission stated that diagnostic test performance is dependent on precise test parameters and thus published references were necessary.

The Aquatic Animals Commission did not accept a Member Country request to add a reference to another real-time PCR method to Section 4.3.1.2.4.3. Taqman real-time PCR method because there was no information on diagnostic sensitivity and specificity in the referenced method. The Commission reiterated that the process of implementing the new chapter template would include a review and update of all diagnostic test methods; at that stage if more information on the proposed test's performance is available, the Commission would consider including it.

In Section 7.2. Definition of confirmed case, the Aquatic Animals Commission did not accept a Member Country proposal to include six more test methods for the detection of WSSV noting that the proposal would be addressed once the new chapter template is implemented. The Commission did not accept a Member Country proposal to not update the definition as it would create too much work for laboratories, noting that this was not an acceptable rationale.

The revised chapter Infection with white spot syndrome virus (Chapter 2.2.8.) is presented in [Annex 22](#) for Member Country comment.

5. Other issues

5.1. New draft chapter for Infection with *Batrachochytrium salamandrivorans* (Chapter 2.1.X.)

In the absence of a Reference Laboratory for *Batrachochytrium salamandrivorans* the Aquatic Animals Commission proposed to request an expert to prepare a draft chapter once the new template has been finalised (see Item 5.7.).

The Aquatic Animals Commission would welcome applications from suitable laboratories to become a Reference Laboratory for *B. salamandrivorans*.

5.2. Epizootic haematopoietic necrosis (Chapter 2.3.1.), Infection with *Gyrodactylus salaris* (Chapter 2.3.3.) and Infection with infectious salmon anaemia virus (Chapter 2.3.5.)

The Aquatic Animals Commission amended Section 2.2.2. Species with incomplete evidence for susceptibility in Chapter 2.3.1. Epizootic haematopoietic necrosis, Chapter 2.3.3. Infection with *Gyrodactylus salaris* and Chapter 2.3.5. Infection with infectious salmon anaemia virus, after consideration of the work of the *ad hoc* Group on Susceptibility of fish species to infection with OIE listed diseases, which had applied the ‘Criteria for listing species as susceptible to infection with a specific pathogen’ (Chapter 1.5.) (see Item 3.1.). The Commission also reviewed comments provided by the relevant OIE Reference Laboratory experts on Section 2.2. Host factors.

The Aquatic Animals Commission also reviewed the three chapters in their entirety and proposed further amendments, in particular to the remainder of Section 2 Disease information and Section 7 Corroborative diagnostic criteria. The Commission also harmonised the titles of the chapters with the name of the disease as listed by the OIE (e.g., Infection with HPR-deleted or HPR0 infectious salmon anaemia virus) and ensured the correct use of the disease name throughout the chapters.

The revised Chapter 2.3.1. is presented in [Annex 23](#) for Member Country comment.

The revised Chapter 2.3.3. is presented in [Annex 24](#) for Member Country comment.

The revised Chapter 2.3.5. is presented in [Annex 25](#) for Member Country comment.

5.3. Infection with infectious hypodermal and haematopoietic necrosis (Chapter 2.2.4.)

As noted in Item 2.7., the *ad hoc* Group on Susceptibility of Crustacean Species to Infection with OIE Listed Diseases had reviewed the assessment of *Macrobrachium rosenbergii* for listing as a susceptible species against the criteria in Chapter 1.5. of the *Aquatic Code*.

The Aquatic Animals Commission agreed with the *ad hoc* Group assessment and proposed that *M. rosenbergii* be added to Section 2.2.2. Species with incomplete evidence for susceptibility for an organism showing ‘pathogen-specific positive polymerase chain reaction (PCR) results, but an active infection has not been demonstrated’.

The revised Sections 2.2.1. and 2.2.2. are presented in [Annex 26](#) for Member Country comment.

5.4. Assessment of kuruma shrimp (*Penaeus japonicus*) as a susceptible species to acute hepatopancreatic necrosis disease (Chapter 2.2.1.)

In response to a Member Country comment that kuruma shrimp (*Penaeus japonicus*) be included in Section 2.2.2. of the *Aquatic Manual* as a ‘Species with incomplete evidence for susceptibility’, the Aquatic Animals Commission had requested that the *ad hoc* Group on Susceptibility of crustacean species to infection with OIE listed diseases assess kuruma shrimp (*P. japonicus*) against the criteria in Chapter 1.5. of the *Aquatic Code*.

The *ad hoc* Group assessed *P. japonicus* for susceptibility to bacteria causing infection with acute hepatopancreatic necrosis disease (AHPND) based on the reference Tinwongger *et al.* (2016). The *ad hoc* Group agreed that the identity of the pathogenic agent had been confirmed in accordance with Article 1.5.5. but that *P. japonicus* did not fulfil criteria A (replication), B (viability/infectivity), C (pathology/clinical signs) or D (location). Regarding criterion C, the *ad hoc* Group noted that although high mortality was reported in Tinwongger *et al.* (2016) there were no other pathological signs specific for AHPND relative to the control group. The *ad hoc* Group agreed that although *P. japonicus* is probably susceptible to the effects of the *Photobacterium* insect-related (Pir) toxins, PirA and PirB, there is insufficient evidence to be conclusive and they therefore allocated a ‘No’ to criterion C.

The *ad hoc* Group agreed that *P. japonicus* did not meet the criteria in Chapter 1.5. for listing in the *Aquatic Code* but agreed it should be included in the *Aquatic Manual* Chapter 2.2.1. Section 2.2.2. Species with incomplete evidence for susceptibility for an organism showing PCR results but an active infection has not been demonstrated.

Reference:

Tinwongger S., Nochiri Y., Thawonsuwan J., Nozaki R., Kondo H., Awasthi S.P., Hinenoya A., Yamasaki S. & Hirono I. (2016). Virulence of acute hepatopancreatic necrosis disease PirAB-like relies on secreted proteins not on gene copy number. *J. Appl. Microbiol.*, 121, 1755–1765.

The assessment for kuruma prawn and the revised Section 2.2.2. of Chapter 2.2.1. are presented in [Annex 27 A](#) and [Annex 27B](#), respectively for Member Country comment.

5.5. Infection with infectious myonecrosis virus (Chapter 2.2.5.)

At the General Session in May 2017, a Member Country had proposed amendments to the reverse-transcriptase (RT) PCR protocol in sub-section ‘RT-PCR for detection of IMNV’ of Section 4.3.1.2.3 of Chapter 2.2.5. Infection with infectious myonecrosis virus. So as not to delay adoption of other important changes to the chapter, the revised chapter was adopted, without any changes to the RT-PCR protocol. In the meantime the Member Country comments were reviewed by an OIE expert. The expert noted that the Member Country did not provide the rationale for the proposed amendments with evidence of equivalency of test performance under the new test conditions, undertaken in parallel with the existing protocol. The Commission agreed that without this information they could not accept the proposal.

5.6. Infection with Taura syndrome virus (Chapter 2.2.7.)

Similar to Item 5.5. above, a Member Country had proposed amendments to the RT-PCR method of Section 4.3.1.2.7.2. of Chapter 2.2.7. Infection with Taura syndrome virus. Again, due to the lack of published data on the equivalency of the test performance with the amended protocol, in parallel with the existing protocol, the OIE expert did not agree with the proposed amendments. The Aquatic Animals Commission agreed that without this information they could not accept the proposal.

5.7. Review of the *Aquatic Manual* disease chapter template proposed by the *ad hoc* Group

The Aquatic Animals Commission reviewed the disease chapter template that had been further amended by the *ad hoc* Group following feedback from the Commission at the February 2017 meeting.

The *ad hoc* Group would further amend the template taking into account feedback provided to them by the Aquatic Animals Commission. At its next meeting in February 2018, the Commission would review the finalised template and the three example chapters, and append these to their meeting report for the information of Member Countries. The Commission agreed that the template would first be applied to the mollusc disease chapters and the task would begin with the aim of providing updated chapters for the Commission to review at its September 2018 meeting.

G. OIE REFERENCE CENTRES

6. Applications for OIE Reference Centre status or changes of experts

The Aquatic Animals Commission recommended acceptance of the following applications for OIE Reference Centre status:

OIE Reference Laboratory for Acute hepatopancreatic necrosis disease

National Chen-Kung University, Center for Shrimp Disease Control and Genetic Improvement, No.500, Sec. 3, Anming Road, Annan Dist., Tainan City 709, Chinese Taipei.

Designated Reference Expert: Dr Grace Chu-Fang Lo.

OIE Reference Laboratory for Infectious haematopoietic necrosis

Animal and Plant Inspection and Quarantine Technical Centre, Shenzhen Exit & Entry Inspection and Quarantine Bureau, Inspection and Quarantine Building, 1011 Fuqiang Road, Futian Qu, Shenzhen City, Guangdong Province, 518045, CHINA (PEOPLE'S REP. OF).

Designated Reference Expert: Dr Hong Liu.

OIE Reference Laboratory for Viral haemorrhagic septicaemia and Infectious haematopoietic necrosis

Pacific Biological Station – Aquatic Animal Health Laboratory (PBS-AAHL), Fisheries & Oceans Canada, 3190 Hammond Bay Road, Nanaimo, BC V9T 6N7, CANADA.

Designated Reference Expert: Dr Kyle Garver.

In February 2017, the Aquatic Animals Commission had approved an application for an OIE Reference Laboratory for AHPND. However, in March 2017, the proposed expert had left the laboratory and so it could not be proposed for adoption in May 2017. The Commission requested that the applicant submit a new application for OIE Reference Laboratory status for AHPND. The same Member Country had also submitted a nomination for a replacement expert at seven OIE Reference Laboratories for crustacean diseases. The Commission requested that the Member Country re-submit the nomination with details of the expert's expertise for each disease given separately.

7. Feedback from the Biological Standards Commission on the brainstorming on Collaborating Centres

At its February 2017 meeting, the Biological Standards Commission had begun to consider the network of OIE Collaborating Centres and how to better engage the network in the goals of the OIE. As a first step, the Biological Standards Commission agreed to identify focus areas for OIE Collaborating Centre activities for future applicants. The aim was to work better with the network of Collaborating Centres and to improve both clarity and opportunities for networking, which is also an integral part of the OIE Sixth Strategic Plan.

At its September 2017 meeting, the Biological Standards Commission further refined the list and submitted it to the Aquatic Animals Commission for comment and amendment. The Biological Standards Commission had identified six focus areas with specific specialties within each topic. The Commission provided feedback on the proposed topics for consideration by the Biological Standards Commission at their February 2018 meeting.

8. Twinning Projects

As of September 2017, two twinning projects have been completed (Canada and Chile for infection with infectious salmon anaemia; USA and China for infectious haematopoietic necrosis) and five projects are currently underway (Norway and Brazil for infection with infectious salmon anaemia; Japan and Indonesia for Koi herpesvirus; USA and Indonesia and USA and Saudi Arabia for shrimp diseases; Denmark and Republic of Korea for viral haemorrhagic septicaemia).

H. OTHER ISSUES

9. New procedure for Self-declaration of disease freedom

The Aquatic Animals Commission was informed that the OIE has developed a 'Procedure for submission of a self-declaration of disease freedom to the OIE. The procedure relates to self-declaration of disease freedom for a country, zone or compartment for OIE listed aquatic and terrestrial animal diseases. It describes the process for the preparation, screening and publication of self-declarations of freedom from any disease, other than those diseases for which the OIE has put in place a specific procedure for official recognition of disease status.

The Aquatic Animals Commission commended the work done by the OIE and reiterated that self-declaration of freedom status is an important topic for Member Countries because an official status recognition process does not exist for OIE listed aquatic animal diseases. The Commission indicated they would provide comments on the draft procedure document noting that there are some points specific to aquatic animal diseases that need to be included in this draft document. The Commission will continue to follow this important issue and requested to be kept informed about ongoing work.

I. WORK PLAN OF THE OIE AQUATIC ANIMAL HEALTH STANDARDS COMMISSION FOR 2017/2018

The Aquatic Animals Commission reviewed and updated its work programme, taking into account Member Country comments, Headquarters' comments, and completed work.

The revised work programme is presented in [Annex 31](#) for Member Country information.

J. ACTIVITIES OF THE MEMBERS OF THE OIE AQUATIC ANIMAL HEALTH STANDARDS COMMISSION

The Aquatic Animals Commission wished to inform Member Countries of activities that Commission members have undertaken in their role as Commission members since their last meeting in February 2017.

Members of the Commission have participated in the following activities:

Dr Ingo Ernst held a teleconference on 16 May 2017 for OIE Delegates and Aquatic Animal Focal Points in the Asia Pacific region. The purpose of the teleconference was to brief Member Countries on the report of the February 2017 meeting of the Aquatic Animals Commission, particularly annexes that had been provided for adoption at the 2017 General Session.

Dr Ingo Ernst represented the OIE at the 16th meeting of the Asia Regional Advisory Group on Aquatic Animal Health which was convened by the Network of Aquaculture Centres in Asia Pacific (Bali, Indonesia, 26–27 August 2017). He also participated in a meeting of intergovernmental organisations, governments and research organisations in Bali on 29 August 2017 and presented the actions taken by the OIE to contribute to the management of tilapia lake virus.

Dr Edmund Peeler attended the expert meeting organised by the European Commission to coordinate the EU response to the report of the February 2017 meeting of the Aquatic Animals Commission. Dr Peeler answered questions, provided clarification and discussed the future work programme of the Commission.

Dr Joanne Constantine represented the Aquatic Animals Commission at a meeting of the *ad hoc* Group on Demonstration of Disease Freedom held in Paris, France, 4–6 July 2017.

Dr Alicia Gallardo Lagno represented the Aquatic Animals Commission at a meeting of the *ad hoc* Group on Aquatic Animal Biosecurity for Aquaculture Establishments, held in Paris, France, from 20–22 June 2017.

Prof. Maxwell Barson represented the OIE at the 'World Aquaculture 2017' in Cape Town (South Africa) from 26 to 30 June 2017 including the Special Workshop on Aquaculture Biosecurity held in parallel from 29 to 30 June and the post-symposium Aquaculture Biosecurity Workshop on 1 and 2 July 2017 where he also presented several papers.

K. NEXT MEETING

The next meeting of the Aquatic Animals Commission is scheduled for 14–21 February 2018 inclusive.

/Annexes

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

Paris, 13–20 September 2017

List of participants

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**MEETING OF THE OIE
AQUATIC ANIMAL HEALTH STANDARDS COMMISSION
Paris, 13–20 September 2017**

Adopted agenda

- A. Meeting with the Director General**
- B. Adoption of the agenda**
- C. Meeting with the President of the OIE Terrestrial Animal Health Standards Commission**
- D. Meeting with the President of the OIE Biological Standards Commission**
- E. OIE Aquatic Animal Health Code**
 - 1. Texts circulated for Member Country comments at the February 2017 meeting
 - 1.1. General comments
 - 1.2. Criteria for listing species as susceptible to infection with a specific pathogen (Chapter 1.5.)
 - 1.3. Criteria to assess the safety of aquatic animal commodities (Chapter 5.4.)
 - 2. Other issues
 - 2.1. User's Guide
 - 2.2. Glossary
 - 2.3. Diseases listed by the OIE (Chapter 1.3.)
 - 2.4. OIE procedures relevant to the Agreement on the Application of Sanitary and Phytosanitary Measures of the World Trade Organization (Chapter 5.3.)
 - 2.5. Amphibian diseases
 - 2.5.1. New draft chapter for Infection with *Batrachochytrium salamandrivorans* (Chapter 8.X.)
 - 2.5.2. Infection with *Batrachochytrium dendrobatidis* (Chapter 8.1.) and Infection with ranavirus (Chapter 8.2.)
 - 2.6. Acute hepatopancreatic necrosis disease (Chapter 9.1.)
 - 2.7. Infection with infectious hypodermal and haematopoietic necrosis virus (Chapter 9.4.)
 - 2.8. Fish disease-specific chapters
 - 2.8.1. Horizontal changes
 - 2.8.2. List of susceptible species
 - 2.9. Article X.X.3.
 - 2.10. Model Articles X.X.8., X.X.9., X.X.10. and X.X.11.
 - 2.10.1. Article X.X.8.
 - 2.10.2. Articles X.X.9. and X.X.10.
 - 2.10.3. Article X.X.11.

Annex 2 (contd)

- 2.11. Technical disease card for tilapia lake virus
- 2.12. Technical disease card for *Batrachochytrium salamandrivorans*
- 3. *Ad hoc* Groups
 - 3.1. Report of the *ad hoc* Group on Susceptibility of fish species to infection with OIE listed diseases
 - 3.2. Report of the *ad hoc* Group on Demonstration of disease freedom
 - 3.3. Report of the *ad hoc* Group on Aquatic animal biosecurity for aquaculture establishments

F. MANUAL OF DIAGNOSTIC TESTS FOR AQUATIC ANIMALS

- 4. Texts circulated for Member Country comments at the February 2017 meeting
 - 4.1. Infection with white spot syndrome virus (Chapter 2.2.8.) – review of Member Country comments
- 5. Other issues
 - 5.1. New draft chapter for Infection with *Batrachochytrium salamandrivorans* (Chapter 2.1.X.)
 - 5.2. Chapter 2.3.1. Epizootic haematopoietic necrosis, Chapter 2.3.3. Infection with *Gyrodactylus salaris* and Chapter 2.3.5. Infection with infectious salmon anaemia virus
 - 5.3. Infection with infectious hypodermal and haematopoietic necrosis (Chapter 2.2.4.)
 - 5.4. Assessment of kuruma shrimp (*Penaeus japonicus*) as a susceptible species to acute hepatopancreatic necrosis disease (Chapter 2.2.1.)
 - 5.5. Infection with infectious myonecrosis virus (Chapter 2.2.5.)
 - 5.6. Infection with Taura syndrome virus (Chapter 2.2.7.)
 - 5.7. Review of the *Aquatic Manual* disease chapter template proposed by the *ad hoc* Group

G. OIE REFERENCE CENTRES

- 6. Applications for OIE Reference Centre status or changes of experts
- 7. Feedback from the Biological Standards Commission on the brainstorming on Collaborating Centres
- 8. Twinning Projects

H. OTHER ISSUES

- 9. New procedure for Self-declaration of disease freedom

I. WORK PLAN OF THE OIE AQUATIC ANIMAL HEALTH STANDARDS COMMISSION FOR 2017/2018**J. ACTIVITIES OF THE MEMBERS OF THE OIE AQUATIC ANIMAL HEALTH STANDARDS COMMISSION****K. NEXT MEETING**

CHAPTER 1.5.

CRITERIA FOR LISTING SPECIES AS SUSCEPTIBLE TO INFECTION WITH A SPECIFIC PATHOGEN

Article 1.5.1.

The purpose of this chapter is to provide criteria for determining which species are listed as susceptible in Article ~~1.5.2.X.X.2.~~ of each *disease-specific* chapter in the *Aquatic Code*.

Article 1.5.2.

Scope

Susceptibility may include clinical or non-clinical *infection* but does not include species that may carry the *pathogenic agent* without replication.

The decision to list an individual a species as susceptible in disease-specific chapters should be based on a finding that the evidence is definite in accordance with Article 1.5.3. All species in a taxonomic group may be listed as susceptible when certain criteria are met in accordance with Article 1.5.9.

However, possible Possible susceptibility of a species is also important information and this should also be included in Section ~~2.2.1. 2.2.2. Species with incomplete evidence for susceptibility~~ entitled «Susceptible host species» of the relevant *disease-specific* chapter of the *Aquatic Manual*, in accordance with Article 1.5.8.

Article 1.5.3.

Approach

A three-stage approach is outlined in this chapter to assess susceptibility of a species to *infection* with a specified *pathogenic agent* and is based on:

- 1) criteria to determine whether the route of transmission is consistent with natural pathways for the *infection* (as described in Article 1.5.4.);
- 2) criteria to determine whether the *pathogenic agent* has been adequately identified (as described in Article 1.5.5.);
- 3) criteria to determine whether the evidence indicates that presence of the *pathogenic agent* constitutes an *infection* (as described in Article 1.5.6.).

Article 1.5.4.

Stage 1: criteria to determine whether the route of transmission is consistent with natural pathways for the infection

The evidence should be classified as transmission through:

- 1) natural occurrence; includes situations where *infection* has occurred without experimental intervention e.g. *infection* in wild or farmed populations; or
- 2) non-invasive experimental procedures; includes cohabitation with infected hosts, *infection* by immersion or ingestion; or
- 3) invasive experimental procedure; includes injection, exposure to unnaturally high loads of pathogen pathogenic agent, or exposure to stressors (e.g. temperature) not encountered in the host's natural or culture environment.

Annex 3 (contd)

Consideration needs to be given to whether experimental procedures (e.g. inoculation, infectivity load) mimic natural pathways for *disease* transmission. Consideration should also be given to environmental factors as these may affect host resistance or transmission of the ~~pathogen~~ *pathogenic agent*.

Article 1.5.5.

Stage 2: criteria to determine whether the pathogenic agent has been adequately identified

The *pathogenic agent* should be identified and confirmed in accordance with the methods described in Section **7 4 (diagnostic methods)** (~~corroborative diagnostic criteria~~) of the relevant *disease-specific* chapter in the *Aquatic Manual*, or other methods that have been demonstrated to be equivalent.

Article 1.5.6.

Stage 3: criteria to determine whether the evidence indicates that presence of the pathogenic agent constitutes an infection

A combination of the following criteria should be used to determine *infection* (see Article 1.5.7.):

- A. the *pathogenic agent* is multiplying in the host, or developing stages of the *pathogenic agent* are present in or on the host;
- B. viable *pathogenic agent* is isolated from the proposed *susceptible species*, or infectivity is demonstrated by way of transmission to naive individuals;
- C. clinical or pathological changes are associated with the *infection*;
- D. the specific location of the ~~pathogen~~ *pathogenic agent* corresponds with the expected target tissues.

The type of evidence to demonstrate *infection* will depend on the *pathogenic agent* and potential host species under consideration.

Article 1.5.7.

Outcomes of the assessment

The decision to list a species as susceptible should be based on a finding of definite evidence. Evidence should be provided for the following:

- 1) transmission has been obtained naturally or by experimental procedures that mimic natural pathways for the *infection* in accordance with Article 1.5.4.;

AND

- 2) the identity of the *pathogenic agent* has been confirmed in accordance with Article 1.5.5.;

AND

- 3) there is evidence of *infection* with the *pathogenic agent* in the suspect host species in accordance with criteria A to D in Article 1.5.6.. Evidence to support criterion A alone is sufficient to determine *infection*. In the absence of evidence to meet criterion A, satisfying at least two of criteria B, C or D would be required to determine *infection*.

Article 1.5.8.

Species for which there is incomplete evidence for susceptibility

The decision to list a species as susceptible in Article 1.5.2. of each *disease-specific* chapter should be based on a finding that the evidence is definite.

However, where there is insufficient incomplete evidence to demonstrate susceptibility of a species through the approach described in Article 1.5.3, ~~because transmission does not mimic natural pathways of infection, or the identity of the pathogenic agent has not been confirmed, or infection is only partially supported, but partial information is available, these species information~~ will be included in Section 2.2.2. Species with incomplete evidence for susceptibility of the relevant disease-specific chapter in the *Aquatic Manual*.

If there is insufficient incomplete evidence to demonstrate susceptibility of a species, the *Competent Authority* should, prior to the implementation of any import health measures for the species, assess the risk of spread of the ~~pathogen~~ pathogenic agent under consideration, in accordance with the recommendations in Chapter 2.1., prior to the implementation of import health measures.

Article 1.5.9.

Listing susceptible species at a taxonomic ranking of genus or higher than species Pathogenic agents with a broad host range

~~For pathogenic agents with that have a broad host range, it may be appropriate for the outcome of the assessment of susceptibility to can be made at a taxonomic ranking higher than species (e.g. genus, family). For a pathogenic agent to be considered to have a broad host range, and thus be a potential candidate for listing susceptible species at a taxonomic ranking of genus or higher, there must be at least one susceptible species within each of three or more host families.~~ It may be appropriate for the outcome of the assessment to be made at a taxonomic classification higher than species for a pathogenic agent that has a broad host range. A pathogenic agent will be considered to have a broad host range when it has been demonstrated as susceptible in at least three families.

~~For pathogenic agents that have a broad host range, 1)A a decision to conclude susceptibility of species at for a taxonomic ranking of genus or higher level above species should only be made where:~~

~~A. susceptibility has been demonstrated in at least one species from within each of three or more families;~~

~~AND~~

~~BA. more than one species within the family taxonomic ranking has been found to be susceptible in accordance with the approach described in Article 1.5.3. criteria above;~~

~~AND~~

~~BCB. no species within the taxonomic group ranking has been found to be refractory to infection.~~

~~AND~~

~~C. The the taxa taxonomic ranking is at chosen should be the lowest level supported by this evidence of points A and B.~~

~~2) Evidence that a species is refractory to infection may include includes:~~

~~A. absence of infection in a species exposed to the pathogenic agent in natural settings where the pathogen pathogenic agent is known to be present and it has causes caused infection in susceptible species;~~

~~B. absence of infection in species exposed to the pathogenic agent through a controlled challenges using experimental procedures.~~

CHAPTER 5.4.

CRITERIA TO ASSESS THE SAFETY OF AQUATIC ANIMAL PRODUCTS COMMODITIES

In the context of this chapter the word 'safety' is applied only to animal health considerations for *listed diseases*.

Article 5.4.1.

Criteria to assess the safety of ~~aquatic animals and aquatic animal products imported (or transited) for any purpose regardless of the disease X status of the exporting~~ for any purpose from a country, zone or compartment not declared free from disease X

~~In all disease chapters, point Point 1 of Article X.X.3. of all disease-specific chapters (Sections 8-11), lists aquatic animals and aquatic animal products that can be imported (or transited) for any purpose regardless of the disease X status of the exporting traded for any purpose from a country, zone or compartment not declared free from disease X. The criteria for inclusion of aquatic animals and aquatic animal products in point 1 of Article X.X.3. are based on the absence of the pathogenic agent in the traded aquatic animals and aquatic animal products or inactivation of the pathogenic agent by treatment or processing.~~

The assessment of the safety of the ~~aquatic animals and aquatic animal products~~ using the criteria relating to treatment or processing can only be undertaken where treatments or processing are well defined. It may not be necessary to provide details of the entire treatment or process undertaken. However, the steps considered critical in the inactivation of the *pathogenic agent* of concern should be detailed.

It is assumed that treatment or processing (i) is done by using standardised protocols, which include the steps considered critical in the inactivation of the *pathogenic agent* of concern; (ii) is conducted in accordance with good manufacturing practices ~~Good Manufacturing Practices~~; and (iii) that any other steps in the treatment, processing and subsequent handling of the *aquatic animal product* do not jeopardise the safety of the traded *aquatic animal product*.

Criteria

For an ~~aquatic animal or aquatic animal product~~ to be considered safe for *international trade* under the provisions of Article X.X.3., it should comply with the following criteria:

- 1) Absence of pathogenic agent in the traded ~~aquatic animal or aquatic animal product~~:
 - a) There is strong evidence that the *pathogenic agent* is not present in the tissues from which the ~~aquatic animal or aquatic animal product~~ is derived.

AND

- b) The water (including ice) used to process or transport the ~~aquatic animal or aquatic animal product~~ is not contaminated with the *pathogenic agent* and the processing prevents cross contamination of the ~~aquatic animal or aquatic animal product to be traded~~.

OR

- 2) Even if the *pathogenic agent* is present in, or contaminates the tissues from which the ~~aquatic animal or aquatic animal product~~ is derived, the treatment or processing methods to produce the ~~aquatic animal or aquatic animal product to be traded~~ inactivate the *pathogenic agent* such as:

- a) physical (e.g. temperature, drying, smoking);

AND/OR

- b) chemical (e.g. iodine, pH, salt, smoke);

AND/OR

- c) biological (e.g. fermentation).

Annex 4 (contd)

Article 5.4.2.

Criteria to assess the safety of ~~aquatic animals or aquatic animal products~~ imported (or transited) for retail trade for human consumption regardless of the disease X status of the exporting ~~from a country, zone or compartment not declared free from disease X~~

In ~~all disease chapters, point~~ Point 1 of Article X.X.12. (amphibian and fish disease-specific chapters) and Article X.X.11. (crustacean, fish and mollusc disease-specific chapters) lists ~~aquatic animals or aquatic animal products~~ for retail trade for human consumption. The criteria for inclusion of ~~aquatic animals or aquatic animal products~~ in point 1 of Article X.X.12. (amphibian and fish disease-specific chapters) and Article X.X.11. (crustacean, fish and mollusc disease-specific chapters) include consideration of the form and presentation of the product, the expected volume of waste tissues generated by the consumer and the likely presence of viable *pathogenic agent* in the waste.

For the purpose of this criterion retail means the selling or provision of ~~the aquatic animals or aquatic animal products~~ directly to the consumer with the intended purpose of human consumption. The retail pathway may also include wholesale distribution of the products provided they are not further processed by the wholesale distributor or the retailer, i.e. are not subjected to actions such as gutting, cleaning, filleting, freezing, thawing, cooking, unpacking, packing or repackaging.

It is assumed that: (i) the ~~aquatic animals or aquatic animal products~~ are used for human consumption only; (ii) waste may not always be handled in an appropriate manner that mitigates the introduction of the *pathogenic agent*; the level of risk is related to the waste disposal practices in each Member's country or territory; (iii) treatment or processing prior to importation is conducted in accordance with good manufacturing practices ~~Good Manufacturing Practices~~, and (iv) any other steps in the treatment, processing and subsequent handling of the ~~aquatic animals or aquatic animal products~~ prior to importation do not jeopardise the safety of the ~~traded aquatic animals or aquatic animal products~~.

Criteria

For ~~aquatic animals or aquatic animal products~~ to be considered safe for *international trade* under the provisions of point 1 of Article X.X.12. (amphibian and fish disease-specific chapters) and Article X.X.11. (crustacean, fish and mollusc disease-specific chapters), it should comply with the following criteria:

- 1) the ~~aquatic animal or aquatic animal product~~ is prepared and packaged for retail trade for human consumption; AND

EITHER

- 2) it includes only a small amount of raw waste tissues generated by the consumer;

OR

- 3) the *pathogenic agent* is not normally found in the waste tissues generated by the consumer.

USER'S GUIDE

A. Introduction

- 1) The OIE *Aquatic Animal Health Code* (hereafter referred to as the *Aquatic Code*) ~~provides~~ establishes standards for the improvement of aquatic animal health worldwide. The *Aquatic Code* also includes standards for the welfare of farmed fish and use of antimicrobial agents in aquatic animals. The purpose of this guide is to advise the Competent Authorities in OIE Member Countries on how to use the *Aquatic Code*.
- 2) Competent Authorities should use the standards in the *Aquatic Code* to develop measures for early detection, internal reporting, notification and control of pathogenic agents in aquatic animals (amphibians, crustaceans, fish and molluscs) and preventing their spread via international trade in aquatic animals and aquatic animal products, while avoiding unjustified sanitary barriers to trade.
- 3) OIE standards are based on the most recent scientific and technical information. Correctly applied, they protect aquatic animal health during the production and trade in aquatic animals and aquatic animal products as well as the welfare of farmed fish.
- 4) The absence of chapters, articles or recommendations on particular pathogenic agents or aquatic animal products does not preclude the application of appropriate sanitary measures by the Competent Authorities, provided they are based on risk analyses conducted in accordance with the *Aquatic Code*.

4bis The year that a chapter was first adopted and the year of last revision are noted at the end of each chapter.

- 5) The complete text of the *Aquatic Code* is available on the OIE website and individual chapters may be downloaded from: <http://www.oie.int>.

B. *Aquatic Code* content

- 1) Key terms and expressions used in more than one chapter in the *Aquatic Code* are defined in the Glossary, where common dictionary definitions are not deemed to be adequate. The reader should be aware of definitions given in the Glossary when reading and using the *Aquatic Code*. Defined terms appear in italics. In the online version of the *Aquatic Code*, a hyperlink leads to the relevant definition.
- 2) The term '(under study)' is found in some rare instances, with reference to an article or part of an article. This means that this part of the text has not been adopted by the World Assembly of OIE Delegates and the particular provisions are thus not part of the *Aquatic Code*.
- 3) The standards in the chapters of Section 1 are designed for the implementation of measures for the surveillance and notification of pathogenic agents. The section includes the criteria for listing aquatic animal diseases, the diseases which are listed by the OIE, procedures for notification to the OIE, and criteria for listing species as susceptible to infection with a specific pathogen.
- 4) The standards in the chapters of Section 2 are designed to guide the importing country in conducting import risk analysis in the absence of OIE standards. The importing country may also use these standards to justify any import measures which are more stringent than that exceed existing OIE standards.
- 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 Competent Authorities of Member Countries to meet their objectives of improving aquatic animal health and welfare of farmed fish, as well as to establish and maintain confidence in their international aquatic animal health certificates.
- 6) The standards in the chapters of Section 4 are designed for the implementation of measures for the prevention and control of pathogenic agents. Measures in this section include zoning, compartmentalisation, disinfection, contingency planning, fallowing, disposal of aquatic animal waste and control of pathogenic agents in aquatic animal feed.

Annex 5 (contd)

- 7) The standards in the chapters of Section 5 are designed for the implementation of general sanitary measures for trade. They address certification and the measures applicable by the exporting, transit and importing countries. A range of model international aquatic animal health certificates is provided to facilitate consistent documentation for international trade.
- 8) The standards in the chapters of Section 6 are designed to ensure the responsible and prudent use of antimicrobial agents in aquatic animals.
- 9) The standards in the chapters of Section 7 are designed for the implementation of welfare measures for farmed fish. The standards cover the general principles for welfare of farmed fish, including during transport, stunning and killing for human consumption, and when killing for disease control purposes.
- 10) The standards in each of the chapters of Sections 8 to 11 are designed to prevent the pathogenic agents of OIE listed diseases from being introduced into an importing country. Each disease chapter includes a list of currently known susceptible species. The standards take into account the nature of the traded commodity, the aquatic animal health status of the exporting country, zone or compartment, and the risk reduction measures applicable to each commodity.

These standards assume that the agent is either not present in the importing country or is the subject of a control or eradication programme. Sections 8 to 11 each relate to amphibian, crustacean, fish and molluscan hosts, respectively.

C. Specific issues

1) Notification

Chapter 1.1. describes Member Countries' obligations under OIE Organic Statutes. Listed diseases, as prescribed in Chapter 1.1., are compulsorily notifiable. Member Countries are encouraged to also provide information to the OIE on other aquatic animal health events of epidemiological significance, including occurrence of emerging diseases.

Chapter 1.2. describes the criteria for the inclusion of a disease listed by the OIE.

Chapter 1.3. specifies the diseases that are listed by the OIE. Diseases are divided into four sections corresponding to amphibian, crustacean, fish and molluscan hosts, respectively.

2)bis Diagnostic tests

Methods for diagnosis of listed diseases are provided in the OIE *Manual of Diagnostic Tests for Aquatic Animals* (hereafter referred to as the *Aquatic Manual*). Experts responsible for laboratory testing should be fully conversant with the methods in the *Aquatic Manual*.

2bis. Freedom from a disease

Article 1.4.6. provides general principles for declaring a country or zone free from infection with a pathogenic agent. This article applies when there is no disease-specific chapter.

2) Pathogen differentiation

Some pathogens have one or more variants. Existence of highly pathogenic variants and the need to differentiate them from more benign variants are recognised in the *Aquatic Code*. When pathogenic agents have strains that are stable, possess characteristics that can be used for diagnostic purposes, and display different levels of pathogenicity, different standards providing protection proportionate to the risk posed by the different strains should be applied. Infection with infectious salmon anaemia virus is the first listed disease for which risk management options based on strain differentiation are provided.

3) Determining the susceptibility of species to listed diseases

~~The *Aquatic Code* proposes the use of criteria to assess the susceptibility of host species to the pathogenic agents of diseases listed in the *Aquatic Code*.~~

Chapter 1.5. provides criteria for determining which species are listed as susceptible in Article X.X.2. of each disease-specific chapter in the *Aquatic Code*. This is important in the aquaculture context, given the large number of existing and new aquaculture species.

This is work in progress and the list of susceptible species in some chapters is yet to be assessed against the criteria in Chapter 1.5.

4) Trade requirements

Aquatic animal health measures related to international trade should be based on OIE standards. A Member Country may authorise the importation of aquatic animals or aquatic animal products into its territory under conditions different from those recommended by the *Aquatic Code*. To scientifically justify **more stringent** measures **that exceed OIE standards**, the importing country should conduct a risk analysis in accordance with OIE standards, as described in Chapter 2.1. Members of the WTO should refer to the Agreement on the Application of Sanitary and Phytosanitary Measures (SPS Agreement).

Chapters 5.1. to 5.3. describe the obligations and ethical responsibilities of importing and exporting countries in international trade. Competent Authorities and all veterinarians and certifying officials directly involved in international trade should be familiar with these chapters. Chapter 5.3. also describes the OIE informal procedure for dispute mediation.

Disease-specific chapters in the *Aquatic Code* include articles listing the aquatic animal products that are considered safe for trade without the imposition of disease-specific sanitary measures, regardless of the status of the exporting country or zone for the pathogenic agent in question. Where such a list is present, importing countries should not require any conditions related to the agent in question with respect to the listed aquatic animal products.

5) Safety of Trade in aquatic animal products for trade commodities

Chapter 5.4. describes the criteria (Articles 5.4.1 and 5.4.2.) used to assess the safety of aquatic animal products commodities that are considered safe for trade regardless of the disease status of the country, zone or compartment without the need for additional risk mitigation measures for the disease.

The aquatic animal products that have been assessed and found to meet these criteria are listed in each disease-specific chapter.

Article X.X.3. lists aquatic animal products that may be imported for any purpose regardless of the disease status of the exporting country, zone or compartment for the disease in question. The inclusion of an aquatic animal product in Article X.X.3. is based on evidence that demonstrates the absence of the pathogenic agent in that product or the inactivation of the pathogenic agent by physical, chemical or biological means.

~~Based on assessments using criteria in Article 5.4.1., in all disease-specific chapters, point 1 of Article X.X.3. lists aquatic animal commodities that may be imported for any purpose from a country, zone or compartment not declared free from the disease in question. The criteria for inclusion of aquatic animal commodities in point 1 of Article X.X.3. are based on the absence of the pathogenic agent or inactivation of the pathogenic agent by treatment or processing.~~

Article X.X.11. (crustaceans, fish and molluscs chapters), Article X.X.12. (amphibian chapters) and Article 10.4.15. (infection with ISAV chapter) list aquatic animal products that may be imported for retail trade for human consumption regardless of the disease status of the exporting country, zone or compartment for the disease in question. The assessment for inclusion of aquatic animal products in these articles is based on the form and presentation of the product, the expected volume of waste tissues generated by the consumer and the likely presence of viable pathogenic agent in the waste.

~~Based on assessments using criteria in Article 5.4.2, in all disease-specific chapters, point 1 of Article X.X.12. (for Chapter 10.4. the relevant Article is 10.4.15.) lists aquatic animal commodities for retail trade for human consumption from a country, zone or compartment not declared free from the disease in question. The criteria for inclusion of aquatic animal commodities in point 1 of Article X.X.12. include consideration of the form and presentation of the product, the expected volume of waste tissues generated by the consumer and the likely presence of viable pathogenic agent in the waste.~~

Annex 5 (contd)6) 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 Aquatic Animal Health Services' ethical approach to the provision of international health certificates and their history in meeting their notification obligations.

International health certificates underpin international trade and provide assurances to the importing country regarding the health status of the aquatic animal products imported. ~~The measures prescribed should take into account the health status of both exporting and importing countries and be based upon the standards in the Aquatic Code.~~

The following steps should be taken when drafting international aquatic animal health certificates:

- a) identify the diseases, from which the importing country is justified in seeking protection because of its own aquatic animal health status. Importing countries should not impose measures in regards to diseases that occur in their own territory but are not subject to official control programmes;
- b) for aquatic animal products capable of transmitting these diseases through international trade, the importing country should apply the relevant articles in the disease-specific chapters. The application of the articles should be adapted to the disease status of the ~~exporting~~ country, zone or compartment of origin. Such a status should be established in accordance with Article 1.4.6. except when articles of the relevant disease chapter specify otherwise;
- c) when preparing international aquatic animal health certificates, the importing country should endeavour to use terms and expressions in accordance with the definitions given in the Glossary. ~~As stated in Article 5.2.3., international~~ International aquatic animal health certificates should be kept as simple as possible and should be clearly worded, to avoid misunderstanding of the importing country's requirements;
- d) Chapter 5.10. provides, as further guidance to Member Countries, model health certificates that should be used as a baseline.

7) Guidance notes for importers and exporters

It is recommended that Competent Authorities prepare 'guidance notes' to assist importers and exporters to understand trade requirements. These notes should identify and explain the trade conditions, including the measures to be applied before and after export and during transport and unloading, and the relevant legal obligations and operational procedures. The guidance notes should advise on all details to be included in the health certification accompanying the consignment to its destination. Exporters should also be reminded of the International Air Transport Association rules governing air transport of aquatic animals and aquatic animal products.

GLOSSARY

AQUATIC ANIMAL HEALTH STATUS

means the status of a country, *zone* or *compartment* with respect to a *disease* in accordance with the criteria listed in the relevant disease-specific chapter or Chapter 1.4, of the *Aquatic Code* ~~dealing with the disease~~.

BIOSECURITY

means a set of management and physical measures designed to ~~reduce~~ mitigate the *risk* of introduction, ~~establishment and spread~~ of *pathogenic agents* to into, from and within or spread within, or release from, ~~an aquatic animal populations~~ population.

BIOSECURITY PLAN

means a ~~plan~~ document that identifies ~~significant~~ potential pathways for the introduction of *pathogenic agents* into, ~~and~~ or spread within, or release from, ~~of disease in a zone, or compartment, or aquaculture establishment~~, and describes the measures ~~which are being, or will be,~~ applied to mitigate the identified risks; to introduce and spread disease, in accordance with taking into consideration the recommendations in the *Aquatic Code*. The plan should also describe how these measures are audited, with respect to both their implementation and their targeting, ~~to ensure that the risks are regularly re-assessed and the measures adjusted accordingly~~.

SELF-DECLARATION OF FREEDOM FROM DISEASE

means declaration by the *Competent Authority* of the Member Country concerned that the country, *zone* or *compartment* is free from a *listed disease* based on implementation of the provisions of the *Aquatic Code* and the *Aquatic Manual*. [NOTE: The Member Country is encouraged to inform the OIE of its claimed status and the OIE may publish the claim but publication does not imply OIE endorsement of the claim.]

SUSCEPTIBLE SPECIES

means a species of *aquatic animals* ~~animal in which infection that have~~ has been demonstrated as susceptible to infection with a specific pathogenic agent, in accordance with Chapter 1.5. by the occurrence of natural cases or by experimental exposure to the pathogenic agent that mimics natural transmission pathways.

CHAPTER 1.3.

DISEASES LISTED BY THE OIE

Preamble: ~~The following diseases in this chapter are have been assessed in accordance with Chapter 1.2. and constitutes are listed by the OIE list of aquatic animal diseases according to the criteria for listing an aquatic animal disease (see Article 1.2.2.).~~

In case of modifications of this list of *aquatic animal diseases* adopted by the World Assembly of Delegates, the new list comes into force on 1 January of the following year.

Article 1.3.1.

The following *diseases* of fish are listed by the OIE:

- ~~Infection with Epizootic epizootic~~ haematopoietic necrosis virus disease
- Infection with *Aphanomyces invadans* (epizootic ulcerative syndrome)
- Infection with *Gyrodactylus salaris*
- Infection with HPR-deleted or HPR0 infectious salmon anaemia virus
- Infection with salmonid alphavirus
- ~~Infectious~~ Infection with infectious haematopoietic necrosis virus
- ~~Koi~~ Infection with koi herpesvirus ~~disease~~
- ~~Infection with red~~ Red sea bream iridovirus ~~iridoviral disease~~
- ~~Spring~~ Infection with spring viraemia of carp virus
- ~~Viral~~ Infection with viral haemorrhagic septicaemia virus

Article 1.3.2.

The following *diseases* of molluscs are listed by the OIE:

- Infection with abalone herpesvirus
- Infection with *Bonamia ostreae*
- Infection with *Bonamia exitiosa*
- Infection with *Marteilia refringens*
- Infection with *Perkinsus marinus*
- Infection with *Perkinsus olseni*
- Infection with *Xenohaliotis californiensis*.

Annex 7 (contd)

Article 1.3.3.

The following *diseases* of crustaceans are listed by the OIE:

- Acute hepatopancreatic necrosis disease
- Infection with *Aphanomyces astaci* (crayfish plague)
- Infection with *Hepatobacter penaei* (necrotising hepatopancreatitis)
- Infection with infectious hypodermal and haematopoietic necrosis virus
- Infection with infectious myonecrosis virus
- Infection with *Macrobrachium rosenbergii* nodavirus (white tail disease)
- Infection with Taura syndrome virus
- Infection with white spot syndrome virus
- Infection with yellow head virus genotype 1.

Article 1.3.4.

The following *diseases* of amphibians are listed by the OIE:

- Infection with *Batrachochytrium dendrobatidis*
 - Infection with *Batrachochytrium salamandrivorans*
 - Infection with *Ranavirus* species
-

CHAPTER 5.3.

OIE PROCEDURES RELEVANT TO THE AGREEMENT ON THE APPLICATION OF SANITARY AND PHYTOSANITARY MEASURES OF THE WORLD TRADE ORGANIZATION

Article 5.3.1.

The Agreement on the Application of Sanitary and Phytosanitary Measures and role and responsibility of the OIE

The *Agreement on the Application of Sanitary and Phytosanitary Measures* (SPS Agreement) specifically encourages the Members of the World Trade Organization to base their *sanitary measures* on international standards, guidelines and recommendations, where they exist. Members may choose to implement sanitary measures that exceed more stringent ~~adopt a higher level of protection than that provided by those in international standards, texts if these are deemed necessary to protect aquatic animal or human health and are scientifically justified by a risk analysis~~ there is a scientific justification or if the level of protection provided by the relevant international texts is considered to be inappropriate. In such circumstances, Members are subject to obligations relating to ~~risk assessment~~ and to should adopt a consistent approach of to risk management.

~~The SPS Agreement encourages Governments to make a wider use of risk analysis: WTO Members shall undertake an assessment as appropriate to the circumstances of the actual risk involved.~~

To promote transparency, ~~The~~ the SPS Agreement, in Article 7, obliges WTO Members to notify changes in, and provide relevant information on, *sanitary measures* ~~which~~ that may, directly or indirectly, affect international trade.

The SPS Agreement recognises the OIE as the relevant international organisation responsible for the development and promotion of international animal health standards, guidelines, and recommendations affecting trade in live aquatic animals and aquatic animal products.

Article 5.3.2.

Introduction ~~on~~ to the judgement determination of the equivalence of sanitary measures

The importation of aquatic animals and aquatic animal products involves a degree of *risk* to ~~the aquatic animal health status and human health status of in an importing country~~. The estimation of that *risk* and the choice of the appropriate *risk management* option(s) are made ~~more~~ difficult by differences among the aquatic animal health management systems and aquatic animal production and processing systems in Member Countries. ~~However, it is now recognised that significantly different animal health and production systems and measures can provide~~ may achieve equivalent aquatic animal and human health protection for the purposes of *international trade*, ~~with benefits to both the importing country and the exporting country~~.

~~These~~ The recommendations in this chapter are intended to assist Member Countries to determine whether *sanitary measures* arising from different ~~animal health and production systems may provide achieve~~ the same level of aquatic animal and human health protection. ~~They discuss principles~~ Principles are provided which might that may be utilised in a judgement determination of equivalence, and outline a step-wise process for trading partners to follow ~~in facilitating a judgement of equivalence~~. These provisions are applicable whether equivalence applies ~~at the level of to~~ specific measures or on a systems-wide basis, and whether equivalence applies to specific areas of trade or aquatic animal products, or in generally general.

Annex 8 (contd)

Article 5.3.3.

General considerations on the judgement determination of the equivalence of sanitary measures

Before trade in *aquatic animals* or their products ~~may occur~~, an *importing country* ~~must~~ should be ~~satisfied~~ assured that its *aquatic animal health status and human health in its territory* will be appropriately protected. In most cases, the *risk management* measures ~~adopted~~ drawn-up will rely in part on judgements made about the *aquatic animal health management* and *aquatic animal* production system(s) in the *exporting country* and the effectiveness of *sanitary measures* ~~procedures applied~~ undertaken there. Systems operating in the *exporting country* may differ from those in the *importing country* and from those in other countries with which the *importing country* has traded. Differences may be ~~with respect to~~ in infrastructure, policies ~~and/or~~ operating procedures, laboratory systems, approaches to control of the pests and diseases present, border security and internal movement controls.

~~International recognition of the legitimacy of different approaches to achieving the importing country's appropriate level of protection (ALOP) has led to the principle of equivalence being included in trade agreements, including the SPS Agreement of the WTO.~~

If trading partners agree that the measures applied achieve the same level of health protection, these measures are considered equivalent. Benefits of applying equivalence may include:

- 1) minimising costs associated with *international trade* by ~~tailoring~~ allowing *sanitary measures* to be tailored animal health measures to local circumstances;
- 2) maximising *aquatic animal* health outcomes for a given level of resource input;
- 3) facilitating trade by achieving the required health protection through less trade restrictive *sanitary measures*; and
- 4) decreased reliance on relatively costly ~~commodity testing and isolation procedures in bilateral or multilateral agreements.~~

The *Aquatic Code* recognises equivalence by recommending alternative *sanitary measures* for many *diseases*. Equivalence may be ~~gained~~ achieved, for example, by enhanced *surveillance* and monitoring, by the use of alternative test, treatment or isolation procedures, or by combinations of the above. To facilitate the judgement determination of equivalence, Member Countries should base their *sanitary measures* on the OIE standards, and guidelines and recommendations of the OIE.

~~It is essential to apply a scientific~~ Member Countries should use *risk analysis* to ~~the extent practicable in~~ establishing the basis for a judgement determination of equivalence.

Article 5.3.4.

Prerequisite considerations ~~in a judgement~~ for the determination of equivalence1) Application of risk assessment

~~Application of the discipline of risk~~ Risk assessment provides a structured basis for judging equivalence among different *sanitary measures* as it allows a comparison ~~close examination to be made~~ of the effect of a measure(s) on a particular step(s) in the importation pathway, ~~and the relative~~ with the effects of a proposed alternative measure(s) on the same or related steps.

A judgement determination of equivalence ~~should~~ needs to assess compare the effectiveness of the *sanitary measures* in terms of its effectiveness against regarding the particular *risk* or group of *risks* against which it ~~the measure is~~ they are designed to protect. Such an assessment may include the following elements: the purpose of the measure, the level of protection achieved by the measure and the contribution the measure makes to achieving the ALOP of the importing country.

2) Categorisation of sanitary measures

Proposals for equivalence may be in terms of a measure comprising consider a single component of a ~~measure~~ (e.g. an isolation or sampling procedure, a test or treatment requirement, a certification procedure) or multiple components (e.g. a production system for a commodity) of a sanitary measure, or a combination of sanitary measures. ~~Multiple components or combinations of measures~~ Sanitary measures Measures may be applied consecutively or concurrently.

~~Sanitary measures are those described in each disease-specific chapter chapter of the Aquatic Code which are used for to manage the risks reduction and are appropriate for particular posed by that disease diseases. Sanitary measures may be applied either alone or in combination and include test requirements, processing requirements, inspection or certification procedures, quarantine confinements, and sampling procedures.~~

For the purposes of ~~judging~~ determining equivalence, sanitary measures can be broadly categorised as:

- a) infrastructure: including the legislative base (e.g. aquatic animal health law) and administrative systems (e.g. organisation of Veterinary Services or Aquatic Animal Health Services national and regional animal health authorities, emergency response organisations);
- b) programme design and/implementation: including documentation of systems, performance and decision criteria, laboratory capability, and provisions for certification, audit and enforcement;
- c) specific technical requirement: including requirements applicable to the use of secure facilities, treatment (e.g. retorting of cans), specific test (e.g. ELISA) and procedures (e.g. pre-export inspection).

A ~~sanitary~~ Sanitary measure(s) proposed for a judgement determination of equivalence may fall into one or more of these categories, which are not mutually exclusive.

In some cases, such as a method for pathogen inactivation, a comparison of specific technical requirements may suffice. In many instances, however, a judgement as to assessment of whether the same level of protection is likely to will be achieved may only be able to be determined through an evaluation of all relevant components of an exporting country's aquatic animal health management systems and aquatic animal production systems. For example, a judgement of equivalence for a specific sanitary measure at the programme design/implementation level may require a prior examination of infrastructure while a judgement of equivalence for a specific measure at the specific technical requirement level may require that the specific measure be judged in its context through examination of infrastructure and programmes.

Article 5.3.5.

Principles for judgement determination of equivalence

~~In conjunction with the above considerations, judgement~~ Determination of the equivalence of sanitary measures should be based on application of the following principles:

- 1) an importing country has the right to set the level of protection it deems appropriate (~~its ALOP~~) in relation to human and animal life and health in its territory; this ~~ALOP~~ may be expressed in qualitative or quantitative terms;
- 2) the importing country should be able to describe the reason for each sanitary measure i.e. the level of protection intended to be achieved by application of the identified measure against a hazard risk;
- 3) an importing country should recognise that sanitary measures different from the ones it has proposed may be capable of providing achieving the same level of protection, in particular, it should consider the existence of free zones or compartments, and of safe safe aquatic animal products;
- 4) the importing country should, upon request, ~~enter into consultations~~ consult with the exporting country with the aim of facilitating a judgement determination of equivalence;
- 5) any sanitary measure or combination of sanitary measures can be proposed for judgement determination of equivalence;

Annex 8 (contd)

- 6) an interactive process should be followed that applies a defined sequence of steps, and utilises an agreed process for exchange of information, so as to limit data collection to that which is necessary, to minimise administrative burden, and to facilitate resolution of claims;
- 7) the *exporting country* should be able to demonstrate objectively how the alternative *sanitary measure(s)* proposed as equivalent will provide the same level of protection;
- 8) the *exporting country* should present a submission for equivalence in a form that facilitates judgement determination by the *importing country*;
- 9) the *importing country* should evaluate submissions for equivalence in a timely, consistent, transparent and objective manner, and in accordance with appropriate *risk assessment* principles;
- 10) the *importing country* should take into account any knowledge of and prior experience with the *Veterinary Authority* or other *Competent Authority* of the *exporting country*;
- 10bis) the importing country should take into account any arrangements it has with other exporting countries on similar issues;
- 10ter) the importing country may also take into account any knowledge of the exporting country's arrangements with other importing countries;
- 11) the *exporting country* should provide access to enable the procedures or systems ~~which that~~ are the subject of the equivalence judgement determination to be examined and evaluated upon request of the *importing country*;
- 12) the *importing country* should be the sole ~~determinant~~ judge of equivalence, but should provide to the *exporting country* a full explanation for its judgement;
- 13) to facilitate a judgement determination of equivalence, Member Countries should base their *sanitary measures* on relevant OIE standards and guidelines, where these exist. However, they may choose to implement sanitary measures that exceed OIE standards if these are scientifically justified by a risk analysis;
- 14) to allow the judgement determination of equivalence to be reassessed if necessary, the *importing country* and the *exporting country* should keep each other informed of significant changes to infrastructure, health status or programmes ~~which that~~ may bear on the judgement determination of equivalence; and
- 15) ~~appropriate technical assistance from an importing country, following a should give positive consideration to a request by an exporting developing country, for appropriate technical assistance that would may facilitate the successful completion of a judgement determination of equivalence.~~

Article 5.3.6.

Sequence of steps to be taken in judgement determination of equivalence

There is no single sequence of steps ~~which that must should~~ be followed in all ~~judgements~~ determinations of equivalence. The steps that trading partners choose will generally depend on the circumstances and their trading experience. ~~Nevertheless, The the~~ interactive sequence of steps described below may be useful for assessing any all sanitary measures irrespective of their categorisation as infrastructure, programme design/ and implementation or specific technical requirement components of an *aquatic animal* health management system or and aquatic animal production system.

This sequence assumes that the *importing country* is meeting its obligations under the WTO SPS Agreement and has in place a transparent measure based either on an international standard or a *risk analysis*.

Recommended steps are:

- 1) the *exporting country* identifies the measure(s) for which it wishes to propose an alternative ~~measure(s)~~, and requests from the *importing country* a reason for its *sanitary measure* in terms of the level of protection intended to be achieved against a ~~hazard(s)~~ risk;

Annex 8 (contd)

- 2) the *importing country* explains the reason for the measure(s), in terms ~~that~~ which would facilitate comparison with an alternative *sanitary measure(s)* and consistent with the principles set out in these provisions;
- 3) the *exporting country* demonstrates the case for equivalence of an alternative *sanitary measure(s)* in a form ~~which~~ that facilitates evaluation analysis by an *importing country*;
- 4) the *exporting country* responds to any technical concerns raised by the *importing country* by providing relevant further information;
- 5) ~~judgement~~ determination of equivalence by the *importing country* should ~~takes~~ into account as appropriate:
 - a) the impact of biological variability and uncertainty;
 - b) the expected effect of the alternative *sanitary measure(s)* ~~on all relevant hazards~~;
 - c) OIE standards and guidelines;
 - d) ~~application of solely qualitative frameworks where it is not possible or reasonable to conduct quantitative~~ the results of a risk assessment;
- 6) the *importing country* notifies the *exporting country* of its judgement and its ~~the~~ underlying reasons within a reasonable period of time. The judgement:
 - a) ~~recognition~~ recognises of the equivalence of the *exporting country's* alternative *sanitary measure(s)*; or
 - b) requests ~~for~~ further information; or
 - c) ~~rejection~~ rejects of the case for equivalence of the alternative *sanitary measure(s)*;
- 7) an attempt should be made to resolve any differences of opinion over judgement of a case, ~~either interim or final~~, by using an agreed mechanism such as to reach consensus (e.g. the OIE informal procedure for dispute mediation), ~~or by referral to an agreed expert (Article 5.3.8.)~~;
- 8) depending on the category of measures involved, the *importing country* and the *exporting country* may informally acknowledge the equivalence or enter into a formal agreement of equivalence ~~agreement~~ giving effect to the judgement ~~or a less formal acknowledgement of the equivalence of a specific measure(s) may suffice~~.

An *importing country* recognising the equivalence of an *exporting country's* alternative *sanitary measure(s)* ~~needs to~~ should ensure that it acts consistently with regard to applications from third countries for recognition of equivalence applying to the same or a very similar measure(s). Consistent action does not mean however that a specific measure(s) proposed by several *exporting countries* should always be judged as equivalent because as a measure(s) should not be considered in isolation but as part of a system of infrastructure, policies and procedures, in the context of the aquatic animal health situation in the exporting country.

Article 5.3.7.

Sequence of steps to be taken in establishing a zone/ or compartment and having it recognised for international trade purposes

The terms 'zone' and 'zoning' in the Aquatic Code have the same meaning as 'region', 'area' and 'regionalisation' in the SPS Agreement of the WTO.

Annex 8 (contd)

~~The requirements for establishing~~ ~~There is no single sequence of steps which should be followed in establishing~~ ~~of a disease-free zone or a compartment declared free of a disease is~~ ~~are described in each disease-specific chapter~~ ~~Chapter 4.1,~~ ~~and should be considered by trading partners when establishing sanitary measures for trade.~~ ~~The steps that the Veterinary Services or Aquatic Animal Health Services of the importing country and the exporting country choose and implement will generally depend on the circumstances existing within the countries and at their borders, and their trading history.~~ ~~The recommended steps are~~ The requirements include:

1. For zoning

- a) The *exporting country* identifies a geographical area within its *territory*, which, based on surveillance, it considers to contain an *aquatic animal subpopulation* with a distinct health status with respect to a specific *disease*. ~~specific diseases based on surveillance.~~
- b) The *exporting country* describes in the *biosecurity plan* for the *zone* the measures ~~which are being, or will be,~~ applied to distinguish such an area epidemiologically from other parts of its territory, in accordance with the recommendations in the *Aquatic Code*.
- c) The *exporting country* provides:
 - i) the above information to the *importing country*, with an explanation of why the area can be treated as an epidemiologically separate *zone* for *international trade* purposes;
 - ii) access to enable the procedures or systems that establish the *zone* to be examined and evaluated upon request by the *importing country*.
- d) The *importing country* determines whether it accepts such an area as a *zone* for the importation of *aquatic animals* ~~and~~ or *aquatic animal products*, taking into account:
 - i) an evaluation of the *exporting country's Veterinary Services or Aquatic Animal Health Services*;
 - ii) the result of a *risk assessment* based on the information provided by the *exporting country* and its own research;
 - iii) its own *aquatic animal* health situation with respect to the *disease(s)* concerned; and
 - iv) other relevant OIE standards or guidelines.
- e) The *importing country* notifies the *exporting country* of its determination judgement and the underlying its reasons, within a reasonable period of time, being:
 - i) recognition of the *zone*; or
 - ii) request for further information; or
 - iii) rejection of the area as a *zone* for *international trade* purposes.
- f) An attempt should be made to resolve any differences over recognition of the *zone*, ~~either in the interim or finally,~~ by using an agreed mechanism ~~to reach consensus~~ such as the OIE informal procedure for dispute mediation (Article 5.3.8.).
- g) The *Veterinary Authorities* or other Competent Authorities of the *importing* and *exporting countries* should enter into an an ~~formal~~ agreement recognising the *zone*.

2. For compartmentalisation

- a) Based on discussions with the relevant industry, the *exporting country* identifies within its territory a *compartment* comprising an *aquatic animal subpopulation* contained in one or more *establishments*, ~~or~~ and other premises operating under common management practices and related to biosecurity plan. The *compartment* contains an identifiable *aquatic animal subpopulation* with a distinct health status with respect to a specific disease(s). The *exporting country* describes how this status is maintained through a partnership between the relevant industry and the *Veterinary Authority or other Competent Authority* of the *exporting country*.
- b) The *exporting country* examines the *compartment's biosecurity plan* and confirms through an audit that:
- i) the *compartment* is epidemiologically closed throughout its routine operating procedures as a result of effective implementation of its *biosecurity plan*; and
 - ii) the *surveillance* and monitoring programme in place is appropriate to verify the status of such a *subpopulation* with respect to ~~such the~~ disease(s) in question.
- c) The *exporting country* describes the *compartment*, in accordance with ~~the recommendations in the Aquatic Code Chapters 4.1. and 4.2.~~
- d) The *exporting country* provides:
- i) the above information to the *importing country*, with an explanation of why such a *subpopulation* can be treated as an epidemiologically separate *compartment* for *international trade* purposes; and
 - ii) access to enable the procedures or systems that establish the *compartment* to be examined and evaluated upon request by the *importing country*.
- e) The *importing country* determines whether it accepts such a *subpopulation* as a *compartment* for the importation of *aquatic animals* ~~or~~ and *aquatic animal products*, taking into account:
- i) an evaluation of the *exporting country's Veterinary Service or Aquatic Animal Health Services*;
 - ii) the result of a *risk assessment* based on the information provided by the *exporting country* and its own research;
 - iii) its own *aquatic animal* health situation with respect to the disease(s) concerned; and
 - iv) other relevant OIE standards or guidelines.
- f) The *importing country* notifies the *exporting country* of its ~~determination~~ judgement and ~~the underlying its~~ reasons, within a reasonable period of time, being:
- i) recognition of the *compartment*, or
 - ii) request for further information; or
 - iii) rejection of such a *subpopulation* as a *compartment* for *international trade* purposes.
- g) An attempt should be made to resolve any differences over recognition of the *compartment*, ~~either in the interim or finally~~, by using an agreed mechanism ~~to reach consensus~~ such as the OIE informal procedure for dispute mediation (Article 5.3.8.).
- h) The *Veterinary Authorities or other Competent Authorities* of the *importing* and *exporting countries* should enter into an agreement recognising the *compartment*.

Annex 8 (contd)

- i) ~~The Veterinary Authority or other Competent Authorities of the exporting country should promptly inform importing countries of any occurrence of a disease in respect of which the compartment was defined.~~

Article 5.3.8.

The OIE informal procedure for dispute mediation

~~The OIE shall maintain its existing a~~ voluntary in-house mechanisms for assisting Member Countries to resolve differences. In-house procedures ~~that which~~ will apply are that:

- 1) Both parties agree to give the OIE a mandate to assist them in resolving their differences.
 - 2) If considered appropriate, the Director General of the OIE recommends an expert, or experts, and a chairman, as requested, agreed by both parties.
 - 3) Both parties agree on the terms of reference and working programme, and to meet all expenses incurred by the OIE.
 - 4) The expert or experts are entitled to seek clarification of any of the information and data provided by either country in the assessment or consultation processes, or to request additional information or data from either country.
 - 5) The expert or experts ~~shall~~ submit a confidential report to the Director General of the OIE, who ~~will~~ then transmits it to both parties.
-

CHAPTER 8.X.

INFECTION WITH *BATRACHOCHYTRIUM*
SALAMANDRIVORANS

Article 8.X.1.

For the purposes of the *Aquatic Code*, infection with *Batrachochytrium salamandrivorans* means *infection* with the *pathogenic agent Batrachochytrium salamandrivorans*, of the Division Chytridiomycota and Order Rhizophydiales.

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

Article 8.X.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.: [alpine newt (*Ichthyosaura alpestris*), blue-tailed fire-bellied newt (*Cynops cyanurus*), fire salamander (*Salamandra salamandra*), eastern newt (*Nothophthalmus viridescens*), French cave salamander (*Hydromantes strinatii*), Italian newt (*Lissotriton italicus*), yellow spotted newt (*Neurergus crocatus*), Japanese fire-bellied newt (*Cynops pyrrhogaster*), northern spectacle salamander (*Salamandrina perspicillata*), Tam Dao salamander (*Paramesotriton deloustali*), rough-skinned newt (*Taricha granulosa*), sardinian brook salamander (*Euproctus platycephalus*) and Spanish ribbed newt (*Pleurodeles waltli*)] (under study).

Article 8.X.3.

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) *Competent Authorities* should not require any 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.X.2. that are intended for any purpose and comply with Article 5.4.1.:
 - 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) mechanically dried amphibian products (i.e. a heat treatment at 100°C for at least 30 minutes or any time/temperature equivalent that has been demonstrated to inactivate *B. salamandrivorans*);
 - e) amphibian skin leather.
- 2) When authorising the importation or transit of *aquatic animal products* of a species referred to in Article 8.X.2., other than those referred to in point 1 of Article 8.X.3., *Competent Authorities* should require the conditions prescribed in Articles 8.X.7. to 8.X.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* of a species not referred to in Article 8.X.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.

Annex 9A (contd)

Article 8.X.4.

Country free from infection with *B. salamandrivorans*

If a country shares a *zone* with one or more other countries, it can only make a *self-declaration of freedom* from infection with *B. salamandrivorans* if all the areas covered by the shared water bodies are declared countries or *zones* free from infection with *B. salamandrivorans* (see Article 8.X.5.).

As described in Article 1.4.6., a country may make a *self-declaration of freedom* from infection with *B. salamandrivorans* if:

- 1) none of the *susceptible species* referred to in Article 8.X.2. are present and *basic biosecurity conditions* have been continuously met for at least the last two years;

OR

- 2) any of the *susceptible species* referred to in Article 8.X.2. are present and the following conditions have been met:
 - a) there has been no occurrence of infection with *B. salamandrivorans* for at least the last ten years despite conditions that are conducive to its clinical expression (as described in the corresponding chapter of the *Aquatic Manual*); and
 - b) *basic biosecurity conditions* have been continuously met for at least the last ten years;

OR

- 3) the infection with *B. salamandrivorans* status prior to *targeted surveillance* is unknown but the following conditions have been met:
 - a) *basic biosecurity conditions* have been continuously met for at least the last two years; and
 - b) *targeted surveillance*, as described in Chapter 1.4., has been in place for at least the last two years without detection of *B. salamandrivorans*;

OR

- 4) it previously made a *self-declaration of freedom* from infection with *B. salamandrivorans* and subsequently lost its free status due to the detection of *B. salamandrivorans* but the following conditions have been met:
 - a) on detection of *B. salamandrivorans*, 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 *B. salamandrivorans*, and appropriate *disinfection* procedures (described in Chapter 4.3.) have been completed; 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 *B. salamandrivorans*; and
 - d) *targeted surveillance*, as described in Chapter 1.4., has been in place for at least the last two years without detection of *B. salamandrivorans*.

In the meantime, part or all of the unaffected area may be declared a free *zone* provided that such a part meets the conditions in point 3 of Article 8.X.5.

Article 8.X.5.

Zone or compartment free from infection with *B. salamandrivorans*

If a *zone* or *compartment* extends over more than one country, it can only be declared a *zone* or *compartment* free from infection with *B. salamandrivorans* if all the relevant *Competent Authorities* confirm that all relevant conditions have been met.

As described in Article 1.4.6., a *zone* or *compartment* within the *territory* of one or more countries not declared free from infection with *B. salamandrivorans* may be declared free by the *Competent Authority* of the country concerned if:

- 1) none of the *susceptible species* referred to in Article 8.X.2. are present in the *zone* or *compartment* and *basic biosecurity conditions* have been continuously met for at least the last two years;

OR

- 2) any of the *susceptible species* referred to in Article 8.X.2. are present in the *zone* or *compartment* and the following conditions have been met:
 - a) there has been no occurrence of infection with *B. salamandrivorans* for at least the last ten years despite conditions that are conducive to its clinical expression (as described in the corresponding chapter of the *Aquatic Manual*); and
 - b) *basic biosecurity conditions* have been continuously met for at least the last ten years;

OR

- 3) the infection with *B. salamandrivorans* status prior to *targeted surveillance* is unknown but the following conditions have been met:
 - a) *basic biosecurity conditions* have been continuously met for at least the last two years; and
 - b) *targeted surveillance*, as described in Chapter 1.4., has been in place, in the *zone* or *compartment*, for at least the last two years without detection of *B. salamandrivorans*;

OR

- 4) it previously made a *self-declaration of freedom* for a *zone* from infection with *B. salamandrivorans* and subsequently lost its free status due to the detection of *B. salamandrivorans* in the *zone* but the following conditions have been met:
 - a) on detection of *B. salamandrivorans*, 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 *B. salamandrivorans*, and the appropriate *disinfection* procedures (as described in Chapter 4.3.) have been completed; 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 *B. salamandrivorans*; and
 - d) *targeted surveillance*, as described in Chapter 1.4., has been in place for at least the last two years without detection of *B. salamandrivorans*.

Annex 9A (contd)

Article 8.X.6.

Maintenance of free status

A country, *zone* or *compartment* that is declared free from infection with *B. salamandrivorans* following the provisions of points 1 or 2 of Articles 8.X.4. or 8.X.5. (as relevant) may maintain its status as free from infection with *B. salamandrivorans* provided that *basic biosecurity conditions* are continuously maintained.

A country, *zone* or *compartment* that is declared free from infection with *B. salamandrivorans* following the provisions of point 3 of Articles 8.X.4. or 8.X.5. (as relevant) may discontinue *targeted surveillance* and maintain its free status provided that conditions that are conducive to clinical expression of infection with *B. salamandrivorans*, as described in the corresponding chapter of the *Aquatic Manual*, and *basic biosecurity conditions* are continuously maintained.

However, for declared free *zones* or *compartments* in infected countries and in all cases where conditions are not conducive to clinical expression of infection with *B. salamandrivorans*, *targeted surveillance* should be continued at a level determined by the *Aquatic Animal Health Service* on the basis of the likelihood of *infection*.

Article 8.X.7.

Importation of aquatic animals or aquatic animal products from a country, zone or compartment declared free from infection with *B. salamandrivorans*

When importing *aquatic animals* of a species referred to in Article 8.X.2., or *aquatic animal products* derived thereof, from a country, *zone* or *compartment* declared free from infection with *B. salamandrivorans*, 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*. The *international aquatic animal health certificate* should state that, on the basis of the procedures described in Articles 8.X.4. or 8.X.5. (as applicable) and 8.X.6., the place of production of the *aquatic animals* or *aquatic animal products* is a country, *zone* or *compartment* declared free from infection with *B. salamandrivorans*.

The *international aquatic animal health certificate* should be in accordance with the Model Certificate in Chapter 5.11.

This article does not apply to *aquatic animal products* listed in point 1 of Article 8.X.3.

Article 8.X.8.

Importation of aquatic animals for aquaculture from a country, zone or compartment not declared free from infection with *B. salamandrivorans*

When importing, for *aquaculture*, *aquatic animals* of species referred to in Article 8.X.2. from a country, *zone* or *compartment* not declared free from infection with *B. salamandrivorans*, the *Competent Authority* of the *importing country* should assess the *risk* in accordance with Chapter 2.1. and consider the *risk* mitigation measures in points 1 and 2 below:

- 1) If the intention is to grow out and harvest the imported *aquatic animals*, consider the following:
 - a) the direct delivery to and lifelong holding of the imported *aquatic animals* in a *quarantine* facility; and
 - b) the treatment of transport water, equipment, effluent and waste material to inactivate *B. salamandrivorans* in accordance with Chapters 4.3., 4.7. and 5.5.

OR

- 2) If the intention is to establish a new stock for *aquaculture*, consider applying the following:
 - 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 *B. salamandrivorans*.

- b) In the *importing country*:
- i) import the F-0 population into a *quarantine* facility;
 - ii) test the F-0 population for *B. salamandrivorans* in accordance with Chapter 1.4. to determine their suitability as broodstock;
 - iii) produce first generation (F-1) population in *quarantine*;
 - iv) culture the F-1 population in *quarantine* under conditions that are conducive to the clinical expression of *B. salamandrivorans*, ~~(as described in Chapter 2.1.X. of the *Aquatic Manual*)~~ and sample and test for *B. salamandrivorans* in accordance with Chapter 1.4. of the *Aquatic Code* and ~~(as described in Chapter 2.1.X. of the *Aquatic Manual*)~~;
 - v) if *B. salamandrivorans* is not detected in the F-1 population, it may be defined as free from infection with *B. salamandrivorans* and may be released from *quarantine*;
 - vi) if *B. salamandrivorans* 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.

Article 8.X.9.

Importation of aquatic animals or aquatic animal products for processing for human consumption from a country, zone or compartment not declared free from infection with *B. salamandrivorans*

When importing, for processing for human consumption, *aquatic animals* of a species referred to in Article 8.X.2., or *aquatic animal products* derived thereof, from a country, *zone* or *compartment* not declared free from infection with *B. salamandrivorans*, the *Competent Authority* of the *importing country* should assess the *risk* and, if justified, require that:

- 1) the consignment is delivered directly to, and held in, *quarantine* or containment facilities until processed into one of the products referred to in point 1 of Article 8.X.3. or in point 1 of Article 8.X.12, or other products authorised by the *Competent Authority*; and
- 2) all water (including ice), equipment, *containers* and packaging material used in transport are treated to ensure inactivation of *B. salamandrivorans* or disposed of in a biosecure manner in accordance with Chapters 4.3., 4.7. and 5.5.; and
- 3) all effluent and waste materials are treated to ensure inactivation of *B. salamandrivorans* or disposed of in a biosecure manner in accordance with Chapters 4.3. and 4.7.

For these *aquatic animals* or *aquatic animal products* Member Countries may wish to consider introducing internal measures to address the *risks* associated with the *aquatic animal* or *aquatic animal product* being used for any purpose other than for human consumption.

Article 8.X.10.

Importation of aquatic animals or aquatic animal products intended for uses other than human consumption, including animal feed and agricultural, industrial, research or pharmaceutical use, from a country, zone or compartment not declared free from infection with *B. salamandrivorans*

When importing *aquatic animals* of a species referred to in Article 8.X.2., or *aquatic animal products* derived thereof, intended for uses other than human consumption, including animal *feed* and agricultural, industrial, research or pharmaceutical use, from a country, *zone* or *compartment* not declared free from infection with *B. salamandrivorans*, the *Competent Authority* of the *importing country* should require that:

- 1) the consignment is delivered directly to, and held in, *quarantine* or containment facilities until processed into one of the products referred to in point 1 of Article 8.X.3. or other products authorised by the *Competent Authority*; and
- 2) all water (including ice), equipment, *containers* and packaging material used in transport are treated to ensure inactivation of *B. salamandrivorans* or disposed of in a biosecure manner in accordance with Chapters 4.3., 4.7. and 5.5.; and

Annex 9A (contd)

- 3) all effluent and waste materials are treated to ensure inactivation of *B. salamandrivorans* or disposed of in a biosecure manner in accordance with Chapters 4.3. and 4.7.

Article 8.X.11.

Importation of aquatic animals intended for use in laboratories or zoos from a country, zone or compartment not declared free from infection with *B. salamandrivorans*

When importing, for use in laboratories and zoos, *aquatic animals* of species referred to in Article 8.X.2. from a country, *zone* or *compartment* not declared free from infection with *B. salamandrivorans*, the *Competent Authority* of the *importing country* should ensure:

- 1) the consignment is delivered directly to, and held in, *quarantine* authorised by the *Competent Authority*; and
- 2) all water (including ice), equipment, *containers* and packaging material used in transport are treated to ensure inactivation of *B. salamandrivorans* or disposed of in a biosecure manner in accordance with Chapters 4.3., 4.7. and 5.5.; and
- 3) all effluent and waste materials from the *quarantine* facilities in the laboratories or zoos are treated to ensure inactivation of *B. salamandrivorans* or disposed of in a biosecure manner in accordance with Chapters 4.3. and 4.7.; and
- 4) the carcasses are disposed of in accordance with Chapter 4.7.

Article 8.X.12.

Importation (or transit) of aquatic animal products for retail trade for human consumption regardless of the infection with *B. salamandrivorans* status of the exporting country, zone or compartment

- 1) *Competent Authorities* should not require any 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 amphibian meat (skin off and fresh or frozen) that has been prepared and packaged for retail trade and comply with Article 5.4.2.

Certain assumptions have been made in assessing the safety of the *aquatic animal products* mentioned above. Member Countries should refer to these assumptions at Article 5.4.2. and consider whether the assumptions apply to their conditions.

For these *aquatic animal products* Member Countries may wish to consider introducing internal measures to address the *risks* associated with the *aquatic animal products* being used for any purpose other than for human consumption.

- 2) When importing *aquatic animal products*, other than those referred to in point 1 above, derived from a species referred to in Article 8.X.2. from a country, *zone* or *compartment* not declared free from infection with *B. salamandrivorans*, the *Competent Authority* of the *importing country* should assess the *risk* and apply appropriate *risk* mitigation measures.

CHAPTER 8.1.
**INFECTION WITH
 BATRACHOCHYTRIUM DENDROBATIDIS**

Article 8.1.1.

For the purposes of the *Aquatic Code*, infection with *Batrachochytrium dendrobatidis* means *infection* with the *pathogenic agent Batrachochytrium dendrobatidis* of the Division Chytridiomycota and Order Rhizophydiales.

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

Article 8.1.2.

Scope

The recommendations in this chapter apply to: all species of *Anura* (frogs and toads), *Caudata* (salamanders, newts and sirens) and *Gymnophiona* (caecilians). The recommendations also apply to any other *susceptible species* referred to in the *Aquatic Manual* when traded internationally.

Article 8.1.3.

Importation or transit of ~~aquatic animals and~~ aquatic animal products for any purpose regardless of the infection with *B. dendrobatidis* status of the exporting country, zone or compartment

- 1) *Competent Authorities* should not require any conditions related to ~~infection with *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* from the species referred to in Article 8.1.2. ~~that~~ which are intended for any purpose and ~~which~~ that comply with Article 5.4.1.:
 - 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 ~~which~~ that has been demonstrated to inactivate *B. dendrobatidis*);
 - c) pasteurised amphibian products that have been subjected to heat treatment at 90°C for at least ten minutes (or any time/temperature equivalent ~~which~~ that has been demonstrated to inactivate *B. dendrobatidis*);
 - d) mechanically dried amphibian products (i.e. a heat treatment of 100°C for at least 30 minutes or any time/temperature equivalent ~~which~~ that has been demonstrated to inactivate *B. dendrobatidis*);
 - e) amphibian skin leather.
- 2) When authorising the importation or transit of ~~aquatic animals and~~ *aquatic animal products* of 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*.

Annex 9B (contd)

- 3) When considering the importation or transit of ~~aquatic animals and aquatic animal products~~ of a species not ~~covered~~ referred to in Article 8.1.2. but which could reasonably be expected to pose a *risk* of ~~spread~~ transmission of ~~infection with~~ *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 ~~assessment~~ analysis.

Article 8.1.4.

Country free from infection with *B. dendrobatidis*

If a country shares a *zone* with one or more other countries, it can only make a *self-declaration of freedom* from infection with *B. dendrobatidis* if all the areas covered by the shared water bodies are declared countries or zones free from the zone are declared infection with *B. dendrobatidis* (see Article 8.1.5.).

As described in Article 1.4.6., a country may make a *self-declaration of freedom* from infection with *B. dendrobatidis* if:

- 1) none of the *susceptible species* referred to in Article 8.1.2. are present and *basic biosecurity conditions* have been continuously met for at least the last two years;

OR

- 2) any of the *susceptible species* referred to in Article 8.1.2. are present and the following conditions have been met:

- a) there has been no ~~observed~~ occurrence of ~~the disease~~ infection with *B. dendrobatidis* for at least the last ten years despite conditions that are conducive to its clinical expression (as described in the corresponding chapter of the *Aquatic Manual*); and
- b) *basic biosecurity conditions* have been continuously met for at least the last ten years;

OR

- 3) the ~~disease~~ infection with *B. dendrobatidis* status prior to *targeted surveillance* is unknown but the following conditions have been met:

- a) *basic biosecurity conditions* have been continuously met for at least the last two years; and
- b) *targeted surveillance*, as described in Chapter 1.4., has been in place for at least the last two years without detection of ~~infection with~~ *B. dendrobatidis*;

OR

- 4) it previously made a *self-declaration of freedom* from infection with *B. dendrobatidis* and subsequently lost its ~~disease~~ free status due to the detection of ~~infection with~~ *B. dendrobatidis* but the following conditions have been met:

- a) on detection of *B. dendrobatidis* ~~the disease~~, 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 ~~have been destroyed or removed from the *infected zone*~~ by means that minimise the likelihood ~~risk~~ of further transmission spread of *B. dendrobatidis* ~~the disease~~, and the appropriate *disinfection* procedures (as described in Chapter 4.3.) have been completed; and

Annex 9B (contd)

- c) previously existing *basic biosecurity conditions* have been reviewed and modified as necessary and have continuously been in place since eradication of infection with *B. dendrobatidis* ~~the disease~~; and
- d) *targeted surveillance*, as described in Chapter 1.4., has been in place for at least the last two years without detection of ~~infection with *B. dendrobatidis*~~.

In the meantime, part or all of the ~~unaffected non-affected~~ area may be declared a free zone provided that such a part meets the conditions in point 3 of Article 8.1.5.

Article 8.1.5.

Zone or compartment free from infection with *B. dendrobatidis*

If a *zone* or *compartment* extends over more than one country, it can only be declared ~~a~~ an infection with *B. dendrobatidis* free zone or compartment free from infection with *B. dendrobatidis* if all the relevant *Competent Authorities* confirm that all relevant conditions have been met.

As described in Article 1.4.6., a *zone* or *compartment* within the *territory* of one or more countries not declared free from infection with *B. dendrobatidis* may be declared free by the *Competent Authority(ies)* of the country(ies) concerned if:

- 1) none of the *susceptible species* referred to in Article 8.1.2. are present in the *zone* or *compartment* and *basic biosecurity conditions* have been continuously met for at least the last two years;

OR

- 2) any of the *susceptible species* referred to in Article 8.1.2. are present in the *zone* or *compartment* and the following conditions have been met;
 - a) there has ~~not~~ been no ~~any observed~~ occurrence of infection with *B. dendrobatidis* ~~the disease~~ for at least the last ten years despite conditions that are conducive to its clinical expression (as described in the corresponding chapter of the *Aquatic Manual*); and
 - b) *basic biosecurity conditions* have been continuously met for at least the last ten years;

OR

- 3) the infection with *B. dendrobatidis* ~~disease~~ status prior to *targeted surveillance* is unknown but the following conditions have been met:
 - a) *basic biosecurity conditions* have been continuously met for at least the last two years; and
 - b) *targeted surveillance*, as described in Chapter 1.4., has been in place, in the *zone* or *compartment*, for at least the last two years without detection of ~~infection with *B. dendrobatidis*~~;

OR

- 4) it previously made a *self-declaration of freedom for a zone* from infection with *B. dendrobatidis* and subsequently lost its ~~disease~~ free status due to the detection of ~~infection with *B. dendrobatidis*~~ but the following conditions have been met:
 - a) on detection of *B. dendrobatidis* ~~the disease~~, the affected area was declared an *infected zone* and a *protection zone* was established; and

Annex 9B (contd)

- b) infected populations ~~within the *infected zone* have been killed and disposed of~~ ~~have been destroyed or removed from the *infected zone*~~ by means that minimise the likelihood ~~risk~~ of further transmission spread of *B. dendrobatidis* ~~the disease~~, and the appropriate *disinfection* procedures (as described in Chapter 4.3.) have been completed; 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 *B. dendrobatidis* ~~the disease~~; and
- d) *targeted surveillance*, as described in Chapter 1.4., has been in place for at least the last two years without detection of ~~infection with *B. dendrobatidis*~~.

Article 8.1.6.

Maintenance of free status

A country, *zone* or *compartment* that is declared free from infection with *B. dendrobatidis* following the provisions of points 1 or 2 of Articles 8.1.4. or 8.1.5. (as relevant) may maintain its status as free from infection with *B. dendrobatidis* provided that *basic biosecurity conditions* are continuously maintained.

A country, *zone* or *compartment* that is declared free from infection with *B. dendrobatidis* following the provisions of point 3 of Articles 8.1.4. or 8.1.5. (as relevant) may discontinue *targeted surveillance* and maintain its free status ~~as free from infection with *B. dendrobatidis*~~ provided that conditions that are conducive to clinical expression of infection with *B. dendrobatidis*, as described in the corresponding chapter of the *Aquatic Manual*, ~~exist~~ and *basic biosecurity conditions* are continuously maintained.

However, for declared free *zones* or *compartments* in infected countries and in all cases where conditions are not conducive to clinical expression of infection with *B. dendrobatidis*, *targeted surveillance* ~~should~~ needs to be continued at a level determined by the *Aquatic Animal Health Service* on the basis of the likelihood of *infection*.

Article 8.1.7.

Importation of aquatic animals ~~and or~~ aquatic animal products from a country, zone or compartment declared free from infection with *B. dendrobatidis*

When importing *aquatic animals* of a species referred to in Article 8.1.2., ~~or and~~ *aquatic animal products* derived thereof, from a country, *zone* or *compartment* declared free from infection with *B. dendrobatidis*, 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*, ~~or a certifying official approved by the importing country~~ The *international aquatic animal health certificate* should state that, ~~certifying that~~, on the basis of the procedures described in Articles 8.1.4. or 8.1.5. (as applicable) and 8.1.6., the place of production of the *aquatic animals* ~~or and~~ *aquatic animal products* is a country, *zone* or *compartment* declared free from infection with *B. dendrobatidis*.

The *international aquatic animal health certificate* should be in accordance with the Model Certificate in Chapter 5.11.

This article does not apply to *aquatic animal products commodities* ~~listed~~ ~~referred to~~ in point 1 of Article 8.1.3.

Article 8.1.8.

Importation of aquatic animals for aquaculture from a country, zone or compartment not declared free from infection with *B. dendrobatidis*

When importing, ~~for aquaculture,~~ *aquatic animals* of a species referred to in Article 8.1.2. from a country, *zone* or *compartment* not declared free from infection with *B. dendrobatidis*, the *Competent Authority* of the *importing country* should assess the *risk* in accordance with Chapter 2.1. and consider the *risk* mitigation measures in points 1 and 2 below.

Annex 9B (contd)

- 1) If the intention is to grow out and harvest the imported *aquatic animals*, consider applying the following:
 - a) the direct delivery to and lifelong holding of the imported *aquatic animals* in a *quarantine* facility; and
 - b) the treatment of all transport water, equipment, effluent and waste materials to inactivate *B. dendrobatidis* in accordance with Chapters 4.3., 4.7. and 5.5.

OR

- 2) If the intention is to establish a new stock for *aquaculture*, consider applying the following:
 - 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 *B. dendrobatidis*.
 - b) In the *importing country*.
 - i) import the F-0 population into a *quarantine* facility;
 - ii) test the F-0 population for *B. dendrobatidis* 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* under conditions that are conducive to the clinical expression of infection with *B. dendrobatidis*, ~~(as described in Chapter 2.1.1. of the *Aquatic Manual*)~~ and sample and test for *B. dendrobatidis* in accordance with Chapter 1.4. *of the Aquatic Code and Chapter 2.1.1. of the Aquatic Manual*;
 - v) if *B. dendrobatidis* is not detected in the F-1 population, it may be defined as free from infection with *B. dendrobatidis* and may be released from *quarantine*;
 - vi) if *B. dendrobatidis* 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.

Article 8.1.9.

Importation of aquatic animals ~~or and~~ aquatic animal products for processing for human consumption from a country, zone or compartment not declared free from infection with *B. dendrobatidis*

When importing, for processing for human consumption, *aquatic animals* of a species referred to in Article 8.1.2., or aquatic animal products derived thereof, from a country, *zone* or *compartment* not declared free from infection with *B. dendrobatidis*, the *Competent Authority* of the *importing country* should assess the *risk* and, if justified, require that:

- 1) the consignment is delivered directly to and held in *quarantine* or containment facilities until processing into one of the products referred to in point 1 of Article 8.1.3., ~~or products described in point 1 of Article 8.1.12.,~~ or other products authorised by the *Competent Authority*; and
- 2) all water (including ice), equipment, containers and packaging material used in transport ~~and all effluent and waste materials from the processing~~ are treated in a manner that to ensures inactivation of *B. dendrobatidis* ~~or is disposed of in a biosecure manner in accordance with Chapters 4.3., 4.7. and 5.5.; and that prevents contact of waste with susceptible species;~~

Annex 9B (contd)

- 3) all effluent and waste materials are treated to ensure inactivation of *B. dendrobatidis* or disposed of in a biosecure manner in accordance with Chapters 4.3. and 4.7.

For these *aquatic animals commodities* or *aquatic animal products* Member Countries may wish to consider introducing internal measures to address the *risks* associated with the *aquatic animal or aquatic animal product commodity* being used for any purpose other than for human consumption.

Article 8.1.10.

Importation of aquatic animals or aquatic animal products intended for uses other than human consumption, including use in animal feed, or for and agricultural, industrial, research or pharmaceutical use, from a country, zone or compartment not declared free from infection with *B. dendrobatidis*

When importing, ~~for use in animal feed or for agricultural, industrial or pharmaceutical use,~~ *aquatic animals* of a species referred to in Article 8.1.2., or *aquatic animal products* derived thereof, intended for uses other than human consumption, including animal feed and agricultural, industrial, research or pharmaceutical use, from a country, zone or compartment not declared free from infection with *B. dendrobatidis*, the *Competent Authority* of the *importing country* should require that:

- 1) the consignment ~~be~~ is delivered directly to, and held in, quarantine or containment facilities until processed into one of the products referred to in point 1 of Article 8.1.3. or other for slaughter and processing into products authorised by the *Competent Authority*; and
- 2) all water (including ice), equipment, containers and packaging material used in transport are treated to ensure inactivation of *B. dendrobatidis* or disposed of in a biosecure manner in accordance with Chapters 4.3., 4.7. and 5.5.; and water and equipment used in transport and all effluent and waste materials from the processing facility be treated in a manner that inactivates *B. dendrobatidis*.
- 3) all effluent and waste materials are treated to ensure inactivation of or disposed of in a biosecure manner in accordance with Chapters 4.3. and 4.7.

~~This article does not apply to *commodities* referred to in point 1 of Article 8.1.3.~~

Article 8.1.11.

Importation of aquatic animals intended for use in laboratories or zoos from a country, zone or compartment not declared free from infection with *B. dendrobatidis*

When importing, for use in laboratories or zoos, *aquatic animals* of a species referred to in Article 8.1.2. from a country, zone or compartment not declared free from infection with *B. dendrobatidis*, the *Competent Authority* of the *importing country* should ensure:

- 1) the consignment ~~is delivered directly to direct delivery to,~~ and lifelong held in, holding in-of the consignment, *quarantine* facilities authorised by the *Competent Authority*; and
- 2) all water (including ice), equipment, containers and packaging material used in transport are treated to ensure inactivation of *B. dendrobatidis* or disposed of in a biosecure manner in accordance with Chapters 4.3., 4.7. and 5.5.; and water and equipment used in transport and all effluent and waste materials from the processing facility be treated in a manner that inactivates *B. dendrobatidis*.
- 3) all effluent and waste materials from the *quarantine* facilities in the laboratories or zoos are treated to ensure inactivation of *B. dendrobatidis* or disposed of in a biosecure manner in accordance with Chapters 4.3. and 4.7.; and
- 2) ~~the treatment of water and equipment used in transport and of all effluent and waste materials in a manner that inactivates *B. dendrobatidis*; and~~
- 34) the carcasses are disposed of in accordance with Chapter 4.7.

Article 8.1.12.

Importation (or transit) of ~~aquatic animals and~~ aquatic animal products for retail trade for human consumption regardless of the infection with *B. dendrobatidis* status of the exporting ~~from a~~ country, zone or compartment ~~not declared free from infection with *B. dendrobatidis*~~

- 1) *Competent Authorities* should not require any conditions related to ~~infection with *B. dendrobatidis*~~, regardless of the infection with *B. dendrobatidis* status of the *exporting country, zone or compartment*, when authorising the importation (or transit) of amphibian meat (skin off and fresh or frozen) that ~~which~~ have been prepared and packaged for retail trade and ~~which~~ comply with Article 5.4.2.

Certain assumptions have been made in assessing the safety of the *aquatic animal products* mentioned above. Member Countries should refer to these assumptions at Article 5.4.2. and consider whether the assumptions apply to their conditions.

For these *aquatic animal products* ~~commodities~~ Member Countries may wish to consider introducing internal measures to address the *risks* associated with the *aquatic animal product* ~~commodity~~ being used for any purpose other than for human consumption.

- 2) When importing ~~*aquatic animals or aquatic animal products*~~, other than those referred to in point 1 above, derived from a of species referred to in Article 8.1.2. from a country, zone or *compartment* not declared free from infection with *B. dendrobatidis*, the *Competent Authority* of the *importing country* should assess the *risk* and apply appropriate *risk* mitigation measures.

CHAPTER 8.2.

INFECTION WITH RANAVIRUS

Article 8.2.1.

For the purposes of the *Aquatic Code*, infection with ranavirus means *infection* with any member virus species of the Genus *Ranavirus* and Family Iridoviridae with the exception of epizootic haematopoietic necrosis virus and European catfish virus.

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

Article 8.2.2.

Scope

The recommendations in this chapter apply to: all species of *Anura* (frogs and toads) and *Caudata* (salamanders and newts). The recommendations also apply to any other *susceptible species* referred to in the *Aquatic Manual* when traded internationally.

Article 8.2.3.

Importation or transit of ~~aquatic animals and~~ aquatic animal products for any purpose regardless of the infection with ranavirus status of the exporting country, zone or compartment

- 1) *Competent Authorities* should not require any conditions related to ~~infection with~~ ranavirus, regardless of the infection with ranavirus status of the *exporting country, zone or compartment*, when authorising the importation or transit of the following *aquatic animal products* derived from a ~~from the~~ species referred to in Article 8.2.2. that which are intended for any purpose and ~~which~~ comply with Article 5.4.1.:
 - 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 all virus species of the genus *Ranavirus* and Family *Iridoviridae* [with the exception of epizootic haematopoietic necrosis virus and European catfish virus]);
 - 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 which has been demonstrated to inactivate all virus species of the genus *Ranavirus* ~~in the~~ and Family *Iridoviridae* [with the exception of epizootic haematopoietic necrosis virus and European catfish virus]);
 - 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 which has been demonstrated to inactivate all virus species of the genus *Ranavirus* ~~in the~~ and Family *Iridoviridae* [with the exception of epizootic haematopoietic necrosis virus and European catfish virus]);
 - d) mechanically dried amphibian products (i.e. a heat treatment at 100°C for at least 30 minutes or any time/temperature equivalent that which has been demonstrated to inactivate all virus species of the genus *Ranavirus* ~~in the~~ and Family *Iridoviridae* [with the exception of epizootic haematopoietic necrosis virus and European catfish virus]).
- 2) When authorising the importation or transit of ~~aquatic animals and~~ *aquatic animal products* of 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 ranavirus status of the *exporting country, zone or compartment*.

Annex 9C (contd)

- 3) When considering the importation or transit of ~~aquatic animals and aquatic animal products~~ of a species not covered referred to in Article 8.2.2. but which could reasonably be expected to pose a risk of transmission spread of infection with ranavirus, 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 assessment.

Article 8.2.4.

Country free from infection with ranavirus

If a country shares a *zone* with one or more other countries, it can only make a *self-declaration of freedom from infection with ranavirus* if all the areas covered by the shared water bodies are declared countries or zones free from the zone are declared infection with ranavirus (see Article 8.2.5.).

As described in Article 1.4.6., a country may make a *self-declaration of freedom from infection with ranavirus* if:

- 1) none of the *susceptible species* referred to in Article 8.2.2. are present and *basic biosecurity conditions* have been continuously met for at least the last two years;

OR

- 2) any of the *susceptible species* referred to in Article 8.2.2. are present and the following conditions have been met:

- a) there has been no ~~observed~~ occurrence of infection with ranavirus the disease for at least the last ten years despite conditions that are conducive to its clinical expression (as described in the corresponding chapter of the *Aquatic Manual*); and
- b) *basic biosecurity conditions* have been continuously met for at least the last ten years;

OR

- 3) the infection with ranavirus disease status prior to *targeted surveillance* is unknown but the following conditions have been met:

- a) *basic biosecurity conditions* have been continuously met for at least the last two years; and
- b) *targeted surveillance*, as described in Chapter 1.4., has been in place for at least the last two years without detection of ~~infection with~~ ranavirus;

OR

- 4) it previously made a *self-declaration of freedom from infection with ranavirus* and subsequently lost its ~~disease~~ free status due to the detection of ~~infection with~~ ranavirus but the following conditions have been met:

- a) on detection of ranavirus the disease, 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 have been destroyed or removed from the infected zone by means that minimise the likelihood risk of further transmission spread of ranavirus the disease, and the appropriate *disinfection* procedures (as described in Chapter 4.3.) have been completed; 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 ranavirus ~~the disease~~; and
- d) *targeted surveillance*, as described in Chapter 1.4., has been in place for at least the last two years without detection of ~~infection with~~ ranavirus.

In the meantime, part or all of the ~~unaffected non-affected~~ area may be declared a free zone provided that such a part meets the conditions in point 3 of Article 8.2.5.

Article 8.2.5.

Zone or compartment free from infection with ranavirus

If a *zone* or *compartment* extends over more than one country, it can only be declared ~~a~~ an infection with ranavirus free zone or compartment free from infection with ranavirus if all the relevant *Competent Authorities* confirm that all relevant conditions have been met.

As described in Article 1.4.6., a *zone* or *compartment* within the *territory* of one or more countries not declared free from infection with ranavirus may be declared free by the *Competent Authority(ies)* of the country(ies) concerned if:

- 1) none of the *susceptible species* referred to in Article 8.2.2. are present in the *zone* or *compartment* and *basic biosecurity conditions* have been continuously met for at least the last two years;

OR

- 2) any of the *susceptible species* referred to in Article 8.2.2. are present in the *zone* or *compartment* and the following conditions have been met:
 - a) there has ~~not~~ been no any observed occurrence of infection with ranavirus ~~the disease~~ for at least the last ten years despite conditions that are conducive to its clinical expression (as described in the corresponding chapter of the *Aquatic Manual*); and
 - b) *basic biosecurity conditions* have been continuously met for at least the last ten years;

OR

- 3) the ~~disease~~ infection with ranavirus status prior to *targeted surveillance* is unknown but the following conditions have been met:
 - a) *basic biosecurity conditions* have been continuously met for at least the last two years; and
 - b) *targeted surveillance*, as described in Chapter 1.4., has been in place, in the *zone* or *compartment*, for at least the last two years without detection of ~~infection with~~ ranavirus;

OR

- 4) it previously made a *self-declaration of freedom* for a *zone* from infection with ranavirus and subsequently lost its ~~disease~~ free status due to the detection of ~~the infection with~~ ranavirus in the *zone* but the following conditions have been met:
 - a) on detection of infection with ranavirus ~~the disease~~, the affected area was declared an *infected zone* and a *protection zone* was established; and

Annex 9C (contd)

- b) infected populations ~~within the *infected zone* have been killed and disposed of~~ ~~have been destroyed or removed from the *infected zone*~~ by means that minimise the likelihood ~~risk~~ of further transmission spread of ranavirus ~~the disease~~, and the appropriate *disinfection* procedures (as described in Chapter 4.3.) have been completed; 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 ranavirus ~~the disease~~; and
- d) *targeted surveillance*, as described in Chapter 1.4., has been in place for at least the last two years without detection of ~~infection with~~ ranavirus.

Article 8.2.6.

Maintenance of free status

A country, *zone* or *compartment* that is declared free from infection with ranavirus following the provisions of points 1 or 2 of Articles 8.2.4. or 8.2.5. (as relevant) may maintain its status as free from infection with ranavirus provided that *basic biosecurity conditions* are continuously maintained.

A country, *zone* or *compartment* that is declared free from infection with ranavirus following the provisions of point 3 of Articles 8.2.4. or 8.2.5. (as relevant) may discontinue *targeted surveillance* and maintain its free status ~~as free from infection with ranavirus~~ provided that conditions that are conducive to clinical expression of infection with ranavirus, as described in the corresponding chapter of the *Aquatic Manual*, ~~exist~~ and *basic biosecurity conditions* are continuously maintained.

However, for declared free *zones* or *compartments* in infected countries and in all cases where conditions are not conducive to clinical expression of infection with ranavirus, *targeted surveillance* ~~needs to~~ should be continued at a level determined by the *Aquatic Animal Health Service* on the basis of the likelihood of *infection*.

Article 8.2.7.

Importation of aquatic animals ~~and~~ or aquatic animal products from a country, zone or compartment declared free from infection with ranavirus

When importing *aquatic animals* of a species referred to in Article 8.2.2., ~~or~~ and *aquatic animal products derived thereof*, from a country, *zone* or *compartment* declared free from infection with ranavirus, 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*. ~~or a certifying official approved by the importing country certifying that,~~ The international aquatic animal health certificate should state that, on the basis of the procedures described in Articles 8.2.4. or 8.2.5. (as applicable) and 8.2.6., the place of production of the *aquatic animals* ~~or~~ and *aquatic animal products* is a country, *zone* or *compartment* declared free from infection with ranavirus.

The international aquatic animal health certificate should be in accordance with the Model Certificate in Chapter 5.11.

This article does not apply to aquatic animal products listed ~~commodities referred~~ to in point 1 of Article 8.2.3.

Article 8.2.8.

Importation of aquatic animals for aquaculture from a country, zone or compartment not declared free from infection with ranavirus

When importing, ~~for aquaculture,~~ *aquatic animals* of a species referred to in Article 8.2.2. from a country, *zone* or *compartment* not declared free from infection with ranavirus, the *Competent Authority* of the *importing country* should assess the *risk* in accordance with Chapter 2.1. and consider the *risk* mitigation measures in points 1 and 2 below.

Annex 9C (contd)

- 1) If the intention is to grow out and harvest the imported *aquatic animals*, consider applying the following:
 - a) the direct delivery to and lifelong holding of the imported *aquatic animals* in a *quarantine* facility; and
 - b) the treatment of all transport water, equipment, effluent and waste materials to inactivate ranavirus in accordance with Chapters 4.3., 4.7. and 5.5.

OR

- 2) If the intention is to establish a new stock for *aquaculture*, consider applying the following:
 - 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 ranavirus.
 - b) In the *importing country*:
 - i) import the F-0 population into a *quarantine* facility;
 - ii) test the F-0 population for ranavirus 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* under conditions that are conducive to the clinical expression of infection with ranavirus, ~~(as described in Chapter 2.1.2. of the *Aquatic Manual*) and~~ sample and test for ranavirus in accordance with Chapter 1.4. of the *Aquatic Code* and Chapter 2.1.2. of the *Aquatic Manual*;
 - v) if ranavirus is not detected in the F-1 population, it may be defined as free from infection with ranavirus and may be released from *quarantine*;
 - vi) if ranavirus 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.

Article 8.2.9.

Importation of aquatic animals ~~or~~ and aquatic animal products for processing for human consumption from a country, zone or compartment not declared free from infection with ranavirus

When importing, for processing for human consumption, *aquatic animals* of a species referred to in Article 8.2.2., or aquatic animal products derived thereof, from a country, zone or compartment not declared free from infection with ranavirus, the *Competent Authority* of the *importing country* should assess the *risk* and, if justified, require that:

- 1) the consignment is delivered directly to, and held in, *quarantine* or containment facilities until processing into one of the products referred to in point 1 of Article 8.2.3.; ~~or products described in point 1 of Article 8.2.12., or other products authorised by the *Competent Authority*;~~ and
- 2) all water (including ice), equipment, containers and packaging material used in transport and all effluent and waste materials from the processing are treated in a manner that ensures inactivation of ranavirus or is disposed of in a biosecure manner that prevents contact of waste with susceptible species in accordance with Chapters 4.3., 4.7. and 5.5.; and

Annex 9C (contd)

- 3) all effluent and waste materials from the holding of the aquatic animals in laboratories or zoos are treated to ensure inactivation of ranavirus or disposed of in a biosecure manner in accordance with Chapters 4.3. and 4.7.

For these aquatic animals or aquatic animal products commodities Member Countries may wish to consider introducing internal measures to address the risks associated with the aquatic animal or aquatic animal product commodity being used for any purpose other than for human consumption.

Article 8.2.10.

Importation of aquatic animals or aquatic animal products intended for uses other than human consumption, including uses in animal feed, or and for agricultural, industrial, research or pharmaceutical use, from a country, zone or compartment not declared free from infection with ranavirus

When importing aquatic animals of the species referred to in Article 8.2.2., or aquatic animal products derived thereof, intended for uses other than human consumption, including in animal feed or and for agricultural, industrial, research or pharmaceutical use, aquatic animals of the species referred to in Article 8.2.2. from a country, zone or compartment not declared free from infection with ranavirus, the *Competent Authority* of the importing country should require that:

- 1) the consignment ~~is to be~~ delivered directly to, and held in, quarantine or containment facilities until processed into one of the products referred to in point 1 of Article 8.2.3. or other for slaughter and processing into products authorised by the Competent Authority; and
- 2) all water (including ice), equipment, containers and packaging material used in transport are treated to ensure inactivation of ranavirus or disposed of in a biosecure manner in accordance with Chapters 4.3., 4.7. and 5.5.; and water and equipment used in transport and all effluent and waste materials from the processing facility be treated in a manner that inactivates ranavirus.
- 3) all effluent and waste materials are treated to ensure inactivation of ranavirus or disposed of in a biosecure manner in accordance with Chapters 4.3. and 4.7.

~~This article does not apply to commodities referred to in point 1 of Article 8.2.3.~~

Article 8.2.11.

Importation of aquatic animals intended for use in laboratories or zoos, from a country, zone or compartment not declared free from infection with ranavirus

When importing, for use in laboratories or zoos, aquatic animals of a species referred to in Article 8.2.2. from a country, zone or compartment not declared free from infection with ranavirus, the *Competent Authority* of the importing country should ensure:

- 1) the ~~consignment is delivered directly to direct delivery to,~~ and lifelong held holding in, of the consignment in quarantine facilities authorised by the *Competent Authority*; and
- 2) all water (including ice), equipment, containers and packaging material used in transport are treated to ensure inactivation of ranavirus or disposed of in a biosecure manner in accordance with Chapters 4.3., 4.7. and 5.5.; and the treatment of water and equipment used in transport and of all effluent and waste materials in a manner that inactivates ranavirus; and
- 3) all effluent and waste materials from the quarantine facilities in the laboratories or zoos are treated to ensure inactivation of ranavirus or disposed of in a biosecure manner in accordance with Chapters 4.3. and 4.7.
- 34) the ~~disposal of carcasses are~~ disposed of in accordance with Chapter 4.7.

Article 8.2.12.

Importation (or transit) of aquatic animals and aquatic animal products for retail trade for human consumption regardless of the infection with ranavirus status of the exporting country, zone or compartment not declared free from infection with ranavirus

- 1) *Competent Authorities* should not require any conditions related to ~~infection with ranavirus~~, regardless of the infection with ranavirus status of the *exporting country, zone or compartment*, when authorising the importation (or transit) of the following *aquatic animal products commodities*, ~~that~~ which have been prepared and packaged for retail trade and ~~which~~ comply with Article 5.4.2.:
 - no *aquatic animal products* listed.
 - 2) When importing ~~aquatic animals or aquatic animal products~~, other than those referred to in point 1 above, derived from a of species referred to in Article 8.2.2. from a country, *zone or compartment* not declared free from infection with ranavirus, the *Competent Authority* of the *importing country* should assess the *risk* and apply appropriate *risk* mitigation measures.
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CHAPTER 9.4.

**INFECTION WITH INFECTIOUS HYPODERMAL AND
HAEMATOPOIETIC NECROSIS VIRUS**

Article 9.4.1.

For the purposes of the *Aquatic Code*, infection with infectious hypodermal and haematopoietic necrosis virus means *infection* with the *pathogenic agent* infectious hypodermal and haematopoietic necrosis virus (IHHNV), of the Genus *Brevidensovirus* and Family Parvoviridae.

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

Article 9.4.2.

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: ~~giant river prawn (*Macrobrachium rosenbergii*) (under study)~~, yellowleg shrimp (*Penaeus californiensis*), giant tiger prawn (*Penaeus monodon*), northern white shrimp (*Penaeus setiferus*), blue shrimp (*Penaeus stylirostris*) and whiteleg shrimp (*Penaeus vannamei*).

[...]

CHAPTER 10.1.

INFECTION WITH THE EPIZOOTIC HAEMATOPOIETIC NECROSIS VIRUS

Article 10.1.1.

For the purposes of the *Aquatic Code*, infection with epizootic haematopoietic necrosis virus (EHN) means infection with the pathogenic agent epizootic haematopoietic necrosis virus EHN virus (EHNV), of the Genus genus *Ranavirus*, of the family and Family Iridoviridae.

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

Article 10.1.2.

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.: black bullhead (*Ameiurus melas*), crimson spotted rainbow fish (*Melanotaenia fluviatilis*), eastern mosquito fish (*Gambusia holbrooki*), European perch (*Perca fluviatilis*), macquarie perch (*Macquaria australasica*), mosquito fish (*Gambusia affinis*), mountain galaxias (*Galaxias olidus*), northern pike (*Esox lucius*), pike-perch (*Sander lucioperca*), redfin perch (*Perca fluviatilis*) and rainbow trout (*Oncorhynchus mykiss*) and silver perch (*Bidyanus bidyanus*). ~~These recommendations also apply to any other susceptible species referred to in the *Aquatic Manual* when traded internationally.~~

Article 10.1.3.

Importation or transit of ~~aquatic animals and aquatic animal products~~ for any purpose regardless of the infection with EHNV epizootic haematopoietic necrosis virus status of the exporting country, zone or compartment

- 1) *Competent Authorities* should not require any conditions related to EHN~~V~~, regardless of the infection with EHN~~V~~ status of the *exporting country, zone or compartment*, when authorising the importation or transit of the following *aquatic animal products* derived from a the species referred to in Article 10.1.2. ~~that~~ which are intended for any purpose and ~~which~~ comply with Article 5.4.1.:
 - a) heat sterilised hermetically sealed fish 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 EHN~~V~~);
 - b) pasteurised fish products that have been subjected to heat treatment at 90°C for ten minutes (or any time/temperature equivalent ~~which~~ that has been demonstrated to inactivate EHN~~V~~);
 - c) mechanically dried eviscerated fish (i.e. a heat treatment at 100°C for at least 30 minutes or any time/temperature equivalent ~~which~~ that has been demonstrated to inactivate EHN~~V~~);
 - d) fish oil;
 - e) fish meal;
 - f) fish skin leather.
- 2) When authorising the importation or transit of ~~aquatic animals and aquatic animal products~~ of a species referred to in Article 10.1.2., other than those referred to in point 1 of Article 10.1.3., *Competent Authorities* should require the conditions prescribed in Articles 10.1.7. to 10.1.44-12, relevant to the infection with EHN~~V~~ status of the *exporting country, zone or compartment*.

Annex 11 (contd)

- 3) When considering the importation or transit of ~~aquatic animals and aquatic animal products~~ of a species not ~~referred to covered~~ in Article 10.1.2. but which could reasonably be expected to pose a risk of transmission spread of EHN~~V~~, 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 analysis~~.

Article 10.1.4.

Country free from infection with EHN~~V~~ ~~the epizootic haematopoietic necrosis virus~~

If a country shares a *zone* with one or more other countries, it can only make a *self-declaration of freedom from infection with EHN~~V~~* if all the areas covered by the shared water bodies are declared countries or *zones* free from infection with EHN~~V~~ (see Article 10.1.5.).

As described in Article 1.4.6., a country may make a *self-declaration of freedom from infection with EHN~~V~~* if:

- 1) none of the *susceptible species* referred to in Article 10.1.2. are present and *basic biosecurity conditions* have been continuously met for at least the last two years;

OR

- 2) any of the *susceptible species* referred to in Article 10.1.2. are present and the following conditions have been met:

- a) there has been no ~~observed~~ occurrence of infection with EHN~~V~~ ~~the disease~~ for at least the last ten years despite conditions that are conducive to its clinical expression (as described in the corresponding chapter of the *Aquatic Manual*); and
- b) *basic biosecurity conditions* have been continuously met for at least the ~~past~~ last ten years;

OR

- 3) the infection with EHN~~V~~ ~~disease~~ status prior to *targeted surveillance* is unknown but the following conditions have been met:

- a) *basic biosecurity conditions* have been continuously met for at least the last two years; and
- b) *targeted surveillance*, as described in Chapter 1.4., has been in place for at least the last two years without detection of EHN~~V~~;

OR

- 4) it previously made a *self-declaration of freedom from infection with EHN~~V~~* and subsequently lost its ~~disease~~ free status due to the detection of EHN~~V~~ but the following conditions have been met:

- a) on detection of EHN~~V~~ ~~the disease~~, 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 ~~have been destroyed or removed from the infected zone~~ by means that minimise the likelihood risk of further spread transmission of EHN~~V~~ ~~the disease~~, and the appropriate *disinfection* procedures (as described in Chapter 4.3.) have been completed; 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 EHN~~V~~ ~~the disease~~; and
- d) *targeted surveillance*, as described in Chapter 1.4., has been in place for at least the last two years without detection of EHN~~V~~.

Annex 11 (contd)

In the meantime, part or all of the unaffected ~~non-affected~~ area may be declared a free *zone* provided that such a part meets the conditions in point 3 of Article 10.1.5.

Article 10.1.5.

Zone or compartment free from infection with EHN~~V the epizootic haematopoietic necrosis virus~~

If a *zone* or *compartment* extends over more than one country, it can only be declared ~~an infection with EHN~~ free a *zone* or *compartment* free from infection with EHN if all the relevant *Competent Authorities* confirm that all relevant conditions have been met.

As described in Article 1.4.6., a *zone* or *compartment* within the *territory* of one or more countries not declared free from infection with EHN may be declared free by the *Competent Authority*(ies) of the country(ies) concerned if:

- 1) none of the *susceptible species* referred to in Article 10.1.2. are present in the *zone* or *compartment* and *basic biosecurity conditions* have been continuously met for at least the last two years;

OR

- 2) any of the *susceptible species* referred to in Article 10.1.2. are present in the *zone* or *compartment* and the following conditions have been met:
 - a) there has been no observed occurrence of infection with EHN for at least the last ten years despite conditions that are conducive to its clinical expression (as described in the corresponding chapter of the *Aquatic Manual*); and
 - b) *basic biosecurity conditions* have been continuously met for at least the last ten years;

OR

- 3) the ~~disease~~ infection with EHN status prior to *targeted surveillance* is unknown but the following conditions have been met:
 - a) *basic biosecurity conditions* have been continuously met for at least the last two years; and
 - b) *targeted surveillance*, as described in Chapter 1.4., has been in place, in the *zone* or *compartment*, for at least the last two years without detection of EHN;

OR

- 4) it previously made a *self-declaration of freedom* for a *zone* from infection with EHN and subsequently lost its ~~disease~~ free status due to the detection of EHN in the *zone* but the following conditions have been met:
 - a) on detection of EHN ~~the disease~~, 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 been destroyed or removed from the infected zone~~ by means that minimise the likelihood ~~risk~~ of further ~~spread~~ transmission of EHN ~~the disease~~, and the appropriate *disinfection* procedures (as described in Chapter 4.3.) have been completed; 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 EHN ~~the disease~~; and
 - d) *targeted surveillance*, as described in Chapter 1.4., has been in place for at least the last two years without detection of EHN.

Annex 11 (contd)

Article 10.1.6.

Maintenance of free status

A country, *zone* or *compartment* that is declared free from infection with EHN_V following the provisions of points 1 or 2 of Articles 10.1.4. or 10.1.5. (as relevant) may maintain its status as free from infection with EHN_V provided that *basic biosecurity conditions* are continuously maintained.

A country, *zone* or *compartment* that is declared free from infection with EHN_V following the provisions of point 3 of Articles 10.1.4. or 10.1.5. (as relevant) may discontinue *targeted surveillance* and maintain its free status as free from EHN_V provided that conditions that are conducive to clinical expression of infection with EHN_V, as described in the corresponding chapter of the *Aquatic Manual*, ~~exist~~ and *basic biosecurity conditions* are continuously maintained.

However, for declared free *zones* or *compartments* in infected countries and in all cases where conditions are not conducive to clinical expression of infection with EHN_V, *targeted surveillance* ~~needs~~ should ~~to~~ be continued at a level determined by the *Aquatic Animal Health Service* on the basis of the likelihood of *infection*.

Article 10.1.7.

Importation of aquatic animals ~~and or~~ aquatic animal products from a country, zone or compartment declared free from infection with EHN_V ~~the epizootic haematopoietic necrosis virus~~

When importing *aquatic animals of a species referred to in Article 10.1.2., or aquatic animal products of species referred to in Article 10.1.2. derived thereof,* from a country, *zone* or *compartment* declared free from infection with EHN_V, 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* ~~or a certifying official approved by the importing country.~~ The *international aquatic animal health certificate* should state that, certifying that, on the basis of the procedures described in Articles 10.1.4. or 10.1.5. (as applicable) and 10.1.6., the place of production of the *aquatic animals or aquatic animal products* is a country, *zone* or *compartment* declared free from infection with EHN_V.

The *international aquatic animal health certificate* should be in accordance with the Model Certificate in Chapter 5.11.

This article does not apply to *aquatic animal products commodities listed* referred to in point 1 of Article 10.1.3.

Article 10.1.8.

Importation of aquatic animals for aquaculture from a country, zone or compartment not declared free from infection with EHN_V ~~the epizootic haematopoietic necrosis virus~~

When importing, for *aquaculture*, *aquatic animals* of a species referred to in Article 10.1.2. from a country, *zone* or *compartment* not declared free from infection with EHN_V, the *Competent Authority* of the *importing country* should assess the *risk* in accordance with Chapter 2.1. and consider the *risk* mitigation measures in points 1 and 2 below.

- 1) If the intention is to grow out and harvest the imported *aquatic animals*, consider applying the following:
 - a) the direct delivery to and lifelong holding of the imported *aquatic animals* in a *quarantine* facility; and
 - b) the treatment of all transport water, equipment, effluent and waste materials to inactivate EHN_V in accordance with Chapters 4.3., 4.7. and 5.5.

OR

- 2) If the intention is to establish a new stock for *aquaculture*, consider applying the following:
 - 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 EHN_V.

Annex 11 (contd)

- b) In the *importing country*:
- i) import the F-0 population into a *quarantine* facility;
 - ii) test the F-0 population for EHN~~V~~ 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* under conditions that are conducive to the clinical expression of EHN~~V~~, ~~(as described in Chapter 2.3.1. of the *Aquatic Manual*)~~, and sample and test for EHN~~V~~ in accordance with Chapter 1.4. of the *Aquatic Code* and ~~(as described in Chapter 2.3.1. of the *Aquatic Manual*)~~;
 - v) if EHN~~V~~ is not detected in the F-1 population, it may be defined as free from infection with EHN~~V~~ and may be released from *quarantine*;
 - vi) if EHN~~V~~ 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.

Article 10.1.9.

Importation of aquatic animals ~~and or~~ aquatic animal products for processing for human consumption from a country, zone or compartment not declared free from infection with EHN~~V~~ ~~the epizootic haematopoietic necrosis virus~~

When importing, for processing for human consumption, ~~*aquatic animals or aquatic animal products*~~ of a species referred to in Article 10.1.2., or *aquatic animal products* derived thereof, from a country, zone or compartment not declared free from infection with EHN~~V~~, the *Competent Authority* of the *importing country* should assess the risk and, if justified, require that:

- 1) the consignment is delivered directly to, and held in, *quarantine* or containment facilities until processing into one of the products referred to in point 1 of Article 10.1.3., ~~or products described or~~ in point 1 of Article 10.1.4-12., or other products authorised by the *Competent Authority*; and
- 2) all water (including ice), equipment, containers and packaging material used in transport are treated to ensure inactivation of EHN~~V~~ or disposed of in a biosecure manner in accordance with Chapters 4.3., 4.7. and 5.5.; and
- 3) all effluent and waste materials are treated to ensure inactivation of EHN~~V~~ or disposed of in a biosecure manner in accordance with Chapters 4.3. and 4.7.

For these *aquatic animals or aquatic animal products* ~~*commodities*~~ Member Countries may wish to consider introducing internal measures to address the risks associated with the *aquatic animals or aquatic animal products* ~~*commodity*~~ being used for any purpose other than for human consumption.

Article 10.1.10.

Importation of aquatic animals or aquatic animal products intended for uses other than human consumption, including use in animal feed, or for and agricultural, industrial, research or pharmaceutical use, from a country, zone or compartment not declared free from infection with EHN~~V~~ ~~the epizootic haematopoietic necrosis virus~~

When importing *aquatic animals* of a species referred to in Article 9.5.2., or *aquatic animal products* derived thereof, intended for uses other than human consumption, including use in animal feed or for and agricultural, industrial, research or pharmaceutical use, *aquatic animals* of species referred to in Article 10.1.2., from a country, zone or compartment not declared free from infection with EHN~~V~~, the *Competent Authority* of the *importing country* should require that:

Annex 11 (contd)

- 1) the consignment is delivered directly to, and held in, in quarantine or containment facilities until processed into one of the products referred to in point 1 of Article 10.1.3. or other facilities for slaughter and processing into products authorised by the Competent Authority; and
- 2) all water (including ice), equipment, containers and packaging material used in transport are treated to ensure inactivation of EHN_V or disposed of in a biosecure manner in accordance with Chapters 4.3., 4.7. and 5.5.; and water used in transport and all effluent and waste materials from the processing are treated in a manner that ensures inactivation of EHN_V
- 3) all effluent and waste materials are treated to ensure inactivation of EHN_V or disposed of in a biosecure manner in accordance with Chapters 4.3. and 4.7.

This article does not apply to ~~commodities~~ referred to in point 1 of Article 10.1.3.

Article 10.1.11.**Importation of aquatic animals intended for use in laboratories or zoos from a country, zone or compartment not declared free from infection with EHN_V**

When importing, for use in laboratories and zoos, aquatic animals of species referred to in Article 10.1.2. from a country, zone or compartment not declared free from infection with EHN_V, the Competent Authority of the importing country should ensure:

- 1) the consignment is delivered directly to, and held in, quarantine facilities authorised by the Competent Authority; and
- 2) all water (including ice), equipment, containers and packaging material used in transport are treated to ensure inactivation of EHN_V or disposed of in a biosecure manner in accordance with Chapters 4.3., 4.7. and 5.5.; and
- 3) all effluent and waste materials from the quarantine facilities in the laboratories or zoos are treated to ensure inactivation of EHN_V or disposed of in a biosecure manner in accordance with Chapters 4.3. and 4.7.; and
- 4) the carcasses are disposed of in accordance with Chapter 4.7.

Article 10.1.14~~2~~.**Importation (or transit) of aquatic animals and aquatic animal products for retail trade for human consumption regardless of the infection with EHN_V status of the exporting from a country, zone or compartment not declared free from infection with the epizootic haematopoietic necrosis virus**

- 1) Competent Authorities should not require any conditions related to EHN_V, regardless of the infection with EHN_V status of the exporting country, zone or compartment, when authorising the importation (or transit) of fish fillets or steaks (chilled or frozen) that which have been prepared and packaged for retail trade and which comply with Article 5.4.2.

Certain assumptions have been made in assessing the safety of the *aquatic animal products* mentioned above. Member Countries should refer to these assumptions at Article 5.4.2. and consider whether the assumptions apply to their conditions.

For these *aquatic animal products* commodities Member Countries may wish to consider introducing internal measures to address the risks associated with the *aquatic animal products* commodity being used for any purpose other than for human consumption.

- 2) When importing ~~aquatic animals or aquatic animal products~~, other than those referred to in point 1 above, derived from a of species referred to in Article 10.1.2. from a country, zone or compartment not declared free from infection with EHN_V, the Competent Authority of the importing country should assess the risk and apply appropriate risk mitigation measures.

CHAPTER 10.3.

INFECTION WITH *GYRODACTYLUS SALARIS*

Article 10.3.1.

For the purposes of the *Aquatic Code*, ~~gyrodactylosis infection with *Gyrodactylus salaris*~~ means *infection with the pathogenic agent *Gyrodactylus salaris*, a viviparous freshwater ectoparasite, *Gyrodactylus salaris* of the Family Gyrodactylidae and (Phylum Platyhelminthes; Class Monogenea).*

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

Article 10.3.2.

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.: Arctic char (*Salvelinus alpinus*), Atlantic salmon (*Salmo salar*), brown trout (*Salmo trutta*), rainbow trout (*Oncorhynchus mykiss*), Arctic char (*Salvelinus alpinus*), North American brook trout (*Salvelinus fontinalis*), grayling (*Thymallus thymallus*), North American lake trout (*Salvelinus namaycush*), and North American brook trout (*Salvelinus fontinalis*) and rainbow trout (*Oncorhynchus mykiss*) brown trout (*Salmo trutta*). The recommendations also apply to other fish species in waters where the parasite is present, because these species may carry the parasite and act as vectors.~~

Article 10.3.3.

Importation or transit of ~~aquatic animals and~~ aquatic animal products for any purpose regardless of the infection with *G. salaris* status of the exporting country, zone or compartment

- 1) *Competent Authorities* should not require any ~~related~~ conditions related to ~~infection with *G. salaris*~~, regardless of the infection with *G. salaris* status of the *exporting country, zone or compartment*, when authorising the importation or transit of the following ~~aquatic animals and aquatic animal products derived from a the species referred to in Article 10.3.2. that which~~ are intended for any purpose and ~~which~~ comply with Article 5.4.1.:
 - a) heat sterilised, hermetically sealed fish 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 *G. salaris*);
 - b) pasteurised fish products that have been subjected to a heat treatment at 63°C for at least 30 minutes (or any time/temperature equivalent ~~which~~ that has been demonstrated to inactivate *G. salaris*);
 - c) mechanically dried, eviscerated fish (i.e. a heat treatment at 100°C for at least 30 minutes or any time/temperature equivalent ~~which~~ that has been demonstrated to inactivate *G. salaris*);
 - d) naturally dried, eviscerated fish (i.e. sun-dried or wind-dried);
 - e) frozen eviscerated fish that have been subjected to minus 18°C or lower temperatures;
 - f) frozen fish fillets or steaks that have been subjected to minus 18°C or lower temperatures;
 - g) chilled eviscerated fish that have been harvested from seawater with a salinity of at least 25 parts per thousand (ppt);
 - h) chilled fish fillets or steaks derived from fish that have been harvested from seawater with a salinity of at least 25 ppt;

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- i) chilled fish products from which the skin, fins and gills have been removed;
 - j) non-viable fish roe;
 - k) fish oil;
 - l) fish *meat*;
 - m) fish skin leather.
- 2) When authorising the importation or transit of ~~aquatic animals and aquatic animal products~~ *aquatic animal products* of a species referred to in Article 10.3.2., other than those referred to in point 1 of Article 10.3.3., *Competent Authorities* should require the conditions prescribed in Articles 10.3.7. to 10.3.11. ~~relevant to the infection with *G. salaris* status of the exporting country, zone or compartment.~~
- 3) When considering the importation or transit of ~~aquatic animals and aquatic animal products~~ *aquatic animal products* of a species not ~~referred to covered~~ in Article 10.3.2. but which could reasonably be expected to pose a *risk of spread* transmission of ~~infection with *G. salaris*~~, 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 analysis~~.

Article 10.3.4.

Country free from infection with *G. salaris*

If a country shares a *zone* with one or more other countries, it can only make a *self-declaration of freedom* from infection with *G. salaris* if all the areas covered by the shared water bodies ~~watercourse(s)~~ are declared countries or *zones* free from infection with *G. salaris* (see Article 10.3.5.).

As described in Article 1.4.6., a country may make a *self-declaration of freedom* from infection with *G. salaris* if:

- 1) none of the *susceptible species* referred to in Article 10.3.2. are present and *basic biosecurity conditions* have been continuously met for at least the last two years;

OR

- 2) any of the *susceptible species* referred to in Article 10.3.2. are present and the following conditions have been met:
- a) there has been no ~~observed~~ occurrence of ~~the infection with *G. salaris* disease~~ for at least the last ten years despite conditions that are conducive to its clinical expression (as described in the corresponding chapter of the *Aquatic Manual*); and
 - b) *basic biosecurity conditions* have been continuously met for at least the last ten years;

OR

- 3) the infection with *G. salaris* disease status prior to *targeted surveillance* is unknown but the following conditions have been met:
- a) *basic biosecurity conditions* have been continuously met for at least the last five years; and
 - b) *targeted surveillance*, as described in Chapter 1.4., has been in place for at least the last five years without detection of ~~infection with *G. salaris*~~;

OR

- 4) it previously made a *self-declaration of freedom* from infection with *G. salaris* and subsequently lost its ~~disease~~ free status due to the detection of ~~infection with *G. salaris*~~ but the following conditions have been met:

Annex 12 (contd)

- a) on detection of ~~the~~ *G. salaris* disease, 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~~ ~~have been destroyed or removed from the *infected zone*~~ by means that minimise the likelihood risk of further ~~spread~~ transmission of the *G. salaris* disease, and the appropriate *disinfection* procedures (as described in Chapter 4.3.) have been completed, or the waters containing the infected fish have been treated by chemicals that kill the parasite; and
- c) previously existing *basic biosecurity conditions* have been reviewed and modified as necessary and have continuously been in place since eradication of the infection with *G. salaris* disease; and
- d) *targeted surveillance*, as described in Chapter 1.4., has been in place for at least the last five years without detection of ~~infection with~~ *G. salaris*.

In the meantime, part or all of the unaffected ~~non-affected~~ area may be declared a free *zone* provided that such a part meets the conditions in point 3 of Article 10.3.5.

Article 10.3.5.

Zone or compartment free from infection with *G. salaris*

If a *zone* or *compartment* extends over more than one country, it can only be declared ~~for~~ a *zone* or *compartment* free from infection with *G. salaris* if all the relevant *Competent Authorities* confirm that all relevant conditions have been met.

As described in Article 1.4.6., a *zone* or *compartment* within the *territory* of one or more countries not declared free from infection with *G. salaris* may be declared free by the *Competent Authority(ies)* of the country(ies) concerned if:

- 1) none of the *susceptible species* referred to in Article 10.3.2. are present in the *zone* or *compartment* and *basic biosecurity conditions* have been continuously met for at least the last two years;

OR

- 2) any of the *susceptible species* referred to in Article 10.3.2. are present in the *zone* or *compartment* and the following conditions have been met:
 - a) there has been no observed occurrence of ~~the infection with *G. salaris* disease~~ for at least the last ten years despite conditions that are conducive to its clinical expression (as described in the corresponding chapter of the *Aquatic Manual*); and
 - b) *basic biosecurity conditions* have been continuously met for at least the last five years;

OR

- 3) a *zone* or *compartment* supplied with seawater with a salinity of at least 25 ppt may be declared free from infection with *G. salaris* provided that no *aquatic animal products* of a species referred to in Article 10.3.2. are introduced from a site of a lesser health status for infection with *G. salaris* during the 14 days prior to any ~~live~~ fish transfers from the *zone* or *compartment*;

OR

- 4) the infection with *G. salaris* disease status prior to *targeted surveillance* is unknown but the following conditions have been met:
 - a) *basic biosecurity conditions* have been continuously met for at least the last ten years; and
 - b) *targeted surveillance*, as described in Chapter 1.4., has been in place, in the *zone* or *compartment*, for at least the last five years without detection of ~~infection with~~ *G. salaris*;

Annex 12 (contd)

OR

- 5) it previously made a *self-declaration of freedom* for a *zone* from infection with *G. salaris* and subsequently lost its ~~disease~~ free status due to the detection of ~~infection with~~ *G. salaris* in the *zone* but the following conditions have been met:
- a) on detection of ~~the~~ *G. salaris* disease, 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 ~~have been destroyed or removed from the *infected zone*~~ by means that minimise the likelihood ~~risk~~ of further ~~spread~~ transmission of ~~the~~ *G. salaris* disease, and the appropriate *disinfection* procedures (as described in Chapter 4.3.) have been completed, or the waters containing the infected fish have been treated by chemicals that kill the parasite; 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 *G. salaris* ~~the disease~~; and
 - d) *targeted surveillance*, as described in Chapter 1.4., has been in place for at least the last five years without detection of ~~infection with~~ *G. salaris*.

Article 10.3.6.

Maintenance of free status

A country, *zone* or *compartment* that is declared free from infection with *G. salaris* following the provisions of points 1 or 2 of Articles 10.3.4. or 10.3.5. (as relevant) may maintain its status as free from infection with *G. salaris* provided that *basic biosecurity conditions* are continuously maintained.

A country, *zone* or *compartment* that is declared free from infection with *G. salaris* following the provisions of point 3 of Article 10.3.4. or point 4 of 10.3.5. (as relevant) may discontinue *targeted surveillance* and maintain its free status as free from infection with *G. salaris* provided that conditions that are conducive to clinical expression of infection with *G. salaris*, as described in the corresponding chapter of the *Aquatic Manual*, ~~exist~~, and *basic biosecurity conditions* are continuously maintained.

However, for declared free *zones* or *compartments* in infected countries and in all cases where conditions are not conducive to clinical expression of infection with *G. salaris*, *targeted surveillance* ~~needs~~ should ~~to~~ be continued at a level determined by the *Aquatic Animal Health Service* on the basis of the likelihood of *infection*.

Article 10.3.7.

Importation of aquatic animals ~~and~~ or aquatic animal products from a country, zone or compartment declared free from infection with *G. salaris*

When importing *aquatic animals* ~~and aquatic animal products~~ of a species referred to in Article 10.3.2., or aquatic animal products derived thereof, from a country, *zone* or *compartment* declared free from infection with *G. salaris*, 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* ~~or a certifying official approved by the importing country~~. The international aquatic animal health certificate should state that, certifying that, on the basis of the procedures described in Articles 10.3.4. or 10.3.5. (as applicable) and 10.3.6., the place of production of the *aquatic animals* or ~~and~~ *aquatic animal products* is a country, *zone* or *compartment* declared free from infection with *G. salaris*.

The *international aquatic animal health certificate* should be in accordance with the Model Certificate in Chapter 5.11.

This article does not apply to *aquatic animal products* listed ~~commodities referred to~~ in point 1 of Article 10.3.3.

Article 10.3.8.

Importation of aquatic animals for aquaculture from a country, zone or compartment not declared free from infection with *G. salaris*

When importing, for aquaculture, aquatic animals of a species referred to in Article 10.3.2. from a country, zone or compartment not declared free from infection with *G. salaris*, the Competent Authority of the importing country should assess the risk in accordance with Chapter 2.1. and consider the risk mitigation measures in points 1 and 2 below.

- 4)- If the intention is to grow out and harvest the imported aquatic animals, consider applying the following:
- a) the direct delivery to and lifelong holding of the imported aquatic animals in a quarantine facility; and
 - b) the treatment of all transport water, equipment, effluent and waste materials to inactivate *G. salaris* in accordance with Chapters 4.3., 4.7. and 5.5.

1) When importing, for aquaculture, live aquatic animals of a species referred to in Article 10.3.2. from a country, zone or compartment not declared free from infection with *G. salaris*, the Competent Authority of the importing country should:

a) require an international aquatic animal health certificate issued by the Competent Authority of the exporting country attesting that:

- i) the aquatic animals have been held, immediately prior to export, in water with a salinity of at least 25 parts per thousand for a continuous period of at least 14 days; and
- ii) no other aquatic animals of the species referred to in Article 10.3.2. have been introduced during that period;

OR

iii) in the case of eyed eggs, the eggs have been disinfected by a method demonstrated to be effective against *G. salaris*;

OR

b) assess the risk and apply risk mitigation measures such as:

- i) the direct delivery to and lifelong holding of the imported aquatic animals in a quarantine facility; and
- ii) if breeding from the imported fish, disinfection of the fertilised eggs by a method demonstrated to be effective against *G. salaris*, and complete separation of the hatched progeny from the imported animals;
- iii) the treatment of all transport water, equipment, effluent and waste materials to inactivate *G. salaris* in accordance with Chapters 4.3., 4.7. and 5.5.

OR

2) If the intention is to establish a new stock for aquaculture, consider applying the following:

- 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 *G. salaris*.

Annex 12 (contd)

- b) In the *importing country*:
- i) import the F-0 population into a *quarantine* facility;
 - ii) test the F-0 population for *G. salaris* 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* under conditions that are conducive to the clinical expression of *G. salaris*, ~~(as described in Chapter 2.3.3. of the *Aquatic Manual*)~~ and sample and test for *G. salaris* in accordance with Chapter 1.4. of the *Aquatic Code* and ~~(as described in Chapter 2.3.3. of the *Aquatic Manual*);~~
 - v) if *G. salaris* is not detected in the F-1 population, it may be defined as free from infection with *G. salaris* and may be released from *quarantine*;
 - vi) if *G. salaris* 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.

Article 10.3.9.

Importation of aquatic animals ~~and or~~ aquatic animal products for processing for human consumption from a country, zone or compartment not declared free from infection with *G. salaris*

When importing, for processing for human consumption, ~~aquatic animals or aquatic animal products~~ of a species referred to in Article 10.3.2., or aquatic animal products derived thereof, from a country, zone or compartment not declared free from infection with *G. salaris*, the *Competent Authority* of the *importing country* should assess the risk and, if justified, require that:

- 1) the consignment is delivered directly to, and held in, *quarantine* or containment facilities until processing into one of the products referred to in point 1 of Article 10.3.3., ~~or products described or~~ in point 1 of Article 10.3.4-12., or other products authorised by the *Competent Authority*; and
- 2) all water (including ice), equipment, containers and packaging material used are treated to ensure inactivation of *G. salaris* or disposed of in a biosecure manner in accordance with Chapters 4.3., 4.7. and 5.5.; and or is disposed in a manner that prevents contact of waste with susceptible species
- 3) all effluent and waste materials are treated to ensure inactivation of ~~HHNV~~ *G. salaris* or disposed of in a biosecure manner in accordance with Chapters 4.3. and 4.7.

For these *aquatic animals* or *aquatic animal products* ~~commodities~~ Member Countries may wish to consider introducing internal measures to address the risks associated with the *aquatic animals* or *aquatic animal products* ~~commodity~~ being used for any purpose other than for human consumption.

Article 10.3.10.

Importation of aquatic animals or aquatic animal products intended for uses other than human consumption, including use in animal feed, or for and agricultural, industrial, research or pharmaceutical use, from a country, zone or compartment not declared free from infection with *G. salaris*

When importing *aquatic animals* of a species referred to in Article 10.3.2., or *aquatic animal products* derived thereof, intended for use uses other than human consumption, including in animal feed, or for and agricultural, industrial, research or pharmaceutical use, ~~*aquatic animals* of species referred to in Article 10.3.2,~~ from a country, zone or compartment not declared free from infection with *G. salaris*, the *Competent Authority* of the *importing country* should require that:

- 1) ~~require~~ an *international aquatic animal health certificate* is issued by the *Competent Authority* of the *exporting country* attesting that the *aquatic animals* have been held, immediately prior to export, in water with a salinity of at least 25 ppt for a continuous period of at least 14 days, and no other *aquatic animals* of ~~the~~ a species referred to in Article 10.3.2. have been introduced during that period;

OR

Annex 12 (contd)

- 2) ~~require that the consignment is be delivered directly to, and held in, in quarantine or containment facilities until processed into one of the products referred to in point 1 of Article 10.3.3. facilities for slaughter and processing into or other products authorised by the Competent Authority; and water used in transport and all effluent and waste materials be treated in a manner that ensures inactivation of *G. salaris*.~~
- 3) all water (including ice), equipment, containers and packaging material used in transport are treated to ensure inactivation of *G. salaris* or disposed of in a biosecure manner in accordance with Chapters 4.3., 4.7. and 5.5.; and
- 4) all effluent and waste materials from the quarantine facilities in the laboratories or zoos are treated to ensure inactivation of *G. salaris* or disposed of in a biosecure manner in accordance with Chapters 4.3. and 4.7.

This article does not apply to ~~commodities~~ referred to in point 1 of Article 10.3.3.

Article 10.3.11.

Importation of aquatic animals intended for use in laboratories or zoos from a country, zone or compartment not declared free from infection with *G. salaris*

When importing, for use in laboratories and zoos, aquatic animals of a species referred to in Article 10.3.2. from a country, zone or compartment not declared free from infection with *G. salaris*, the Competent Authority of the importing country should ensure:

- 1) the consignment is delivered directly to, and held in, quarantine facilities authorised by the Competent Authority; and
- 2) all water (including ice), equipment, containers and packaging material used in transport are treated to ensure inactivation of *G. salaris* or disposed of in a biosecure manner in accordance with Chapters 4.3., 4.7. and 5.5.; and
- 3) all effluent and waste materials from the quarantine facilities in the laboratories or zoos are treated to ensure inactivation of *G. salaris* or disposed of in a biosecure manner in accordance with Chapters 4.3. and 4.7.; and
- 4) the carcasses are disposed of in accordance with Chapter 4.7.

Article 10.3.14~~2~~.

Importation (or transit) of ~~aquatic animals and aquatic animal products for retail trade for human consumption regardless of the infection with *G. salaris* status of the exporting from a country, zone or compartment not declared free from infection with *G. salaris*~~

- 1) ~~Competent Authorities should not require any conditions related to infection with *G. salaris*, regardless of the infection with *G. salaris* status of the exporting country, zone or compartment, when authorising the importation (or transit) the following aquatic animal products commodities that which have been prepared and packaged for retail trade and which comply with Article 5.4.2.:~~
 - no aquatic animal products listed.
- 2) When importing ~~aquatic animals or aquatic animal products~~, other than those referred to in point 1 above, derived from a ~~of~~ species referred to in Article 10.3.2. from a country, zone or compartment not declared free from infection with *G. salaris*, the Competent Authority of the importing country should assess the risk and apply appropriate risk mitigation measures.

CHAPTER 10.4.

INFECTION WITH INFECTIOUS SALMON ANAEMIA VIRUS

Article 10.4.1.

For the purposes of the *Aquatic Code*, infection with infectious salmon anaemia virus (ISAV) means *infection with the pathogenic agent* HPR0 (non-deleted highly polymorphic region) or HPR-deleted *infectious salmon anaemia virus* (ISAV) of the *Genus* ~~genus~~ *Isavirus* ~~of and the family~~ *Family* Orthomyxoviridae. Both genotypes should be notified in accordance with Chapter 1.1. of the Aquatic Code.

There is a link between non-pathogenic HPR0 ISAV and pathogenic HPR-deleted ISAV, with some *outbreaks* potentially occurring as a result of the emergence of HPR-deleted from HPR0.

The provisions in this chapter are provided in recognition of three possible levels of disease status with respect to ISAV:

- 1) HPR0 ISAV and HPR-deleted ISAV free;
- 2) HPR0 ISAV endemic (but HPR-deleted ISAV free);
- 3) HPR0 ISAV and HPR-deleted ISAV endemic.

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

Article 10.4.2.

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.: Atlantic salmon (*Salmo salar*), brown trout (*Salmo trutta*) and rainbow trout (*Oncorhynchus mykiss*). ~~These recommendations also apply to any other susceptible species referred to in the Aquatic Manual when traded internationally.~~

Article 10.4.3.

Importation or transit of ~~aquatic animals and~~ aquatic animal products for any purpose regardless of the infection with ISAV ~~infectious salmon anaemia virus~~ status of the exporting country, zone or compartment

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

- 1) *Competent Authorities* should not require any conditions related to ~~infection with~~ ISAV, regardless of the infection with ISAV status of the *exporting country, zone or compartment*, when authorising the importation or transit of the following *aquatic animal products* derived from a ~~the~~ species referred to in Article 10.4.2. that ~~which~~ are intended for any purpose and ~~which~~ comply with Article 5.4.1.:
 - a) heat sterilised, hermetically sealed fish 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 ISAV);
 - b) pasteurised fish products that have been subjected to a heat treatment at 90°C for at least ten minutes (or to any time/temperature equivalent ~~which~~ that has been demonstrated to inactivate ISAV);
 - c) mechanically dried, eviscerated fish (i.e. a heat treatment at 100°C for 30 minutes or any time/temperature equivalent ~~which~~ that has been demonstrated to inactivate ISAV);

Annex 13 (contd)

- d) fish oil;
 - e) fish meal;
 - f) fish skin leather.
- 2) When authorising the importation or transit of ~~aquatic animals and aquatic animal products~~ of a species referred to in Article 10.4.2., other than those referred to in point 1 of Article 10.4.3., *Competent Authorities* should require the conditions prescribed in Articles 10.4.10. to 10.4.16-17, relevant to the infection with ISAV 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 referred to covered in Article 10.4.2. but which could reasonably be expected to pose a *risk of spread transmission of infection with ISAV*, 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 analysis~~.

Article 10.4.4.

Country free from infection with ISAV ~~infectious salmon anaemia virus~~

In this article, all statements referring to a country free from infection with ISAV are for any detectable ISAV, including HPR0 ISAV.

If a country shares a *zone* with one or more other countries, it can only make a *self-declaration of freedom* from infection with ISAV if all the areas covered by the shared water bodies are declared countries or *zones* free from infection with ISAV (see Article 10.4.6.)

As described in Article 1.4.6., a country may make a *self-declaration of freedom* from infection with ISAV if:

- 1) none of the *susceptible species* referred to in Article 10.4.2. are present and *basic biosecurity conditions* have been continuously met for at least the last two years;

OR

- 2) the ~~disease~~ infection with ISAV status prior to *targeted surveillance* is unknown but the following conditions have been met:
- a) *basic biosecurity conditions* have been continuously met for at least the last two years; and
 - b) *targeted surveillance*, as described in Chapter 1.4., has been in place for at least the last two years without detection of ~~infection with~~ ISAV;

OR

- 3) it previously made a *self-declaration of freedom* from infection with ISAV and subsequently lost its ~~disease~~ free status due to the detection of ~~infection with~~ ISAV but the following conditions have been met:
- a) on detection of the ISAV ~~disease~~, 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 have been destroyed or removed from the infected zone~~ by means that minimise the likelihood ~~risk~~ of further ~~spread transmission of ISAV the disease~~, and the appropriate *disinfection* procedures (as described in Chapter 4.3.) have been completed; 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 ISAV ~~the disease~~; and

Annex 13 (contd)

- d) *targeted surveillance*, as described in Chapter 1.4., has been in place for at least the last two years without detection of ~~infection with~~ ISAV.

In the meantime, part or all of the unaffected ~~non-affected~~ area may be declared a free zone provided that such a part meets the conditions in point 3 of Article 10.4.6.

The pathway for *self-declaration of freedom* from infection with ISAV HPR0 based on absence of clinical ~~disease~~ expression of infection with ISAV (referred to as historical freedom in Article 1.4.6.) cannot be achieved because infection with ISAV HPR0 is unlikely to cause any clinical signs.

Article 10.4.5.

Country free from infection with HPR-deleted ISAV ~~infectious salmon anaemia virus~~

In this article, all statements refer to a country free from infection with HPR-deleted ISAV but not necessarily free from infection with HPR0 ISAV.

If a country shares a zone with one or more other countries, it can only make a *self-declaration of freedom* from infection with HPR-deleted ISAV if all the areas covered by the shared water bodies are declared countries or zones free from infection with HPR-deleted ISAV (see Article 10.4.7.)

As described in Article 1.4.6., a country may make a *self-declaration of freedom* from infection with HPR-deleted ISAV if:

- 1) any of the *susceptible species* referred to in Article 10.4.2. are present and the following conditions have been met:
 - a) there has been no ~~observed~~ occurrence of infection with HPR-deleted ISAV for at least the last ten years despite conditions that are conducive to clinical expression (as described in the corresponding chapter of the *Aquatic Manual*); and
 - b) *basic biosecurity conditions* have been continuously met for at least the last ten years;

OR

- 2) the infection with HPR-deleted ISAV ~~disease~~ status prior to *targeted surveillance* is unknown but the following conditions have been met:
 - a) *basic biosecurity conditions* have been continuously met for at least the last two years; and
 - b) *targeted surveillance*, as described in Chapter 1.4., has been in place for at least the last two years without detection of ~~infection with~~ HPR-deleted ISAV;

OR

- 3) it previously made a *self-declaration of freedom* from infection with HPR-deleted ISAV and subsequently lost its ~~disease~~ free status due to the detection of ~~infection with~~ HPR-deleted ISAV but the following conditions have been met:
 - a) on detection of ~~infection with~~ HPR-deleted ISAV, 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 ~~been destroyed or removed from the infected zone~~ by means that minimise the likelihood ~~risk~~ of further spread transmission of HPR-deleted ISAV, and the appropriate *disinfection* procedures (as described in Chapter 4.3.) have been completed; and
 - c) previously existing *basic biosecurity conditions* have been reviewed and modified as necessary and have continuously been in place since eradication of ~~the~~ infection with HPR-deleted ISAV ~~disease~~; and

Annex 13 (contd)

- d) *targeted surveillance*, as described in Chapter 1.4., has been in place for at least the last two years without detection of ~~infection with~~ HPR-deleted ISAV.

In the meantime, part or all of the ~~unaffected non-affected~~ area may be declared a free zone provided that such a part meets the conditions in point 3 of Article 10.4.7.

Article 10.4.6.

Zone or compartment free from infection with ISAV ~~infectious salmon anaemia virus~~

In this article, all statements referring to a *zone* or *compartment* free from infection with ISAV are for any detectable ISAV, including HPR0 ISAV.

If a *zone* or *compartment* extends over more than one country, it can only be declared a *zone* or *compartment* free from infection with ISAV if all the relevant *Competent Authorities* confirm that all relevant conditions have been met.

As described in Article 1.4.6., a *zone* or *compartment* within the *territory* of one or more countries not declared free from infection with ISAV may be declared free by the *Competent Authority(ies)* of the country(ies) concerned if:

- 1) none of the *susceptible species* referred to in Article 10.4.2. are present in the *zone* or *compartment* and *basic biosecurity conditions* have been continuously met for at least the last two years;

OR

- 2) the ~~infection with ISAV disease~~ status prior to *targeted surveillance* is unknown but the following conditions have been met:

- a) *basic biosecurity conditions* have been continuously met for at least the last two years; and
 b) *targeted surveillance*, as described in Chapter 1.4., has been in place for at least the last two years without detection of ~~infection with~~ ISAV;

OR

- 3) it previously made a *self-declaration of freedom* for a *zone* from infection with ISAV and subsequently lost its ~~disease-free~~ status due to the detection of ~~infection with~~ ISAV in the *zone* but the following conditions have been met:

- a) on detection of ~~infection with~~ ISAV, 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 have been destroyed or removed from the infected zone~~ by means that minimise the likelihood ~~risk~~ of further ~~spread~~ transmission of the ISAV disease, and the appropriate *disinfection* procedures (as described in Chapter 4.3.) have been completed; and
 c) previously existing *basic biosecurity conditions* have been reviewed and modified as necessary and have continuously been in place since eradication of ~~the infection with ISAV disease~~; and
 d) *targeted surveillance*, as described in Chapter 1.4., has been in place for at least the last two years without detection of ~~infection with~~ ISAV.

Article 10.4.7.

Zone or compartment free from infection with HPR-deleted ISAV ~~infectious salmon anaemia virus~~

In this article, all statements refer to a *zone* or *compartment* free from infection with HPR-deleted ISAV but not necessarily free from infection with HPR0 ISAV.

Annex 13 (contd)

If a *zone* or *compartment* extends over more than one country, it can only be declared a *zone* or *compartment* free from infection with HPR-deleted ISAV if all the relevant *Competent Authorities* confirm that all relevant conditions have been met.

As described in Article 1.4.6., a *zone* or *compartment* within the *territory* of one or more countries not declared free from infection with HPR-deleted ISAV may be declared free by the *Competent Authority(ies)* of the country(ies) concerned if:

- 1) any of the *susceptible species* referred to in Article 10.4.2. are present in the *zone* or *compartment* and the following conditions have been met:
 - a) there has been no observed occurrence of infection with HPR-deleted ISAV for at least the last ten years despite conditions that are conducive to its clinical expression (as described in the corresponding chapter of the *Aquatic Manual*); and
 - b) *basic biosecurity conditions* have been continuously met for at least the last ten years;

OR

- 2) the infection with ~~HPR-deleted ISAV disease~~ status prior to *targeted surveillance* is unknown but the following conditions have been met:
 - a) *basic biosecurity conditions* have been continuously met for at least the last two years; and
 - b) *targeted surveillance*, as described in Chapter 1.4., has been in place, in the *zone* or *compartment*, for at least the last two years without detection of ~~infection with~~ HPR-deleted ISAV;

OR

- 3) it previously made a *self-declaration of freedom* for a *zone* from infection with HPR-deleted ISAV and subsequently lost its ~~disease~~ free status due to the detection of ~~infection with~~ HPR-deleted ISAV in the *zone* but the following conditions have been met:
 - a) on detection of ~~infection with~~ HPR-deleted ISAV, 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~~ ~~have been destroyed or removed from the infected zone~~ by means that minimise the likelihood ~~risk~~ of further ~~spread~~ transmission of the ISAV disease, and the appropriate *disinfection* procedures (as described in Chapter 4.3.) have been completed; and
 - c) previously existing *basic biosecurity conditions* have been reviewed and modified as necessary and have continuously been in place since eradication of ~~the infection with HPR-deleted ISAV disease~~; and
 - d) *targeted surveillance*, as described in Chapter 1.4., has been in place for at least two years without detection of ~~infection with~~ HPR-deleted ISAV.

Article 10.4.8.

Maintenance of free status for infection with ISAV ~~infectious salmon anaemia virus~~

In this article, all statements referring to a country, *zone* or *compartment* free from infection with ISAV are for any detectable ISAV, including HPR0 ISAV.

A country, *zone* or *compartment* that is declared free from infection with ISAV following the provisions of point 1 of Articles 10.4.4. or 10.4.6. (as relevant) may maintain its status as free from infection with ISAV provided that *basic biosecurity conditions* are continuously maintained.

Annex 13 (contd)

A country, *zone* or *compartment* that is declared free from infection with ISAV following the provisions of point 2 of Articles 10.4.4. or 10.4.6. (as relevant) may maintain its status as free from infection with ISAV provided that *targeted surveillance* is continued at a level determined by the *Aquatic Animal Health Service* on the basis of the likelihood of *infection*, and *basic biosecurity conditions* are continuously maintained.

Article 10.4.9.

Maintenance of free status for infection with HPR-deleted ISAV ~~infectious salmon anaemia virus~~

In this article, all statements refer to a country, *zone* or *compartment* free from infection with HPR-deleted ISAV, but not necessarily free from infection with HPR0 ISAV.

A country, *zone* or *compartment* that is declared free from infection with HPR-deleted ISAV following the provisions of points 1 or 2 of Articles 10.4.5. or 10.4.7. (as relevant) may maintain its status as free from infection with HPR-deleted ISAV provided that *basic biosecurity conditions* are continuously maintained.

A country, *zone* or *compartment* that is declared free from infection with HPR-deleted ISAV following the provisions of point 3 of Articles 10.4.5. or 10.4.7. (as relevant) may discontinue *targeted surveillance* and maintain its free status provided that conditions that are conducive to clinical expression of infection with HPR-deleted ISAV, as described in the corresponding chapter of the *Aquatic Manual*, ~~exist~~ and *basic biosecurity conditions* are continuously maintained.

However, for declared free *zones* or *compartments* in an infected country and in all cases where conditions are not conducive to clinical expression of infection with HPR-deleted ISAV, *targeted surveillance* ~~needs should~~ to be continued at a level determined by the *Aquatic Animal Health Service* on the basis of the likelihood of *infection*.

Article 10.4.10.

Importation of aquatic animals ~~and or~~ aquatic animal products from a country, zone or compartment declared free from infection with ISAV ~~infectious salmon anaemia virus~~

In this article, all statements referring to a country, *zone* or *compartment* free from infection with ISAV includes HPR deleted ISAV and HPR0 ISAV.

When importing *aquatic animals* ~~and aquatic animal products~~ of a species referred to in Article 10.4.2., or aquatic animal products derived thereof, from a country, *zone* or *compartment* 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* ~~or a certifying official approved by the importing country~~. The international aquatic animal health certificate should state that, certifying that, on the basis of the procedures described in Articles 10.4.4. or 10.4.6. (as applicable) and 10.4.8., the place of production of the *aquatic animals* ~~and or~~ *aquatic animal products* is a country, *zone* or *compartment* declared free from infection with ISAV.

The *international aquatic animal health certificate* should be in accordance with the Model Certificate in Chapter 5.11.

This article does not apply to aquatic animal products commodities referred to listed in point 1 of Article 10.4.3.

Article 10.4.11.

Importation of aquatic animals ~~and or~~ aquatic animal products from a country, zone or compartment declared free from infection with HPR-deleted ISAV ~~infectious salmon anaemia virus~~

In this article, all statements refer to a country, *zone* or *compartment* free from infection with HPR-deleted ISAV, but not necessarily free from infection with HPR0 ISAV.

When importing *aquatic animals* and *aquatic animal products* of a species referred to in Article 10.4.2., or aquatic animal products derived thereof, from a country, zone or compartment declared free from infection with HPR-deleted 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* ~~or a certifying official approved by the importing~~. The international aquatic animal health certificate should state that, certifying that, on the basis of the procedures described in Articles 10.4.5. or 10.4.7. (as applicable) and 10.4.9., the place of production of the *aquatic animals* ~~and or~~ *aquatic animal products* is a country, zone or compartment declared free from infection with HPR-deleted ISAV.

The *international aquatic animal health certificate* should be in accordance with the Model Certificate in Chapter 5.11.

This article does not apply to aquatic animal products commodities referred to listed in point 1 of Article 10.4.3.

Article 10.4.12.

Importation of aquatic animals for aquaculture from a country, zone or compartment not declared free from infection with ISAV infectious salmon anaemia virus

In this article, all statements referring to infection with ISAV are for any detectable ISAV, including HPR0 ISAV.

When importing, for aquaculture, aquatic animals or aquatic animal products 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 the *risk* mitigation measures in points 1 and 2 below.

- 1) If the intention is to grow out and harvest the imported *aquatic animals*, consider applying the following:
 - a) the direct delivery to and lifelong holding of the imported *aquatic animals* in a *quarantine* facility; and
 - b) the treatment of all transport water, equipment, effluent and waste materials to inactivate ISAV in accordance with Chapters 4.3., 4.7. and 5.5.

OR

- 2) If the intention is to establish a new stock for *aquaculture*, consider applying the following:
 - a) In the *exporting country*:
 - i) identify potential source populations and evaluate their *aquatic animal* health records;
 - ii) test source populations in accordance with Chapter 1.4. and select a founder population (F-0) of *aquatic animals* with a high health status for infection with ISAV.
 - b) In the *importing country*:
 - i) import the F-0 population into a *quarantine* facility;
 - ii) test the F-0 population for ISAV in accordance with Chapter 1.4. to determine their suitability as broodstock;
 - iii) produce a first generation (F-1) population in *quarantine*;
 - iv) culture the F-1 population in *quarantine* under conditions that are conducive to the clinical expression of ISAV, ~~(as described in Chapter 2.3.5. of the *Aquatic Manual*)~~ and sample and test for ISAV in accordance with Chapter 1.4. ~~of the *Aquatic Code* and (as described in Chapter 2.3.5. 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.

Annex 13 (contd)

Article 10.4.13.

Importation of aquatic animals ~~and or~~ aquatic animal products for processing for human consumption from a country, zone or compartment not declared free from infection with ISAV ~~infectious salmon anaemia virus~~

In this article, all statements referring to infection with ISAV are for any detectable ISAV, including HPR0 ISAV.

When importing, for processing for human consumption, ~~aquatic animals or aquatic animal products~~ of a species referred to in Article 10.1.2., or aquatic animal products derived thereof, from a country, zone or compartment not declared free from infection with ISAV, the *Competent Authority* of the *importing country* should assess the *risk* and, if justified, require that:

- 1) the consignment is delivered directly to, and held in, quarantine or containment facilities until processing into one of the products referred to in point 1 of Article 10.4.3., or products described in point 1 of Article 10.4.15-16., or other products authorised by the *Competent Authority*; and
- 2) 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 4.3., 4.7. and 5.5.; and all effluent and waste materials from the processing are treated in a manner that ensures inactivation of ISAV or is disposed in a manner that prevents contact of waste with susceptible species; and
- 3) all effluent and waste materials are treated to ensure inactivation of ISAV or disposed of in a biosecure manner in accordance with Chapters 4.3. and 4.7.

For these aquatic animals or aquatic animal products ~~commodities~~ Member Countries may wish to consider introducing internal measures to address the *risks* associated with the aquatic animals or aquatic animal products ~~commodity~~ being used for any purpose other than for human consumption.

Article 10.4.14.

Importation of aquatic animals or aquatic animal products intended for use uses other than human consumption, including in animal feed, or for and agricultural, industrial, research or pharmaceutical use, from a country, zone or compartment not declared free from infection with ISAV ~~infectious salmon anaemia virus~~

In this article, all statements referring to infection with ISAV are for any detectable ISAV, including HPR0 ISAV.

When importing aquatic animals of a species referred to in Article 10.4.2., or aquatic animal products derived thereof, intended for use uses other than human consumption, including in animal feed, or for and agricultural, industrial, research or pharmaceutical use, ~~aquatic animals of 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 require that:

- 1) the consignment is delivered directly to, and held in, quarantine or containment facilities until processed into one of the products referred to in point 1 of Article 10.4.3. or other for slaughter and processing into products authorised by the *Competent Authority*; and
- 2) all water (including ice), equipment, containers and packaging material used are treated to ensure inactivation of ISAV or disposed of in a biosecure manner in accordance with Chapters 4.3., 4.7. and 5.5.; and water used in transport and all effluent and waste materials from the processing are treated in a manner that ensures inactivation of ISAV.
- 3) all effluent and waste materials are treated to ensure inactivation of ISAV or disposed of in a biosecure manner in accordance with Chapters 4.3. and 4.7.

~~This article does not apply to commodities referred to in point 1 of Article 10.4.3.~~

Article 10.4.15.**Importation of aquatic animals intended for use in laboratories or zoos from a country, zone or compartment not declared free from infection with infection with ISAV**

When importing, for use in laboratories and zoos, aquatic animals of species referred to in Article 8.X.2. from a country, zone or compartment not declared free from infection with ISAV, the Competent Authority of the importing country should ensure:

- 1) the consignment is delivered directly to, and held in, quarantine facilities authorised by the Competent Authority; and
- 2) 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 4.3., 4.7. and 5.5.; and
- 3) all effluent and waste materials from the quarantine facilities in the laboratories or zoos are treated to ensure inactivation of ISAV or disposed of in a biosecure manner in accordance with Chapters 4.3. and 4.7.; and
- 4) the carcasses are disposed of in accordance with Chapter 4.7.

Article 10.4.156.**Importation (or transit) of aquatic animals and aquatic animal products for retail trade for human consumption regardless of the infection with ISAV status of the exporting from a country, zone or compartment not declared free from infection with infectious salmon anaemia virus**

In this article, all statements referring to infection with ISAV includes HPR deleted ISAV and HPR0 ISAV.

- 1) *Competent Authorities* should not require any conditions related to infection with ISAV, regardless of the infection with ISAV status of the *exporting country, zone or compartment*, when authorising the importation (or transit) of fish fillets or steaks (frozen or chilled) ~~which that~~ have been prepared and packaged for retail trade and ~~which~~ comply with Article 5.4.2.

Certain assumptions have been made in assessing the safety of the *aquatic animal products* mentioned above. Member Countries should refer to these assumptions at Article 5.4.2. and consider whether the assumptions apply to their conditions.

For these ~~commodities~~ *aquatic animal products* Member Countries may wish to consider introducing internal measures to address the *risks* associated with the ~~commodity~~ *aquatic animal products* being used for any purpose other than for human consumption.

- 2) When importing ~~aquatic animals or~~ *aquatic animal products*, other than those referred to in point 1 above, ~~of~~ derived from 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* and apply appropriate *risk* mitigation measures.

Article 10.4.167.**Importation of disinfected eggs for aquaculture from a country, zone or compartment not declared free from infection with ISAV** ~~infectious salmon anaemia virus~~

In this article, all statements referring to infection with ISAV are for any detectable ISAV, including HPR0 ISAV.

Annex 13 (contd)

- 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 the *risk* associated with at least:
 - a) the infection with ISAV status of the water to be used during the *disinfection* of the eggs;
 - b) the ~~level~~ prevalence of infection with ISAV in broodstock (ovarian fluid and milt); and
 - c) the temperature and pH of the water to be used for *disinfection*.
- 2) If the *Competent Authority* of the *importing country* concludes that the importation is acceptable, it should apply the following *risk* mitigation measures including:
 - a) the eggs should be disinfected prior to importing, in accordance with recommendations in Chapter 4.4. or those specified by the *Competent Authority* of the *importing country*; and
 - b) between *disinfection* and the import, eggs should not come into contact with anything which may affect their health status.

The *Competent Authority* may wish to consider internal measures, such as renewed *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* ~~or a certifying official approved by the *importing country*~~ certifying that the procedures described in point 2 of this article have been fulfilled.

CHAPTER 10.2.

INFECTION WITH *APHANOMYCES INVADANS* (EPIZOOTIC ULCERATIVE SYNDROME)

Article 10.2.1.

For the purposes of the *Aquatic Code*, infection with *Aphanomyces invadans* means all *infections* caused by the pathogenic agent *Aphanomyces invadans* (syn. *A. piscicida*). The *disease* was previously referred to as epizootic ulcerative syndrome.

~~Standards for diagnostic tests are described~~ Information on methods for diagnosis is provided in the *Aquatic Manual*.

Article 10.2.2.

Scope

The recommendations in this chapter apply to: yellowfin seabream (*Acantopagrus australis*), climbing perch (*Anabas testudineus*), eels (*Anguillidae*), bagrid catfishes (*Bagridae*), silver perch (*Bidyanus bidyanus*), Atlantic menhaden (*Brevoortia tyrannus*), jacks (*Caranx* spp.), catla (*Catla catla*), striped snakehead (*Channa striatus*), mrigal (*Cirrhinus mrigala*), torpedo-shaped catfishes (*Clarius* spp.), halfbeaks flying fishes (*Exocoetidae*), tank goby (*Glossogobius giuris*), marble goby (*Oxyeleotris marmoratus*), gobies (*Gobiidae*), rohu (*Labeo rohita*), rhinofishes (*Labeo* spp.), barramundi and giant sea perch (*Lates calcarifer*), striped mullet (*Mugil cephalus*), mullets (*Mugilidae*) (*Mugil* spp. and *Liza* spp.), ayu (*Plecoglossus altivelis*), pool barb (*Puntius sophore*), barcoo grunter (*Scortum barcoo*), sand whiting (*Sillago ciliata*), wells catfishes (*Siluridae*), snakeskin gourami (*Trichogaster pectoralis*), common archer fish (*Toxotes chatareus*), silver barb (*Puntius gonionotus*), spotted scat (*Scatophagus argus*), giant gourami (*Osphronemus goramy*), dusky flathead (*Platycephalus fuscus*), spiny turbot (*Psettodes* sp.), Tairiku-baratanago (*Rhodeus ocellatus*), Keti-Bangladeshi (*Rohtee* sp.), rudd (*Scaridinius erythrophthalmus*), theraon (*Terapon* sp.) and three-spot gouramy (*Trichogaster trichopterus*). These recommendations also apply to any other *susceptible species* referred to in the *Aquatic Manual* when traded internationally.

Article 10.2.3.

Importation or transit of ~~aquatic animals and~~ aquatic animal products for any purpose regardless of the infection with *A. invadans* status of the exporting country, zone or compartment

- 1) *Competent Authorities* should not require any conditions related to ~~infection with *A. invadans*~~, regardless of the infection with *A. invadans* status of the *exporting country, zone or compartment*, when authorising the importation or transit of the following ~~aquatic animals and aquatic animal products derived from the a~~ species referred to in Article 10.2.2. ~~that which~~ are intended for any purpose and ~~which~~ comply with Article 5.4.1.:
 - a) heat sterilised hermetically sealed fish products (i.e. a heat treatment at 121°C for at least 3.6 minutes or any time/temperature equivalent) that has been demonstrated to inactivate *A. invadans*;
 - b) pasteurised fish products that have been subjected to heat treatment at 90°C for at least ten minutes (or any time/temperature equivalent ~~which~~ that has been demonstrated to inactivate *A. invadans*);
 - c) mechanically dried eviscerated fish (i.e. a heat treatment at 100°C for at least 30 minutes or any time/temperature equivalent ~~which~~ that has been demonstrated to inactivate *A. invadans*);
 - d) fish oil;
 - e) fish meal;
 - f) frozen eviscerated fish;
 - g) frozen fish fillets or steaks.

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- 2) When authorising the importation or transit of ~~aquatic animals and aquatic animal products~~ of a species referred to in Article 10.2.2., other than those referred to in point 1 of Article 10.2.3., *Competent Authorities* should require the conditions prescribed in Articles 10.2.7. to 10.2.12~~4~~. relevant to infection with *A. invadans* status of the *exporting country, zone or compartment*.
- 3) When considering the importation or transit of ~~aquatic animals and aquatic animal products from an exporting country, zone or compartment~~ not declared free from infection with *A. invadans* of a species not covered referred to in Article 10.2.2. but which could reasonably be expected to pose a risk of transmission spread of infection with *A. invadans*, the *Competent Authority* should conduct a *risk analysis* in accordance with the recommendations in Chapter 2.1. The *exporting country* should be informed of the outcome of this analysis assessment.

Article 10.2.4.

Country free from infection with *A. invadans*

If a country shares a *zone* with one or more other countries, it can only make a *self-declaration of freedom* from infection with *A. invadans* if all the areas covered by the shared water bodies are declared countries or *zones* free from infection with *A. invadans* (see Article 10.2.5.).

As described in Article 1.4.6., a country may make a *self-declaration of freedom* from infection with *A. invadans* if:

- 1) a country where there has been no ~~observed~~ occurrence of infection with *A. invadans* for at least the last ten years despite conditions that are conducive to its clinical expression, as described in the corresponding chapter of the *Aquatic Manual*, may make a *self-declaration of freedom* from infection with *A. invadans* when *basic biosecurity conditions* have been continuously met in the country for at least the last ten years;

OR

- 2) the infection with *A. invadans* disease status prior to *targeted surveillance* is unknown but the following conditions have been met:
 - a) *basic biosecurity conditions* have been continuously met for at least the last two years; and
 - b) *targeted surveillance*, as described in Chapter 1.4., has been in place for at least the last two years without detection of ~~infection with *A. invadans*~~;

OR

- 3) it previously made a *self-declaration of freedom* from infection with *A. invadans* and subsequently lost its ~~disease~~ free status due to the detection of ~~infection with *A. invadans*~~ but the following conditions have been met:
 - a) on detection of *A. invadans* ~~the disease~~, 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 ~~have been destroyed or removed from the *infected zone*~~ by means that minimise the likelihood risk of further transmission spread of *A. invadans* ~~the disease~~, and the appropriate *disinfection* procedures (as described in Chapter 4.3.) have been completed; 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 *A. invadans* ~~the disease~~; and
 - d) *targeted surveillance*, as described in Chapter 1.4., has been in place for at least the last two years without detection of ~~infection with *A. invadans*~~.

In the meantime, part or all of the unaffected non-affected area may be declared a free *zone* provided that such a part meets the conditions in point 2 of Article 10.2.5.

Article 10.2.5.

Zone or compartment free from infection with *A. invadans*

If a *zone* or *compartment* extends over more than one country, it can only be declared a *zone* or *compartment* free from infection with *A. invadans* if all the relevant *Competent Authorities* confirm that all relevant conditions have been met.

As described in Article 1.4.6., a *zone* or *compartment* within the *territory* of one or more countries not declared free from infection with *A. invadans* may be declared free by the *Competent Authority(ies)* of the country(ies) concerned if:

- 1) a *zone* or *compartment* where the species referred to in Article 10.2.2. are present but there has been no ~~observed~~ occurrence of infection with *A. invadans* ~~the disease~~ for at least the last ten years despite conditions that are conducive to its clinical expression, as described in the corresponding chapter of the *Aquatic Manual*, may be declared free from infection with *A. invadans* when *basic biosecurity conditions* have been continuously met in the *zone* or *compartment* for at least the last ten years;

OR

- 2) the infection with *A. invadans* ~~disease~~ status prior to *targeted surveillance* is unknown but the following conditions have been met:
 - a) *basic biosecurity conditions* have been continuously met for at least the last two years; and
 - b) *targeted surveillance*, as described in Chapter 1.4., has been in place, in the *zone* or *compartment*, for at least the last two years without detection of ~~infection with *A. invadans*~~;

OR

- 3) it previously made a *self-declaration of freedom* for a *zone* from infection with *A. invadans* and subsequently lost its ~~disease~~ free status due to the detection of ~~infection with *A. invadans*~~ in the *zone* but the following conditions have been met:
 - a) on detection of *A. invadans* ~~the disease~~, 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~~ ~~have been destroyed or removed from the *infected zone*~~ by means that minimise the likelihood ~~risk~~ of further transmission ~~spread~~ of *A. invadans* ~~the disease~~, and the appropriate *disinfection* procedures (as described in Chapter 4.3.) have been completed; 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 *A. invadans* ~~the disease~~; and
 - d) *targeted surveillance*, as described in Chapter 1.4., has been in place for at least the last two years without detection of ~~infection with *A. invadans*~~.

Article 10.2.6.

Maintenance of free status

A country, *zone* or *compartment* that is declared free from infection with *A. invadans* following the provisions of point 1 of Articles 10.2.4. or 10.2.5. (as relevant) may maintain its status as free from infection with *A. invadans* provided that *basic biosecurity conditions* are continuously maintained.

A country, *zone* or *compartment* that is declared free from infection with *A. invadans* following the provisions of point 2 of Articles 10.2.4. or 10.2.5. (as relevant) may discontinue *targeted surveillance* and maintain its free status ~~as free from infection with *A. invadans*~~ provided that conditions that are conducive to clinical expression of infection with *A. invadans*, as described in the corresponding chapter of the *Aquatic Manual*, ~~exist~~ and *basic biosecurity conditions* are continuously maintained.

Annex 14 (contd)

However, for declared free zones or compartments in infected countries and in all cases where conditions are not conducive to clinical expression of infection with *A. invadans*, ~~targeted surveillance needs to~~ should be continued at a level determined by the *Aquatic Animal Health Service* on the basis of the likelihood of infection.

Article 10.2.7.

Importation of aquatic animals ~~and or~~ aquatic animal products from a country, zone or compartment declared free from infection with *A. invadans*

When importing aquatic animals of a species referred to in Article 10.2.2., or and aquatic animal products of species referred to in Article 10.2.2. derived thereof, from a country, zone or compartment declared free from infection with *A. invadans*, 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* ~~or a certifying official approved by the importing country~~. The international aquatic animal health certificate should state that certifying that, on the basis of the procedures described in Articles 10.2.4. or 10.2.5. (as applicable) and 10.2.6., the place of production of the aquatic animals or and aquatic animal products is a country, zone or compartment declared free from infection with *A. invadans*.

The international aquatic animal health certificate should be in accordance with the Model Certificate in Chapter 5.11.

This article does not apply to aquatic animal products listed commodities referred to in point 1 of Article 10.2.3.

Article 10.2.8.

Importation of aquatic animals for aquaculture from a country, zone or compartment not declared free from infection with *A. invadans*

When importing, for aquaculture, aquatic animals of a species referred to in Article 10.2.2. from a country, zone or compartment not declared free from infection with *A. invadans*, the *Competent Authority* of the *importing country* should assess the *risk* in accordance with Chapter 2.1. and consider the *risk* mitigation measures in points 1 and 2 below.

- 1) If the intention is to grow out and harvest the imported *aquatic animals*, consider applying the following:
 - a) the direct delivery to and lifelong holding of the imported *aquatic animals* in a *quarantine* facility; and
 - b) the treatment of all transport water, equipment, effluent and waste materials to inactivate *A. invadans* in accordance with Chapters 4.3., 4.7. and 5.5.

OR

- 2) If the intention is to establish a new stock for aquaculture, consider applying the following:
 - 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 *A. invadans*.
 - b) In the *importing country*:
 - i) import the F-0 population into a *quarantine* facility;
 - ii) test the F-0 population for *A. invadans* in accordance with Chapter 1.4. to determine their suitability as broodstock;
 - iii) produce a first generation (F-1) population in *quarantine*;

Annex 14 (contd)

- iv) culture the F-1 population in quarantine under conditions that are conducive to the clinical expression of infection with *A. invadans*, ~~(as described in Chapter 2.3.2. of the Aquatic Manual)~~ and sample and test for *A. invadans* in accordance with Chapter 1.4. of the Aquatic Code and Chapter 2.3.2. of the Aquatic Manual;
- v) if *A. invadans* is not detected in the F-1 population, it may be defined as free from infection with *A. invadans* and may be released from *quarantine*;
- vi) if *A. invadans* 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.

Article 10.2.9.

Importation of aquatic animals ~~and~~ or aquatic animal products for processing for human consumption from a country, zone or compartment not declared free from infection with *A. invadans*

When importing, for processing for human consumption, ~~aquatic animals or aquatic animal products~~ of a species referred to in Article 10.2.2., or aquatic animal products derived thereof, from a country, zone or compartment not declared free from infection with *A. invadans*, the *Competent Authority* of the *importing country* should assess the *risk* and, if justified, require that:

- 1) the consignment is delivered directly to, and held, in *quarantine* or containment facilities until processing into one of the products referred to in point 1 of Article 10.2.3., ~~or products described in point 1 of Article 10.2.11.,~~ or other products authorised by the *Competent Authority*; and
- 2) all water (including ice), equipment, containers and packaging material used in transport are treated to ensure inactivation of *A. invadans* or disposed of in a biosecure manner in accordance with Chapters 4.3., 4.7. and 5.5.; and ~~and all effluent and waste materials from the processing are treated in a manner that ensures inactivation of *A. invadans* or is disposed in a manner that prevents contact of waste with susceptible species.~~
- 3) all effluent and waste materials are treated to ensure inactivation of *A. invadans* or disposed of in a biosecure manner in accordance with Chapters 4.3. and 4.7.

For these aquatic animals or aquatic animal products ~~commodities~~ Member Countries may wish to consider introducing internal measures to address the *risks* associated with the aquatic animals or aquatic animal products ~~commodity~~ being used for any purpose other than for human consumption.

Article 10.2.10.

Importation of aquatic animals or aquatic animal products intended for uses other than human consumption, including use in animal feed, or for ~~and~~ agricultural, industrial, research or pharmaceutical use, from a country, zone or compartment not declared free from infection with *A. invadans*

When importing aquatic animals of a species referred to in Article 10.2.2., or aquatic animal products derived thereof, intended for uses other than human consumption, including use in animal feed or for ~~and~~ agricultural, industrial, research or pharmaceutical use, aquatic animals of species referred to in Article 10.2.2., from a country, zone or compartment not declared free from infection with *A. invadans*, the *Competent Authority* of the *importing country* should require that:

- 1) the consignment is delivered directly to, and held in, quarantine or containment facilities until processed into one of the products referred to in point 1 of Article 10.2.3. or other facilities for slaughter and processing into products authorised by the *Competent Authority*; and
- 2) all water (including ice), equipment, containers and packaging material used in transport are treated to ensure inactivation of *A. invadans* or disposed of in a biosecure manner in accordance with Chapters 4.3., 4.7. and 5.5.; and ~~and all effluent and waste materials from the processing are treated in a manner that ensures inactivation of *A. invadans* or is disposed in a manner that prevents contact of waste with susceptible~~

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- 3) all effluent and waste materials are treated to ensure inactivation of *A. invadans* or disposed of in a biosecure manner in accordance with Chapters 4.3. and 4.7.

This Article does not apply to ~~commodities~~ referred to in point 1 of Article 10.2.3.

Article 10.2.11.**Importation of aquatic animals intended for use in laboratories or zoos from a country, zone or compartment not declared free from infection with *A. invadans***

When importing, for use in laboratories and zoos, *aquatic animals* of a species referred to in Article 10.2.2. from a country, zone or compartment not declared free from infection with *A. invadans*, the *Competent Authority* of the importing country should ensure:

- 1) the consignment is delivered directly to, and held, in *quarantine* facilities authorised by the *Competent Authority*; and
- 2) all water (including ice), equipment, *containers* and packaging material used in transport are treated to ensure inactivation of *A. invadans* or disposed of in a biosecure manner in accordance with Chapters 4.3., 4.7. and 5.5.; and
- 3) all effluent and waste materials from the *quarantine* facilities in the laboratories or zoos are treated to ensure inactivation of *A. invadans* or disposed of in a biosecure manner in accordance with Chapters 4.3. and 4.7.; and
- 4) the carcasses are disposed of in accordance with Chapter 4.7.

Article 10.2.12+**Importation (or transit) of ~~aquatic animals and aquatic animal products~~ for retail trade for human consumption regardless of the infection with *A. invadans* status of the exporting ~~from a country, zone or compartment not declared free from infection with *A. invadans*~~**

- 1) *Competent Authorities* should not require any conditions related to ~~infection with *A. invadans*~~, regardless of the infection with *A. invadans* status of the *exporting country, zone or compartment*, when authorising the importation (or transit) of fish fillets or steaks (chilled) ~~that which~~ have been prepared and packaged for retail trade and ~~which~~ comply with Article 5.4.2.

Certain assumptions have been made in assessing the safety of the *aquatic animal products* mentioned above. Member Countries should refer to these assumptions at Article 5.4.2. and consider whether the assumptions apply to their conditions.

For these *aquatic animal products* ~~commodities~~ Member Countries may wish to consider introducing internal measures to address the *risks* associated with the *aquatic animal products* ~~commodity~~ being used for any purpose other than for human consumption.

- 2) When importing ~~*aquatic animals*~~ or *aquatic animal products*, other than those referred to in point 1 above, derived from a ~~of~~ species referred to in Article 10.2.2. from a country, zone or compartment not declared free from infection with *A. invadans*, the *Competent Authority* of the *importing country* should assess the *risk* and apply appropriate *risk* mitigation measures.

CHAPTER 10.5.

INFECTION WITH SALMONID ALPHAVIRUS

Article 10.5.1.

General provisions

For the purposes of the *Aquatic Code*, infection with salmonid alphavirus means *infection* with any subtype of the pathogenic agent salmonid alphavirus (SAV), of the ~~g~~Genus *Alphavirus* ~~of and the f~~Family *Togaviridae*.

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

Article 10.5.2.

Scope

The recommendations in this chapter apply to: Atlantic salmon (*Salmo salar*), brown trout (*Salmo trutta*) and rainbow trout (*Onchorynchus mykiss*). These recommendations also apply to any other *susceptible species* referred to in the *Aquatic Manual* when traded internationally.

Article 10.5.3.

Importation or transit of ~~aquatic animals and~~ aquatic animal products for any purpose regardless of the infection with SAV salmonid alphavirus status of the exporting country, zone or compartment

- 1) *Competent Authorities* should not require any conditions related to ~~infection with~~ SAV, regardless of the infection with SAV status of the *exporting country, zone or compartment* when authorising the importation or transit of the following *aquatic animal products* derived from ~~the a~~ species referred to in Article 10.5.2. that ~~which are~~ intended for any purpose and complying with Article 5.4.1.:
 - a) heat sterilised, hermetically sealed fish 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 SAV;
 - b) pasteurised fish products that have been subjected to a heat treatment at 90°C for at least ten minutes (or to any time/temperature equivalent which that has been demonstrated to inactivate SAV);
 - c) mechanically dried, eviscerated fish (i.e. a heat treatment at 100°C for 30 minutes or any time/temperature equivalent which that has been demonstrated to inactivate SAV);
 - d) fish oil;
 - e) fish *meal*;
 - f) fish skin leather.
- 2) When authorising the importation or transit of ~~aquatic animals and~~ *aquatic animal products* of a species referred to in Article 10.5.2., other than those referred to in point 1 of Article 10.5.3., *Competent Authorities* should require the conditions prescribed in Articles 10.5.7. to 10.5.132. relevant to the infection with SAV 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~~ referred to in Article 10.5.2. but which could reasonably be expected to pose a *risk of transmission spread of infection with* SAV, 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 assessment.

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Article 10.5.4.

Country free from infection with SAV ~~salmonid alphavirus~~

If a country shares a *zone* with one or more other countries, it can only make a *self-declaration of freedom* from infection with SAV if all the areas covered by the shared water bodies are declared countries or *zones* free from infection with SAV (see Article 10.5.5.).

As described in Article 1.4.6., a country may make a *self-declaration of freedom* from infection with SAV if:

- 1) none of the *susceptible species* referred to in Article 10.5.2. are present and *basic biosecurity conditions* have been continuously met for at least the last two years;

OR

- 2) any of the *susceptible species* referred to in Article 10.5.2. are present and the following conditions have been met:
 - a) there has been no ~~observed~~ occurrence of infection with SAV ~~the disease~~ for at least the last ten years despite conditions that are conducive to clinical expression (as described in the corresponding chapter of the *Aquatic Manual*); and
 - b) *basic biosecurity conditions* have been continuously met for at least the ~~past~~ last ten years;

OR

- 3) the ~~disease~~ infection with SAV status prior to *targeted surveillance* is unknown but the following conditions have been met:
 - a) *basic biosecurity conditions* have been continuously met for at least the last two years; and
 - b) *targeted surveillance*, as described in Chapter 1.4., has been in place for at least the last two years without detection of infection with SAV;

OR

- 4) it previously made a *self-declaration of freedom* from infection with SAV and subsequently lost its ~~disease~~ free status due to the detection of ~~infection with~~ SAV but the following conditions have been met:
 - a) on detection of SAV ~~the disease~~, 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 ~~have been destroyed or removed from the infected zone~~ by means that minimise the likelihood ~~risk~~ of further transmission spread of SAV ~~the disease~~, and the appropriate *disinfection* procedures (as described in Chapter 4.3.) have been completed; 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 SAV ~~the disease~~; and
 - d) *targeted surveillance*, as described in Chapter 1.4., has been in place for at least the last two years without detection of ~~infection with~~ SAV.

In the meantime, part or all of the unaffected ~~non-affected~~ area may be declared a free *zone* provided that such a part meets the conditions in point 3 of Article 10.5.5.

Article 10.5.5.

Zone or compartment free from infection with SAV salmonid alphavirus

If a *zone* or *compartment* extends over more than one country, it can only be declared a *zone* or *compartment* free from infection with SAV if all the relevant *Competent Authorities* confirm that all relevant conditions have been met.

As described in Article 1.4.6., a *zone* or *compartment* within the *territory* of one or more countries not declared free from infection with SAV may be declared free by the *Competent Authority(ies)* of the country(ies) concerned if:

- 1) none of the *susceptible species* referred to in Article 10.5.2. are present in the *zone* or *compartment* and *basic biosecurity conditions* have been continuously met for at least the last two years;

OR

- 2) any of the *susceptible species* referred to in Article 10.5.2. are present in the *zone* or *compartment* and the following conditions have been met:
 - a) there has been no ~~observed~~ occurrence of infection with SAV ~~the disease~~ for at least the last ten years despite conditions that are conducive to its clinical expression (as described in the corresponding chapter of the *Aquatic Manual*); and
 - b) *basic biosecurity conditions* have been continuously met for at least the last ten years;

OR

- 3) the infection with SAV ~~disease~~ status prior to *targeted surveillance* is unknown but the following conditions have been met:
 - a) *basic biosecurity conditions* have been continuously met for at least the last two years; and
 - b) *targeted surveillance*, as described in Chapter 1.4., has been in place, in the *zone* or *compartment*, for at least the last two years without detection of infection with SAV;

OR

- 4) it previously made a *self-declaration of freedom* for a *zone* from infection with SAV and subsequently lost its ~~disease~~ free status due to the detection of ~~infection with~~ SAV in the *zone* but the following conditions have been met:
 - a) on detection of ~~infection with~~ SAV, 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 ~~have been destroyed or removed from the infected zone~~ by means that minimise the likelihood risk of further transmission spread of SAV the disease, and the appropriate *disinfection* procedures (as described in Chapter 4.3.) have been completed; 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 SAV ~~the disease~~; and
 - d) *targeted surveillance*, as described in Chapter 1.4., has been in place for at least the last two years without detection of ~~infection with~~ SAV.

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Article 10.5.6.

Maintenance of free status ~~for infection with salmonid alphavirus~~

A country, *zone* or *compartment* that is declared free from infection with SAV following the provisions of points 1 or 2 of Articles 10.5.4. or 10.5.5. (as relevant) may maintain its status as free from infection with SAV provided that *basic biosecurity conditions* are continuously maintained.

A country, *zone* or *compartment* that is declared free from infection with SAV following the provisions of point 3 of Articles 10.5.4. or 10.5.5. (as relevant) may discontinue *targeted surveillance* and maintain its free status as free from infection with SAV provided that conditions that are conducive to clinical expression of infection with SAV, as described in the corresponding chapter of the *Aquatic Manual*, ~~exist~~ and *basic biosecurity conditions* are continuously maintained.

However, for declared free *zones* or *compartments* in an infected country and in all cases where conditions are not conducive to clinical expression of infection with SAV, *targeted surveillance* ~~needs to~~ should be continued at a level determined by the *Aquatic Animal Health Service* on the basis of the likelihood of *infection*.

Article 10.5.7.

Importation of aquatic animals ~~and/or~~ aquatic animal products from a country, zone or compartment declared free from infection with SAV salmonid alphavirus

When importing aquatic animals of a species referred to in Article 10.5.2., or and aquatic animal products of species referred to in Article 10.2.2. derived thereof. from a country, *zone* or *compartment* declared free from infection with SAV, 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 or a certifying official approved by the importing country.~~ The international aquatic animal health certificate should state that certifying that, on the basis of the procedures described in Articles 10.5.4. or 10.5.5. (as applicable) and 10.5.6., the place of production of the *aquatic animals* or and *aquatic animal products* is a country, *zone* or *compartment* declared free from infection with SAV.

The international aquatic animal health certificate should be in accordance with the Model Certificate in Chapter 5.11.

This article does not apply to aquatic animal products listed commodities referred to in point 1 of Article 10.5.3.

Article 10.5.8.

Importation of aquatic animals for aquaculture from a country, zone or compartment not declared free from infection with SAV salmonid alphavirus

When importing, for aquaculture, *aquatic animals* of a species referred to in Article 10.5.2. from a country, *zone* or *compartment* not declared free from infection with SAV, the *Competent Authority* of the *importing country* should assess the *risk* in accordance with Chapter 2.1. and consider the *risk* mitigation measures in points 1 and 2 below.

- 1) If the intention is to grow out and harvest the imported *aquatic animals*, consider applying the following:
 - a) the direct delivery to and lifelong holding of the imported *aquatic animals* in a *quarantine* facility; and
 - b) the treatment of all transport water, equipment, effluent and waste materials to inactivate SAV in accordance with Chapters 4.3., 4.7. and 5.5.

OR

Annex 15 (contd)

- 2) If the intention is to establish a new stock for *aquaculture*, consider applying the following:
- 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 SAV.
 - b) In the *importing country*:
 - i) import the F-0 population into a *quarantine* facility;
 - ii) test the F-0 population for SAV 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* under conditions that are conducive to the clinical expression of infection with SAV, (as described in Chapter 2.3.6. of the *Aquatic Manual*) and sample and test for SAV in accordance with Chapter 1.4. of the *Aquatic Code* and Chapter 2.3.6. of the *Aquatic Manual*.
 - v) if SAV is not detected in the F-1 population, it may be defined as free from infection with SAV and may be released from *quarantine*;
 - vi) if SAV 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.

Article 10.5.9.

Importation of aquatic animals ~~and~~ or aquatic animal products for processing for human consumption from a country, zone or compartment not declared free from infection with SAV salmonid alphavirus

When importing, for processing for human consumption, ~~*aquatic animals or aquatic animal products*~~ of a species referred to in Article 10.5.2., or aquatic animal products derived thereof, from a country, zone or compartment not declared free from infection with infection with SAV, the *Competent Authority* of the *importing country* should assess the *risk* and, if justified, require that:

- 1) the consignment is delivered directly to, and held, in *quarantine* or containment facilities until processing into one of the products referred to in point 1 of Article 10.5.3., ~~or products described in point 1 of Article 10.5.11.~~, or other products authorised by the *Competent Authority*; and
- 2) all water (including ice), equipment, containers and packaging material used in transport are treated to ensure inactivation of SAV or disposed of in a biosecure manner in accordance with Chapters 4.3., 4.7. and 5.5.; and all effluent and waste materials from the processing are treated in a manner that ensures inactivation of SAV or is disposed in a manner that prevents contact of waste with susceptible species.
- 3) all effluent and waste materials are treated to ensure inactivation of SAV or disposed of in a biosecure manner in accordance with Chapters 4.3. and 4.7.

For these *aquatic animals or aquatic animal products* ~~commodities~~ Member Countries may wish to consider introducing internal measures to address the *risks* associated with the *aquatic animal or aquatic animal product commodity* being used for any purpose other than for human consumption.

Article 10.5.10.

Importation of aquatic animals or aquatic animal products intended for uses other than human consumption, including use in animal feed, ~~or for~~ and agricultural, industrial, research or pharmaceutical use, from a country, zone or compartment not declared free from infection with SAV salmonid alphavirus

When importing *aquatic animals of a species referred to in Article 10.5.2., or aquatic animal products derived thereof, intended for uses other than human consumption, including use in animal feed or for and* agricultural, industrial, research or pharmaceutical use, ~~*aquatic animals of species referred to in Article 10.5.2.*~~ from a country, zone or compartment not declared free from infection with SAV, the *Competent Authority* of the *importing country* should require that:

Annex 15 (contd)

- 1) the consignment is delivered directly to, and held in, quarantine or containment facilities until processed into one of the products referred to in point 1 of Article 10.5.3. or other facilities for slaughter and processing into products authorised by the Competent Authority; and
- 2) all water (including ice), equipment, containers and packaging material used in transport are treated to ensure inactivation of SAV or disposed of in a biosecure manner in accordance with Chapters 4.3., 4.7. and 5.5.; and all effluent and waste materials from the processing are treated in a manner that ensures inactivation of SAV or is disposed in a manner that prevents contact of waste with susceptible species.
- 3) all effluent and waste materials are treated to ensure inactivation of SAV or disposed of in a biosecure manner in accordance with Chapters 4.3. and 4.7.

This article does not apply to ~~commodities~~ referred to in point 1 of Article 10.5.3.

Article 10.5.11.**Importation of aquatic animals intended for use in laboratories or zoos from a country, zone or compartment not declared free from infection with SAV**

When importing, for use in laboratories and zoos, aquatic animals of a species referred to in Article 10.5.2. from a country, zone or compartment not declared free from infection with SAV, the Competent Authority of the importing country should ensure:

- 1) the consignment is delivered directly to, and held in, quarantine facilities authorised by the Competent Authority; and
- 2) all water (including ice), equipment, containers and packaging material used in transport are treated to ensure inactivation of SAV or disposed of in a biosecure manner in accordance with Chapters 4.3., 4.7. and 5.5.; and
- 3) all effluent and waste materials from the quarantine facilities in the laboratories or zoos are treated to ensure inactivation of SAV or disposed of in a biosecure manner in accordance with Chapters 4.3. and 4.7.; and
- 4) the carcasses are disposed of in accordance with Chapter 4.7.

Article 10.5.12+**Importation (or transit) of aquatic animals and aquatic animal products for retail trade for human consumption regardless of the infection with SAV status of the exporting ~~from a country, zone or compartment not declared free from infection with SAV~~ salmonid alphavirus**

- 1) *Competent Authorities* should not require any conditions related to ~~infection with~~ SAV, regardless of the infection with SAV status of the *exporting country, zone or compartment*, when authorising the importation (or transit) of fish fillets or steaks (chilled) ~~which that~~ have been prepared and packaged for retail trade and ~~which~~ comply with Article 5.4.2.

Certain assumptions have been made in assessing the safety of the *aquatic animal products* mentioned above. Member Countries should refer to these assumptions at Article 5.4.2. and consider whether the assumptions apply to their conditions.

For these *aquatic animal products* ~~commodities~~ Member Countries may wish to consider introducing internal measures to address the *risks* associated with the *aquatic animal products* ~~commodity~~ being used for any purpose other than for human consumption.

- 2) When importing ~~aquatic animals or aquatic animal products~~, other than those referred to in point 1 above, derived from a ~~of~~ species referred to in Article 10.5.2. from a country, zone or compartment not declared free from infection with SAV, the *Competent Authority* of the *importing country* should assess the *risk* and apply appropriate *risk* mitigation measures.

Article 10.5.1~~32~~.**Importation of disinfected eggs for aquaculture from a country, zone or compartment not declared free from infection from infection with SAV ~~salmonid alphavirus~~**

- 1) When importing disinfected eggs of the species referred to in Article 10.5.2. for *aquaculture*, from a country, zone or *compartment* not declared free from infection with SAV, the *Competent Authority* of the *importing country* should assess the *risk* associated with at least:
 - a) the infection with SAV status of the water to be used during the *disinfection* of the eggs;
 - b) the ~~level~~ prevalence of infection with SAV in broodstock; and
 - c) the temperature and pH of the water to be used for *disinfection*.

- 2) If the *Competent Authority* of the *importing country* concludes that the importation is acceptable, it should apply the following *risk* mitigation measures including:
 - a) the eggs should be disinfected prior to importing, in accordance with recommendations in Chapter 4.4. or those specified by the *Competent Authority* of the *importing country*; and
 - b) between *disinfection* and the import, eggs should not come into contact with anything which may affect their health status.

The *Competent Authority* may wish to consider internal measures, such as renewed *disinfection* of the eggs upon arrival in the *importing country*.

- 3) When importing disinfected eggs of the species referred to in Article 10.5.2. for *aquaculture*, from a country, zone or *compartment* not declared free from infection with SAV, 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* ~~or a certifying official approved by the importing country~~ certifying that the procedures described in point 2 of this article have been fulfilled.

CHAPTER 10.6.

INFECTION WITH INFECTIOUS HAEMATOPOIETIC NECROSIS VIRUS

Article 10.6.1.

For the purposes of the *Aquatic Code*, infection with infectious haematopoietic necrosis virus (IHNV) means infection with the pathogenic agent infectious haematopoietic necrosis virus (IHNV), of the Genus Novirhabdovirus of and the Family Rhabdoviridae.

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

Article 10.6.2.

Scope

The recommendations in this chapter apply to: rainbow trout or steelhead (*Oncorhynchus mykiss*), the Pacific salmon species (chinook [*Oncorhynchus tshawytscha*], sockeye [*Oncorhynchus nerka*], chum [*Oncorhynchus keta*], masou [*Oncorhynchus masou*], pink [*Oncorhynchus rhodurus*] and coho [*Oncorhynchus kisutch*]), and Atlantic salmon (*Salmo salar*). These recommendations also apply to any other *susceptible species* referred to in the *Aquatic Manual* when traded internationally.

Article 10.6.3.

Importation or transit of ~~aquatic animals and~~ aquatic animal products for any purpose regardless of the infection with IHNV ~~infectious haematopoietic necrosis~~ status of the exporting country, zone or compartment

- 1) *Competent Authorities* should not require any conditions related to IHNV, regardless of the infection with IHNV status of the *exporting country, zone or compartment*, when authorising the importation or transit of the following *aquatic animal products* derived from a ~~the~~ species referred to in Article 10.6.2. that ~~which~~ are intended for any purpose and ~~which~~ comply with Article 5.4.1.:
 - a) heat sterilised, hermetically sealed fish 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 IHNV);
 - b) pasteurised fish products that have been subjected to a heat treatment at 90°C for at least ten minutes (or any time/temperature equivalent ~~which~~ that has been demonstrated to inactivate IHNV);
 - c) mechanically dried, eviscerated fish (i.e. a heat treatment at 100°C for at least 30 minutes or any time/temperature equivalent ~~which~~ that has been demonstrated to inactivate IHNV);
 - d) fish oil;
 - e) fish *meal*;
 - f) fish skin leather.
- 2) When authorising the importation or transit of ~~aquatic animals and~~ *aquatic animal products* of a species referred to in Article 10.6.2., other than those referred to in point 1 of Article 10.6.3., *Competent Authorities* should require the conditions prescribed in Articles 10.6.7. to 10.6.132. relevant to the infection with IHNV 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~~ referred to in Article 10.6.2. but which could reasonably be expected to pose a *risk of transmission spread* of IHNV, 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 ~~assessment~~.

Annex 16 (contd)

Article 10.6.4.

Country free from infection with IHNV ~~infectious haematopoietic necrosis~~

If a country shares a *zone* with one or more other countries, it can only make a *self-declaration of freedom* from infection with IHNV if all the areas covered by the shared water bodies are declared countries or *zones* free from infection with IHNV (see Article 10.6.5.).

As described in Article 1.4.6., a country may make a *self-declaration of freedom* from infection with IHNV if:

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

OR

- 2) any of the *susceptible species* referred to in Article 10.6.2. are present and the following conditions have been met:
 - a) there has been no ~~observed~~ occurrence of infection with IHNV ~~the disease~~ for at least the last ten years despite conditions that are conducive to its clinical expression (as described in the corresponding chapter of the *Aquatic Manual*); and
 - b) *basic biosecurity conditions* have been continuously met for at least the last ten years;

OR

- 3) the ~~disease~~ infection with IHNV status prior to *targeted surveillance* is unknown but the following conditions have been met:
 - a) *basic biosecurity conditions* have been continuously met for at least the last two years; and
 - b) *targeted surveillance*, as described in Chapter 1.4., has been in place for at least the last two years without detection of IHNV;

OR

- 4) it previously made a *self-declaration of freedom* from infection with IHNV and subsequently lost its ~~disease~~ free status due to the detection of IHNV but the following conditions have been met:
 - a) on detection of IHNV ~~the disease~~, 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 ~~have been destroyed or removed from the infected zone~~ by means that minimise the likelihood ~~risk~~ of further transmission spread of IHNV ~~the disease~~, and the appropriate *disinfection* procedures (as described in Chapter 4.3.) have been completed; 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 IHNV ~~the disease~~; and
 - d) *targeted surveillance*, as described in Chapter 1.4., has been in place for at least the last two years without detection of IHNV.

In the meantime, part or all of the unaffected ~~non-affected~~ area may be declared a free *zone* provided that such a part meets the conditions in point 3 of Article 10.6.5.

Article 10.6.5.

Zone or compartment free from infection with IHNV ~~infectious haematopoietic necrosis~~

If a *zone* or *compartment* extends over more than one country, it can only be declared ~~an IHNV~~ a free *zone* or *compartment* if all the relevant *Competent Authorities* confirm that all relevant conditions have been met.

As described in Article 1.4.6., a *zone* or *compartment* within the *territory* of one or more countries not declared free from infection with IHNV may be declared free by the *Competent Authority(ies)* of the country(ies) concerned if:

- 1) none of the *susceptible species* referred to in Article 10.6.2. are present in the *zone* or *compartment* and *basic biosecurity conditions* have been continuously met for at least the last two years;

OR

- 2) any of the *susceptible species* referred to in Article 10.6.2. are present in the *zone* or *compartment* and the following conditions have been met:
 - a) there has been no ~~observed~~ occurrence of infection with IHNV ~~the disease~~ for at least the last ten years despite conditions that are conducive to its clinical expression (as described in the corresponding chapter of the *Aquatic Manual*); and
 - b) *basic biosecurity conditions* have been continuously met for at least the last ten years;

OR

- 3) the ~~disease~~ infection with IHNV status prior to *targeted surveillance* is unknown but the following conditions have been met:
 - a) *basic biosecurity conditions* have been continuously met for at least the last two years; and
 - b) *targeted surveillance*, as described in Chapter 1.4., has been in place, in the *zone* or *compartment*, for at least the last two years without detection of IHNV;

OR

- 4) it previously made a *self-declaration of freedom* for a *zone* from infection with IHNV and subsequently lost its ~~disease~~ free status due to the detection of IHNV in the *zone* but the following conditions have been met:
 - a) on detection of IHNV ~~the disease~~, 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 ~~have been destroyed or removed from the infected zone~~ by means that minimise the *likelihood* ~~risk~~ of further *transmission* spread of IHNV ~~the disease~~, and the appropriate *disinfection* procedures (as described in Chapter 4.3.) have been completed; 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 IHNV ~~the disease~~; and
 - d) *targeted surveillance*, as described in Chapter 1.4., has been in place for at least the last two years without detection of IHNV.

Annex 16 (contd)

Article 10.6.6.

Maintenance of free status

A country, *zone* or *compartment* that is declared free from infection with IHNV following the provisions of points 1 or 2 of Articles 10.6.4. or 10.6.5. (as relevant) may maintain its status as free from infection with IHNV provided that *basic biosecurity conditions* are continuously maintained.

A country, *zone* or *compartment* that is declared free from infection with IHNV following the provisions of point 3 of Articles 10.6.4. or 10.6.5. (as relevant) may discontinue *targeted surveillance* and maintain its free status ~~as free from IHNV~~ provided that conditions that are conducive to clinical expression of infection with IHNV, as described in the corresponding chapter of the *Aquatic Manual*, ~~exist~~ and *basic biosecurity conditions* are continuously maintained.

However, for declared free *zones* or *compartments* in infected countries and in all cases where conditions are not conducive to clinical expression of infection with IHNV, ~~targeted surveillance needs to~~ should be continued at a level determined by the *Aquatic Animal Health Service* on the basis of the likelihood of *infection*.

Article 10.6.7.

Importation of aquatic animals ~~and/or~~ aquatic animal products from a country, zone or compartment declared free from infection with IHNV ~~infectious haematopoietic necrosis~~

When importing aquatic animals of a species referred to in Article 10.6.2., or ~~and~~ aquatic animal products of species referred to in Article 10.2.2. derived thereof. from a country, *zone* or *compartment* declared free from infection with IHNV, 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 or a certifying official approved by the importing country.~~ The *international aquatic animal health certificate* should state that certifying that, on the basis of the procedures described in Articles 10.6.4. or 10.6.5. (as applicable) and 10.6.6., the place of production of the aquatic animals ~~or and~~ aquatic animal products is a country, *zone* or *compartment* declared free from infection with IHNV.

The *international aquatic animal health certificate* should be in accordance with the Model Certificate in Chapter 5.11.

This article does not apply to aquatic animal products listed ~~commodities referred to~~ in point 1 of Article 10.6.3.

Article 10.6.8.

Importation of aquatic animals for aquaculture from a country, zone or compartment not declared free from infection with IHNV ~~infectious haematopoietic necrosis~~

When importing, for aquaculture, aquatic animals of a species referred to in Article 10.6.2. from a country, *zone* or *compartment* not declared free from infection with IHNV, the *Competent Authority* of the *importing country* should assess the *risk* in accordance with Chapter 2.1. and consider the *risk* mitigation measures in points 1 and 2 below.

- 1) If the intention is to grow out and harvest the imported *aquatic animals*, consider applying the following:
 - a) the direct delivery to and lifelong holding of the imported *aquatic animals* in a *quarantine* facility; and
 - b) the treatment of all transport water, equipment, effluent and waste materials to inactivate IHNV in accordance with Chapters 4.3., 4.7. and 5.5.

OR

Annex 16 (contd)

- 2) If the intention is to establish a new stock for *aquaculture*, consider applying the following:
- 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 IHNV.
 - b) In the *importing country*:
 - i) import the F-0 population into a *quarantine* facility;
 - ii) test the F-0 population for IHNV 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* under conditions that are conducive to the clinical expression of IHNV, ~~(as described in Chapter 2.3.4. of the *Aquatic Manual*)~~ and sample and test for IHNV in accordance with Chapter 1.4. of the *Aquatic Code* and Chapter 2.3.4. of the *Aquatic Manual*;
 - v) if IHNV is not detected in the F-1 population, it may be defined as free from infection with IHNV and may be released from *quarantine*;
 - vi) if IHNV 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.

Article 10.6.9.

Importation of aquatic animals ~~and or~~ aquatic animal products for processing for human consumption from a country, zone or compartment not declared free from infection with IHNV ~~infectious haematopoietic necrosis~~

When importing, for processing for human consumption, ~~*aquatic animals or aquatic animal products*~~ of a species referred to in Article 10.6.2., or aquatic animal products derived thereof, from a country, zone or compartment not declared free from infection with IHNV, the *Competent Authority* of the *importing country* should assess the risk and, if justified, require that:

- 1) the consignment is delivered directly to, and held in, *quarantine* or containment facilities until processing into one of the products referred to in point 1 of Article 10.6.3., ~~or products described in point 1 of Article 10.6.11.~~, or other products authorised by the *Competent Authority*; and
- 2) all water (including ice), equipment, containers and packaging material used in transport are treated to ensure inactivation of IHNV or disposed of in a biosecure manner in accordance with Chapters 4.3., 4.7. and 5.5.; and all effluent and waste materials from the processing are treated in a manner that ensures inactivation of IHNV or is disposed in a manner that prevents contact of waste with susceptible species.
- 3) all effluent and waste materials from the holding of the aquatic animals in laboratories or zoos are treated to ensure inactivation of IHNV or disposed of in a biosecure manner in accordance with Chapters 4.3. and 4.7.

For these ~~*aquatic animals or aquatic animal products commodities*~~ Member Countries may wish to consider introducing internal measures to address the risks associated with the *aquatic animals or aquatic animal products commodity* being used for any purpose other than for human consumption.

Article 10.6.10.

Importation of aquatic animals or aquatic animal products intended for uses other than human consumption, including use in animal feed, or for and agricultural, industrial, research or pharmaceutical use, from a country, zone or compartment not declared free from infection with IHNV ~~infectious haematopoietic necrosis~~

When importing *aquatic animals of a species referred to in Article 10.6.2., or aquatic animal products derived thereof, intended for uses other than human consumption, including use in animal feed or for and* agricultural, industrial, research or pharmaceutical use, ~~*aquatic animals of species referred to in Article 10.6.2.*~~ from a country, zone or compartment not declared free from infection with IHNV, the *Competent Authority* of the *importing country* should require that:

Annex 16 (contd)

- 1) the consignment is delivered directly to, and held in, quarantine or containment facilities until processed into one of the products referred to in point 1 of Article 10.6.3. or other facilities for slaughter and processing into products authorised by the Competent Authority; and
- 2) all water (including ice), equipment, containers and packaging material used in transport are treated to ensure inactivation of IHN~~V~~ or disposed of in a biosecure manner in accordance with Chapters 4.3., 4.7. and 5.5.; and ~~and all effluent and waste materials from the processing are treated in a manner that ensures inactivation of IHN~~V~~ or is disposed in a manner that prevents contact of waste with susceptible~~
- 3) all effluent and waste materials are treated to ensure inactivation of IHN~~V~~ or disposed of in a biosecure manner in accordance with Chapters 4.3. and 4.7.

This article does not apply to ~~commodities~~ referred to in point 1 of Article 10.6.3.

Article 10.6.11.**Importation of aquatic animals intended for use in laboratories or zoos from a country, zone or compartment not declared free from infection with SAV**

When importing, for use in laboratories and zoos, aquatic animals of a species referred to in Article 10.6.2. from a country, zone or compartment not declared free from infection with IHN~~V~~, the Competent Authority of the importing country should ensure:

- 1) the consignment is delivered directly to, and held in, quarantine facilities authorised by the Competent Authority; and
- 2) all water (including ice), equipment, containers and packaging material used in transport are treated to ensure inactivation of IHN~~V~~ or disposed of in a biosecure manner in accordance with Chapters 4.3., 4.7. and 5.5.; and
- 3) all effluent and waste materials from the quarantine facilities in the laboratories or zoos are treated to ensure inactivation of IHN~~V~~ or disposed of in a biosecure manner in accordance with Chapters 4.3. and 4.7.; and
- 4) the carcasses are disposed of in accordance with Chapter 4.7.

Article 10.6.12.**Importation (or transit) of ~~aquatic animals and aquatic animal products~~ for retail trade for human consumption regardless of the infection with IHN~~V~~ status of the exporting ~~from a country, zone or compartment not declared free from infection with IHN~~V~~~~ infectious haematopoietic necrosis**

- 1) Competent Authorities should not require any conditions related to IHN~~V~~, regardless of the infection with IHN~~V~~ status of the exporting country, zone or compartment, when authorising the importation (or transit) of fish fillets or steaks (chilled) ~~that which~~ have been prepared and packaged for retail trade and ~~which~~ comply with Article 5.4.2.

Certain assumptions have been made in assessing the safety of the *aquatic animal products* mentioned above. Member Countries should refer to these assumptions at Article 5.4.2. and consider whether the assumptions apply to their conditions.

For these *aquatic animal products* commodities Member Countries may wish to consider introducing internal measures to address the *risks* associated with the *aquatic animal products* commodity being used for any purpose other than for human consumption.

- 2) When importing ~~aquatic animals or aquatic animal products~~, other than those referred to in point 1 above, derived from a ~~of~~ species referred to in Article 10.6.2. from a country, zone or compartment not declared free from infection with IHN~~V~~, the Competent Authority of the importing country should assess the risk and apply appropriate risk mitigation measures.

Article 10.6.1~~3~~2.**Importation of disinfected eggs for aquaculture from a country, zone or compartment not declared free from infection with IHNV ~~infectious haematopoietic necrosis~~**

- 1) When importing disinfected eggs of the species referred to in Article 10.6.2. for *aquaculture*, from a country, zone or compartment not declared free from infection with IHNV, the *Competent Authority* of the *importing country* should assess the *risk* associated with at least:
 - a) the infection with IHN virus status of the water to be used during the *disinfection* of the eggs;
 - b) the prevalence of infection with IHNV virus in broodstock (ovarian fluid and milt); and
 - c) the temperature and pH of the water to be used for *disinfection*.

- 2) If the *Competent Authority* of the *importing country* concludes that the importation is acceptable, it should apply the following *risk* mitigation measures including:
 - a) the eggs should be disinfected prior to importing, in accordance with recommendations in Chapter 4.4. or those specified by the *Competent Authority* of the *importing country*; and
 - b) between *disinfection* and the import, eggs should not come into contact with anything which may affect their health status.

The *Competent Authority* may wish to consider internal measures, such as renewed *disinfection* of the eggs upon arrival in the *importing country*.

- 3) When importing disinfected eggs of species referred to in Article 10.6.2. for *aquaculture*, from a country, zone or compartment not declared free from infection with IHNV, 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* ~~or a certifying official approved by the importing country~~ certifying that the procedures described in point 2 of Article 10.6.12. have been fulfilled.

CHAPTER 10.7.

INFECTION WITH KOI HERPESVIRUS DISEASE

Article 10.7.1.

For the purposes of the *Aquatic Code*, infection with koi herpesvirus disease (KHVD) means *infection with the pathogenic agent viral species koi herpesvirus (KHV) tentatively placed in the sub-family Genus Cyprinid Cyprinivirus herpesvirus of the -and Family Alloherpesviridae.*

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

Article 10.7.2.

Scope

The recommendations in this chapter apply to: common carp (*Cyprinus carpio carpio*), ghost carp (*Cyprinus carpio goi*), koi carp (*Cyprinus carpio koi*) and common carp hybrids (e.g. *Cyprinus carpio* x *Carassius auratus*). These recommendations also apply to any other *susceptible species* referred to in the *Aquatic Manual* when traded internationally.

Article 10.7.3.

Importation or transit of ~~aquatic animals and~~ aquatic animal products for any purpose regardless of the infection with KHV koi herpesvirus disease status of the exporting country, zone or compartment

- 1) *Competent Authorities* should not require any conditions related to KHVD, regardless of the infection with KHVD status of the *exporting country, zone or compartment*, when authorising the importation or transit of the following *aquatic animal products* derived from a ~~the~~ species referred to in Article 10.7.2. that ~~which~~ are intended for any purpose and ~~which~~ comply with Article 5.4.1.:
 - a) heat sterilised hermetically sealed fish 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 KHV);
 - b) pasteurised fish products that have been subjected to heat treatment at 90°C for at least ten minutes (or to any time/temperature equivalent ~~which~~ that has been demonstrated to inactivate KHV);
 - c) mechanically dried eviscerated fish (i.e. a heat treatment at 100°C for at least 30 minutes (or any time/temperature equivalent ~~which~~ that has been demonstrated to inactivate KHV);
 - d) fish oil;
 - e) fish meal.
- 2) When authorising the importation or transit of ~~aquatic animals and~~ *aquatic animal products* of a species referred to in Article 10.7.2., other than those referred to in point 1 of Article 10.7.3., *Competent Authorities* should require the conditions prescribed in Articles 10.7.7. to 10.7.12~~4~~. relevant to the infection with KHVD 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~~ referred to in Article 10.7.2. but which could reasonably be expected to pose a *risk of transmission spread* of KHVD, 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 ~~assessment~~.

Annex 17 (contd)

Article 10.7.4.

Country free from infection with KHV ~~koi herpesvirus disease~~

If a country shares a *zone* with one or more other countries, it can only make a *self-declaration of freedom from infection with KHV* if all the areas covered by the shared water bodies are declared countries or *zones* free from infection with KHV (see Article 10.7.5.).

As described in Article 1.4.6., a country may make a *self-declaration of freedom from infection with KHV* if:

- 1) none of the *susceptible species* referred to in Article 10.7.2. are present and *basic biosecurity conditions* have been continuously met for at least the last two years;

OR

- 2) any of the *susceptible species* referred to in Article 10.7.2. are present and the following conditions have been met:
 - a) there has been no ~~observed~~ occurrence of infection with KHV ~~the disease~~ for at least the last ten years despite conditions that are conducive to its clinical expression (as described in the corresponding chapter of the *Aquatic Manual*); and
 - b) *basic biosecurity conditions* have been continuously for at least the last ten years;

OR

- 3) the ~~disease~~ infection with KHV status prior to *targeted surveillance* is unknown but the following conditions have been met:
 - a) *basic biosecurity conditions* have been continuously met for at least the last two years; and
 - b) *targeted surveillance*, as described in Chapter 1.4., has been in place for at least the last two years without detection of KHV;

OR

- 4) it previously made a *self-declaration of freedom from infection with KHV* and subsequently lost its ~~disease~~ free status due to the detection of KHV but the following conditions have been met:
 - a) on detection of ~~the KHV disease~~, 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 have been destroyed or removed from the infected zone~~ by means that minimise the likelihood risk of further transmission spread of KHV ~~the disease~~, and the appropriate *disinfection* procedures (as described in Chapter 4.3.) have been completed; and
 - c) previously existing *basic biosecurity conditions* have been reviewed and modified as necessary and have continuously been in place since eradication of ~~the infection with KHV-disease~~; and
 - d) *targeted surveillance*, as described in Chapter 1.4., has been in place for at least the last two years without detection of KHV.

In the meantime, part or all of the ~~unaffected non-affected~~ area may be declared a free *zone* provided that such a part meets the conditions in point 3 of Article 10.7.5.

Article 10.7.5.

Zone or compartment free from infection with KHV ~~koi herpesvirus disease~~

If a *zone* or *compartment* extends over more than one country, it can only be declared a ~~KHV~~ free *zone* or *compartment* free from infection with KHV if all the relevant *Competent Authorities* confirm that all relevant conditions have been met.

Annex 17 (contd)

As described in Article 1.4.6., a *zone* or *compartment* within the *territory* of one or more countries not declared free from infection with KHVD may be declared free by the *Competent Authority*(ies) of the country(ies) concerned if:

- 1) none of the *susceptible species* referred to in Article 10.7.2. are present in the *zone* or *compartment* and *basic biosecurity conditions* have been continuously met for at least the last two years;

OR

- 2) any of the *susceptible species* referred to in Article 10.7.2. are present in the *zone* or *compartment* and the following conditions have been met:
 - a) there has been no ~~observed~~ occurrence of infection with KHV ~~the disease~~ for at least the last ten years despite conditions that are conducive to its clinical expression (as described in the corresponding chapter of the *Aquatic Manual*); and
 - b) *basic biosecurity conditions* have been continuously met for at least the last ten years;

OR

- 3) the ~~disease~~ infection with KHV status prior to *targeted surveillance* is unknown but the following conditions have been met:
 - a) *basic biosecurity conditions* have been continuously met for at least the last two years; and
 - b) *targeted surveillance*, as described in Chapter 1.4., has been in place, in the *zone* or *compartment*, for at least the last two years without detection of KHVD;

OR

- 4) it previously made a *self-declaration of freedom* for a *zone* from infection with KHVD and subsequently lost its ~~disease~~ free status due to the detection of KHV in the *zone* but the following conditions have been met:
 - a) on detection of KHV ~~the disease~~, 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 ~~have been destroyed or removed from the infected zone~~ by means that minimise the likelihood ~~risk~~ of further transmission spread of KHV ~~the disease~~, and the appropriate *disinfection* procedures (as described in Chapter 4.3.) have been completed; 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 KHV ~~the disease~~; and
 - d) *targeted surveillance*, as described in Chapter 1.4., has been in place for at least the last two years without detection of KHVD.

Article 10.7.6.

Maintenance of free status

A country, *zone* or *compartment* that is declared free from infection with KHVD following the provisions of points 1 or 2 of Articles 10.7.4. or 10.7.5. (as relevant) may maintain its status as free from infection with KHVD provided that *basic biosecurity conditions* are continuously maintained.

A country, *zone* or *compartment* that is declared free from infection with KHVD following the provisions of point 3 of Articles 10.7.4. or 10.7.5. (as relevant) may discontinue *targeted surveillance* and maintain its free status ~~as free from KHVD~~ provided that conditions that are conducive to clinical expression of infection with KHVD, as described in the corresponding chapter of the *Aquatic Manual*, ~~exist~~, and *basic biosecurity conditions* are continuously maintained.

Annex 17 (contd)

However, for declared free zones or compartments in infected countries and in all cases where conditions are not conducive to clinical expression of infection with KHVD, ~~targeted surveillance needs to~~ should be continued at a level determined by the *Aquatic Animal Health Service* on the basis of the likelihood of *infection*.

Article 10.7.7.

Importation of aquatic animals ~~and~~ or aquatic animal products from a country, zone or compartment declared free from infection with KHV koi herpesvirus disease

When importing aquatic animals of a species referred to in Article 10.7.2., or ~~and~~ aquatic animal products of species referred to in Article 10.2.2. derived thereof, from a country, zone or compartment declared free from infection with KHVD, 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* ~~or a certifying official approved by the importing country~~. The international aquatic animal health certificate should state that certifying that, on the basis of the procedures described in Articles 10.7.4. or 10.7.5. (as applicable) and 10.7.6., the place of production of aquatic animals ~~or~~ and aquatic animal products is a country, zone or compartment declared free from infection with KHVD.

The international aquatic animal health certificate should be in accordance with the Model Certificate in Chapter 5.11.

This article does not apply to aquatic animal products listed ~~commodities referred to~~ in point 1 of Article 10.7.3.

Article 10.7.8.

Importation of aquatic animals for aquaculture from a country, zone or compartment not declared free from infection with KHV koi herpesvirus disease

When importing, for aquaculture, aquatic animals of a species referred to in Article 10.7.2. from a country, zone or compartment not declared free from infection with KHVD., the *Competent Authority* of the *importing country* should assess the *risk* in accordance with Chapter 2.1. and consider the *risk* mitigation measures in points 1 and 2 below.

- 1) If the intention is to grow out and harvest the imported *aquatic animals*, consider applying the following:
 - a) the direct delivery to and lifelong holding of the imported *aquatic animals* in a *quarantine* facility; and
 - b) the treatment of all transport water, equipment, effluent and waste materials to inactivate KHV in accordance with Chapters 4.3., 4.7. and 5.5.

OR

- 2) If the intention is to establish a new stock for *aquaculture*, consider applying the following:
 - 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 KHVD.
 - b) In the *importing country*:
 - i) import the F-0 population into a *quarantine* facility;
 - ii) test the F-0 population for KHV 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* under conditions that are conducive to the clinical expression of KHVD, ~~(as described in Chapter 2.3.7. of the Aquatic Manual)~~ and sample and test for KHV in accordance with Chapter 1.4. of the Aquatic Code and Chapter 2.3.7. of the Aquatic Manual.

Annex 17 (contd)

- v) if KHV is not detected in the F-1 population, it may be defined as free from infection with KHV and may be released from *quarantine*;
- vi) if KHV 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.

Article 10.7.9.

Importation of aquatic animals ~~and~~ or aquatic animal products for processing for human consumption from a country, zone or compartment not declared free from infection with KHV koi herpesvirus disease

When importing, for processing for human consumption, ~~aquatic animals or aquatic animal products of a species referred to in Article 10.7.2., or aquatic animal products derived thereof,~~ from a country, zone or compartment not declared free from infection with KHV, the *Competent Authority* of the *importing country* should assess the *risk* and, if justified, require that:

- 1) the consignment is delivered directly to, and held in, *quarantine* or containment facilities until processing into one of the products referred to in point 1 of Article 10.7.3., ~~or products described~~ products described in point 1 of Article 10.7.11., or other products authorised by the *Competent Authority*; and
- 2) all water (including ice), equipment, containers and packaging material used in transport are treated to ensure inactivation of KHV or disposed of in a biosecure manner in accordance with Chapters 4.3., 4.7. and 5.5.; and all effluent and waste materials from the processing are treated in a manner that ensures inactivation of KHV or is disposed in a manner that prevents contact of waste with susceptible species.
- 3) all effluent and waste materials are treated to ensure inactivation of KHV or disposed of in a biosecure manner in accordance with Chapters 4.3. and 4.7.

For these aquatic animals or aquatic animal products commodities Member Countries may wish to consider introducing internal measures to address the *risks* associated with the aquatic animals or aquatic animal products commodity being used for any purpose other than for human consumption.

Article 10.7.10.

Importation of aquatic animals or aquatic animal products intended for uses other than human consumption, including use in animal feed, or for and agricultural, industrial, research or pharmaceutical use, from a country, zone or compartment not declared free from infection with KHV koi herpesvirus disease

When importing aquatic animals of a species referred to in Article 10.7.2., or aquatic animal products derived thereof, intended for uses other than human consumption, including use in animal feed or for and agricultural, industrial, research or pharmaceutical use, ~~aquatic animals of species referred to in Article 10.2.2,~~ from a country, zone or compartment not declared free from infection with KHV, the *Competent Authority* of the *importing country* should require that:

- 1) the consignment is delivered directly to, and held in, quarantine or containment facilities until processed into one of the products referred to in point 1 of Article 10.7.3. or other facilities for slaughter and processing into products authorised by the *Competent Authority*; and
- 2) all water (including ice), equipment, containers and packaging material used in transport are treated to ensure inactivation of KHV or disposed of in a biosecure manner in accordance with Chapters 4.3., 4.7. and 5.5.; and all effluent and waste materials from the processing are treated in a manner that ensures inactivation of KHV or is disposed in a manner that prevents contact of waste with susceptible
- 3) all effluent and waste materials are treated to ensure inactivation of KHV or disposed of in a biosecure manner in accordance with Chapters 4.3. and 4.7.

~~This article does not apply to commodities referred to in point 1 of Article 10.7.3.~~

Annex 17 (contd)Article 10.7.11.**Importation of aquatic animals intended for use in laboratories or zoos from a country, zone or compartment not declared free from infection with KHV**

When importing, for use in laboratories and zoos, aquatic animals of a species referred to in Article 10.7.2. from a country, zone or compartment not declared free from infection with KHV, the Competent Authority of the importing country should ensure:

- 1) the consignment is delivered directly to, and held in, quarantine facilities authorised by the Competent Authority; and
- 2) all water (including ice), equipment, containers and packaging material used in transport are treated to ensure inactivation of KHV or disposed of in a biosecure manner in accordance with Chapters 4.3., 4.7. and 5.5.; and
- 3) all effluent and waste materials from the quarantine facilities in the laboratories or zoos are treated to ensure inactivation of KHV or disposed of in a biosecure manner in accordance with Chapters 4.3. and 4.7.; and
- 4) the carcasses are disposed of in accordance with Chapter 4.7.

Article 10.7.12.**Importation (or transit) of aquatic animals and aquatic animal products for retail trade for human consumption regardless of the infection with KHV status of the exporting from a country, zone or compartment not declared free from infection with KHV koi herpesvirus disease**

- 1) *Competent Authorities* should not require any conditions related to KHVD, regardless of the infection with KHVD status of the *exporting country, zone or compartment*, when authorising the importation (or transit) of fish fillets or steaks (chilled) that which have been prepared and packaged for retail trade and which comply with Article 5.4.2.

Certain assumptions have been made in assessing the safety of the *aquatic animal products* mentioned above. Member Countries should refer to these assumptions at Article 5.4.2. and consider whether the assumptions apply to their conditions.

For these aquatic animal products commodities Member Countries may wish to consider introducing internal measures to address the *risks* associated with the aquatic animal products commodity being used for any purpose other than for human consumption.

- 2) When importing ~~aquatic animals~~ or *aquatic animal products*, other than those referred to in point 1 above, derived from a of species referred to in Article 10.7.2. from a country, zone or *compartment* not declared free from the infection with KHVD, the *Competent Authority* of the *importing country* should assess the *risk* and apply appropriate *risk* mitigation measures.

CHAPTER 10.8.

INFECTION WITH RED SEA BREAM IRIDOVIRUS
IRIDOVIRAL DISEASE

Article 10.8.1.

For the purposes of the *Aquatic Code*, infection with red sea bream iridovirus iridoviral disease (RSIVD) means infection with the pathogenic agent red sea bream iridovirus (RSIV) of the Genus *Megalocytiavirus* and Family Iridoviridae.

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

Article 10.8.2.

Scope

The recommendations in this chapter apply to: red sea bream (*Pagrus major*), yellowtail (*Seriola quinqueradiata*), amberjack (*Seriola dumerili*), sea bass (*Lateolabrax* sp. and *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.). These recommendations also apply to any other *susceptible species* referred to in the *Aquatic Manual* when traded internationally.

Article 10.8.3.

Importation or transit of aquatic animals and aquatic animal products for any purpose regardless of the infection with RSIV red sea bream iridoviral disease status of the exporting country, zone or compartment

- 1) *Competent Authorities* should not require any conditions related to RSIVD, regardless of the infection with RSIVD-status of the *exporting country, zone or compartment*, when authorising the importation or transit of the following *aquatic animal products* derived from the a species referred to in Article 10.8.2. that which are intended for any purpose and which comply with Article 5.4.1.:
 - a) heat sterilised hermetically sealed fish 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 RSIV);
 - b) pasteurised fish products that have been subjected to heat treatment at 90°C for at least ten minutes (or any time/temperature equivalent which that has been demonstrated to inactivate RSIV);
 - c) mechanically dried eviscerated fish (i.e. a heat treatment at 100°C for at least 30 minutes (or any time/temperature equivalent which that has been demonstrated to inactivate RSIV);
 - d) fish oil;
 - e) fish *meal*;
 - f) fish skin leather.
- 2) When authorising the importation or transit of ~~aquatic animals and aquatic animal products~~ of a species referred to in Article 10.8.2., other than those referred to in point 1 of Article 10.8.3., *Competent Authorities* should require the conditions prescribed in Articles 10.8.7. to 10.8.12~~4~~. relevant to the infection with RSIVD 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~~ referred to in Article 10.8.2. but which could reasonably be expected to pose a risk of transmission spread of RSIVD, 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 assessment.

Annex 18 (contd)

Article 10.8.4.

Red sea bream iridovirus free eCountry free from infection with RSIV

If a country shares a *zone* with one or more other countries, it can only make a *self-declaration of freedom from infection with RSIV* if all the areas covered by the shared water bodies are declared countries or *zones* free from infection with RSIV (see Article 10.8.5.).

As described in Article 1.4.6., a country may make a *self-declaration of freedom from infection with RSIV* if:

- 1) none of the *susceptible species* referred to in Article 10.8.2. are present and *basic biosecurity conditions* have been continuously met for at least the last two years;

OR

- 2) any of the *susceptible species* referred to in Article 10.8.2. are present and the following conditions have been met:
 - a) there has been no ~~observed~~ occurrence of infection with RSIV ~~the disease~~ for at least the last ten years despite conditions that are conducive to its clinical expression (as described in the corresponding chapter of the *Aquatic Manual*), and
 - b) *basic biosecurity conditions* have been continuously met for at least the last ten years;

OR

- 3) the ~~disease~~ infection with RSIV status prior to *targeted surveillance* is unknown but the following conditions have been met:
 - a) *basic biosecurity conditions* have been continuously met for at least the last two years; and
 - b) *targeted surveillance*, as described in Chapter 1.4., has been in place for at least the last two years without detection of RSIV;

OR

- 4) it previously made a *self-declaration of freedom from infection with RSIV* and subsequently lost its ~~disease~~ free status due to the detection of RSIV but the following conditions have been met:
 - a) on detection of RSIV ~~the disease~~, 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 ~~have been destroyed or removed from the infected zone~~ by means that minimise the likelihood ~~risk~~ of further transmission ~~spread~~ of RSIV ~~the disease~~, and the appropriate *disinfection* procedures (as described in Chapter 4.3.) have been completed; 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 RSIV ~~the disease~~; and
 - d) *targeted surveillance*, as described in Chapter 1.4., has been in place for at least the last two years without detection of RSIV.

In the meantime, part or all of the unaffected ~~non-affected~~ area may be declared a free *zone* provided that such a part meets the conditions in point 3 of Article 10.8.5.

Article 10.8.5.

Red sea bream iridoviral diseases free zone or free compartment free from infection with RSIV

If a *zone* or *compartment* extends over more than one country, it can only be declared a ~~RSIV-free zone~~ or *compartment free from infection with RSIV* if all the relevant *Competent Authorities* confirm that all relevant conditions have been met.

As described in Article 1.4.6., a *zone* or *compartment* within the *territory* of one or more countries not declared free from infection with RSIV may be declared free by the *Competent Authority(ies)* of the country(ies) concerned if:

- 1) none of the *susceptible species* referred to in Article 10.8.2. are present in the *zone* or *compartment* and *basic biosecurity conditions* have been met continuously for at least the last two years;

OR

- 2) any of the *susceptible species* referred to in Article 10.8.2. are present in the *zone* or *compartment* and the following conditions have been met:
 - a) there has been no ~~observed~~ occurrence of infection with RSIV ~~the disease~~ for at least the last ten years despite conditions that are conducive to its clinical expression (as described in the corresponding chapter of the *Aquatic Manual*); and
 - b) *basic biosecurity conditions* have been continuously met for at least the last ten years;

OR

- 3) the ~~disease~~ infection with RSIV status prior to *targeted surveillance* is unknown but the following conditions have been met:
 - a) *basic biosecurity conditions* have been continuously met for at least the last two years; and
 - b) *targeted surveillance*, as described in Chapter 1.4., has been in place, in the *zone* or *compartment*, for at least the last two years without detection of RSIV;

OR

- 4) it previously made a *self-declaration of freedom* for a *zone* from infection with RSIV and subsequently lost its ~~disease~~ free status due to the detection of RSIV in the *zone* but the following conditions have been met:
 - a) on detection of ~~RSIV/the disease~~, 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 ~~have been destroyed or removed from the infected zone~~ by means that minimise the likelihood ~~risk~~ of further transmission spread of ~~RSIV/the disease~~, and the appropriate *disinfection* procedures (as described in Chapter 4.3.) have been completed; and
 - c) previously existing *basic biosecurity conditions* have been reviewed and modified as necessary and have continuously been in place since eradication of ~~the disease~~ infection with RSIV; and
 - d) *targeted surveillance*, as described in Chapter 1.4., has been in place for at least the last two years without detection of RSIV.

Annex 18 (contd)

Article 10.8.6.

Maintenance of free status

A country, *zone* or *compartment* that is declared free from infection with RSIV~~D~~ following the provisions of points 1 or 2 of Articles 10.8.4. or 10.8.5. (as relevant) may maintain its status as free from infection with RSIV~~D~~ provided that *basic biosecurity conditions* are continuously maintained.

A country, *zone* or *compartment* that is declared free from infection with RSIV~~D~~ following the provisions of point 3 of Articles 10.8.4. or 10.8.5. (as relevant) may discontinue *targeted surveillance* and maintain its free status ~~as free from RSIV~~D~~~~ provided that conditions that are conducive to clinical expression of infection with RSIV~~D~~, as described in the corresponding chapter of the *Aquatic Manual*, ~~exist~~ and *basic biosecurity conditions* are continuously maintained.

However, for declared free *zones* or *compartments* in infected countries and in all cases where conditions are not conducive to clinical expression of infection with RSIV~~D~~, *targeted surveillance* ~~needs to~~ should be continued at a level determined by the *Aquatic Animal Health Service* on the basis of the likelihood of *infection*.

Article 10.8.7.

Importation of aquatic animals ~~and/or~~ aquatic animal products from a country, zone or compartment declared free from infection with RSIV-red sea bream iridoviral disease

When importing aquatic animals of a species referred to in Article 10.8.2., or ~~and aquatic animal products of species referred to in Article 10.2.2. derived thereof,~~ from a country, *zone* or *compartment* declared free from infection with RSIV~~D~~, 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 or a certifying official approved by the importing country.~~ The *international aquatic animal health certificate* should state that ~~certifying that~~, on the basis of the procedures described in Articles 10.8.4. or 10.8.5. (as applicable) and 10.8.6., the place of production of the *aquatic animals* ~~or~~ and *aquatic animal products* is a country, *zone* or *compartment* declared free from infection with RSIV~~D~~.

The *international aquatic animal health certificate* should be in accordance with the Model Certificate in Chapter 5.11.

This article does not apply to aquatic animal products listed ~~commodities referred to~~ in point 1 of Article 10.8.3.

Article 10.8.8.

Importation of aquatic animals for aquaculture from a country, zone or compartment not declared free from infection with RSIV-red sea bream iridoviral disease

When importing, ~~for aquaculture,~~ *aquatic animals* of a species referred to in Article 10.8.2. from a country, *zone* or *compartment* not declared free from infection with RSIV~~D~~, the *Competent Authority* of the *importing country* should assess the *risk* in accordance with Chapter 2.1. and consider the *risk* mitigation measures in points 1 and 2 below.

- 1) If the intention is to grow out and harvest the imported *aquatic animals*, consider applying the following:
 - a) the direct delivery to and lifelong holding of the imported *aquatic animals* in a *quarantine* facility; and
 - b) the treatment of all transport water, equipment, effluent and waste materials to inactivate RSIV in accordance with Chapters 4.3., 4.7. and 5.5.

OR

Annex 18 (contd)

- 2) If the intention is to establish a new stock for *aquaculture*, consider applying the following:
- 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 RSIV.
 - b) In the *importing country*:
 - i) import the F-0 population into a *quarantine* facility;
 - ii) test the F-0 population for RSIV 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* under conditions that are conducive to the clinical expression of RSIV, ~~(as described in Chapter 2.3.8. of the *Aquatic Manual*)~~ and sample and test for RSIV in accordance with Chapter 1.4. of the *Aquatic Code* and Chapter 2.3.8. of the *Aquatic Manual*;
 - v) if RSIV is not detected in the F-1 population, it may be defined as free from infection with RSIV and may be released from *quarantine*;
 - vi) if RSIV 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.

Article 10.8.9.

Importation of aquatic animals and or aquatic animal products for processing for human consumption from a country, zone or compartment not declared free from infection with RSIV ~~red sea bream iridoviral disease~~

When importing, for processing for human consumption, *aquatic animals* ~~or aquatic animal products~~ of a species referred to in Article 10.8.2., or aquatic animal products derived thereof, from a country, zone or compartment not declared free from RSIV, the *Competent Authority* of the *importing country* should assess the *risk* and, if justified, require that:

- 1) the consignment is delivered directly to, and held in, *quarantine* or containment facilities until processing into one of the products referred to in point 1 of Article 10.8.3., ~~or products described in point 1 of Article 10.8.11.~~, or other products authorised by the *Competent Authority*; and
- 2) all water (including ice), equipment, containers and packaging material used in transport are treated to ensure inactivation of RSIV or disposed of in a biosecure manner in accordance with Chapters 4.3., 4.7. and 5.5.; and all effluent and waste materials from the processing are treated in a manner that ensures inactivation of RSIV or is disposed in a manner that prevents contact of waste with susceptible species.
- 3) all effluent and waste materials are treated to ensure inactivation of RSIV or disposed of in a biosecure manner in accordance with Chapters 4.3. and 4.7.

For these *aquatic animals* or aquatic animal products ~~commodities~~ Member Countries may wish to consider introducing internal measures to address the *risks* associated with the *aquatic animals* or aquatic animal products ~~commodity~~ being used for any purpose other than for human consumption.

Article 10.8.10.

Importation of aquatic animals or aquatic animal products intended for uses other than human consumption, including use in animal feed, or for and agricultural, industrial, research or pharmaceutical use, from a country, zone or compartment not declared free from infection with RSIV ~~red sea bream iridoviral disease~~

When importing *aquatic animals* of a species referred to in Article 10.8.2., or aquatic animal products derived thereof, intended for uses other than human consumption, including use in animal feed or for and agricultural, industrial, research or pharmaceutical use, *aquatic animals* of species referred to in Article 10.2.2., from a country, zone or compartment not declared free from infection with RSIV, the *Competent Authority* of the *importing country* should require that:

Annex 18 (contd)

- 1) the consignment is delivered directly to, and held in, *quarantine or containment facilities until processed into one of the products referred to in point 1 of Article 10.8.3. or other facilities for slaughter and processing into products authorised by the Competent Authority*; and
- 2) all water (including ice), equipment, *containers and packaging material used in transport are treated to ensure inactivation of RSIV or disposed of in a biosecure manner in accordance with Chapters 4.3., 4.7. and 5.5.; and* and all effluent and waste materials from the processing are treated in a manner that ensures inactivation of RSIV or is disposed in a manner that prevents contact of waste with *susceptible*
- 3) all effluent and waste materials are treated to ensure inactivation of RSIV or disposed of in a biosecure manner in accordance with Chapters 4.3. and 4.7.

This article does not apply to ~~commodities~~ referred to in point 1 of Article 10.8.3.

Article 10.8.11.**Importation of aquatic animals intended for use in laboratories or zoos from a country, zone or compartment not declared free from infection with RSIV**

When importing, for use in laboratories and zoos, *aquatic animals of a species referred to in Article 10.8.2. from a country, zone or compartment not declared free from infection with RSIV, the Competent Authority of the importing country should ensure:*

- 1) the consignment is delivered directly to, and held in, *quarantine facilities authorised by the Competent Authority*; and
- 2) all water (including ice), equipment, *containers and packaging material used in transport are treated to ensure inactivation of IHNV or disposed of in a biosecure manner in accordance with Chapters 4.3., 4.7. and 5.5.; and*
- 3) all effluent and waste materials from the *quarantine facilities in the laboratories or zoos are treated to ensure inactivation of RSIV or disposed of in a biosecure manner in accordance with Chapters 4.3. and 4.7.; and*
- 4) the carcasses are disposed of in accordance with Chapter 4.7.

Article 10.8.12.**Importation (or transit) of ~~aquatic animals and aquatic animal products for retail trade for human consumption regardless of the infection with RSIV status of the exporting~~ from a country, zone or compartment ~~not declared free from infection with RSIV~~ ~~red sea bream iridoviral disease~~**

- 1) Competent Authorities should not require any RSIV-related conditions, regardless of the infection with RSIV status of the exporting country, *zone or compartment*, when authorising the importation (or transit) of fish fillets or steaks (chilled) ~~that which~~ have been prepared and packaged for retail trade and ~~which~~ comply with Article 5.4.2.

Certain assumptions have been made in assessing the safety of the *aquatic animal products* listed above. Member Countries should refer to these assumptions at Article 5.4.2. and consider whether the assumptions apply to their conditions.

For these *aquatic animal products* ~~commodities~~ Member Countries may wish to consider introducing internal measures to address the risks associated with the *aquatic animal products* ~~commodity~~ being used for any purpose other than for human consumption.

Annex 18 (contd)

- 2) When importing ~~aquatic animals~~ or *aquatic animal products*, other than those referred to in point 1 above, derived from a ~~of~~ species referred to in Article 10.8.2. from a country, *zone* or *compartment* not declared free from infection with RSIVD, the *Competent Authority* of the *importing country* should assess the *risk* and apply appropriate *risk* mitigation measures.
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CHAPTER 10.9.

**INFECTION WITH
SPRING VIRAEMIA OF CARP VIRUS**

Article 10.9.1.

For the purposes of the *Aquatic Code*, infection with spring viraemia of carp virus (SVC) means *infection* with the *pathogenic agent* viral species SVC virus (SVCV) ~~tentatively placed in the genus *Vesiculovirus* of the family Rhabdoviridae~~ *Sprivirus* of the family Rhabdoviridae.

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

Article 10.9.2.

Scope

The recommendations in this chapter apply to: common carp (*Cyprinus carpio carpio*) and koi carp (*Cyprinus carpio koi*), crucian carp (*Carassius carassius*), sheatfish (also known as European catfish or wels) (*Silurus glanis*), silver carp (*Hypophthalmichthys molitrix*), bighead carp (*Aristichthys nobilis*), grass carp (white amur) (*Ctenopharyngodon idellus*), goldfish (*Carassius auratus*), orfe (*Leuciscus idus*), and tench (*Tinca tinca*). These recommendations also apply to any other *susceptible species* referred to in the *Aquatic Manual* when traded internationally.

Article 10.9.3.

Importation or transit of ~~aquatic animals and~~ aquatic animal products for any purpose regardless of the infection with SVCV spring viraemia of carp status of the exporting country, zone or compartment

- 1) *Competent Authorities* should not require any conditions related to SVCV, regardless of the infection with SVCV status of the *exporting country, zone or compartment*, when authorising the importation or transit of the following *aquatic animal products* derived from the a species referred to in Article 10.9.2. ~~that~~ which are intended for any purpose and ~~which~~ comply with Article 5.4.1.:
 - a) heat sterilised hermetically sealed fish products (i.e. a heat treatment at 121°C for at least 3.6 minutes or equivalent that has been demonstrated to inactivate SVCV);
 - b) pasteurised fish products that have been subjected to heat treatment at 90°C for at least ten minutes (or any time/temperature equivalent ~~which~~ that has been demonstrated to inactivate SVCV);
 - c) mechanically dried eviscerated fish (i.e. a heat treatment at 100°C for at least 30 minutes or any time/temperature equivalent ~~which~~ that has been demonstrated to inactivate SVCV);
 - d) fish oil;
 - e) fish meal.
- 2) When authorising the importation or transit of ~~aquatic animals and~~ *aquatic animal products* of a species referred to in Article 10.9.2., other than those referred to in point 1 of Article 10.9.3., *Competent Authorities* should require the conditions prescribed in Articles 10.9.7. to 10.9.14. relevant to the infection with SVCV 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~~ referred to in Article 10.9.2. but which could reasonably be expected to pose a *risk of transmission spread* of SVCV, 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 ~~assessment~~.

Annex 19 (contd)

Article 10.9.4.

Country free from infection with SVCV ~~spring viraemia of carp~~

If a country shares a *zone* with one or more other countries, it can only make a *self-declaration of freedom* from infection with SVCV if all the areas covered by the shared water bodies are declared countries or *zones* free from infection with SVCV (see Article 10.9.5.).

As described in Article 1.4.6., a country may make a *self-declaration of freedom* from infection with SVCV if:

- 1) none of the *susceptible species* referred to in Article 10.9.2. are present and *basic biosecurity conditions* have been continuously met for at least the last two years;

OR

- 2) any of the *susceptible species* referred to in Article 10.9.2. are present and the following conditions have been met:
 - a) there has been no ~~observed~~ occurrence of infection with SVCV ~~the disease~~ for at least the last ten years despite conditions that are conducive to its clinical expression (as described in the corresponding chapter of the *Aquatic Manual*); and
 - b) *basic biosecurity conditions* have been continuously met for at least the last ten years;

OR

- 3) ~~the disease~~ infection with SVCV status prior to *targeted surveillance* is unknown but the following conditions have been met:
 - a) *basic biosecurity conditions* have been continuously met for at least the last two years; and
 - b) *targeted surveillance*, as described in Chapter 1.4., has been in place for at least the last two years without detection of SVCV;

OR

- 4) it previously made a *self-declaration of freedom* from infection with SVCV and subsequently lost its ~~disease~~ free status due to the detection of SVCV but the following conditions have been met:
 - a) on detection of SVCV ~~the disease~~, 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 ~~have been destroyed or removed from the infected zone~~ by means that minimise the ~~likelihood~~ *risk* of further transmission spread of SVCV ~~the disease~~, and the appropriate *disinfection* procedures (as described in Chapter 4.3.) have been completed; 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 SVCV ~~the disease~~; and
 - d) *targeted surveillance*, as described in Chapter 1.4., has been in place for at least the last two years without detection of SVCV.

In the meantime, part or all of the unaffected non-affected area may be declared a free *zone* provided that such a part meets the conditions in point 3 of Article 10.9.5.

Article 10.9.5.

Zone or compartment free from infection with SVCV ~~spring viraemia of carp~~

If a *zone* or *compartment* extends over more than one country, it can only be declared ~~a~~ an SVC-free zone or *compartment* free from infection with SVCV if all the relevant *Competent Authorities* confirm that all relevant conditions have been met.

As described in Article 1.4.6., a *zone* or *compartment* within the *territory* of one or more countries not declared free from infection with SVCV may be declared free by the *Competent Authority(ies)* of the country(ies) concerned if:

- 1) none of the *susceptible species* referred to in Article 10.9.2. are present in the *zone* or *compartment* and *basic biosecurity conditions* have been continuously met for at least the last two years;

OR

- 2) any of the *susceptible species* referred to in Article 10.9.2. are present in the *zone* or *compartment* and the following conditions have been met:
 - a) there has been no ~~observed~~ occurrence of infection with SVCV ~~the disease~~ for at least the last ten years despite conditions that are conducive to its clinical expression (as described in the corresponding chapter of the *Aquatic Manual*); and
 - b) *basic biosecurity conditions* have been continuously met for at least the last ten years;

OR

- 3) the ~~disease~~ infection with SVCV status prior to *targeted surveillance* is unknown but the following conditions have been met:
 - a) *basic biosecurity conditions* have been continuously met for at least the last two years; and
 - b) *targeted surveillance*, as described in Chapter 1.4., has been in place, in the *zone* or *compartment*, for at least the last two years without detection of SVCV;

OR

- 4) it previously made a *self-declaration of freedom* for a *zone* from infection with SVCV and subsequently lost its ~~disease~~ free status due to the detection of SVCV in the *zone* but the following conditions have been met:
 - a) on detection of SVCV ~~the disease~~, 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 ~~have been destroyed or removed from the infected zone~~ by means that minimise the likelihood ~~risk~~ of further transmission spread of SVCV ~~the disease~~, and the appropriate *disinfection* procedures (as described in Chapter 4.3.) have been completed; 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 SVCV ~~the disease~~; and
 - d) *targeted surveillance*, as described in Chapter 1.4., has been in place for at least the last two years without detection of SVCV.

Annex 19 (contd)

Article 10.9.6.

Maintenance of free status

A country, *zone* or *compartment* that is declared free from infection with SVCV following the provisions of points 1 or 2 of Articles 10.9.4. or 10.9.5. (as relevant) may maintain its status as free from infection with SVCV provided that *basic biosecurity conditions* are continuously maintained.

A country, *zone* or *compartment* that is declared free from infection with SVCV following the provisions of point 3 of Articles 10.9.4. or 10.9.5. (as relevant) may discontinue *targeted surveillance* and maintain its free status ~~as free from SVC~~ provided that conditions that are conducive to clinical expression of infection with SVCV, as described in the corresponding chapter of the *Aquatic Manual*, ~~exist~~ and *basic biosecurity conditions* are continuously maintained.

However, for declared free *zones* or *compartments* in infected countries and in all cases where conditions are not conducive to clinical expression of infection with SVCV, *targeted surveillance* ~~needs to~~ should be continued at a level determined by the *Aquatic Animal Health Service* on the basis of the likelihood of *infection*.

Article 10.9.7.

Importation of aquatic animals ~~and/or~~ aquatic animal products from a country, zone or compartment declared free from infection with SVCV ~~spring viraemia of carp~~

When importing aquatic animals of a species referred to in Article 10.9.2., or ~~and~~ aquatic animal products of species referred to in Article 10.2.2. derived thereof. from a country, *zone* or *compartment* declared free from infection with SVCV, 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 or a certifying official approved by the importing country.~~ The *international aquatic animal health certificate* should state that certifying that, on the basis of the procedures described in Articles 10.9.4. or 10.9.5. (as applicable) and 10.9.6., the place of production of the aquatic animals ~~or~~ and aquatic animal products is a country, *zone* or *compartment* declared free from infection with SVCV.

The *international aquatic animal health certificate* should be in accordance with the Model Certificate in Chapter 5.11.

This article does not apply to aquatic animal products listed ~~commodities referred to~~ in point 1 of Article 10.9.3.

Article 10.9.8.

Importation of aquatic animals for aquaculture from a country, zone or compartment not declared free from infection with SVCV ~~spring viraemia of carp~~

When importing, ~~for aquaculture,~~ aquatic animals of a species referred to in Article 10.9.2. from a country, *zone* or *compartment* not declared free from infection with SVCV, the *Competent Authority* of the *importing country* should assess the *risk* in accordance with Chapter 2.1. and consider the *risk* mitigation measures in points 1 and 2 below.

- 1) If the intention is to grow out and harvest the imported *aquatic animals*, consider applying the following:
 - a) the direct delivery to and lifelong holding of the imported *aquatic animals* in a *quarantine* facility; and
 - b) the treatment of all transport water, equipment, effluent and waste materials to inactivate SVCV in accordance with Chapters 4.3., 4.7. and 5.5.

OR

Annex 19 (contd)

- 2) If the intention is to establish a new stock for *aquaculture*, consider applying the following:
- 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 SVCV.
 - b) In the *importing country*:
 - i) import the F-0 population into a *quarantine* facility;
 - ii) test the F-0 population for SVCV 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* under conditions that are conducive to the clinical expression of SVCV, ~~(as described in Chapter 2.3.9. of the *Aquatic Manual*)~~ and sample and test for SVCV in accordance with Chapter 1.4. of the *Aquatic Code* and Chapter 2.3.9. of the *Aquatic Manual*;
 - v) if SVCV is not detected in the F-1 population, it may be defined as free from infection with SVCV and may be released from *quarantine*;
 - vi) if SVCV 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.

Article 10.9.9.

Importation of aquatic animals ~~and or~~ aquatic animal products for processing for human consumption from a country, zone or compartment not declared free from infection with SVCV ~~spring viraemia of carp~~

When importing, for processing for human consumption, ~~*aquatic animals* or *aquatic animal products*~~ of a species referred to in Article 10.9.2., ~~or *aquatic animal products* derived thereof~~, from a country, zone or compartment not declared free from infection with SVCV, the *Competent Authority* of the *importing country* should assess the *risk* and, if justified, require that:

- 1) the consignment is delivered directly to, and held in, *quarantine* or containment facilities until processing into one of the products referred to in point 1 of Article 10.9.3., ~~or products described in point 1 of Article 10.9.11.~~, or other products authorised by the *Competent Authority*; and
- 2) all water (including ice), equipment, containers and packaging material used in transport are treated to ensure inactivation of SVCV or disposed of in a biosecure manner in accordance with Chapters 4.3., 4.7. and 5.5.; and all effluent and waste materials from the processing are treated in a manner that ensures inactivation of SVCV or is disposed in a manner that prevents contact of waste with susceptible species.
- 3) all effluent and waste materials are treated to ensure inactivation of SVCV or disposed of in a biosecure manner in accordance with Chapters 4.3. and 4.7.

For these *aquatic animals* or *aquatic animal products* ~~commodities~~ Member Countries may wish to consider introducing internal measures to address the *risks* associated with the *aquatic animals* or *aquatic animal products* ~~commodity~~ being used for any purpose other than for human consumption.

Article 10.9.10.

Importation of aquatic animals or aquatic animal products intended for uses other than human consumption, including use in animal feed, or for and agricultural, industrial, research or pharmaceutical use, from a country, zone or compartment not declared free from infection with SVCV ~~spring viraemia of carp~~

When importing *aquatic animals* of a species referred to in Article 10.9.2., or *aquatic animal products* derived thereof, intended for uses other than human consumption, including use in animal feed or for and agricultural, industrial, research or pharmaceutical use, ~~*aquatic animals* of species referred to in Article 10.2.2.~~ from a country, zone or compartment not declared free from infection with SVCV, the *Competent Authority* of the *importing country* should require that:

Annex 19 (contd)

- 1) the consignment is delivered directly to, and held in, quarantine or containment facilities until processed into one of the products referred to in point 1 of Article 10.9.3. or other facilities for slaughter and processing into products authorised by the Competent Authority; and
- 2) all water (including ice), equipment, containers and packaging material used in transport are treated to ensure inactivation of SVCV or disposed of in a biosecure manner in accordance with Chapters 4.3., 4.7. and 5.5.; and all effluent and waste materials from the processing are treated in a manner that ensures inactivation of SVCV or is disposed in a manner that prevents contact of waste with susceptible
- 3) all effluent and waste materials are treated to ensure inactivation of SVCV or disposed of in a biosecure manner in accordance with Chapters 4.3. and 4.7.

This article does not apply to commodities referred to in point 1 of Article 10.9.3.

Article 10.9.11.**Importation of aquatic animals intended for use in laboratories or zoos from a country, zone or compartment not declared free from infection with SVCV**

When importing, for use in laboratories and zoos, aquatic animals of a species referred to in Article 10.11.2. from a country, zone or compartment not declared free from infection with SVCV, the Competent Authority of the importing country should ensure:

- 1) the consignment is delivered directly to, and held in, quarantine facilities authorised by the Competent Authority; and
- 2) all water (including ice), equipment, containers and packaging material used in transport are treated to ensure inactivation of SVCV or disposed of in a biosecure manner in accordance with Chapters 4.3., 4.7. and 5.5.; and
- 3) all effluent and waste materials from the quarantine facilities in the laboratories or zoos are treated to ensure inactivation of SVCV or disposed of in a biosecure manner in accordance with Chapters 4.3. and 4.7.; and
- 4) the carcasses are disposed of in accordance with Chapter 4.7.

Article 10.9.12.**Importation (or transit) of aquatic animals and aquatic animal products for retail trade for human consumption regardless of the infection with SVCV status of the exporting from a country, zone or compartment not declared free from infection with SVCV spring viraemia of carp**

- 1) Competent Authorities should not require any conditions related to SVCV, regardless of the infection with SVCV status of the exporting country, zone or compartment, when authorising the importation or (or transit) of fish fillets or steaks (chilled) that which have been prepared and packaged for retail trade and which comply with Article 5.4.2.

Certain assumptions have been made in assessing the safety of the *aquatic animal products* mentioned above. Member Countries should refer to these assumptions at Article 5.4.2. and consider whether the assumptions apply to their conditions.

For these aquatic animal products commodities Member Countries may wish to consider introducing internal measures to address the risks associated with the aquatic animal products commodity being used for any purpose other than for human consumption.

Annex 19 (contd)

- 2) When importing ~~aquatic animals or~~ *aquatic animal products*, other than those referred to in point 1 above, derived from a ~~of~~ of species referred to in Article 10.9.2. from a country, *zone* or *compartment* not declared free from infection with SVCV, the *Competent Authority* of the *importing country* should assess the *risk* and apply appropriate *risk* mitigation measures.
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CHAPTER 10.10.

INFECTION WITH VIRAL HAEMORRHAGIC SEPTICAEMIA VIRUS

Article 10.10.1.

For the purposes of the *Aquatic Code*, infection with viral haemorrhagic septicaemia virus (VHS) means *infection with the pathogenic agent VHS viral haemorrhagic septicaemia virus (VHSV, synonym: Egtved virus)*, of the Genus *Novirhabdovirus* and Family Rhabdoviridae.

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

Article 10.10.2.

Scope

The recommendations in this chapter apply to: rainbow trout (*Oncorhynchus mykiss*), brown trout (*Salmo trutta*), grayling (*Thymallus thymallus*), white fish (*Coregonus* spp.), pike (*Esox lucius*), turbot (*Scophthalmus maximus*), herring and sprat (*Clupea* spp.), Pacific salmon (*Oncorhynchus* spp.), Atlantic cod (*Gadus morhua*), Pacific cod (*Gadus macrocephalus*), haddock (*Gadus aeglefinus*) and rockling (*Onos mustelus*). These recommendations also apply to any other *susceptible species* referred to in the *Aquatic Manual* when traded internationally.

Article 10.10.3.

Importation or transit of ~~aquatic animals and~~ aquatic animal products for any purpose regardless of the infection with VHSV viral haemorrhagic septicaemia status of the exporting country, zone or compartment

- 1) *Competent Authorities* should not require any conditions related to VHSV, regardless of the infection with VHSV status of the *exporting country, zone or compartment*, when authorising the importation or transit of the following *aquatic animal products* derived from ~~the~~ a species referred to in Article 10.10.2. that ~~which~~ are intended for any purpose and ~~which~~ comply with Article 5.4.1.:
 - a) heat sterilised, hermetically sealed fish 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 VHSV);
 - b) pasteurised fish products that have been subjected to a heat treatment at 90°C for at least ten minutes (or to any time/temperature equivalent ~~which~~ that has been demonstrated to inactivate VHSV);
 - c) mechanically dried, eviscerated fish (i.e. a heat treatment at 100°C for at least 30 minutes or any time/temperature equivalent ~~which~~ that has been demonstrated to inactivate VHSV);
 - d) naturally dried, eviscerated fish (i.e. sun-dried or wind-dried);
 - e) fish oil;
 - f) fish *meal*;
 - g) fish skin leather.
- 2) When authorising the importation or transit of ~~aquatic animals and~~ *aquatic animal products* of a species referred to in Article 10.10.2., other than those referred to in point 1 of Article 10.10.3., *Competent Authorities* should require the conditions prescribed in Articles 10.10.7. to 10.10.13. relevant to the infection with VHSV status of the *exporting country, zone or compartment*.

Annex 20 (contd)

- 3) When considering the importation or transit of ~~aquatic animals and aquatic animal products~~ of a species not covered ~~referred to~~ in Article 10.10.2. but which could reasonably be expected to pose a risk of transmission spread of VHSV, 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 assessment.

Article 10.10.4.

Country free from infection with VHSV ~~viral haemorrhagic septicaemia~~

If a country shares a *zone* with one or more other countries, it can only make a *self-declaration of freedom* from infection with VHSV if all the areas covered by the shared water bodies are declared countries or *zones* free from infection with VHSV (see Article 10.10.5.).

As described in Article 1.4.6., a country may make a *self-declaration of freedom* from infection with VHSV if:

- 1) a country where the species referred to in Article 10.10.2. are present but there has been no ~~observed~~ occurrence of infection with VHSV ~~the disease~~ for at least the last ten years despite conditions that are conducive to its clinical expression, as described in the corresponding chapter of the *Aquatic Manual*, may make a *self-declaration of freedom* from infection with VHSV when *basic biosecurity conditions* have been continuously met in the country for at least the last ten years;

OR

- 2) the ~~disease~~ infection with VHSV status prior to *targeted surveillance* is unknown but the following conditions have been met:
- a) *basic biosecurity conditions* have been continuously met for at least the last two years; and
 - b) *targeted surveillance*, as described in Chapter 1.4., has been in place for at least the last two years without detection of VHSV;

OR

- 3) it previously made a *self-declaration of freedom* from infection with VHSV and subsequently lost its ~~disease~~ free status due to the detection of VHSV but the following conditions have been met:
- a) on detection of VHSV ~~the disease~~, 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 ~~have been destroyed or removed from the infected zone~~ by means that minimise the likelihood risk of further transmission spread of VHSV ~~the disease~~, and the appropriate *disinfection* procedures (as described in Chapter 4.3.) have been completed; 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 VHSV ~~the disease~~; and
 - d) *targeted surveillance*, as described in Chapter 1.4., has been in place for at least the last two years without detection of VHSV.

In the meantime, part or all of the unaffected non-affected area may be declared a free *zone* provided that such a part meets the conditions in point 2 of Article 10.10.5.

Article 10.10.5.

Zone or compartment free from infection with VHSV ~~viral haemorrhagic septicaemia~~

If a *zone* or *compartment* extends over more than one country, it can only be declared a ~~VHS free~~ *zone* or *compartment* free from infection with VHSV if all the relevant *Competent Authorities* confirm that all relevant conditions have been met.

Annex 20 (contd)

As described in Article 1.4.6., a *zone* or *compartment* within the *territory* of one or more countries not declared free from infection with VHSV may be declared free by the *Competent Authority*(ies) of the country(ies) concerned if:

- 1) a *zone* or *compartment* where the species referred to in Article 10.10.2. are present but there has been no ~~observed~~ occurrence of infection with VHSV ~~the disease~~ for at least the last ten years despite conditions that are conducive to its clinical expression, as described in the corresponding chapter of the *Aquatic Manual*, may be declared free from infection with VHSV when *basic biosecurity conditions* have been continuously met in the *zone* or *compartment* for at least the last ten years;

OR

- 2) the ~~disease~~ infection with VHSV status prior to *targeted surveillance* is unknown but the following conditions have been met:
 - a) *basic biosecurity conditions* have been continuously met for at least the last two years; and
 - b) *targeted surveillance*, as described in Chapter 1.4., has been in place, in the *zone* or *compartment*, for at least the last two years without detection of VHSV;

OR

- 3) it previously made a *self-declaration of freedom* for a *zone* from infection with VHSV and subsequently lost its ~~disease~~ free status due to the detection of VHSV in the *zone* but the following conditions have been met:
 - a) on detection of VHSV ~~the disease~~, 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~~ ~~have been destroyed or removed from the infected zone~~ by means that minimise the likelihood ~~risk~~ of further transmission spread of VHSV ~~the disease~~, and the appropriate *disinfection* procedures (as described in Chapter 4.3.) have been completed; 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 VHSV ~~the disease~~; and
 - d) *targeted surveillance*, as described in Chapter 1.4., has been in place for at least the last two years without detection of VHSV.

Article 10.10.6.

Maintenance of free status

A country, *zone* or *compartment* that is declared free from infection with VHSV following the provisions of point 1 of Articles 10.10.4. or 10.10.5. (as relevant) may maintain its status as free from infection with VHSV provided that *basic biosecurity conditions* are continuously maintained.

A country, *zone* or *compartment* that is declared free from infection with VHSV following the provisions of point 2 of Articles 10.10.4. or 10.10.5. (as relevant) may discontinue *targeted surveillance* and maintain its free status ~~as free from VHS~~ provided that conditions that are conducive to clinical expression of infection with VHSV, as described in the corresponding chapter of the *Aquatic Manual*, ~~exist~~ and *basic biosecurity conditions* are continuously maintained.

However, for declared free *zones* or *compartments* in infected countries and in all cases where conditions are not conducive to clinical expression of infection with VHSV, *targeted surveillance* ~~needs to~~ should be continued at a level determined by the *Aquatic Animal Health Service* on the basis of the likelihood of *infection*.

Annex 20 (contd)

Article 10.10.7.

Importation of aquatic animals and aquatic animal products from a country, zone or compartment declared free from infection with VHSV ~~viral haemorrhagic septicaemia~~

When importing aquatic animals of a species referred to in Article 10.10.2., or ~~and aquatic animal products of species referred to in Article 10.2.2. derived thereof,~~ from a country, zone or compartment declared free from infection with VHSV, 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* ~~or a certifying official approved by the importing country.~~ The international aquatic animal health certificate should state that certifying that, on the basis of the procedures described in Articles 10.10.4. or 10.10.5. (as applicable) and 10.10.6., the place of production of the aquatic animals ~~or and~~ aquatic animal products is a country, zone or compartment declared free from infection with VHSV.

The international aquatic animal health certificate should be in accordance with the Model Certificate in Chapter 5.11.

This article does not apply to aquatic animal products listed commodities referred to in point 1 of Article 10.10.3.

Article 10.10.8.

Importation of aquatic animals for aquaculture from a country, zone or compartment not declared free from infection with VHSV ~~viral haemorrhagic septicaemia~~

When importing, for *aquaculture*, *aquatic animals* of a species referred to in Article 10.10.2. from a country, zone or compartment not declared free from infection with VHSV, the *Competent Authority* of the *importing country* should assess the *risk* in accordance with Chapter 2.1. and consider the *risk* mitigation measures in points 1 and 2 below.

- 1) If the intention is to grow out and harvest the imported *aquatic animals*, consider applying the following:
 - a) the direct delivery to and lifelong holding of the imported *aquatic animals* in a *quarantine* facility; and
 - b) the treatment of all transport water, equipment, effluent and waste materials to inactivate KHV in accordance with Chapters 4.3., 4.7. and 5.5.

OR

- 2) If the intention is to establish a new stock for *aquaculture*, consider applying the following:
 - 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 VHSV.
 - b) In the *importing country*:
 - i) import the F-0 population into a *quarantine* facility;
 - ii) test the F-0 population for VHSV 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 under conditions that are conducive to the clinical expression of VHSV, (as described in Chapter 2.3.10. of the Aquatic Manual) and sample and test for VHSV in accordance with Chapter 1.4. of the Aquatic Code and Chapter 2.3.10. of the Aquatic Manual.
 - v) if VHSV is not detected in the F-1 population, it may be defined as free from infection with VHSV, and may be released from *quarantine*;

Annex 20 (contd)

- vi) if VHSV 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.

Article 10.10.9.

Importation of aquatic animals ~~and~~ or aquatic animal products for processing for human consumption from a country, zone or compartment not declared free from infection with VHSV ~~viral haemorrhagic septicaemia~~

When importing, for processing for human consumption, ~~aquatic animals or aquatic animal products~~ of a species referred to in Article 10.10.2., ~~or aquatic animal products derived thereof,~~ from a country, zone or compartment not declared free from infection with VHSV, the *Competent Authority* of the *importing country* should assess the risk and, if justified, require that:

- 1) the consignment is delivered directly to, and held in, *quarantine* or containment facilities until processing into one of the products referred to in point 1 of Article 10.10.3., ~~or products described in point 1 of Article 10.10.11.,~~ or other products authorised by the *Competent Authority*; and
- 2) all water (including ice), equipment, containers and packaging material used in transport are treated to ensure inactivation of VHSV or disposed of in a biosecure manner in accordance with Chapters 4.3., 4.7. and 5.5.; ~~and all effluent and waste materials from the processing are treated in a manner that ensures inactivation of VHSV or is disposed in a manner that prevents contact of waste with susceptible species.~~
- 3) all effluent and waste materials are treated to ensure inactivation of VHSV or disposed of in a biosecure manner in accordance with Chapters 4.3. and 4.7.

For these aquatic animals or aquatic animal products ~~commodities~~ Member Countries may wish to consider introducing internal measures to address the risks associated with the aquatic animals or aquatic animal products ~~commodity~~ being used for any purpose other than for human consumption.

Article 10.10.10.

Importation of aquatic animals or aquatic animal products intended for uses other than human consumption, including use in animal feed, or for and agricultural, industrial, research or pharmaceutical use, from a country, zone or compartment not declared free from infection with VHSV ~~viral haemorrhagic septicaemia~~

When importing aquatic animals of a species referred to in Article 10.10.2., or aquatic animal products derived thereof, intended for uses other than human consumption, including use in animal feed or for and agricultural, industrial, research or pharmaceutical use, ~~aquatic animals of species referred to in Article 10.10.2,~~ from a country, zone or compartment not declared free from infection with VHSV, the *Competent Authority* of the *importing country* should require that:

- 1) the consignment is delivered directly to, and held in, *quarantine* or containment facilities until processed into one of the products referred to in point 1 of Article 10.10.3. or other facilities for slaughter and processing into products authorised by the *Competent Authority*; and
- 2) all water (including ice), equipment, containers and packaging material used in transport are treated to ensure inactivation of VHSV or disposed of in a biosecure manner in accordance with Chapters 4.3., 4.7. and 5.5.; ~~and all effluent and waste materials from the processing are treated in a manner that ensures inactivation of VHSV or is disposed in a manner that prevents contact of waste with susceptible~~
- 3) all effluent and waste materials are treated to ensure inactivation of VHSV or disposed of in a biosecure manner in accordance with Chapters 4.3. and 4.7.

This article does not apply to ~~commodities~~ referred to in point 1 of Article 10.10.3.

Article 10.10.11.

Importation of aquatic animals intended for use in laboratories or zoos from a country, zone or compartment not declared free from infection with VHSV

When importing, for use in laboratories and zoos, aquatic animals of a species referred to in Article 10.10.2. from a country, zone or compartment not declared free from infection with VHSV, the *Competent Authority* of the *importing country* should ensure:

Annex 20 (contd)

- 1) the consignment is delivered directly to, and held in, *quarantine* facilities authorised by the *Competent Authority*; and
- 2) all water (including ice), equipment, *containers* and packaging material used in transport are treated to ensure inactivation of VHSV or disposed of in a biosecure manner in accordance with Chapters 4.3., 4.7. and 5.5.; and
- 3) all effluent and waste materials from the *quarantine* facilities in the laboratories or zoos are treated to ensure inactivation of VHSV or disposed of in a biosecure manner in accordance with Chapters 4.3. and 4.7.; and
- 4) the carcasses are disposed of in accordance with Chapter 4.7.

Article 10.10.12~~4~~.

Importation (or transit) of aquatic animals and aquatic animal products for retail trade for human consumption regardless of the infection with VHSV status of the exporting ~~from a country, zone or compartment not declared free from infection with VHSV~~ viral haemorrhagic septicaemia

- 1) *Competent Authorities* should not require any conditions related to VHSV, regardless of the infection with VHSV, status of the *exporting country, zone or compartment*, when authorising the importation or (or transit) of fish fillets or steaks (chilled) that which have been prepared and packaged for retail trade and ~~which~~ comply with Article 5.4.2.

Certain assumptions have been made in assessing the safety of the *aquatic animal products* mentioned above. Member Countries should refer to these assumptions at Article 5.4.2. and consider whether the assumptions apply to their conditions.

For these *aquatic animal products* ~~commodities~~ Member Countries may wish to consider introducing internal measures to address the *risks* associated with the *aquatic animal products* ~~commodity~~ being used for any purpose other than for human consumption.

- 2) When importing ~~*aquatic animals*~~ or *aquatic animal products*, other than those referred to in point 1 above, derived from a ~~of~~ species referred to in Article 10.10.2. from a *country, zone or compartment* not declared free from infection with VHSV, the *Competent Authority* of the *importing country* should assess the *risk* and apply appropriate *risk* mitigation measures.

Article 10.10.13~~2~~.

Importation of disinfected eggs for aquaculture from a country, zone or compartment not declared free from infection with VHSV ~~viral haemorrhagic septicaemia~~

- 1) When importing disinfected eggs of the species referred to in Article 10.10.2. for *aquaculture*, from a *country, zone or compartment* not declared free from infection with VHSV, the *Competent Authority* of the *importing country* should assess the *risk* associated with at least:
 - a) ~~the VHS virus~~ VHSV status of the water to be used during the *disinfection* of the eggs;
 - b) the prevalence of infection with ~~VHSV virus~~ VHSV in broodstock (ovarian fluid and milt); and
 - c) the temperature and pH of the water to be used for *disinfection*.
- 2) If the *Competent Authority* of the *importing country* concludes that the importation is acceptable, it should apply the following *risk* mitigation measures including:
 - a) the eggs should be disinfected prior to importing, in accordance with recommendations in Chapter 4.4. or those specified by the *Competent Authority* of the *importing country*; and
 - b) between *disinfection* and the import, eggs should not come into contact with anything which may affect their health status.

The *Competent Authority* may wish to consider internal measures, such as renewed *disinfection* of the eggs upon arrival in the *importing country*.

Annex 20 (contd)

- 3) When importing disinfected eggs of the species referred to in Article 10.10.2. for *aquaculture*, from a country, zone or *compartment* not declared free from infection with VHSV, 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* ~~or a certifying official approved by the *importing country*~~ certifying that the procedures described in point 2 of Article 10.10.12. have been fulfilled.
-

Model Articles X.X.8., X.X.9., X.X.10. and X.X.11.

Article X.X.8.

[...]

- 2) If the intention is to establish a new stock for *aquaculture*, consider applying the following:
- 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 pathogenic agent X.
 - b) In the *importing country*:
 - i) import the F-0 population into a *quarantine* facility;
 - ii) test the F-0 population for pathogenic agent 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 F-1 population in *quarantine* under conditions that are conducive to the clinical expression of pathogenic agent X, ~~(as described in Chapter X.X.X. of the *Aquatic Manual*)~~ and sample and test for pathogenic agent X in accordance with Chapter 1.4. of the *Aquatic Code* and ~~(as described in Chapter X.X.X. of the *Aquatic Manual*)~~;
 - v) if pathogenic agent X is not detected in the F-1 population, it may be defined as free from infection with pathogenic agent X and may be released from *quarantine*;
 - vi) if pathogenic agent 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.

Article X.X.9.

Importation of aquatic animals ~~and/or~~ aquatic animal products for processing for human consumption from a country, zone or compartment not declared free from infection with pathogenic agent X

When importing, for processing for human consumption, ~~*aquatic animals or aquatic animal products*~~ of a species referred to in Article X.X.2., or *aquatic animal products* derived thereof, from a country, zone or compartment not declared free from infection with pathogenic agent X, the *Competent Authority* of the *importing country* should assess the *risk* and, if justified, require that:

- 1) the consignment is delivered directly to, and held in, *quarantine* or containment facilities until processing into one of the products referred to in point 1 of Article X.X.3., ~~or products described or~~ in point 1 of Article X.X.12./13, or other products authorised by the *Competent Authority*; and
- 2) all water (including ice), equipment, containers and packaging material used in transport are treated to ensure inactivation of pathogenic agent X or disposed of in a biosecure manner in accordance with Chapters 4.3., 4.7. and 5.5.; and ~~and all effluent and waste materials from the processing are treated in a manner that ensures inactivation of pathogenic agent X or is disposed of in a manner that prevents contact of waste with susceptible species.~~

Annex 21 (contd)

- 3) all effluent and waste materials are treated to ensure inactivation of pathogenic agent X or disposed of in a biosecure manner in accordance with Chapters 4.3. and 4.7.

For these aquatic animals or aquatic animal products ~~commodities~~ Member Countries may wish to consider introducing internal measures to address the *risks* associated with the aquatic animals or aquatic animal products ~~commodity~~ being used for any purpose other than for human consumption.

Article X.X.10.

Importation of aquatic animals or aquatic animal products intended for uses other than human consumption, including use in animal feed, or for and agricultural, industrial, research or pharmaceutical use, from a country, zone or compartment not declared free from infection with pathogenic agent X

When importing aquatic animals of a species referred to in Article X.X.2., or aquatic animal products derived thereof, intended for uses other than human consumption, including for use in animal feed or for and agricultural, industrial, research or pharmaceutical use, aquatic animals of a species referred to in Article X.X.2. or aquatic animal products derived thereof, from a country, zone or compartment not declared free from infection with pathogenic agent X, the *Competent Authority* of the *importing country* should require that:

- 1) the consignment is delivered directly to, and held in, quarantine or containment facilities until processed into one of the products referred to in point 1 of Article X.X.3. or other facilities for slaughter and processing into products authorised by the Competent Authority; and
- 2) all water (including ice), equipment, containers and packaging material used in transport are treated to ensure inactivation of pathogenic agent X or disposed of in a biosecure manner in accordance with Chapters 4.3., 4.7. and 5.5.; and all effluent and waste materials from the processing are treated in a manner that ensures inactivation of pathogenic agent X or is disposed in a manner that prevents contact of waste with susceptible species.
- 3) all effluent and waste materials are treated to ensure inactivation of pathogenic agent X or disposed of in a biosecure manner in accordance with Chapters 4.3. and 4.7.

~~This article does not apply to commodities referred to in point 1 of Article X.X.3.~~

Article X.X.11.

Importation of aquatic animals intended for use in laboratories or zoos from a country, zone or compartment not declared free from infection with pathogenic agent X

When importing, for use in laboratories and zoos, aquatic animals of a species referred to in Article X.X.2. from a country, zone or compartment not declared free from infection with pathogenic agent X, the Competent Authority of the importing country should ensure:

- 1) the consignment is delivered directly to, and held in, quarantine facilities authorised by the Competent Authority; and
- 2) all water (including ice), equipment, containers and packaging material used in transport are treated to ensure inactivation of pathogenic agent X or disposed of in a biosecure manner in accordance with Chapters 4.3., 4.7. and 5.5.; and
- 3) all effluent and waste materials from the quarantine facilities in the laboratories or zoos are treated to ensure inactivation of pathogenic agent X or disposed of in a biosecure manner in accordance with Chapters 4.3. and 4.7.; and
- 4) the carcasses are disposed of in accordance with Chapter 4.7.

CHAPTER 2.2.7.

INFECTION WITH WHITE SPOT SYNDROME VIRUS DISEASE

1. Scope

For the purpose of this chapter, Infection with disease (WSD) is considered to be infection with white spot syndrome virus (WSSV) means infection with the pathogenic agent white spot syndrome virus (WSSV), Family *Nimaviridae*, Genus *Whispovirus*.

2. Disease information

2.1. Agent factors

Various WSSV isolates with small genetic polymorphisms have been identified (variants). It should be realised, however, that as the *Nimaviridae* is a newly recognised family, the species concept will be subject to change after existing and new isolates have been studied in more detail.

2.1.1. Aetiological agent, agent strains

WSSV was assigned by the International Committee on Taxonomy of Viruses (ICTV) as the only member of the genus *Whispovirus* within the *Nimaviridae* family. Virions of WSSV are ovoid or ellipsoid to bacilliform in shape, have a regular symmetry, and measure 80–120 nm in diameter and 250–380 nm in length. Most notable is the thread- or A flagella-like extension (appendage) may be observed at one end of the virion. Today, although various geographical isolates with genotypic variability have been identified, they are all classified as a single species (white spot syndrome virus) within the genus *Whispovirus* (Lo *et al.*, 2012).

2.1.2. Survival outside the host

The agent is viable for at least 30 days at 30°C in seawater under laboratory conditions (Momoyama *et al.*, 1998); and is viable in ponds for at least 3–4 days (Nakano *et al.*, 1998). Laboratory emulations of drainable and non-drainable ponds suggest that the virus is no longer infective after 21 days of sun-drying or after 40 days in waterlogged pond sediment (Satheesh Kumar *et al.*, 2013).

2.1.3. Stability of the agent (effective inactivation methods)

The agent is inactivated in <120 minutes at 50°C and <1 minute at 60°C (Nakano *et al.*, 1998).

In laboratory studies, WSSV was inactivated under following conditions:

Heat: 55°C for 90 minutes, 70°C for 5 minutes (Chang *et al.*, 1998); 50°C for 60 minutes; 60°C for 1 minute; 70°C for 0.2 minutes (Nakano *et al.*, 1998).

Desiccation: WSSV adsorbed onto the filter paper and allowed to dry subsequently was inactivated in 1 hour at 30°C and in 3 hours at 26°C (Maeda *et al.*, 1998, Nakano *et al.*, 1998).

pH: pH 3 for 60 minutes; pH 12 for 10 minutes (Chang *et al.*, 1998, Balasubramanian *et al.*, 2006).

Ultraviolet light: Total dose of 9.30×10^5 $\mu\text{Ws}/\text{cm}^2$ (Chang *et al.*, 1998).

Ozone: Total residual oxidants concentration of 0.5 $\mu\text{g ml}^{-1}$ for 10 minutes (Chang *et al.*, 1998).

Sodium hypochlorite: Total free chlorine concentration of 100 ppm for 10 minutes (Chang *et al.*, 1998).

Benzalkonium chloride: 100 ppm for 10 minutes (Balasubramanian *et al.*, 2006).

Iodophore: Total free iodine concentration of 100 ppm for 10 minutes (Chang *et al.*, 1998).

Annex 22 (contd)**2.1.4. Life cycle**

In-vitro studies with primary cell culture and *in-vivo* studies with postlarvae (PL) show that the replication cycle is approximately 20 hours at 25°C (Chang *et al.*, 1996; Chen *et al.*, 2011; Wang *et al.*, 2000).

2.2. Host factors

WSSV has an extremely wide host range. The virus can infect a wide range of aquatic crustaceans especially decapod, including marine, brackish and freshwater prawns, crabs, crayfish and lobsters (Maeda *et al.*, 2000).

2.2.1. Susceptible host species

Of all of the species that have been tested to date, no decapod (order Decapoda) crustacean from marine and brackish or freshwater sources has been reported to be refractory resistant to infection with WSSV (Flegel, 1997; Lightner, 1996; Lo & Kou, 1998; Maeda *et al.*, 2000; Stentiford *et al.*, 2009).

2.2.2. Susceptible stages of the host

All life stages are potentially susceptible, from eggs to broodstock (Lightner, 1996; Venegas *et al.*, 1999). WSSV genetic material has been detected in reproductive organs (Lo *et al.*, 1997), but susceptibility of the gametes to WSSV infection has not been determined definitively.

2.2.3. Species or subpopulation predilection (probability of detection)

The best life stages of crustaceans for detection of infection with WSSV are late PL stages, juveniles and adults. Probability of detection can be increased by exposure to stressful conditions (e.g. eye-stalk ablation, spawning, moulting, changes in salinity, temperature or pH, and during plankton blooms).

2.2.4. Target organs and infected tissue

The major targets of infection with WSSV are tissues of ectodermal and mesodermal embryonic origin, especially the cuticular epithelium and subcuticular connective tissues (Momoyama *et al.*, 1994; Wongteerasupaya *et al.*, 1995). Although WSSV infects the underlying connective tissue in the shrimp hepatopancreas and midgut, the tubular epithelial cells of these two organs are of endodermal origin, and they do not become infected.

2.2.5. Persistent infection with lifelong carriers

Many decapod species have been shown to be subclinically infected with WSSV and are thought to be carriers of disease Persistent infection occurs commonly and lifelong infection has been shown (Lo & Kou, 1998). Viral loads during persistent infection can be extremely low and are very hard to detect even by sensitive methods such as real-time and nested PCR.

2.2.6. Vectors

The virus can be transmitted from host to host and does not need a biological vector.

2.2.7. Known or suspected wild aquatic animal carriers

Wild decapods known to be reservoirs of infection with WSSV include *Mysis* sp. (Huang *et al.*, 1995a), *Acetes* sp., *Alpheus* sp., *Callinassa* sp., *Exopalaemon* sp., *Helice* sp., *Hemigrapsus* sp., *Macrophthalmus* sp., *Macrophthel* sp., *Metaplex* sp., *Orithyia* sp., *Palaemonoidea* sp., *Scylla* sp., *Sesarma* sp., *Stomatopoda* sp. and (He & Zhou, 1996; Lei *et al.*, 2002). These species can be easily infected by WSSV and may express the disease under suitable environmental conditions. However, non-decapodal crustaceans, such as copepods (Huang *et al.*, 1995a), rotifers (Yan *et al.*, 2004), *Artemia salina* (Chang *et al.*, 2002), *Balanus* sp. (Lei *et al.*, 2002), and *Tachypleidue* sp. (He & Zhou, 1996) may be apparently healthy carrier animals become wild aquatic animal carriers by latent infection without disease. Other marine molluscs, polychaete worms (Vijayan *et al.*, 2005), as well as non-crustacean aquatic arthropods such as sea slaters (*Isopoda*) and Euphydradae insect larvae can mechanically carry the virus without evidence of infection (Lo & Kou, 1998).

2.3. Disease pattern

Infection with WSSV sometimes causes clinical disease ~~and sometimes not~~ (Tsai *et al.*, 1999), depending on factors as yet poorly understood but related to species tolerance and environmental triggers. With an appropriate infection dose to allow sufficient time before mortality, animals susceptible to disease show large numbers of virions circulating in the haemolymph (Lo *et al.*, 1997), but this may also occur for tolerant species that show no mortality. Thus, high viral loads *per se* do not cause disease or mortality for all susceptible species.

2.3.1. Transmission mechanisms

Infection with WSSV can be transmitted ~~vertically (trans-ovum)~~, horizontally by consumption of infected tissue (e.g. cannibalism, predation, etc.), and by water-borne routes. Transmission of infection with WSSV can occur from apparently healthy animals in the absence of disease. Dead and moribund animals can be a source of disease transmission (Lo & Kou, 1998).

True vertical transmission (intra-ovum) of WSSV to the progeny has not been demonstrated.

2.3.2. Prevalence

Prevalence of infection with WSSV is highly variable, from <1% in infected wild populations to up to 100% in captive populations (Lo & Kou, 1998).

2.3.3. Geographical distribution

~~WSD~~ Infection with WSSV has been identified from crustaceans in China (People's Rep. of), Japan, Korea (Rep. of), South-East Asia, South Asia, the Indian Continent, the Mediterranean (Stentiford & Lightner, 2011), the Middle East, and the Americas. ~~WSD-free Zones~~ and compartments free from infection with WSSV are known within these regions (Lo *et al.*, 2012).

2.3.4. Mortality and morbidity

All penaeid shrimp species are highly susceptible to infection with WSSV, often resulting in high mortality. Crabs, crayfish, freshwater prawns, spiny lobsters and clawed lobsters are susceptible to infection with WSSV, but morbidity and mortality as a consequence of infection are highly variable (Lo & Kou, 1998). High level infections with WSSV are known in some decapods in the absence of clinical disease.

2.3.5. Environmental factors

Disease outbreaks may be induced by stressors, such as rapid changes in salinity. Water temperature has a profound effect on disease expression, with average water temperatures of between 18 and 30°C being conducive to ~~WSD~~ WSSV outbreaks (Song *et al.*, 1996; Vidal *et al.*, 2001). Under experimental challenge conditions, WSSV-induced mortality in shrimp is reduced at temperatures above 32°C (Vidal *et al.*, 2001).

2.4. Control and prevention

Although the underlying mechanism remains unknown, laboratory experiments have shown that 'vaccinated' shrimp and crayfish have better survival rates after WSSV challenge. It was first shown that *Penaeus japonicus* shrimp that survived natural and experimental WSSV infections displayed resistance to subsequent challenge with WSSV (Venegas *et al.*, 2000). Later studies showed that intramuscular injection of inactivated WSSV virions or recombinant structural protein, (VP28), provided shrimp with some protection against experimental WSSV infection. Furthermore, shrimp fed with food pellets coated with inactivated bacteria over-expressing VP28 showed better survival rates after WSSV challenge (Witteveldt *et al.*, 2004). However, although these results seemed promising, the protection was effective only when the shrimp were infected with a low dosage of WSSV. Also, the effect usually lasted for only a few days, or in the case of crayfish, for about 20 days. Another potential means of protecting shrimp against infection with WSSV ~~infection~~ is to use RNA interference (RNAi). WSSV gene-specific double-stranded (ds) RNAs produced strong anti-WSSV activity, protecting the shrimp against infection with WSSV ~~infection~~, but the same study showed that long dsRNA induced both sequence-dependent and independent anti-viral responses in shrimp (Robalino *et al.*, 2005). A more recent study ~~even~~ showed that even oral administration of bacterially expressed VP28 dsRNA could protect shrimp against infection with WSSV ~~infection~~ (Sarathi *et al.*, 2008). ~~To date,~~ However, although dsRNA technology continues to be explored, there are still no field trial data for either the vaccination or the RNAi approach.

Annex 22 (contd)**2.4.1. Vaccination**

No consistently effective vaccination methods have been developed for infection with WSSV.

2.4.2. Chemotherapy

~~No scientifically confirmed reports for infection with WSSV. No published or validated methods.~~

2.4.3. Immunostimulation

Several reports have shown that beta-glucan, vitamin C, seaweed extracts (fucoidan) and other immunostimulants may improve resistance to infection with WSSV-WSD (Chang *et al.*, 2003; Chotigeat *et al.*, 2004).

2.4.4. Resistance breeding

~~No significant improvements. Progress in breeding *P. vannamei* for resistance to infections with WSSV have has been reported for infections with WSSV (Cuéllar-Anjel *et al.*, 2012; Huang *et al.*, 2012).~~

2.4.5. Restocking with resistant species

Not applicable for infection with WSSV-WSD.

2.4.6. Blocking agents

There are no efficient blocking agents that can be recommended at this time. rVP28 has an effect, but it cannot yet be used as a practical blocking agent.

2.4.7. Disinfection of eggs and larvae

For transovum transmission, disinfection of egg is likely to be effective (Lo & Kou, 1998), but this has not yet been confirmed in formal scientific trials.

2.4.8. General husbandry practices

A number of husbandry practices have been used successfully to manage infection with WSSV-WSD, such as avoiding stocking in the cold season, use of specific pathogen free (SPF) or polymerase chain reaction (PCR)-negative seed stocks, ~~and~~ use of biosecure water and culture systems (Withyachumnarkul, 1999) and polyculture of shrimp and fish (He *et al.*, unpublished data).

3. Sampling**3.1. Selection of individual specimens**

Samples of moribund shrimp or shrimp that show clinical signs (see Section 4.1.1) or exhibit behavioural changes (Section 4.1.2) should be selected for ~~WSSV~~ detection of infection with WSSV.

3.2. Preservation of samples for submission

See Chapter 2.2.0 *General information* (for diseases of crustaceans) for guidance on preservation of samples for the intended test method.

3.3. Pooling of samples

~~Samples taken for molecular or antibody-based test methods for WSD may be combined as pooled samples of no more than five specimens per pooled sample of juveniles or subadults. However, for eggs, larvae and PL, pooling of larger numbers (e.g. 150 or more eggs or larvae or 50 to 150 PL depending on their size/age) may be necessary to obtain sufficient sample material. See also chapter 2.2.0.~~

The effect of pooling on diagnostic sensitivity has not been evaluated, therefore larger life stages should be processed and tested individually. However, small life stages, especially PL or specimens up to 0.5 g, can be pooled to obtain enough material for molecular testing.

3.4. Best organs or tissues

Tissue tropism analysis from both experimentally infected shrimp and wild-captured brooders shows that tissues originating from the ectoderm and mesoderm, especially the cuticular epithelium and subcuticular connective tissues, as well as other target tissues (e.g. antennal gland, haematopoietic organ, etc.), are the main target tissues for infection with WSSV. Samples of or from the pleopods, gills, haemolymph, stomach or abdominal muscle are recommended for submission (Lo *et al.*, 1997).

For non-destructive non-lethal sampling and screening by PCR, it is recommended to submit (a small piece of) gill, (a small aliquot of) haemolymph or (a small piece of) pleopod are suitable tissues. There is also some evidence to suggest that an ablated eyestalk would be a good alternative, provided that the compound eye is removed prior to submission.

Please see section 4.3.1.2.4.1 for details of the sample procedure.

3.5. Samples/tissues that are not suitable

Although WSSV infects the underlying connective tissue in the shrimp hepatopancreas and midgut, the columnar epithelial cells of these two organs are of endodermal embryonic origin (Lo *et al.*, 1997), and they are not appropriate tissues for detection. The compound eye may contain a PCR inhibitor (Lo *et al.*, 1997) and it is therefore not suitable for PCR-based diagnosis.

4. Diagnostic methods

4.1. Field diagnostic methods

4.1.1. Clinical signs

White spots embedded within the exoskeleton are the most commonly observed clinical sign. In most shrimp, these spots range from barely visible to 3 mm in diameter, and they sometimes coalesce into larger plates. However, it should be noted that environmental stress factors, such as high alkalinity, or bacterial disease can also cause white spots on the carapace of shrimp, and that moribund shrimp with infection with WSSV WSD may in fact have few, if any, white spots. Therefore, the appearance of white spots is absolutely not a good reliable diagnostic sign of infection with WSSV infection. Furthermore, other crustaceans, such as most crayfish, are often reported to show no sign of white spots when infected with WSSV. High degrees of colour variation with a predominance of reddish or pinkish discoloured shrimp are seen in diseased populations.

WSSV infections can be subclinical or manifest as clinical disease. Penaeid shrimp in aquaculture will generally show clinical signs associated with high morbidity and mortality. Some animals may die without showing any clinical signs. Non-penaeid species (e.g. crab, lobster) generally have subclinical infections under natural conditions.

4.1.2. Behavioural changes

The affected animals can show lethargy, decreased or absent feed consumption and abnormal swimming behaviour – slow swimming, swimming on side, swimming near water surface and gathering around edges of rearing units (Corbel *et al.*, 2001, Sahul Hameed *et al.*, 1998, 2001). The presence of white spots does not always mean that the condition is terminal. For instance, under non-stressful conditions, infected shrimp that have white spots may survive indefinitely. A very high mortality rate in the shrimp population can be expected within a few days of the onset of behavioural signs. However, if the shrimp also appear lethargic, if their colour changes to pink or reddish-brown, if they gather around the edges of ponds/tanks at the water surface, or if there is a rapid reduction in food consumption, then a very high mortality rate in the shrimp population can be expected within a few hours to a few days of the onset of these signs.

4.2. Clinical methods

4.2.1. Gross pathology

See In addition to the clinical and behavioural signs in Section 4.1.1 and 4.1.2 above, the following gross pathology has been reported in clinically affected penaeid shrimp: loosened attachment of the carapace with underlying cuticular epithelium (Sánchez-Paz, 2010) such that the carapace can be easily removed (Wen-Bin Zhan, 1998); empty gastro-intestinal tract due to anorexia (Escobedo-Bonilla, 2008); delayed clotting of haemolymph (Heidarieh, 2013); excessive fouling of gills (Wu *et al.*, 2013) and exoskeleton.

4.2.2. Clinical chemistry

Haemolymph withdrawn from WSSV-infected shrimp always has a delayed (or sometimes completely absent) clotting reaction.

4.2.3. Microscopic pathology

4.2.3.1. Wet mounts

Demonstration of hypertrophied nuclei in squash preparations of the gills and/or cuticular epithelium, which may be stained or unstained.

4.2.3.1.1 T-E staining

A T-E staining solution may be prepared from Trypan blue 0.6%, Eosin Y 0.2%, NaCl 0.5%, phenol 0.5%, and glycerol 20% (Huang & Yu, 1995) and used as follows:

- i) Place a piece of lesion tissue (e.g. a piece of gill or stomach epithelium without the cuticle) on a slide and mince with a scalpel.
- ii) Add 1–2 drops of the T-E staining solution to the minced tissue, mix and allow to stain for 3–5 minutes.
- iii) Lay a cover glass over the stained tissue and cover with several pieces of absorbent paper. Use a thumb to squash the mince into a single layer of cells.

If the sample was taken from a heavily infected shrimp, it should be easy to see the hypertrophied nuclei and intranuclear eosinophilic or vacuolation-like inclusion bodies under a 400–1000× light microscope.

4.2.3.2. Smears

Demonstration of aggregates of WSSV virions in unstained smear preparations of haemolymph by dark-field microscopy.

NOTE: This is the simplest of the microscopic techniques and is recommended for people with limited expertise in diagnosing infection with WSSV. The aggregates appear as small reflective spots of 0.5 µm in diameter (Momoyama *et al.*, 1995).

4.2.3.3. Fixed sections

Histological changes commonly reported with WSSV infection in susceptible species include: hypertrophied nuclei with marginated chromatin material in virus-infected cells; eosinophilic to pale basophilic (with haematoxylin & eosin stain) stained intranuclear viral inclusions within hypertrophied nuclei and multifocal necrosis associated with pyknotic and karyorrhectic nuclei in affected tissues of ectodermal and mesodermal origin. The infection with infectious hypodermal and hematopoietic necrosis virus, another DNA virus, produces similar inclusions that need to be differentiated from those of WSSV. Histological demonstration of pathognomonic inclusion bodies in target tissues.

4.2.3.4. In-situ hybridisation

Use of WSSV-specific DNA probes with histological sections to demonstrate the presence of WSSV nuclei acid in infected cells.

4.2.3.5. Immunohistochemistry

Use of WSSV-specific antibodies with histological sections or wet mounts to demonstrate the presence of WSSV antigen in infected cells.

4.2.4. Electron microscopy/cytopathology

Demonstration of the virus in tissue sections or in semi-purified negatively stained virus preparations (e.g. from haemolymph). See Section 2.1.1 for virion morphology.

4.3. Agent detection and identification methods

4.3.1. Direct detection methods

Not reported.

4.3.1.1. Microscopic methods

See Section 4.2.3 above.

4.3.1.1.1. Wet mounts

See Section 4.2.4 above.

4.3.1.1.2. Smears

See Section 4.2.5 above.

4.3.1.1.3. Fixed sections

See Section 4.2.3 above.

4.3.1.2. Agent isolation and identification

4.3.1.2.1. Bioassay method

If SPF shrimp are available, the following bioassay method is based on Nunan *et al.* (1998) and Durand *et al.* (2000), is suitable for WSSV diagnosis.

- i) For bioassay, remove the pleopods from shrimp suspected of being infected with WSSV ~~infection~~ and homogenise in TN buffer (0.02 M Tris/HCl, 0.4 M NaCl, pH 7.4).
- ii) Following centrifugation at 1000 **g** for 10 minutes, dilute the supernatant fluid 1/10 with 2% NaCl and filter (0.2 µm filter)
- iii) Inject 0.2 ml of inoculum into the dorso-lateral aspect of the fourth abdominal segment of indicator shrimp (e.g. SPF *P. vannamei* at the juvenile stage), injecting between the tergal plates into the muscle of the third abdominal segment.
- iv) Examine moribund shrimp grossly or by using the methods described above. If at 3–5 days after inoculation there are still no moribund shrimp and all test results are negative, then it is safe to conclude that the bioassay results are negative.

4.3.1.2.2. Cell culture/artificial media

WSSV can be isolated from primary cultures of lymphoid or ovary cells, However, it is NOT recommended to use cell culture as a routine isolation method because of: 1) the high risk of contamination, and, 2) the composition of the medium varies depending on the tissue type, host species and experimental purpose; that is, to date there is no standard or recognised medium that can be recommended. As primary cell culture is so difficult to initiate and maintain for virus isolation purposes, bioassay should be the primary means for virus propagation

Annex 22 (contd)4.3.1.2.3. *Antibody-based antigen detection methods*

Both polyclonal and monoclonal antibodies raised against either the virus or a recombinant viral structural protein have been used in various immunological assays including western blot analysis, immunodot assay, indirect fluorescent antibody test (IFAT), immunohistochemistry (IHC) or enzyme-linked immunosorbent assay (ELISA) to detect WSSV (Huang *et al.*, 1995a; Poulos *et al.*, 2001; Sithigorngul *et al.*, 2006; Yoganandhan *et al.*, 2004). Antibody-based methods can be fast, convenient and applicable to field use, but as they have only about the same sensitivity as 1-step PCR, they are recommended only to confirm acute infection with WSSV-WSD.

4.3.1.2.4. *Molecular techniques*4.3.1.2.4.1 *Polymerase chain reaction (PCR)*

The PCR protocol described here is from Lo *et al.* 1996a and b, and uses sampling methods from Lo *et al.* 1997. It is recommended for all situations where infection with WSSV diagnosis is required. A positive result in the first step of this standard protocol implies a serious infection with WSSV-infection, whereas, when a positive result is obtained in the second amplification step only, a latent or carrier-state infection is indicated. Alternative PCR assays have also been developed (e.g. Numan & Lightner, 2011), but before use they should first be compared with the protocol described here.

PCR commercial kits are available for WSSV detection diagnosis and are acceptable provided they have been validated as fit for such purpose. Please consult the OIE Register for kits that have been certified by the OIE (<http://www.oie.int/en/our-scientific-expertise/certification-of-diagnostic-tests/the-register-of-diagnostic-tests/>).

DNA extraction

- i) Collect 100–200 mg shrimp tissue (pleopod of live juvenile to subadult shrimp, postlarvae 11 upwards [PL11 up] with removed heads, or whole PL10, or use 100 µl haemolymph) in a 1.5 ml microfuge tube with 600 µl lysis solution (100 mM NaCl, 10 mM Tris/HCl, pH 8, 25 mM EDTA [ethylene diamine tetra-acetic acid], 0.5% SLS [sodium N-laurylsarcosinate] or 2% SDS [sodium dodecyl sulphate], and 0.5 mg ml⁻¹ proteinase K added just before use). For non-destructive screening, pleopods can be removed using red-hot forceps. For this procedure, the animal should be wrapped in a wet towel such that only the organ to be excised is left exposed.
- ii) Using a disposable stick, homogenise the tissue in the tube thoroughly.
- iii) After homogenisation, incubate at 65°C for 1 hour.
- iv) Add 5 M NaCl to a final concentration of 0.7 M. Next, slowly add 1/10 volume of N-cetyl N,N,N-trimethylammonium bromide (CTAB)/NaCl solution (10% CTAB in 0.7 M NaCl) and mix thoroughly.

NOTE: In addition to the CTAB extraction method described here, commercial extraction kits are often used as part of normal surveillance activities.

- v) Incubate at 65°C for 10 minutes, and then, at room temperature, add an equal volume of chloroform/isoamyl alcohol (24/1) and mix gently. Centrifuge at 13,000 g for 5 minutes and then transfer the aqueous solution (upper layer) to a fresh 1.5 ml tube and add an equal volume of phenol.
- vi) Mix gently and centrifuge at 13,000 g for 5 minutes. Collect the upper layer solution and repeat the phenol extraction process once or twice.
- vii) Transfer the final upper layer to a new tube, mix gently with two volumes of chloroform/isoamyl alcohol (24/1) and centrifuge at 13,000 g for 5 minutes.
- viii) Transfer the upper layer to a new tube and precipitate DNA by adding two volumes of 95% or absolute ethanol followed by standing at –20°C for 30 minutes or –80° C for 15 minutes.
- ix) Centrifuge at 13,000 g for 30 minutes and discard the ethanol. Wash the DNA pellet with 70% ethanol, dry and resuspend in 100 µl sterilised double-distilled water at 65°C for 15 minutes.
- x) Use 1 µl of this DNA solution for one PCR.

Annex 22 (contd)

Note: the following nested PCR procedures are well established and provide reliable diagnostic results under the specified conditions. Care should be taken, however, to ensure that DNA samples are prepared from the recommended organs, and that the PCR temperature is accurately applied (particularly for annealing, the recommended temperature is 62°C). To prevent the possibility of false positive results, it is important to adhere to the specified procedures, especially when they are used to test new candidate hosts such as *Cherax quadricarinatus* (Claydon *et al.*, 2004), as well as *Procambarus clarkii* (red swamp crayfish) and *Procambarus zonangulus* (Southern white river crayfish). For diagnosed incidences of infection with WSSV in a new host or in a previously free zone, DNA sequencing should be used to confirm the positive results.

First-step PCR

- i) Add 1 µl DNA template solution (containing about 0.1–0.3 µg DNA) to a PCR tube containing 100 µl of reaction mixture (10 mM Tris/HCl, pH 8.8, 50 mM KCl, 1.5 mM MgCl₂, 0.1% Triton X-100, 200 µM of each dNTP, 100 pmol of each primer, 2 units of heat-stable DNA polymerase).
- ii) The outer primer sequences are 146F1, 5'-ACT-ACT-AAC-TTC-AGC-CTA-TCTAG-3' and 146R1, 5'-TAA-TGC-GGG-TGT-AAT-GTT-CTT-ACG-A-3'.
- iii) The PCR profile is one cycle of 94°C for 4 minutes, 55°C for 1 minute, and 72°C for 2 minutes, followed by 39 cycles of 94°C for 1 minute, 55°C for 1 minute, and 72°C for 2 minutes and a final 5-minute extension at 72°C. The WSSV-specific amplicon from this reaction is 1447 bp. The sensitivity is approximately 20,000 copies of a plasmid template.

Second step of the (nested) PCR

This second step is necessary for the detection of infection with WSSV in shrimp at the carrier stage.

- i) Add 10 µl of the first-step PCR product to 90 µl of a PCR cocktail with the same composition as above except that it contains the second (inner) primer pair: 146F2 (5'-GTA-ACT-GCC-CCT-TCC-ATC-TCC-A-3') and 146R2 (5'-TAC-GGC-AGC-TGC-TGC-ACC-TTG-T-3').
- ii) Use the same PCR amplification protocol as above. The WSSV-specific amplicon from this reaction is 941 bp. The overall sensitivity of both steps is approximately 20 copies of a WSSV plasmid template.
- iii) To visualise, electrophorese 10 µl PCR products on 1% agarose gels containing ethidium bromide at a concentration of 0.5 µg ml⁻¹.
- iv) Decapod-specific primers (143F 5'-TGC-CTT-ATC-AGCTNT-CGA-TTG-TAG-3' and 145R 5'-TTC-AGN-TTT-GCA-ACC-ATA-CTT-CCC-3' yielding an 848 bp amplicon; N represents G, A, T, or C) should be used in control reactions to verify the quality of the extracted DNA and the integrity of the PCR. In the penaeid shrimp *P. aztecus*, the PCR product generated by this decapod-specific primer pair corresponds to nucleotide sequence 352–1200 of the 18s rRNA. The decapod 18s RNA sequence is highly conserved and produces a similar sized PCR product in almost all decapods. A positive control (WSSV DNA template) and negative controls (no template and shrimp DNA template) should be included in every assay.

4.3.1.2.4.2 DNA sequencing of PCR products

For confirmation of suspected new host of WSSV, the DNA fragment amplified-The amplicon from the two-step nested diagnostic PCR should be sequenced. The cloning and sequencing protocols described here are according to Claydon *et al.* (2004).

Note: to save time and money, it is acceptable to sequence the PCR amplicon directly. If a positive result is obtained, then go to step iv below. In the event that only band[s] of unexpected size are obtained, then the sample should be tested again using the cloning and sequencing procedures described below.

- i) Excise the DNA fragments selected for further analysis from the agarose gels and purify them using any of the commercially available PCR clean up kits.
- ii) Faint Ligase amplicons can be cloned into vector plasmids and clone prior to sequencing if required the construct. Amplify and purify the recombinant plasmid for DNA sequencing.

Annex 22 (contd)

- iii) Use suitable primers to amplify sequence the inserted amplicon, and then subject the amplified product to DNA sequencing.
- iv) Compare the sequences obtained with available databases using the Basic Local Alignment Search Tool (BLAST) to determine approximate phylogenetic affiliations.

4.3.1.2.4.3 Taqman real-time PCR method

The protocol described here is from Durand & Lightner (2002). This detection method is highly specific to WSSV, is extremely sensitive (four copies) and has a wide dynamic range (seven logs).

Construction of positive control vector and preparation of standard curve

The DNA fragment of 69 bp amplified by the forward and reverse primers (indicated below) is cloned in pGEM-T easy or other suitable vectors, and then confirmed by sequencing. The plasmid DNA is purified by any commercial plasmid extraction kits and the concentration is determined by using a spectrophotometer or other methods. The gene copy number is determined according to the molar mass derived from the plasmid DNA containing the 69 bp insert. The plasmid DNAs are then serially diluted tenfold to generate standard curves ranging from 10^2 - 10^1 to 10^7 copies.

DNA extraction

DNA extraction should be performed according to the above protocol described for PCR (4.3.1.2.4.1) or by using a commercial kit. The concentration of purified DNA can be determined by spectrophotometer or by other methods.

Real-time PCR

The TaqMan assay is carried out using the TaqMan Universal PCR Master Mix, which contains AmpliTaq Gold DNA polymerase, AmpErase UNG, dNTPs with dUTP and optimised buffer components (PE Applied Biosystems, Foster City, CA, USA¹). Primer sequences are WSS1011F: 5'-TGG-TCC-CGT-CCT-CAT-CTC-AG-3', WSS1079R: 5'-GCT-GCC-TTG-CCG-GAA-ATT-A-3', Taqman Probe: 5'-AGC-CAT-GAA-GAA-TGC-CGT-CTA-TCA-CAC-A-3'.

- i) Add a sample of 10–50 ng of DNA to set up a 25 µl reaction mixture containing 0.3 µM of each primer and 0.15 µM of TaqMan probe.
- ii) The PCR profile is one cycle of 50°C for 2 minutes for AmpErase uracil-N-glycosylase (UNG) and 95°C for 10 minutes for activation of AmpliTaq, followed by 40 cycles of 95°C for 15 seconds and 60°C for 1 minute.
- iii) To determine the WSSV copy number of the extracted DNA samples, the samples are subjected to PCR reaction alongside the serially diluted plasmid DNA standard. After reaction, the software accompanying the PCR system automatically determines the Ct value for each PCR sample. Based on the Ct values, the software calculates the standard curve for standard dilution and determines the WSSV copy number for the DNA samples by extrapolating values from the standard curve.

4.3.1.2.4.4. In-situ hybridisation (ISH) method

The protocol described here is based on that developed by Nunan & Lightner (1997).

- i) Fix moribund shrimp with Davidson's AFA fixative for 24–48 hours.
- ii) Embed the tissues in paraffin and cut into 5 µm sections. Place sections on to positively charged microscope slides.
- iii) Heat the slide on a hot plate at 65°C for 30 minutes.
- iv) Deparaffinise, rehydrate and then treat for 2–30 minutes (depending on tissue type) with 100 µg ml⁻¹ proteinase K in Tris/NaCl/EDTA (TNE) buffer at 37°C.

¹ Reference to specific commercial products as examples does not imply their endorsement by the OIE. This applies to all commercial products referred to in this *Aquatic Manual*.

Annex 22 (contd)

- v) Post-fix the slides by chilling in pre-cooled 0.4% formaldehyde for 5 minutes at 4°C and wash the slides in 2 × standard saline citrate (SSC; 1 × SSC = 150 mM NaCl, 15 mM tri-sodium citrate, pH 7.0) at room temperature.
- vi) Pre-hybridise the slides with pre-hybridisation solution (50% formamide, 0.2% Ficoll 400, 0.2% polyvinylpyrrolidone, 0.2% bovine serum albumin, 5 × SSC, 1 mM EDTA, 50 mM Tris/HCl, pH 8) for 30 minutes at 42°C.
- vii) Follow with hybridisation with the 1447 bp WSSV-specific PCR amplicon (or with any other WSSV-specific PCR amplicon; see Section 4.3.1.2.3.1 “First-step PCR” above) that has been labelled with digoxigenin. It is recommended that the probe be labelled by incorporating DIG-dNTP by the PCR method. Optimum concentration should be determined by testing and adjusting until a high specific signal is obtained against a low background.
- viii) For hybridisation, boil the probe for 10 minutes and immediately place on ice. Dilute the probe to 30–50 ng ml⁻¹ in pre-hybridisation solution and apply 500 µl to each slide.
- ix) Put the slide on a hotplate at 85–95°C for 6–10 minutes (make sure that it does not reach boiling point), quench slides on ice for 5 minutes and then transfer to a humid chamber for 16–20 hours at 42°C.
- x) After hybridisation, wash the slides twice for 15 minutes each time with 2 × SSC at room temperature, twice for 5 minutes with 1 × SSC at 37°C, and twice for 5 minutes with 0.5 × SSC at 37°C.
- xi) For hybridisation detection, wash slides with maleic acid buffer (100 mM maleic acid, 150 mM NaCl, pH 7.5) for 5 minutes at room temperature.
- xii) Block the slides with blocking solution (2% normal goat serum and 0.3% Triton X-100 in maleic acid buffer) for 30 minutes at 37°C.
- xiii) Add 250 µl anti-DIG alkaline phosphatase (AP)-conjugated antibody solution (1 µl ml⁻¹ anti-DIG/AP-Fab fragment in maleic acid buffer containing 1% normal goat serum and 0.3% Triton X-100) to each slide, and incubate at 37°C for 30 minutes.
- xiv) Wash the slides twice with maleic acid buffer for 10 minutes each and once with detection buffer (100 mM Tris/HCl, 100 mM NaCl, pH 9.5) at room temperature.
- xv) Add 500 µl development solution (prepare immediately before use by adding 45 µl NBT salt solution [75 mg ml⁻¹ in 70% dimethylformamide], 35 µl 5-bromo-4-chloro-3-indoyl phosphate, toluidinum salt [X-phosphate] solution [50 mg ml⁻¹ in dimethylformamide] and 1 ml 10% PVA to 9 ml of detection buffer) to each slide and incubate in the dark in a humid chamber for 1–3 hours.
- xvi) Stop the reaction by washing the slides in TE buffer (10 mM Tris/HCl, 1 mM EDTA, pH 8.0) for 15 minutes at room temperature. Wash the slides in distilled water for ten dips, counterstain the slides in 0.5% aqueous Bismarck Brown Y for approximately 5 minutes and then rinse with water. Wet mount using aqueous mounting media for observation immediately or dehydrate the slides and mount with mounting media for long-term preservation.
- xvii) Mount the slides with cover-slips and examine with a bright field microscope. Positive hybridisation appears as a dark blue to black precipitate against the yellow to brown counterstain.

4.3.1.2.4.5. Loop-mediated isothermal amplification (LAMP) method

The protocol described here is from Kono *et al.* (2004). The LAMP method is sensitive and rapid, and it amplifies the target nucleic acids under isothermal conditions, therefore needing no sophisticated machine for thermal cycling.

DNA extraction

DNA extraction could be performed according to the above protocol described for PCR (4.3.1.2.4.1) or by other suitable methods or by commercial kits.

Annex 22 (contd)*LAMP reaction*

- i) Add DNA to a tube to set up a 25 µl reaction mixture (20 mM Tris/HCl, pH 8.8, 10 mM KCl, 8 mM MgSO₄, 10 mM (NH₄)₂SO₄, 0.1% Tween 20, 0.8M Betaine, 1.4 mM of each dNTP, 40 pmol of WSSV-FIP and -BIP primers, 5 pmol of WSSV-F3 and -B3 primers).
- ii) The primer sequences are WSSV-FIP: 5'-GGG-TCG-TCG-AAT-GTT-GCC-CAT-TTT-GCC-TAC- GCA-CCA-ATC-TGT-G-3', WSSV-BIP: 5'-AAA-GGA-CAA-TCC-CTC-TCC-TGC-GTT-TTA-GAA-CGG-AAG-AAA-CTG-CC-TT-3', WSSV-F3: ACG-GAC-GGA-GGA-CCC-AAA-TCG-A-3', WSSV-B3: 5'-GCC-TCT-GCA-ACA-TCC-TTT-CC-3'.
- iii) Heat the mixture at 50°C for 5 minutes and at 95°C for 5 minutes, then chill on ice, and add 1 µl (8 U) of *Bst* DNA polymerase.
- iv) Incubate the mixture at 65°C for 60 minutes, and then terminate the reaction at 80°C for 10 minutes.
- v) To visualise, electrophorese 2 µl LAMP reaction products on 2% agarose gels containing ethidium bromide at a concentration of 0.5 µg ml⁻¹. This reaction produces WSSV-specific LAMP products with multiple bands of various sizes from approximately 200 bp to the loading well.

Reliable LAMP commercial kits may be an alternative for WSSV diagnosis.

4.3.1.2.5. *Agent purification*

The WSSV virion can be purified as described previously with slight modifications (Xie *et al.*, 2005). Briefly, collect five or six moribund crayfish or shrimp (20–25 g each) at 3 days to 1 week post-infection. Homogenise all tissues excluding the hepatopancreas for 2 minutes using a mechanical homogeniser in 1200 ml TNE buffer (50 mM Tris/HCl, 400 mM NaCl, 5 mM EDTA, pH 8.5) containing protease inhibitors (1 mM phenylmethylsulfonyl fluoride, 1 mM benzamidine, and 1 mM Na₂S₂O₅). Centrifuge at 3500 *g* for 5 minutes. Save the supernatant and rehomogenise the pellet in 1200 ml TNE buffer. Filter the pooled supernatant through a nylon net (400 mesh) and centrifuge at 30,000 *g* for 30 minutes. Discard the supernatant and carefully rinse out the upper loose layer (pink) of the pellet using a Pasteur pipette. Resuspend the lower compact layer (grey) in 10 ml TM buffer (50 mM Tris/HCl, 10 mM MgCl₂, pH 7.5). Pool the crude virus suspension and centrifuge at 3000 *g* for 5 minutes. Centrifuge the supernatant again at 30,000 *g* for 20 minutes. Remove the supernatant and pink loose layer and resuspend the white pellet in 1.2 ml TM buffer containing 0.1% NaN₃. Transfer to a 1.5-ml Eppendorf tube. Centrifuge the suspension three to five times at 650 *g* for 5 minutes each time to remove pink impurities. Finally, store the milk-like pure virus suspension at 4°C until use.

4.3.2. **Serological methods**

None developed.

5. Rating of tests against purpose of use

The methods currently available for targeted surveillance and diagnosis of infection with WSSV are listed in Table 5.1. The designations used in the Table indicate: a = the method is the recommended method for reasons of availability, utility, and diagnostic specificity and sensitivity; b = the method is a standard method with good diagnostic sensitivity and specificity; c = the method has application in some situations, but cost, accuracy, or other factors severely limits its application; and d = the method is presently not recommended for this purpose. These are somewhat subjective as suitability involves issues of reliability, sensitivity, specificity and utility. Although not all of the tests listed as category A or B have undergone formal standardisation and validation, their routine nature and the fact that they have been used widely without dubious results, makes them acceptable.

Table 5.1. Methods for targeted surveillance and diagnosis

Method	Targeted surveillance				Presumptive diagnosis	Confirmatory diagnosis
	Larvae	PLs	Juveniles	Adults		
Gross signs	d	d	c	c	c	d
Bioassay	d	d	d	d	c	b
Direct LM Wet mounts and smears	d	d	c	c	c	e-d
Histopathology	d	c	c	c	a	c
Transmission EM	d	d	d	d	d	a
Antibody-based assays	d	d	c	c	a	b
<i>In-situ</i> DNA probes	d	d	c	c	a	a
Real-time PCR	e-c	b	a	a	a	a
Conventional PCR	d	c	b	b	b	a
Sequence	d	d	d	d	d	a
LAMP	d	d	a	a	a	a

PLs = postlarvae; LM = light microscopy; EM = electron microscopy;
PCR = polymerase chain reaction; LAMP = loop-mediated isothermal amplification.

6. Test(s) recommended for targeted surveillance to declare freedom from infection with white spot syndrome virus disease

Two-step PCR and sequencing are the recommended methods for declaring freedom, only for juveniles and adults and possibly PLs. Two-step PCR negative results are required. Where a two-step PCR positive result cannot be confirmed as infection with WSSV by sequencing, this also counts as a negative result.

Real-time PCR is the recommended test for targeted surveillance to declare freedom from **infection with white spot disease syndrome virus**.

Annex 22 (contd)**7. Corroborative diagnostic criteria****7.1. Definition of suspect case**

For juvenile and adult shrimp: gross signs of WSD (See Sections 4.1.1 and 4.1.2 above).

For shrimp at any life stage (larva to adult): mortality.

For shrimp and crab at any life stage (larva to adult): hypertrophied nuclei in squash preparations of gill and/or cuticular epithelium; unusual aggregates in haemolymph by dark-field microscopy; inclusion bodies in histological sections in target tissues.

Infection with WSSV is suspected if at least one of the following criteria is met:

1. Gross pathology consistent with infection with WSSV
2. Histopathology consistent with infection with WSSV
3. Positive conventional PCR result
4. Positive real-time PCR result
5. Positive LAMP result

7.2. Definition of confirmed case

Suspect cases should first be checked by PCR or LAMP. If in a previously WSSV-free country/zone/compartiment, where PCR results are positive, they should be confirmed by sequencing. Histopathology, probes and electron microscopy also can be used to confirm the case.

Infection with WSSV is considered to be confirmed if one or more of the following criteria are met:

1. Histopathology consistent with WSSV and positive *in-situ* hybridisation test
2. Positive conventional PCR results from and two positive conventional PCRs and conventional PCR targeting a two different regions of the WSSV genome with sequence analysis consistent with WSSV
3. Positive real-time PCR results and positive conventional PCR targeting a different region of the WSSV genome with sequence analysis consistent with WSSV and conventional PCR targeting a two different regions of the WSSV genome
4. Positive LAMP results and positive conventional PCR targeting a different region of the WSSV genome with sequence analysis consistent with WSSV and conventional PCR targeting a two different regions of the WSSV genome

For confirmation of an index case in a previously free zone or country, sequence analysis of conventional PCR amplicons is required.

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* *

NB: There is an OIE Reference Laboratory for infection with white spot syndrome virus-disease (see Table at the end of this *Aquatic Manual* or consult the OIE web site for the most up-to-date list: <http://www.oie.int/en/our-scientific-expertise/reference-laboratories/list-of-laboratories/>). Please contact the OIE Reference Laboratories for any further information on for infection with white spot syndrome virus-disease

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

CHAPTER 2.3.1.

INFECTION WITH EPIZOOTIC HAEMATOPOIETIC NECROSIS VIRUS

1. Scope

For the purpose of this chapter, Infection with epizootic haematopoietic necrosis virus means is considered to be systemic clinical or subclinical infection of finfish with the pathogenic agent epizootic haematopoietic necrosis virus (EHNV) of the Genus *Ranavirus* of the and Family Iridoviridae.

2. Disease information

2.1. Agent factors

2.1.1. Aetiological agent, agent strains

EHNV is a member of the genus *Ranavirus* in the Family Iridoviridae with the type species Frog virus 3 (FV3) (Chinchar *et al.*, 2005). Other species include Bohle virus (BIV), European catfish virus (ECV), European sheatfish virus (ESV) and Santee-Cooper ranavirus. Caution should be taken when speaking of ECV and ESV as two separate viruses because the scientific literature (Hyatt *et al.*, 2000) indicates they are isolates of the same virus. There are many other tentative species in this Genus. *Ranaviruses* have been isolated from healthy or diseased frogs, salamanders and reptiles in America, Europe and Australia (Chinchar., 2002; Drury *et al.*, 2002; Fijan *et al.*, 1991; Hyatt *et al.*, 2002; Speare & Smith, 1992; Whittington *et al.*, 2010; Wolf *et al.*, 1968; Zupanovic *et al.*, 1998). *Ranaviruses* have large (150–180 nm), icosahedral virions, a double-stranded DNA genome 150–170 kb, and replicate in both the nucleus and cytoplasm with cytoplasmic assembly (Chinchar *et al.*, 2005). They possess common antigens that can be detected by several techniques.

Since the recognition of disease due to EHNV in Australia in 1986, similar systemic necrotising iridovirus syndromes have been reported in farmed fish. These include catfish (*Ictalurus melas*) in France (ECV) (Pozet *et al.*, 1992), sheatfish (*Silurus glanis*) in Germany (ESV) (Ahne *et al.*, 1989; 1990), turbot (*Scophthalmus maximus*) in Denmark (Bloch & Larsen, 2009) and others in Finland (Ariel *et al.*, 1999).

EHNV and ECV are distinct viruses that can be differentiated using genomic analysis (Ahne *et al.*, 1998; Holopainen *et al.*, 2009; Hyatt *et al.*, 2000; Mao *et al.*, 1996; 1997; Marsh *et al.*, 2002). This enables epidemiological separation of disease events in finfish in Australia (EHNV) and Europe (ECV), and differentiation of these from ranavirus occurrences in frogs (FV3 and BIV). However, many ranavirus isolates have not been characterised to this level.

2.1.2. Survival outside the host

EHNV is extremely resistant to drying and, in water, can survive for months (Langdon, 1989). It can persist in frozen fish tissues for more than 2 years (Langdon, 1989) and frozen fish carcasses for at least a year (Whittington *et al.*, 1996). For these reasons, it is presumed that EHNV would persist for months to years on a fish farm in water and sediment, as well as on plants and equipment.

2.1.3. Stability of the agent (effective inactivation methods)

EHNV is susceptible to 70% ethanol, 200 mg litre⁻¹ sodium hypochlorite or heating to 60°C for 15 minutes (Langdon, 1989). Data for the inactivation of an amphibian ranavirus may also be relevant: 150 mg/litre chlorhexidine and 200 mg/litre potassium peroxymonosulphate were effective after 1 minute contact time (Bryan *et al.*, 2009). If it is first dried, EHNV in cell culture supernatant is resistant to heating (Whittington *et al.*, 2010).

Annex 23 (contd)**2.1.4. Life cycle**

The route of infection is unknown but fish are susceptible experimentally following bath exposure. The virus infects a range of cell types including hepatocytes, haematopoietic cells and endothelial cells in many organs (Reddacliff & Whittington, 1996). Virus is shed into water from infected tissues and carcasses as they disintegrate.

2.2. Host factors**2.2.1. Susceptible host species**

Natural EHNV infections are known from only two teleost species, roach (*Perca fluviatilis*) and rainbow trout (*Oncorhynchus mykiss*) (Langdon, 1989; Langdon *et al.*, 1986; 1987; 1988), however, a number of other finfish species are susceptible to EHNV experimentally. Individuals of the following species have died after bath inoculation: Species that fulfil the criteria for listing as susceptible to infection with EHNV according to Chapter 1.5. of the Aquatic Animal Health Code (Aquatic Code) include: black bullhead (*Ameiurus melas*), crimson spotted rainbow fish (*Melanotaenia fluviatilis*), eastern mosquito fish (*Gambusia holbrooki*), European perch (*Perca fluviatilis*), macquarie perch (*Macquaria australasica*), mosquito fish (*Gambusia affinis*), mountain galaxias (*Galaxias olidus*), northern pike (*Esox lucius*), pike-perch (*Sander lucioperca*), rainbow trout (*Oncorhynchus mykiss*) and silver perch (*Bidyanus bidyanus*).

Macquarie perch (*Macquaria australasica*), silver perch (*Bidyanus bidyanus*), mosquito fish (*Gambusia affinis*) and mountain galaxias (*Galaxias olidus*). Some species, for example goldfish (*Carassius auratus*) and common carp (*Cyprinus carpio*), are resistant (Langdon, 1989). European studies have shown that black bullhead (*Ameiurus melas*) and pike (*Esox lucius*) are susceptible to EHNV by bath exposure (Bang Jensen *et al.*, 2009; Gobbo *et al.*, 2010).

2.2.2. Species with incomplete evidence for susceptibility

Species for which there is incomplete evidence for susceptibility according to Chapter 1.5. of the Aquatic Code include: none known.

2.2.3. Susceptible stages of the host

Susceptible stages of the host are all age classes of rainbow trout and ~~redfin-European~~ perch.

2.2.4. Species or subpopulation predilection (probability of detection)

Clinical signs are usually more obvious in fingerlings and juvenile fish than adults of both rainbow trout and ~~redfin-European~~ perch.

2.2.5. Target organs and infected tissue

Target organs and tissues infected with the virus are: liver, kidney, spleen and other parenchymal tissues. It is not known if EHNV can be detected in gonadal tissues, ovarian fluid or milt or whether these tissues are suitable for surveillance of broodstock.

2.2.65. Persistent infection with lifelong carriers

2.2.65.1. Rainbow trout

The high case fatality rate and low prevalence of infection with EHNV infection in natural infections in rainbow trout means that the recruitment rate of carriers is likely to be very low (<2%) (Whittington et al., 1994). Persistent infection with very small numbers of infectious virions was detected in one clinically healthy rainbow trout fingerling 63 days after intraperitoneal inoculation (Whittington & Reddacliff, 1995), but the significance of this observation is unclear because of the artificial route of infection. EHNV has been detected in grower-growout fish, but as histopathological lesions consistent with infection with EHNV were also present there was active infection rather than a carrier state (Whittington et al., 1999). Too few broodstock samples have been examined to be certain that broodstock are not infected (Whittington et al., 1994). Anti-EHNV serum antibodies were not detected in 0+ fingerlings during or after an outbreak but were detected in a low proportion of 1+ to 2+ grower-growout fish, hence, it is uncertain whether these were survivors of the outbreak (Whittington et al., 1994; Whittington et al., 1999). There are data for European stocks of rainbow trout in experimental infections where potential carriers were identified (Ariel & Bang Jensen, 2009).

2.2.65.2. Redfin-European perch

This species is extremely susceptible to infection with EHNV and it seems unlikely that it is a suitable reservoir host in Australia (Whittington & Reddacliff, 1995). However, there is some conflicting evidence. EHNV, or a related ranavirus, was isolated from 2 of 40 apparently healthy adult redfin-European perch during epizootics in juveniles in Victoria, Australia (Langdon & Humphrey, 1987), but as the incubation period extends for up to 28 days (Whittington & Reddacliff, 1995), these fish may have been in the preclinical phase. Several ranavirus isolates have been obtained from redfin-European perch in Victoria at times when there was no obvious epizootic, and some apparently healthy redfin-European perch in Victoria had serum antibodies against EHNV or a related virus (Whittington & Hyatt, unpublished data). Furthermore, there are data for European stocks of redfin-European perch in experimental infections where the virulence of EHNV appeared to be lower than in Australia (Ariel & Bang Jensen, 2009).

2.2.65.3. Murray cod

This species may be a suitable carrier as infection without disease has occurred after bath inoculation (Langdon, 1989).

2.2.65.4. Rainbow trout and Atlantic salmon

These species may be a suitable carrier as infection without disease has occurred after intraperitoneal or bath inoculation (Langdon, 1989).

2.2.65.5. Pike

This species may be a suitable carrier based on limited trials with fry (Bang Jensen et al., 2009).

2.2.76. Vectors

Since EHNV is a resistant virus, it may be transferred on nets, boats and other equipment, or in fish used for bait by recreational fishers. Birds are potential mechanical vectors for EHNV, it being carried in the gut, on feathers, feet and the bill. Piscivorous birds feed on affected juvenile redfin perch and the gastrointestinal contents of these birds may contain EHNV (Whittington et al., 1994). However, the virus is likely to may be inactivated at typical avian body temperatures (40–44°C). Nevertheless, the spread of EHNV by regurgitation of ingested material within a few hours of feeding is possible (Whittington et al., 1994).

2.2.87. Known or suspected wild aquatic animal carriers

None known.

Annex 23 (contd)

2.3. Disease pattern

2.3.1. Transmission mechanisms

Rainbow trout: EHNV has spread between rainbow trout farms by transfer of infected fingerlings and probably transport water (Langdon *et al.*, 1988; Whittington *et al.*, 1994; 1999). ~~It is assumed that consignments of fish contain a low proportion of individuals with progressive subclinical or clinical infection, rather than carrier fish.~~ The low prevalence of infection in rainbow trout means that active infection can easily go unrecognised in a population and be spread by trading fish. There are no data on possible vertical transmission of EHNV on or within ova, and disinfection protocols for ova have not been evaluated. EHNV has not yet been isolated from ovarian tissues or from broodstock. Annual recurrence in farmed rainbow trout may be due to reinfection of successive batches of fish from wild ~~redfin-European~~ perch present in the same catchment.

~~Redfin-European perch:~~ The occurrence of infection with EHNV in ~~redfin-European~~ perch in widely separated river systems and impoundments, and its upstream progression, indicates that EHNV is spread by means other than water; mechanisms include translocation of live fish or bait by recreational fishers. ~~Redfin-European~~ perch migrations in Australia are uncertain (see also Section 2.2.6 Vectors).

2.3.2. Prevalence

Rainbow trout: ~~the clinical~~ disease is generally difficult to identify-detect with very low mortality rates and infection with EHNV may be present on a farm without causing suspicion. During outbreaks, EHNV has been detected by virus isolation in 60–80% of moribund or dead fish, but in only 0–4% of in-contact, clinically healthy fish. The 99% confidence limits for the prevalence of subclinical infection are 0–8% (Whittington *et al.*, 1994; 1999). The virus could not be found at all in surviving cohorts after an outbreak. Anti-EHNV antibodies were detected in ~~grower-growout~~ fish at low prevalence (0.7%, 95% confidence limits 0.02% to 3.7%).

~~Redfin-European perch:~~ the disease is recognised by epizootic mortality in fish of any age affecting a very large proportion of the population with dramatic population decline (Langdon *et al.*, 1986; 1987; Whittington *et al.*, 1996). Typically, fingerling and juvenile fish are affected in endemic areas, but in newly infected areas adults are also affected. When the disease is first recognised in an area there is a dramatic population decline (Langdon *et al.*, 1986; 1987; Whittington *et al.*, 1996).

The studies above were conducted prior to the availability of real-time PCR assays, which may have greater diagnostic sensitivity and reveal higher prevalence in subclinically infected populations.

2.3.3. Geographical distribution

Rainbow trout: infection with EHNV is known only from fish farms located in the Murrumbidgee and Shoalhaven river catchments in New South Wales, Australia (Whittington *et al.*, 2010). ~~However, some farms within this region have remained free of the disease (Whittington *et al.*, 1999).~~

~~Redfin-European perch:~~ infection with EHNV is endemic in south-eastern Australia, but there is a discontinuous distribution (Whittington *et al.*, 2010). The ~~disease-infection~~ occurs in many small and large impoundments in Victoria and since 1986 has spread progressively upstream in the Murrumbidgee river catchment through New South Wales and the Australian Capital Territory. Similar spread has been observed in the Murray River in South Australia (Whittington *et al.*, 1996).

2.3.4. Mortality and morbidity

Rainbow trout: It appears that under natural conditions EHNV is poorly infective but has a high case fatality rate. infection with EHNV may be present on a farm without causing suspicion because the mortality rate may not rise above the usual background rate. infection with EHNV has most often been reported in young fingerlings <125 mm fork length with daily mortality of less than 0.2% and total mortality of up to 4%. However, rainbow trout of all ages may be susceptible, although infection has not yet been seen in broodstock (Whittington *et al.*, 1994; 1999). There is a low direct economic impact because of the low mortality rate. ~~In keeping with the natural pattern of disease, rainbow trout were resistant to bath exposure in $10^{2.2}$ TCID₅₀ (50% tissue culture infective dose) ml⁻¹ (Whittington & Reddacliff, 1995), while only 1 of 7 became infected after bath inoculation for 1 hour in 10^3 TCID₅₀ ml⁻¹ (Langdon *et al.*, 1988).~~ Differences in susceptibility between European and Australian stocks of rainbow trout may exist (Ariel & Bang Jensen, 2009).

~~Redfin-European perch~~: There is a very high rate of infection and mortality in natural outbreaks that, over time, leads to loss in wild fish populations (Langdon *et al.*, 1986; 1987; Whittington *et al.*, 1996). Experimental bath inoculation with as few as 0.08 TCID₅₀ ml⁻¹ was lethal, and doses too low to be detected by virus isolation in BF-2 cells were fatal by intraperitoneal inoculation (Whittington & Reddacliff, 1995). Differences in susceptibility between European and Australian stocks of ~~redfin-European perch~~ may exist (Ariel & Bang Jensen, 2009).

2.3.5. Environmental factors

~~Rainbow trout: Natural~~ Outbreaks appear to be related to poor husbandry, particularly overcrowding, inadequate water flow and fouling of tanks with feed. ~~Water quality parameters are suboptimal, and intercurrent diseases, including skin diseases caused by protozoa and fungi, and systemic bacterial infection are common.~~ Damage to skin may provide a route of entry for EHNV. Outbreaks have been seen on farms at water temperatures ranging from 11 to 20°C (Whittington *et al.*, 1994; 1999). The incubation period after intraperitoneal inoculation was 3–10 days at 19–21°C compared with 14–32 days at 8–10°C (Whittington & Reddacliff, 1995).

~~Redfin-European perch~~: Natural epizootics of infection with EHNV affecting juvenile and adult ~~redfin-European perch~~ occur mostly in summer (Langdon *et al.*, 1986; 1987; Whittington *et al.*, 1994). It has been assumed that the disease in juvenile fish is related to the annual appearance of large numbers of non-immune young fish and their subsequent exposure to the virus while schooling in shallow waters; adults are uncommonly involved in these outbreaks. It is possible that environmental temperature is the trigger for outbreaks as juvenile fish feed in warm shallow waters on planktonic fauna, whereas adults feed on benthic invertebrates and larger prey in deeper cooler water (Whittington & Reddacliff, 1995). Experimentally the incubation period ranged from 10 to 28 days at 12–18°C compared with 10–11 days at 19–21°C, and adult perch were refractory to infection at temperatures below 12°C (Whittington & Reddacliff, 1995). European stocks of ~~redfin-European perch~~ also displayed temperature-dependent susceptibility (Ariel & Bang Jensen, 2009).

2.4. Control and prevention

2.4.1. Vaccination

None available.

2.4.2. Chemotherapy

None available.

2.4.3. Immunostimulation

Not tested.

2.4.4. Resistance breeding

There has been no formal breeding programme for resistant strains of susceptible species. However, experimental trials using a bath exposure have shown that European perch from water bodies in New South Wales, Australia with previous EHNV infections showed lower mortality compared with European perch from neighbouring and distant water bodies in Australia that have no previous history of EHNV (Becker *et al.*, 2016). ~~Not tested.~~

2.4.5. Restocking with resistant species

Not tested.

2.4.6. Blocking agents

Not tested.

Annex 23 (contd)**2.4.7. Disinfection of eggs and larvae**

Not tested.

2.4.8. General husbandry practices

Disease control in rainbow trout at the farm level relies on reducing the impact of infection by maintaining low stocking rates and adequate water quality. ~~The mechanism of protection may be through maintenance of healthy integument.~~

Investigations on one rainbow trout farm indicated that ponds with high stocking rates and low water flow, and thus poorer water quality, may result in higher levels of clinical disease compared with ponds on the same farm with lower stocking rates and higher water flow (Whittington *et al.*, 1994). Disease control in rainbow trout at the farm level relies on reducing the impact of infection by maintaining low stocking rates and adequate water quality. The mechanism of protection may be through maintenance of healthy integument (Whittington *et al.*, 1994).

3. Sampling**3.1. Selection of individual specimens**

A simple method for preparation of fish tissues for cell culture and enzyme-linked immunosorbent assay (ELISA) has been validated (Whittington & Steiner, 1993).

Bath large fish for 30 seconds in 70% ethanol; bath fingerlings for 5 seconds in 70% ethanol then rinse in sterile water. Dissect fish aseptically in a Class II biosafety cabinet.

Large fish (>60 mm fork length): remove 0.1 g liver, kidney, spleen (\pm other organs in specific situations) and place in sterile 1.5 ml tubes. Tubes suitable for use with pestles for grinding tissues (see below) are available, but standard 1.5 ml tubes may be suitable. In some situations liver, kidney and spleen may be pooled in a single tube (see Section 3.3).

Medium fish (30–60 mm fork length): scrape all viscera into the tube.

Small fish (<30 mm fork length): remove head and tail, place rest of fish into the tube.

3.2. Preservation of samples for submission

For cell culture and ELISA, freeze tubes containing tissues at -20°C to -80°C until needed.

For light microscopic examination, fix tissues in 10% neutral buffered formalin.

3.3. Pooling of samples

The effect of pooling tissues from multiple fish on the sensitivity of diagnostic tests has not been evaluated. ~~However, tissues for virus isolation are commonly pooled in lots of 5 or 10 individual fish per test.~~

3.4. Best organs or tissues

Liver, anterior kidney, spleen.

3.5. Samples/tissues that are not suitable

Inappropriate tissues include gonads, gonadal fluids, milt and ova, since there is no evidence of reproductive tract infection.

4. Diagnostic methods

4.1. Field diagnostic methods

4.1.1. Clinical signs

There are no specific clinical signs. Fish are found dead. There may be clinical evidence of poor husbandry practices, such as overcrowding and suboptimal water quality manifesting as skin, fin and gill lesions (Reddacliff & Whittington, 1996).

4.1.2. Behavioural changes

Moribund fish may have loss of equilibrium, flared opercula and may be dark in colour (Reddacliff & Whittington, 1996).

4.2. Clinical methods

4.2.1. Gross pathology

There may be no gross lesions or nonspecific lesions on the skin, fins and gill. A small proportion of fish may have enlargement of kidney, liver or spleen. There may be focal white to yellow lesions in the liver corresponding to areas of necrosis (Reddacliff & Whittington, 1996).

4.2.2. Clinical chemistry

Not applicable.

4.2.3. Microscopic pathology

Acute focal, multifocal or locally extensive coagulative or liquefactive necrosis of liver, haematopoietic kidney and spleen are commonly seen in routine haematoxylin and eosin (H&E)-stained sections of formalin-fixed material. A small number of basophilic intracytoplasmic inclusion bodies may be seen, particularly in areas immediately surrounding necrotic areas in the liver and kidney. Necrotic lesions may also be seen in heart, pancreas, gastrointestinal tract, gill and pseudobranch (Reddacliff & Whittington, 1996).

4.2.4. Wet mounts

Not applicable.

4.2.5. Smears

Not tested.

4.2.6. Electron microscopy/cytopathology

Affected tissues (e.g. kidney liver and spleen) contain cells exhibiting necrosis. Cells contain conspicuous cytoplasmic inclusions that are rarefied areas of the cytoplasm in which the viruses are assembled. Within the cytoplasm, aggregates (paracrystalline arrays) of large (175 nm ± 6 nm) nonenveloped icosahedral viruses are apparent; single viruses are also present. Complete viruses (containing electron-dense cores) bud/egress from the infected cells through the plasma membrane. The nuclei of infected cells are frequently located peripherally and are distorted in shape.

Annex 23 (contd)**4.3. Agent detection and identification methods****4.3.1. Direct detection methods****4.3.1.1. Microscopic methods**

Light microscopy: routine methods can be used for tissue fixation, such as in 10% buffered neutral formalin, paraffin embedding, preparation of 10 µm sections and staining with H&E to demonstrate tissue necrosis and basophilic intracytoplasmic inclusion bodies. These inclusion bodies are indicative but not confirmatory for infection with EHNV. Formalin-fixed paraffin-embedded sections can also be stained using an immunoperoxidase method (see below) to identify EHNV antigen associated with necrotic lesions.

Electron microscopy: Ultrathin routine sectioning methods can be used for preparation of tissues and cell cultures (Eaton *et al.*, 1991) to demonstrate tissue necrosis, presence of viruses and virus inclusion bodies. Tissues and cells fixed with an alternative fixation and embedding regime can be used for antigen detection (Hyatt, 1991).

Negative contrast electron microscopy: supernatants from dounce homogenised tissues (10% [w/v]) and cell cultures can be used to detect viruses. Ranaviruses have a definitive appearance. They vary in diameter (150–180 nm) and have a limiting cell-derived (plasma membrane) envelope that surrounds a capsid of skewed symmetry. Underlying the capsid is a *de novo* membrane that itself surrounds a core containing the double-stranded (ds) DNA and minor proteins. These preparations can also be used to confirm ranavirus antigenicity (Eaton *et al.*, 1991).

4.3.1.1.1. Wet mounts

Not applicable.

4.3.1.1.2. Smears

Not applicable.

4.3.1.1.3. Fixed sections

See Section 4.3.1.1 on microscopic methods.

4.3.1.2. Agent isolation and identification*4.3.1.2.1. Cell culture/artificial media**Preparation of fish tissues for virus isolation and ELISA*

A simple method for preparation of fish tissues for cell culture and ELISA has been validated (Whittington & Steiner, 1993) (see sampling Section 3.1).

- i) Freeze tubes containing tissues at –80°C until needed.
- ii) Add 0.5 ml of homogenising medium (minimal essential medium Eagle, with Earle's salts with glutamine) [MEM] with 200 International Units [IU] ml⁻¹ penicillin, 200 µg ml⁻¹ streptomycin and 4 µg ml⁻¹ amphotericin B) to each tube. Grind tissue to a fine mulch with a sterile fitted pestle.
- iii) Add another 0.5 ml of homogenising medium to each tube and mix with a pestle.
- iv) Add three sterile glass beads to each tube (3 mm diameter) and close the lid of the tube.
- v) Vortex the suspension vigorously for 20–30 seconds and place at 4°C for 2 hours.
- vi) Vortex the suspension again as above and centrifuge for 10 minutes at 2500 **g** in a benchtop microcentrifuge.
- vii) Transfer the supernatant, now called clarified tissue homogenate, to a fresh sterile tube. Homogenates may be frozen at –80°C until required for virus isolation and ELISA.

Cell culture/artificial media

~~Cell culture is the gold standard test but is costly and time consuming.~~ EHNV grows well in many fish cell lines including BF-2 (bluegill fry ATCC CCL 91), FHM (fathead minnow; ATCC CCL 42), EPC (epithelioma papulosum cyprini [Cinkova *et al.*, 2010]), and CHSE-214 (Chinook salmon embryo cell line; ATCC CRL 1681) at temperatures ranging from 15 to 22°C (Crane *et al.*, 2005). Incubation temperatures of 20°C or 24°C result in higher titres than 15°C; 22°C and BF-2 EPC or CHSE-214 cells are recommended to maximise titres, which might be important for the detection of low numbers of viruses in fish tissues (Ariel *et al.*, 2009). BF-2 cells are preferred by the OIE Reference Laboratory with an incubation temperature of 22°C ~~both before and after inoculation with virus has been recommended for many years.~~ The procedure for BF-2 cells is provided below. A procedure for CHSE-214 cells is provided under immunoperoxidase staining below (Section 4.3.1.2.2). The identity of viruses in cell culture is determined by immunostaining, ELISA, immuno-electron microscopy, polymerase chain reaction (PCR) or other methods.

Samples: tissue homogenates.

Cell culture technical procedure: cells are cultured (in flasks, tubes or multi-well plates) with growth medium (MEM + 10% fetal calf serum [FCS] with 100 IU ml⁻¹ penicillin, 100 µg ml⁻¹ streptomycin and 2 µg ml⁻¹ amphotericin B). The cells are incubated until almost confluent at 22°C, which can take up to 4 days depending on the seeding rate. Medium is changed to a maintenance medium (MEM with 2% FCS and 100 IU ml⁻¹ penicillin, 100 µg ml⁻¹ streptomycin and 2 µg ml⁻¹ amphotericin B) on the day of inoculation. A 1/10 dilution using homogenising medium is made of single or pooled homogenates. Each culture is inoculated with 100 µl of sample per ml of culture medium. This represents a final 1/100 dilution of a 0.1 mg ml⁻¹ tissue homogenate. A further 1/10 dilution is made representing a final 1/1000 dilution, and two cultures are inoculated. No adsorption step is used. As an alternative, two to three cultures can be inoculated directly with 10 µl undiluted homogenate per ml of culture medium. Note that a high rate of cell toxicity or contamination often accompanies the use of a large undiluted inoculum. The cultures are incubated at 22°C in an incubator for 6 days. Cultures are read at day 3 and day 6. Cultures are passed at least once to detect samples with low levels of virus. On day 6, the primary cultures (P1) are frozen overnight at -20°C, thawed, gently mixed and then the culture supernatant is inoculated onto fresh cells as before (P2), i.e. 100 µl P1 supernatant per ml culture medium. Remaining P1 supernatants are transferred to sterile 5 ml tubes and placed at 4°C for testing by ELISA or PCR or another means to confirm the cause of cytopathic effect (CPE) as EHNV. P2 is incubated as above, and a third pass is conducted if necessary.

Interpretation of results

CPE is well developed and consists of focal lysis surrounded by rounded granular cells. This change extends rapidly to involve the entire monolayer, which detaches and disintegrates.

4.3.1.2.2. Antibody-based antigen detection methods

It should be noted that polyclonal antibodies used in all related methods (immunoperoxidase, antigen-capture ELISA and immunoelectron microscopy) cross-react with all known ranaviruses except Santee Cooper ranaviruses (Ahne *et al.*, 1998; Cinkova *et al.*, 2010; Hedrick *et al.*, 1992; Hyatt *et al.*, 2000).

4.3.1.2.2.1. Detection of EHNV using immunoperoxidase staining of infected cell cultures

Principle of the test: EHNV replicates within cultured cells. The addition of a mild detergent permeabilises the cells allowing an affinity purified rabbit antibody to bind to intracellular viral proteins. EHNV is detected by a biotinylated anti-species antibody and a streptavidin-peroxidase conjugate. The addition of a substrate results in 'brick-red' staining in areas labelled with antibodies.

Samples: tissue homogenates.

Operating characteristics: when performed as described in this protocol, the staining is conspicuous and specific. However, the test has not been validated with respect to sensitivity or reproducibility.

Preparation of cells: the procedure described below is for CHSE-214 cells. Other recommended cell lines can also be used.

Annex 23 (contd)

- i) CHSE-214, 24-well plates are seeded the day before use with 250,000 cells/well (or 4 million cells in 40 ml of growth medium per plate) in 1.5 ml of growth medium (Earle's MEM with non-essential amino acids [EMEM], 10% FCS, 10 mM N-2-hydroxyethyl-piperazine-N-2-ethanesulfonic acid [HEPES], 2 mM glutamine, 100 IU penicillin and 100 µg streptomycin) and incubated in 5% CO₂ at 22°C overnight. (NOTE: cultures must be nearly confluent and have healthy dividing cells prior to use.)
- ii) Discard the medium, inoculate each well with 150 µl of a 10% suspension of ground tissue (e.g. liver, kidney or spleen), incubate for 1 hour (22°C) then add 1.5 ml of fresh maintenance medium (as for growth medium except 2% FCS) and return to the incubator (22°C).
- iii) Observe cultures for CPE. If no CPE occurs by day 10, pass the cultures on to fresh CHSE cells by collecting the cells and medium and adding 150 µl to the cells of the fresh plate; note that cells are not freeze-thawed. There is no need to discard the existing medium, just return the new plate to the incubator (22°C). Again, observe daily for CPE.
- iv) Fix cells (add 50 µl for 96-well plate cultures with 200 µl culture medium/well or 400 µl (for 24-well plate cultures with 1.6 ml culture medium/well) of a 20% formalin solution to each well), without discarding the culture medium when CPE is first observed. After incubation (22°C) for 1 hour at room temperature (RT), the medium/formalin mixture is discarded and the wells are rinsed twice with PBS-A (phosphate buffered saline, Ca⁺⁺ and Mg⁺⁺ free) to remove the formalin. More PBS-A is added if the plates are to be stored at 4°C.

Protocol

- i) Dilute primary anti-EHNV antibody and normal serum to working strength as described below (fixation protocol for immunocytochemistry) for the relevant agent in 1% skim milk (SM) solution (PBS-A [SM]) to the volume required for the test.
- ii) Remove PBS-A from wells (with fixed cell cultures) and wash wells twice with 0.05% (v/v) PBS/Tween 20 (PBST). Add 50 µl of primary antibody solutions to each well in a 96-well plate well or 200 µl in a 24-well plate well. Incubate on a plate shaker at 100–200 rpm at RT (22–24°C) for 15–30 minutes or without shaking at 37°C for 1 hour.
- iii) Dilute biotinylated anti-species serum (secondary antibody) in 0.1% SM solution as described in the fixation protocol (below) for the relevant agent to the volume required for the test.
- iv) Remove primary antibody solution and wash wells three times with PBST. Add secondary antibody to all wells. Incubate on a plate shaker at 100–200 rpm at RT for 15–30 minutes or without shaking at 37°C for 1 hour.
- v) Dilute streptavidin–peroxidase conjugate in 0.1% SM solution for the relevant agent to the volume required for the test.
- vi) Remove secondary antibody from wells and wash wells three times with PBST. Add conjugate to each well. Incubate on a plate shaker at 100–200 rpm at RT for 15–30 minutes or without shaking at 37°C for 1 hour.
- vii) Prepare stock substrate of 3-amino-9-ethylcarbazole (AEC) solution: dissolve one AEC tablet (20 mg) in 2.5 ml of dimethyl formamide.
- viii) Remove conjugate from wells. Wash (three times) with PBST.
- ix) Dilute dissolved AEC stock in 47.5 ml of acetate buffer (4.1 ml anhydrous sodium acetate in 1 litre of deionised water; the pH is adjusted to 5.0 with glacial acetic acid). Just before use, add 25 µl 30% hydrogen peroxide to AEC solution then add to each well. Incubate at RT for 20 minutes.
- x) Remove substrate solution and wash wells twice with deionised water to stop reaction.
- xi) To visualise all cells counterstain with Mayer's haematoxylin (50 µl/well or 200 µl/well) for 1 minute and rinse with deionised water.
- xii) Add 50 µl Scott's tap water and rinse with deionised water and air dry.

Interpretation of the results

Positive reaction: granular-like, focal, brick-red staining of cells indicates presence of virus identified by the diagnostic antibody.

Negative reaction: no red staining apparent – all cells should be stained pale blue due to counterstain.

Background staining: nongranular, nonfocal, more generalised, pale, pinkish staining may occur throughout the culture. This background staining could be caused by any number of reasons, e.g. nonspecific antibody reaction with nonviral components, inefficient washing, and expiration of other reagents.

*Reagents for immunocytochemistry tests**20% Formaldehyde (PBS-A) saline*

Formalin (36–38% formaldehyde)	54 ml
Distilled water	36 ml
10 × PBS-A	10 ml

10 × PBS-A

To make up 1 litre of 10 × PBS-A use:

NaCl	80.0 g
Na ₂ HPO ₄	11.5 g
KCl	2.0 g
KH ₂ PO ₄	2.0 g
Distilled water	1.0 litre

NOTE: some salts are supplied with extra water groups. If using these reagents adjust the masses to ensure the appropriate mass of salt is added, e.g. for Na₂HPO₄·2H₂O add 15 g instead of 11.5 g (156 mw/120 mw × 11.5 g = 14.95 g) to remove the effect of the water molecules.

4.3.1.2.2.2 Detection of EHNV using antigen-capture ELISA

Antigen-capture ELISA has been validated to detect EHNV in cell cultures and directly in fish tissue homogenates. The analytical sensitivity is 10³ to 10⁴ TCID₅₀ ml⁻¹. Specificity approaches 100% and sensitivity for direct detection in fish tissues is 60% relative to the gold standard of virus isolation in BF-2 cells (Hyatt *et al.*, 1991; Whittington & Steiner, 1993) and unpublished data). ELISA is useful for both diagnosis and certification. Neutralisation tests cannot be used to identify EHNV because neutralising antibodies are not produced following immunisation of mammals or fish. Mouse monoclonal antibodies produced against EHNV are directed against major capsid protein (MCP) epitopes and are non-neutralising (unpublished data). Rabbit-anti-EHNV antibodies have been developed for use in antigen-capture ELISA, immunoperoxidase staining and immunoelectron microscopy (Hengstberger *et al.*, 1993; Hyatt *et al.*, 1991; Reddacliff & Whittington, 1996). Reagents and protocols are available from the reference laboratory.

Samples: tissue homogenate samples prepared using a validated the protocol (see below), and cell cultures.

Principle of the test: EHNV particles are captured from the sample by an affinity purified rabbit antibody that is coated to the plate. EHNV is detected by a second antibody and a peroxidase-labelled conjugate using the chromogen ABTS (2,2'-azino-di-[3-ethyl-benzthiazoline]-6-sulphonic acid). The enzyme is inactivated after 20 minutes and the resulting optical density (OD) is compared with standards.

Operating characteristics: the protocol is based on published procedures (Hyatt *et al.*, 1991; Steiner *et al.*, 1991; Whittington, 1992; Whittington & Steiner, 1993). When performed as described in this protocol, the operating characteristics of the test are as given in Table 4.1. The precision of the assay is <10% coefficient of variation, measured as variation in the OD of the controls between plates and over time, when the recommended normalisation procedure is followed.

Annex 23 (contd)

Table 4.1. EHNV ELISA operating characteristics compared with the ~~gold standard of cell culture virus isolation~~ in BF-2 cells

Sample	Positive-negative cut-off**	Sensitivity %	Specificity %
Tissues of fish*	OD 0.5	60	>99
Tissue culture supernatants with cytopathic effect (BF2 cells)	OD 0.3	>99	>99

*~~Redfin~~ European perch and rainbow trout only. Higher background OD occurs with golden perch. There are no data for other species.

** these cut-offs are determined by the OIE Reference Laboratory for EHNV and will vary with the batch of control antigen. Values above are for batch 86/8774-4-5-01.

Test components and preparation of reagents

- i) Flat bottom microtitre plates are required.
- ii) Affinity purified rabbit anti-EHNV immunoglobulin and sheep anti-EHNV antiserum reagents are supplied in freeze-dried form. Reconstitute using 1 ml of purified water and allow the vial to stand at RT for 2 minutes. Mix the vial very gently. These reagents are stable when stored at -20°C for at least 4 years. For routine use in ELISA, it is recommended that working stocks of both antibodies be prepared as a 1/10 dilution in Tris saline glycerol merthiolate (TSGM; formula at end of this section). These are stable at -20°C for at least 5 years and do not solidify at this temperature.
- iii) The peroxidase labelled anti-sheep immunoglobulin conjugate (commercial reagent, KPL #14-23-06; 0.5 mg) is supplied as a freeze-dried powder. This reagent has displayed remarkable consistency in activity between different lots over a period of 15 years. The product should be reconstituted in sterile 50% glycerol water, dispensed in 150 μl aliquots and stored at -20°C as undiluted stock. A working stock is prepared by adding 900 μl of TSGM to 100 μl of undiluted stock. The working stock is also stored at -20°C and is stable for at least 1 year. New batches of this conjugate should be titrated against an older batch using standard protocols.
- iv) EHNV control antigen, heat-inactivated, is supplied as freeze-dried powder. Reconstitute in 1 ml sterile water and store in small aliquots at -20°C . Prepare dilutions using PBSTG (PBS + Tween + gelatin) on the same day the test is performed. Control EHNV antigen dilutions (A, B, D and F) cover the range of the signal response of the assay and enable a normalisation procedure to be undertaken.

Equipment

An automatic plate washer is recommended although plates can be washed by hand. The assay is sensitive to plate washing conditions. If the OD of the controls is unexpectedly low, and the conjugate and other reagents are within date, the plate washer should be adjusted so that washing pressure during filling of wells and aspiration of wells is minimised.

An automatic plate reader is recommended although plates can be read by eye.

Precision calibrated pipettes (e.g. Gilson) should be used to prepare dilutions of all reagents and to load reagents into microtitre plate wells.

Protocol

- i) Coat a 96-well ELISA plate (100 μl /well) with affinity purified rabbit-anti-EHNV diluted 1/12,800 in borate coating buffer. Incubate overnight at 4°C .
- ii) Wash plate five times with wash buffer (Milli-Q [MQ] purified water plus 0.05% Tween 20). Note that distilled and deionised water can also be used in this and all other steps.
- iii) Prepare a blocking solution: warm the solutions in a microwave oven or water bath to dissolve the gelatin, then cool to RT.
- iv) Block remaining binding sites using blocking solution (100 μl /well) (1% [w/v] gelatin in PBSTG diluent [PBS, 0.05% [v/v] Tween 20, 0.1% [w/v] gelatin]). Incubate at RT for 30 minutes.

Annex 23 (contd)

- v) Wash plate five times as above.
- vi) Work in a Class II biological safety cabinet. Dilute the control antigen (see below) in PBSTG and add to the lower right-hand corner of the plate. Add tissue homogenate samples or culture supernatant samples and control antigens at 100 µl/well. All samples and controls are added to duplicate wells. Incubate for 90 minutes at RT.

The control antigens are dilutions of a heat killed cell culture supernatant of EHNV 86/8774. The controls are expected to give the following OD, although there will be some variation from laboratory to laboratory and ±10% variation should therefore be allowed:

Control	Dilution in PBS*	OD (405 nm)*
A	1/5	>2.0
B	1/40	1.90
D	1/200	0.68
F	1/3000	0.16

*These dilutions and OD values are determined by the OIE Reference Laboratory for infection with EHNV and will vary with the batch of control antigen. The values above are for batch 86/8774-4-5-01. The positive-negative cut-off for clarified tissue homogenate samples from ~~redfin~~ European perch and rainbow trout in this ELISA is approximated by the OD value of control D on each plate.

- vii) Wash the plate by hand to avoid contamination of the plate washer. Work in a Class II cabinet. Aspirate wells using a multichannel pipette. Rinse the plate twice.
- viii) Wash the plate five times on the plate washer, as above.
- ix) Add the second antibody sheep-anti-EHNV diluted 1/32,000 in PBSTG (100 µl/well). Incubate for 90 minutes at RT.
- x) Wash the plate five times on the plate washer.
- xi) Add the conjugate diluted 1/1500 in PBSTG (100 µl/well). Incubate for 90 minutes at RT.
- xii) Wash the plate five times on the plate washer.
- xiii) Add ABTS substrate (22 ml ABTS + 10 µl H₂O₂) (100 µl/well) and place the plate on a plate shaker. Time this step from the moment substrate is added to the first wells of plate 1. Incubate for 20 minutes.
- xiv) Immediately add ABTS stop solution (50 µl/well), shake the plate briefly and read OD at 405 nm. Calculate mean ELISA OD of duplicate wells. Calculate the coefficient of variation of the duplicates: samples with CV >15% should be retested if the mean OD lies near the positive-negative cut-off.

Normalisation of data and decision limit quality control

If it is desired to normalise data from plate to plate and over time, or to undertake decision limit quality control, the following procedure can be followed. Run control antigens in ELISA on at least five occasions over a period of 3 weeks (a total of 20 separate ELISA plates). Calculate the mean OD for each control antigen. Then, for each plate subsequently used, calculate a plate correction factor (PCF) as follows:

PCF = (mean OD control A/actual OD + mean OD control B/actual OD + mean OD control D/actual OD + mean OD control F/actual OD)/4. Multiply the actual mean OD of each sample by the PCF for that plate and report these values.

PCF is allowed to vary between 0.8 and 1.2, which approximates to a coefficient of variation of 10%. Values outside this range suggest that a plate needs to be retested. Plots of PCF over time provide a ready means for monitoring the stability of reagents, procedural variations and operator errors. This QC method has been validated for antigen capture ELISA.

Annex 23 (contd)*Buffers and other reagents**Borate coating buffer*

Boric acid	6.18 g
Disodium tetraborate ($\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$)	9.54 g
NaCl	4.38 g
MQ water to	1 litre
Autoclave	

10 x phosphate buffered saline

NaCl	80.00 g
KCl	2.00 g
Na_2HPO_4	11.50 g
KH_2PO_4	2.00 g
MQ water to	900 ml
Adjust pH to 7.2 with HCl or NaOH; make up to 1 litre	
Autoclave	

For working strength dilute 1/10 and recheck pH.

For storage of powder in jars, make up twice the above quantity of powder; store; to make up add 1.8 litres MQW, pH, make up to 2 litres.

ABTS

Citrate phosphate buffer	
Citric acid	21.00 g
Na_2HPO_4	14.00 g
MQ water to 800 ml; adjust pH to 4.2; make up to 1 litre	
ABTS	0.55 g
Citrate phosphate buffer to	1 litre
Dispense in 22-ml aliquots and freeze.	
Immediately prior to use add 10 μl H_2O_2 per 22-ml aliquot.	

ABTS stop solution (0.01% NaN_3 in 0.1 M citric acid)

Citric acid	10.5 g
MQW to	500 ml
Add 50 mg sodium azide or 1 ml of 5% solution.	

*KPL Conjugate #14-23-06²**TSGM cryoprotectant*

10 x Tris/saline, pH 7.4	50 ml
Glycerol	250 ml
Sterile purified water to	500 ml
Autoclave	
Add 10% Merthiolate	1 ml
Store in dark bottle at 4°C.	

10 x Tris/saline (250 mM Tris, 1.5 M NaCl)

Tris	15.14 g
NaCl	43.83 g
Sterile purified water	500 ml
pH adjust to	7.4

2 Reagent Supplier: Bio-Mediq DPC Australia, P.O. Box 106, Doncaster, Victoria 3108, Australia; Tel.: (+61-3) 9840 2767; Fax: (+61-3) 9840 2767. Visit: www.kpl.com for links to worldwide network distributors. Reference to specific commercial products as examples does not imply their endorsement by the OIE. This applies to all commercial products referred to in this *Aquatic Manual*.

4.3.1.2.2.3. Immunoelectron microscopy

Gold-labelling of sections containing tissues or cell cultures

Principle of the test: cell cultures, tissues and/or tissue homogenates can be used for examination by electron microscopy. Conventional electron microscopy (examination of ultra-thin sections) will generate data on virus structure and morphogenesis. Negative contrast electron microscopy will produce images that can be used to examine the particulate structure of the virus. The use of ranavirus-specific antibodies and conjugated gold with these preparations permits both ultrastructure and antigenicity to be examined (Hyatt, 1991). These collective data enable classification to the genus Ranavirus.

Cell cultures and tissues

- i) Fix tissues or cell cultures as described in Drury *et al.*, 2002. Briefly, 2.5% (v/v) buffered glutaraldehyde (cacodylate or phosphate) is used to fix cells for 40 minutes. Following primary fixation the cells are rinsed in the same buffer (3 x 20 minutes), post-fixed in 1% (w/v) buffered osmium tetroxide (1 hour), washed (3 x 5 minutes) in double-distilled/reverse osmosis (RO) water, dehydrated through graded alcohol (70–100%) and infiltrated and embedded in an epoxy resin (e.g. Spurr's or epon). For gold labelling of ultra-thin resin sections, attention must be given to fixation and embedding regimes. For example, cells should be fixed in 0.25% (v/v) glutaraldehyde with 2–4% paraformaldehyde. No secondary fixation is used and the cells are infiltrated and embedded in an acrylic resin such as LR White.
- ii) Following fixation and embedding, cut and transfer ultrathin sections onto filmed nickel grids.
- iii) Cut sections from the appropriate blocks.
- iv) Block in 2% (w/v) skim milk powder in PBS-A (10 minutes).
- v) Block free aldehydes with 0.1 M glycine in PBS-A (20 minutes).
- vi) Wash in PBS-A (3 x 1 minutes). This is an optional step used only if there is an excess of free aldehydes (a high background may be indicative of this).
- vii) If protein A-gold is not being used then block in normal species serum – this serum should be homologous to that complexed to gold. Recommended dilution is approximately 1/40 (10 minutes).
- viii) Incubate in primary antibody. If incubation details are unknown then perform initial reactions with 1/100 to 1/2700 dilutions (with three-fold dilutions). Dilute antibodies in 1% (v/v) cold water fish gelatin in PBS-A, (60 minutes, RT).
- ix) Rinse in 1% (v/v) coldwater fish gelatin in PBS-A, (6 x 3 minutes).
- x) Incubate in gold-labelled secondary antibody or protein A-gold or protein G-gold. Suggested dilution 1/40 in a PBS-A containing 1% (w/v) bovine serum albumin (BSA), 0.1% (v/v) Tween 20 and 0.1% (v/v) Triton X, 60 minutes, RT.
- xi) Rinse in PBS-A (6 x 3 minutes, RT).
- xii) Post-fix in 2.5% (v/v) glutaraldehyde in PBS-A (5 minutes, RT).
- xiii) Rinse in water (RO) (3 x 3 minutes, RT).
- xiv) Dry on filter paper (type not critical).
- xv) Stain in uranyl acetate and lead acetate.

Interpretation of results

Viruses within the cytoplasm of infected cells will be specifically gold-labelled. Viruses will be located singularly, within assembly bodies (inclusion bodies) and within paracrystalline arrays.

Gold-labelling of virus particles (viruses adsorbed to grids)

- i) Dounce homogenise 10% (w/v) liver, kidney or spleen and clarify (5 minutes, 2500 g).
- ii) Adsorb the supernatant (from homogenate or cell cultures) to grid substrate.
- iii) Use carbon-coated 200 mesh gold grids.

Annex 23 (contd)

- iv) Fix the sample with 0.1% (v/v) glutaraldehyde and 1% (v/v) Nonidet P40 (NP40) in PBS (2 minutes).
- v) Wash in PBS (3 × 3 minutes).
- vi) Block with 5% (v/v) cold water fish gelatin (Sigma) in PBS (10 minutes) followed with incubation buffer (PBS/0.1% cold water fish gelatin).
- vii) Incubate with antibody (affinity purified rabbit anti-EHNV, Lot No. M708; supplied by the OIE Reference Laboratory; suggested dilution 1/500) for 1 hour, at RT.
- viii) Wash grids (6 × 3 minutes) in incubation buffer.
- ix) Incubate with 10 nm protein A-gold (for dilution, refer to suppliers recommendation) for 1 hour, at RT.
- x) Wash (6 × 3 minutes).
- xi) Fix with 2.5% glutaraldehyde (5 minutes).
- xii) Wash with distilled water (3 × 3 minutes) and stain with 2% phosphotungstic acid (pH 6.8) for 1 minute.

Interpretation of results

The inclusion of NP40 will permit antibodies and protein A-gold to penetrate the outer membrane and react with the underlying capsid. Labelling should be specific for the virus. Non-EHNV affinity purified rabbit serum (1/500) should be included as a negative control.

4.3.1.2.2.4. Immunohistochemistry (immunoperoxidase stain)

Samples: formalin-fixed paraffin-embedded tissue sections.

Technical procedure

The following protocol is intended for the qualitative demonstration of EHNV antigens in formalin-fixed paraffin-embedded tissue sections (Reddacliff & Whittington, 1996). It assumes that antigens may have become cross linked and therefore includes a protease digestion step that may be omitted if unfixed samples are examined. A commercial kit (DAKO[®] LSAB K0679) with peroxidase-labelled streptavidin and a mixture of biotinylated anti-rabbit/anti-mouse/anti-goat immunoglobulins as link antibodies is used for staining. Other commercially supplied reagents are also used. For convenience these are also supplied by DAKO³. The primary affinity purified rabbit anti-EHNV antibody (Lot No. M708) is supplied freeze-dried by the OIE Reference Laboratory.

- i) Cut 5 µm sections and mount on SuperFrost[®] Plus G/Edge slides (Menzel-Glaser, HD Scientific Cat. No. HD 041300 72P3). Mark around the section with a diamond pencil to limit the spread of reagents.
- ii) Deparaffinise the section:
Preheat slides in a 60°C incubator for 30 minutes.
Place slides in a xylene bath and incubate for 5 minutes. Repeat once. Note that xylene replacements can be used without deleterious effects.
Tap off excess liquid and place slides in absolute ethanol for 3 minutes. Repeat once.
Tap off excess liquid and place slides in 95% ethanol for 3 minutes. Repeat once.
Tap off excess liquid and place slides in distilled or deionised water for 30 seconds.
- iii) Expose antigens using a protease treatment. Flood slide with proteinase K (5–7 µg ml⁻¹) and incubate for 20 minutes (ready-to-use solution, DakoCytomation Cat. No. S3020). Rinse slide by immersing three times in water. Place in a PBST bath for 5 minutes (PBS pH 7.2, 0.05% [v/v] Tween 20). Tap off the excess wash solution and carefully wipe around the section.

3 Dako Cytomation California Inc., 6392 via Real, Carpinteria, CA 93013, USA, Tel.: (+1-805) 566 6655, Fax: (+1-805) 566 6688; Dako Cytomation Pty Ltd, Unit 4, 13 Lord Street, Botany, NSW 2019, Australia, Fax: (+61-2) 9316 4773; Visit www.dakocytomation.com for links to other countries.

Annex 23 (contd)

- iv) Perform the immunostaining reaction using the Universal DAKO LSAB[®]+ Kit, Peroxidase (DakoCytomation Cat No. K0679). Ensuring the tissue section is completely covered, add the following reagents to the slide. Avoid drying out.
- v) 3% hydrogen peroxide: cover the section and incubate for 5 minutes. Rinse gently with PBST and place in a fresh wash bath.
- vi) Primary antibody (affinity purified rabbit anti-EHNV 1:/1500 Lot No. M708) and negative control reagent (non-immune rabbit serum at a dilution of 1/1500) on a second slide. Cover the section and incubate for 15 minutes. Rinse slides.
- vii) Link: cover the section and incubate for 15 minutes. Rinse slides.
- viii) Streptavidin peroxidase: cover the section and incubate for 15 minutes. Rinse slides.
- ix) Substrate–chromogen solution: cover the section and incubate for 5 minutes. Rinse slides gently with distilled water.
- x) Counterstain by placing slides in a bath of DAKO[®] Mayer's Haematoxylin for 1 minute (Lillie's Modification, Cat. No. S3309). Rinse gently with distilled water. Immerse 10 times into a water bath. Place in distilled or deionised water for 2 minutes.
- xi) Mount and cover-slip samples with an aqueous-based mounting medium (DAKO[®] Faramount Aqueous Mounting Medium Cat. No. S3025).

Interpretation of results

EHNV antigen appears as a brown stain in the areas surrounding degenerate and necrotic areas in parenchymal areas. There should be no staining with negative control rabbit serum on the same section.

Availability of test and reagents: antibody reagents and test protocols are available from the OIE Reference Laboratory.

4.3.1.2.3. Molecular techniques

Although several conventional PCR or quantitative real-time PCR methods have been described, none has been validated according to OIE guidelines for primary detection of EHNV or other ranaviruses in fish tissues. However, identification of ranavirus at genus and species level is possible using several published PCR strategies. In the first method described here, two PCR assays using MCP primers are used with restriction analysis to detect and rapidly differentiate EHNV from the European (ECV), North American (FV3) and other Australian ranaviruses (BIV) (Marsh *et al.*, 2002). This can be completed in less than 24 hours at relatively low cost. In the second method described here, a single MCP PCR assay is used to generate a 580 bp product, which is then sequenced to identify the type of ranavirus. Alternatively, PCR of the DNA polymerase gene and neurofilament triplet H1-like protein genes can be used (Holopainen *et al.*, 2011) (this method is not described in this chapter).

Samples: virus from cell culture or direct analysis of tissue homogenate.

4.3.1.2.3.1. PCR and restriction endonuclease analysis (REA): technical procedure

Amplified product from PCR assay MCP-1 digested with Pflm I enables differentiation of Australian iridoviruses (EHNV and BIV) from non-Australian iridoviruses (FV3, Americas; and ECV, Europe). Amplified product from PCR assay MCP-2 digested with Hinc II, Acc I and Fnu4H I (individually) enables differentiation of EHNV and BIV (Australia) from each other and from FV3 (Americas) and ECV (Europe).

Preparation of reagents

EHNV-purified DNA and BIV-purified DNA PCR control reagents are supplied by the reference laboratory in freeze-dried form. Reconstitute using 0.5 ml of Tris-EDTA (TE) buffer (10 mM Tris/HCl, 1 mM EDTA, pH 8.0) and allow the vial to stand at RT for 2 minutes. Mix the vial very gently. For routine use, as a PCR control, it is recommended that working stocks be prepared as a 1/10 dilution in TE buffer (pH 8.0). Aliquots of 250 µl should be stored at –20°C. Each aliquot is sufficient for at least 50 reactions (1 to 5 µl added to cocktail) and has a minimum shelf life of 6 months from date of diluting.

Annex 23 (contd)

Primers M151 and M152 (MCP-1, 321 bp), M153 and M154 (MCP-2, 625 bp) are supplied in working strength ($100 \text{ ng } \mu\text{l}^{-1}$) and should be stored at -20°C . Primers can also be ordered from commercial suppliers. For primer sequences, refer to Table 4.2.

Table 4.2. MCP-1 and MCP-2 primer sequences

PCR assay	Primer	Sequence	Product size	Gene location
MCP-1	M151	AAC-CCG-GCT-TTC-GGG-CAG-CA	321 bp	266–586
	M152	CGG-GGC-GGG-GTT-GAT-GAG-AT		
MCP-2	M153	ATG-ACC-GTC-GCC-CTC-ATC-AC	625 bp	842–1466
	M154	CCA-TCG-AGC-CGT-TCA-TGA-TG		

PCR cocktail

Amplification reactions in a final volume of $50 \mu\text{l}$ (including $5 \mu\text{l}$ DNA sample) contain $2.5 \mu\text{l}$ (250 ng) of each working primer, $200 \mu\text{M}$ of each of the nucleotides dATP, dTTP, dGTP and dCTP, $5 \mu\text{l}$ of $10 \times$ PCR buffer (66.6 mM Tris/HCl, 16.6 mM $(\text{NH}_4)_2\text{SO}_4$, 2.5 mM MgCl_2 , 1.65 mg ml^{-1} BSA, 10 mM beta-mercaptoethanol) and 2 U Taq polymerase. Instructions on preparation of $10 \times$ PCR buffer are included in Table 4.3.

Table 4.3. $10 \times$ PCR buffer preparation

Ingredients	Amount	Final concentration in $50 \mu\text{l}$ PCR mix
Tris	4.050 g	66.6 mM
Ammonium sulphate	1.100 g	16.6 mM
BSA (albumin bovine fraction V fatty acid free)	0.825 g	1.65 mg ml^{-1}
Magnesium chloride	1.25 ml	2.5 mM
TE buffer (sterile)	50 ml	

NOTE: alternative commercial buffers may also be used.

Two negative controls are included, one comprising PCR cocktail only and the second containing $5 \mu\text{l}$ TE buffer.

The MCP-1 and MCP-2 reactions have the following profile: 1 cycle of denaturation at 94°C for 3 minutes, followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing at 50°C for 30 seconds and extension at 72°C for 1 minute; a final extension of 72°C for 5 minutes, and cooling to 4°C .

NOTE: the annealing temperature may be increased to 60 or 62°C to reduce nonspecific amplification when the assay is used to test fish tissues.

PCR results are assessed by electrophoresis in 2% agarose gels stained with ethidium bromide. EHNV PCR control DNA ($1/10$ working stock) should give a result similar in intensity to the 10–3 band in both cases.

Restriction endonuclease analysis (REA)

PCR amplicons are subjected to REA with the enzymes described in Table 4.4. All endonucleases should be used according to the manufacturers' instructions. REA reactions are prepared by adding $4 \mu\text{l}$ of PCR product, 2 U of the appropriate restriction endonuclease, $1.6 \mu\text{l}$ of buffer (supplied with each restriction endonuclease), $1.6 \mu\text{l}$ of $100 \mu\text{g ml}^{-1}$ BSA (for PflM I and Hinc II) and made up to a final volume of $16 \mu\text{l}$ with sterile purified water. Restriction digests are incubated for 2–4 hours at the recommended temperatures and assessed by agarose gel electrophoresis in 3% gels. The predicted band sizes after restriction are given in Table 4.4.

Table 4.4. Restriction endonuclease analysis of ranavirus MCP amplicons

PCR Assay	Restriction enzyme	Predicted band sizes after restriction (bp)	Pattern applies to
MCP-1 (321bp)	<i>PfiI</i>	321	EHN, BIV
		131, 190	FV3, WV
MCP-2 (625bp)	<i>HincII</i>	100, 138, 387	EHN
		100, 525	BIV, FV3
		100, 240, 285	WV
	<i>AccI</i>	238, 387	EHN
		625	BIV, ESV, ECV, WV
		164, 461	FV3, GV
	<i>Fnu4HI</i>	33, 38, 44, 239, 271	EHN
		3, 33, 38, 44, 108, 399	BIV
		3, 38, 44, 108, 432	FV3, GV
		3, 9, 38, 44, 108, 151, 272	ESV, ECV
3, 44, 71, 108, 399		WV	

Aliquot into 500 µl volumes and store at -20°C. For a working solution, add 3.5 µl of beta-mercaptoethanol per 500 µl 10 × buffer. Any remaining buffer should be discarded after preparing the PCR cocktail.

The sensitivity of PCR in diagnostic applications directly on fish tissues is being evaluated.

Detailed protocols to enable completion of the test, worksheets and purified control EHN DNA are available from the OIE Reference Laboratory.

4.3.1.2.3.2. Alternative PCR and sequencing for viral identification

In this assay two primers, a reverse primer (5'-AAA-GAC-CCG-TTT-TGC-AGC-AAA-C-3') and a forward primer (5'-CGC-AGT-CAA-GGC-CTT-GAT-GT-3'), are used for amplification of the target MCP sequence (580 base pairs [bp]) of EHN DNA by PCR. This PCR procedure can be used for the specific detection of ranaviruses from ~~redfin~~ European perch, rainbow trout, sheatfish, catfish, guppy fish (*Poecilia reticulata*), doctor fish (*Labroides dimidiatus*) and a range of amphibian ranaviruses (Hyatt *et al.*, 2000). Nucleic acid (1 µl) is added to Taq polymerase buffer containing 0.1 µM of each primer, 2.5 U Taq polymerase (Promega) and 2.5 mM MgCl₂. The mixture is incubated in an automatic thermal cycler programmed for 35 cycles at 95°C for 60 seconds, 55°C for 60 seconds, and 72°C for 60 seconds, and finally held at 72°C for 15 minutes. Amplified DNA (580 bp) is analysed by agarose gel electrophoresis, excised and sequenced using a range of standard technologies). Each viral species is identified by its unique DNA sequence available from GenBank. Samples can be submitted to the OIE reference laboratory for specific identification.

4.3.1.2.4. Agent purification

Purification of EHN has been described (Hyatt *et al.*, 1991; Steiner *et al.*, 1991) and a protocol is available from the reference laboratory.

Annex 23 (contd)**4.3.2. Serological methods**

Neutralising antibodies have not been detected in fish or mammals exposed to EHN. Indirect ELISA for detection of antibodies induced following exposure to EHN has been described for rainbow trout and ~~redfin~~ European perch (Whittington *et al.*, 1994; 1999; Whittington & Reddacliff, 1995). The sensitivity and specificity of these assays in relation to a ~~gold~~ standard test are not known and interpretation of results is currently difficult. Protocols and specific anti-immunoglobulin reagents required to conduct these tests are available from the reference laboratory.

5. Rating of tests against purpose of use

The methods currently available for surveillance, detection, and diagnosis of infection with EHN are listed in Table 5.1. The designations used in the Table indicate: a = the method is the recommended method for reasons of availability, utility, and diagnostic specificity and sensitivity; b = the method is a standard method with good diagnostic sensitivity and specificity; c = the method has application in some situations, but cost, accuracy, or other factors severely limits its application; d = the method is presently not recommended for this purpose; and NA = not applicable. These are somewhat subjective as suitability involves issues of reliability, sensitivity, specificity and utility. Although not all of the tests listed as category a or b have undergone formal standardisation and validation (see Chapter 1.1.2 *Principles and methods of validation of diagnostic assays for infectious diseases*), their routine nature and the fact that they have been used widely without dubious results, makes them acceptable.

Table 5.1. Methods for targeted surveillance and diagnosis

Method	Targeted surveillance				Presumptive diagnosis	Confirmatory diagnosis
	Ova/ milt	Fry/ fingerlings	Juveniles	Adults		
Gross signs	n/a	d	d	d	d	d
Histopathology	n/a	d	d	d	b	d
Immunoperoxidase stain <u>Immunohistochemistry</u>	n/a	c	c	c	c	c
Transmission EM	n/a	d	d	d	c	b
Immuno-EM	n/a	d	d	d	c	b
Cell culture	n/a	a	a	a	a	b
Antigen-capture ELISA	n/a	a	a	a	a	a
Antibody-capture ELISA	n/a	d	d	c	c	d
PCR-REA	n/a	d	a	d	e	a
PCR sequence analysis	n/a	d	d	d	c	a

EM = electron microscopy; ELISA = enzyme-linked immunosorbent assay; PCR = polymerase chain reaction; REA = restriction endonuclease analysis; n/a = not applicable.

6. Test(s) recommended for targeted surveillance to declare freedom from epizootic haematopoietic necrosis

The test recommended for targeted surveillance is cell culture, and antigen-capture ELISA. Serology (antibody-capture ELISA) might also play a useful role in surveys to identify infected trout populations.

Statistically valid sampling practices need to be used and the correct organs/samples need to be collected;

Standardised tests of specified sensitivity and specificity should be used. This restricts certification testing to cell culture, the gold standard test, and antigen capture ELISA.

The chances of detecting EHNV infection in apparently healthy rainbow trout is extremely low, even where disease is active in the same population, because the prevalence of infection is low and there is a high case fatality rate. For practical purposes, EHNV can only be detected in fish that are clinically affected or that have died with the infection. From a random sample of live rainbow trout it would be possible to misclassify a farm as being free of EHNV even during an outbreak of the disease because the prevalence of infection is generally very low. Consequently, the examination of 'routine' mortalities is recommended (Whittington *et al.*, 1999).

During a low grade outbreak of disease in rainbow trout, the prevalence of EHNV among mortalities may be 60–80% and the contribution of EHNV to 'background' mortality is high enough to enable detection of the virus in the absence of overt disease in the population. For EHNV detection and certification purposes the population of interest is 'the population of mortalities' and sampling rates can be selected to detect at least one EHNV infected individual at a given level of confidence given a certain prevalence of infection and test sensitivity (Cannon & Roe, 1982; Simon & Schill, 1984). During an outbreak of EHNV the virus was detected in at least 2% of dead fish (Whittington *et al.*, 1999). For this reason, assume a prevalence of 2% for sampling of EHNV for certification purposes. The antigen capture ELISA used to screen tissue homogenates for EHNV has a sensitivity of at least 60% compared with cell culture (Whittington & Steiner, 1993). The sample size required from a very large population of 'routine' mortalities (Whittington *et al.*, 1999) to provide 95% confidence in detecting at least one infected individual using a test of 60% sensitivity is approximately 250. In practice, 'routine' mortalities should be collected daily and stored in plastic bags in groups of 20 at –20°C until a sample of 250 has been gathered. Where possible, young age classes should be selected to simplify dissections and tissue processing. Individual clarified homogenates that are positive in antigen capture ELISA are then subjected to cell culture to confirm the presence of EHNV. This is an economical approach as it greatly reduces the number of cell cultures required. Alternatively, cell culture could be used and samples from five fish pooled to reduce costs.

Serology might also play a useful role in surveys to identify infected trout populations. Assuming 1% prevalence of seropositive grower fish on an endemically infected farm, a sample of 300 fish would be required to be 95% certain of detecting at least one infected individual (Cannon & Roe, 1982). Further research is required to confirm the validity of this approach.

7. Corroborative diagnostic criteria

7.1. Definition of suspect case

Finfish, apparently healthy, moribund or dead in which parenchymal tissues contain histological evidence of focal, multifocal or locally extensive liquefactive or coagulative necrosis with or without intracytoplasmic basophilic inclusion bodies.

The presence of EHNV shall be suspected if at least one of the following criteria is met:

- i) Histopathology consistent with EHNV, with or without clinical signs of disease;
- ii) CPE typical of EHNV in cell cultures;
- iii) Positive conventional PCR result;
- iv) Positive antigen capture ELISA.

7.2. Definition of confirmed case

The presence of EHNV is considered to be confirmed if, in addition to the criteria in Section 7.1, one or more of the following criteria are met:

- i) EHNV isolation is carried out in cell culture followed by virus identification by either an antibody-based test (immunoperoxidase stain, ELISA, neutralisation test, immunohistochemistry) and/or conventional PCR followed by sequencing of the amplicon;

Annex 23 (contd)

- ii) EHNV is detected in histological sections by immunoassay using specific anti-EHNV antibodies;
- iii) Detection of EHNV in tissue preparations by conventional PCR followed by sequencing of the amplicon.

~~Finfish, apparently healthy, moribund or dead in which parenchymal tissues contain histological evidence of focal, multifocal or locally extensive liquefactive or coagulative necrosis with or without intracytoplasmic basophilic inclusion bodies and/or in which EHNV is demonstrated by the following means:~~

~~1. Characteristic CPE in cell culture and cell culture is positive for EHNV in immunoperoxidase test or antigen-capture ELISA or PCR,~~

~~or~~

~~2. Tissues positive in antigen-capture ELISA or immunoperoxidase stain or immunoelectron microscopy or PCR~~

~~And for both 1 and 2,~~

~~3. Sequence consistent with EHNV is demonstrated by PCR-REA or PCR-sequencing.~~

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Annex 23 (contd)

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Annex 23 (contd)

NB: There is an OIE Reference Laboratory for infection with Epizootic haematopoietic necrosis virus (EHNV) (see Table at the end of this *Aquatic Manual* or consult the OIE web site for the most up-to-date list: <http://www.oie.int/en/our-scientific-expertise/reference-laboratories/list-of-laboratories/>).

Please contact the OIE Reference Laboratories for any further information on infection with EHNV.

The OIE Reference Laboratory can supply purified EHNV DNA, heat killed EHNV antigen and polyclonal antibodies against EHNV together with technical methods.

A fee is charged for the reagents to cover the costs of operating the laboratory.

NB: FIRST ADOPTED IN 1995 AS EPIZOOTIC HAEMATOPOIETIC NECROSIS; MOST RECENT UPDATES ADOPTED IN 2012.

CHAPTER 2.3.3

INFECTION WITH *GYRODACTYLUS SALARIS*

1. Scope

Infection with *Gyrodactylus salaris* means infection with the pathogenic agent *Gyrodactylus salaris* (*G. salaris*) of the Genus *Gyrodactylus* and Family Gyrodactylidae, (Platyhelminthes; Monogenea) is a viviparous freshwater parasite that may cause infection in Atlantic salmon (*Salmo salar*).

2. Disease information

2.1. Agent factors

2.1.1. Aetiological agent, agent strains

Several strains or clades of *G. salaris* have been identified on the basis of genotyping with the mitochondrial cytochrome oxidase 1 (CO1) marker (Hansen *et al.*, 2003; 2007b; Meiniälä *et al.*, 2002; 2004). Although there does not seem to be any correspondence between strains as identified by CO1 and pathogenicity (Hansen *et al.*, 2007a), all strains recovered from Atlantic salmon that have been studied in laboratory experiments, so far, are highly pathogenic to strains of Atlantic salmon. ~~Recently,~~ Strains non-pathogenic to salmon ~~were have been~~ recovered from non-anadromous Arctic charr (*Salvelinus alpinus*) in Norway (Olstad *et al.*, 2007a; Robertsen *et al.*, 2007) and from rainbow trout (*Oncorhynchus mykiss*) in Denmark (Jørgensen *et al.*, 2007; Lindenstrøm *et al.*, 2003).

2.1.2. Survival outside the host

Survival of detached parasites is temperature ~~dependant~~ dependent, e.g. about 24 hours at 19°C, 54 hours at 13°C, 96 hours at 7°C and 132 hours at 3°C (Olstad *et al.*, 2006). Likewise, survival attached to a dead host is temperature ~~dependant~~ dependent: *G. salaris* can survive on dead Atlantic salmon for 72, 142 and 365 hours at 18, 12 and 3°C, respectively (Olstad *et al.*, 2006).

2.1.3. Stability of the agent (effective inactivation methods)

Gyrodactylus salaris is known to survive between all temperatures of between 0°C to and 25°C. Tolerance to temperatures above 25°C is unknown. It is not resistant to freezing. *Gyrodactylus salaris* is sensitive to desiccation ~~not drought resistant and must be surrounded by water for survival~~. *Gyrodactylus salaris* dies after a few days at pH≤5. It is more sensitive to low pH (5.1<pH<6.4) in association with aluminium and zinc than the host Atlantic salmon (Poléo *et al.*, 2004; Soleng *et al.*, 2000) (see also Section 2.4.2).

2.1.4. Life cycle

Gyrodactylus salaris is an obligate parasite with a direct life cycle. Parasites give birth to live offspring, and there are no other life stages ~~eggs, resting stages, specialised transmission stages or intermediate hosts~~.

2.2. Host factors

2.2.1. Susceptible host species

Species that fulfil the criteria for listing a species as susceptible to infection with *G. salaris* according to Chapter 1.5. of the Aquatic Animal Health Code (Aquatic Code) include: Arctic char (*Salvelinus alpinus*), Atlantic salmon (*Salmo salar*), brown trout (*Salmo trutta*), grayling (*Thymallus thymallus*), North American brook trout (*Salvelinus fontinalis*) and rainbow trout (*Oncorhynchus mykiss*).

Annex 24 (contd)

Gyrodactylus salaris is an ectoparasite mainly on Atlantic salmon (*Salmo salar*), but can survive and reproduce on several salmonids, such as rainbow trout (*Oncorhynchus mykiss*), Arctic charr (*Salvelinus alpinus*), North American brook trout (*Salvelinus fontinalis*), grayling (*Thymallus thymallus*), North American lake trout (*Salvelinus namaycush*) and brown trout (*Salmo trutta*) (in declining order of susceptibility).

Strains of Atlantic salmon have shown variable susceptibility to *G. salaris* (Bakke *et al.*, 2002). The Baltic strains have been considered resistant. However, this has only been shown for salmon from the Russian River Neva, the Swedish River Torneälven and the Finnish landlocked Lake Saima population. Salmon from the Baltic Swedish River Indalsälven are almost as susceptible as the Norwegian salmon and salmon from the Scottish River Conon (Bakke *et al.*, 2004). Salmon from other Baltic rivers have shown intermediate susceptibility.

2.2.2. Species with incomplete evidence for susceptibility

Species for which there is incomplete evidence for susceptibility according to Chapter 1.5. of the Aquatic Code include: nil.

2.2.32. Susceptible stages of the host

All stages of the host are susceptible but mortality has only been observed in fry and parr stages.

2.2.43. Species or subpopulation predilection (probability of detection)

Not applicable.

2.2.54. Target organs and infected tissue

Gyrodactylus salaris usually occurs on the fins of ~~most~~ infected Atlantic salmon, but site preference is dependent on intensity of infection (Jensen & Johnsen, 1992; Mo, 1992). Parasites are also commonly found on the body and less commonly on the gills. On other hosts, the distribution may be different, but in general on some host species the parasite is relatively less abundant on the fins and relatively more common on the body compared with Atlantic salmon.

2.2.65. Persistent infection with lifelong carriers

Not applicable.

2.2.76. Vectors

Not applicable.

2.3. Disease pattern

2.3.1. Transmission mechanisms

Gyrodactylus salaris has spread between rivers and farms mainly by the transport/restocking translocation of live fish. ~~Migrating fish migrating swimming through brackish water can also spread~~ cause the parasite to be spread between rivers (see also Section 2.3.5). Rivers with susceptible Atlantic salmon located near rivers with infected populations are at great risk of infection if these rivers are located within the same brackish water system. If *G. salaris* is introduced into a farm/tank with susceptible Atlantic salmon, there is a high probability that all fish in the farm will become infected, depending on the layout of the farm. ~~Rivers with susceptible Atlantic salmon located near infected rivers are at great risk of infection if these rivers are located within the same brackish water system.~~

2.3.2. Prevalence

Prevalence in susceptible strains of Atlantic salmon reaches close to 100% in parr in rivers (Appleby & Mo, 1997; Johnsen & Jensen, 1991); and farms reaches similarly prevalence in farmed Atlantic salmon (in freshwater) rises to close to 100% within a short time after introduction of the parasite. Prevalence in resistant strains in rivers and farms is unknown. Prevalence in other susceptible species is usually much lower and can be below 10% (e.g. in farmed rainbow trout; Buchmann & Bresciani, 1997).

2.3.3. Geographical distribution

Gyrodactylus salaris is restricted in its distribution to Europe. It has been recovered from farmed Atlantic salmon or farmed rainbow trout in several (mainly northern) European countries. In the wild, the parasite has been found on wild salmonids, mainly Atlantic salmon parr, in rivers in Russia, Sweden and Norway. Infection with *G. salaris* is more common in farmed rainbow trout than previously thought, and is likely to be present in more countries than those currently known. In 2006, infection with *G. salaris* was reported from fish farms in Italy (Paladini *et al.*, 2009) and, in 2007, from fish farms in Poland (Rokicka *et al.*, 2007) and Macedonia (Ziřtara *et al.*, 2007). In 2009, *G. salaris* was identified by the OIE Reference Laboratory, from fish farms in Romania. ~~Great Britain~~ The United Kingdom and Ireland have been demonstrated to be free of the parasite.

2.3.4. Mortality and morbidity

Mortality ~~in farmed susceptible Atlantic salmon fry and parr~~ can be 100% ~~in susceptible farmed Atlantic salmon~~ if not treated. Mortality in wild Atlantic salmon fry and parr in Norwegian rivers can be as high as 98%, with an average of about 85%. Mortality in other susceptible host species is usually low or not observed.

2.3.5. Environmental factors

Although *G. salaris* mainly lives in fresh water, it reproduces normally at salinities up to 5–6 ppt. Survival at higher salinities is temperature dependent. For example at 1.4°C, *G. salaris* may survive for 240 hours, 78 hours and 42 hours at 10 ppt, 15 ppt and 20 ppt salinity, respectively, while at 12°C it may survive for 72 hours, 24 hours and 12 hours at the same three salinities, respectively (Soleng & Bakke, 1997).

2.4. Control and prevention

2.4.1. Vaccination

Vaccines are not available.

2.4.2. Chemotherapy

Gyrodactylus salaris is sensitive to changes in the chemical composition of the water. It is sensitive to the most commonly used chemicals for bath treatment of farmed salmon parr and salmon eggs (e.g. high salinity salt water, formaldehyde and compounds containing chlorine and iodine). Furthermore, *G. salaris* is sensitive to acidic solutions (pH 5.0–6.0) of aluminium sulphate ($[Al_2(SO_4)_3] \cdot nH_2O$) (Soleng *et al.*, 1999). As ~~AlS~~ aluminium sulphate is less toxic to fish than to *G. salaris* in moderately acidified waters, this chemical has been used in attempts to eradicate the parasite from river systems in Norway.

2.4.3. Immunostimulation

Immunostimulation is not available.

2.4.4. Resistance breeding

In laboratory experiments, selected breeding has resulted in increased survival among the offspring (Salte *et al.*, 2010). However, selected breeding has not been applied to wild salmon stocks, mainly because the stock will remain infected and thus the parasite may spread to more rivers.

2.4.5. Restocking with resistant species

Restocking with resistant strains of Atlantic salmon (e.g. Baltic Neva strain) in affected rivers is not compatible with existing strain management of Atlantic salmon.

2.4.6. Blocking agents

Not applicable.

2.4.7. Disinfection of eggs and larvae

Eggs that are transferred from infected farms should be disinfected (iodine-containing compounds have been used).

Annex 24 (contd)**2.4.8. General husbandry practices**

The general recommended husbandry practices for avoiding the spread of infective agents between units in freshwater fish farms apply to *G. salaris*. Equipment (e.g. fish nets) used in one unit should not be used in another without adequate disinfection.

3. Sampling**3.1. Selection of individual specimens**

In cases where sampling is performed and infection is not suspected, a random sample with an adequate number of fish should be taken from, for example, a river. In farms, if fish show clinical signs of infection (as described in Section 4.1.1), these fish should be selected.

3.2. Preservation of samples for submission

Fish should be killed immediately and should not be allowed to dry out before preservation. Whole fish should be preserved in ~~96–80~~–100% EtOH in bottles large enough to provide excess space and preservative. The concentration of EtOH after preservation should not be below 70%. As a rule of thumb this concentration is obtained if the proportion of fish to EtOH does not exceed 1:9. If the concentration is lower, the mucous and epidermis may disintegrate and *Gyrodactylus* specimens, even if they are preserved, may drop off. Bottles should have an opening wide enough to avoid the possibility of scraping off *Gyrodactylus* specimens when fish are put into the bottle or when taken out for examination. Bottles should be stored in a horizontal position until the tissue is fixed/preserved to prevent the fish curling. This facilitates examination of the fish as they can easily be turned with a pair of forceps under the microscope. When preservation of the fish is complete, the bottles can be stored in a vertical position.

As *G. salaris* is common on fins of Atlantic salmon, fins cut off from the body and stored in EtOH as described above can also be submitted. This is especially suitable for larger fish and under field conditions where, for example, transport is limited.

3.3. Pooling of samples

~~Samples from a river or a farm~~ can be pooled, although each fish is subsequently examined and analysed separately. Fins of fish from a farm or a river can be pooled and are also examined and analysed separately, but in this instance each fin cannot be related to a certain fish host.

3.4. Best organs or tissues

Fish can be examined as whole specimens either live under anaesthesia (for example, with MS222), freshly killed, or preserved. In addition, fresh or preserved fins can be examined. The same examination method (see Section 4.3.1) is used in all cases. Examination of live, anaesthetised fish is very time-consuming and not recommended.

Instead of examining the whole fish, the fins can be examined (by the method described in Section 4.3.1). When Norwegian salmon parr are infected, almost all fish have at least one *G. salaris* on one of the fins. On some fish, *G. salaris* specimens may occur on the body or head, including the nostrils, the gills and the mouth cavity. The distribution of *G. salaris* on fins and other parts of the fish varies among fish species and seems to vary among salmon strains.

3.5. Samples/tissues that are not suitable

Dead fish, stored on ice, are not acceptable for *Gyrodactylus* examination, even if the fish are kept separately in plastic bags, etc. The parasites quickly ~~soon~~ die if not covered in water, and as these parasites do not have an exoskeleton, dead parasites disintegrate quickly. If such dead fish are rinsed in water, *Gyrodactylus* specimens may be found in the sediment. However, if specimens are not found in the sediment, it cannot be concluded that the fish were uninfected. ~~Examination of formaldehyde-fixed fish is not recommended for reasons of operator safety.~~ Formaldehyde-fixed *Gyrodactylus* specimens are also very difficult to identify morphologically and are unsuitable for DNA analysis.

4. Diagnostic methods

4.1. Field diagnostic methods

4.1.1. Clinical signs

Usually there are no clinical signs in fish with one or up to a few tens of parasite specimens.

In the early disease phase, increased flashing (fish scratch their skin on the substrate) is typical. Later, fish may become greyish because of increased mucous production and the fins may be eroded. Diseased fish are lethargic and are usually found in slower-moving water.

4.1.2. Behavioural changes

Flashing is common among moderate to heavily infected farmed fish as they scratch their skin on the bottom or wall of a tank or pond. Heavily infected fish may have reduced activity and stay in low current areas.

4.2. Clinical methods

4.2.1. Gross pathology

Heavily infected fish may become greyish as a result of increased mucification, and at a later stage the dorsal and pectoral fins may become whitish as a result of increased thickness (mainly hypertrophy) of the epidermis.

Heavily infected fish may have eroded fins, especially dorsal, tail and pectoral fins, because of parasite feeding.

Secondary fungal infections (*Saprolegnia* spp.) are commonly observed in fish with infection with *G. salaris*.

4.2.2. Clinical chemistry

Not applicable.

4.2.3. Microscopic pathology

Not applicable.

4.2.4. Wet mounts

Scrapings (wet mounts) from skin or fins can be used to detect *Gyrodactylus* specimens on infected fish. In these cases, with high intensity infestation, hundreds or thousands of *Gyrodactylus* specimens are present all over the body and fins. Preparations of wet mounts are usually not suitable for identification of *Gyrodactylus* to the species level and other preparations for morphological or DNA analysis must be made (see below). If the number of *Gyrodactylus* specimens is low, the chances of detecting the parasites by scrapings are limited.

4.2.5. Smears

Not applicable.

4.2.6. Fixed sections

Not applicable.

4.2.7. Electron microscopy/cytopathology

Not applicable.

Annex 24 (contd)**4.3. Agent detection and identification methods****4.3.1. Direct detection methods**

Detection of *Gyrodactylus* and identification of *G. salaris* is a two-step process. Firstly, parasite specimens are observed using optical equipment and secondly, parasites are identified, usually on an individual basis using other equipment and methods.

Optical equipment must be used to detect *Gyrodactylus*. In the case of a suspected outbreak of infection with *G. salaris* where only light microscopy is available, wet mounts can be used to detect *Gyrodactylus* specimens. However, it is strongly advised not to use this method in a surveillance programme as the presumed specificity and sensitivity is very low (value not known) and, therefore, the number of fish examined ~~is needs to be unreasonably high~~.

Fish can be examined as live whole specimens (under anaesthesia), freshly killed or preserved/~~fixed~~. The same examination method (see below) is used in all cases. Examination of live, anaesthetised fish is very time-consuming and not recommended. ~~Examination of formaldehyde-fixed fish is not recommended for reasons of operator safety.~~ *Gyrodactylus* specimens fixed in formaldehyde are also very difficult to identify and are not suitable for DNA analysis. Instead of examining the whole fish, the fins can be examined (by the method described below). When parr of very susceptible Atlantic salmon strains ~~parr~~ are infected/infested, almost all fish have at least one *G. salaris* on one of the fins. On some fish, *G. salaris* specimens may occur on the body or head, including the nares-nostrils, the gills and the mouth cavity. The distribution of *G. salaris* on fins and other parts of the fish varies among fish species and the distribution also seems to vary among salmon strains.

Live anaesthetised fish, freshly cut fins or EtOH-preserved fish or fins should be examined under a binocular dissecting microscope with good illumination. The fish should be placed in a box and completely covered in fresh water. Preserved fish can also be examined in EtOH. Living parasites are more easily detected by their movements, thus disturbing light refraction on the skin of the fish should be avoided. Live *Gyrodactylus* are colourless while EtOH-preserved *Gyrodactylus* specimens are usually only slightly opaque. If the dissecting microscope is illuminated from above, the bottom of the microscope stage should be black. This will increase the contrast and the parasites will be detected more easily. The whole surface of the fish, including gills and mouth cavity, must be examined. It is best to use two forceps for this process. The fins of relatively small fish, usually less than 10 cm, can also be studied using illumination through the bottom of the microscope stage. This way, *Gyrodactylus* specimens on the fins can usually be easily observed.

~~If examination is carried out in EtOH, the use of gloves should be considered. For operator protection purposes, the dissecting microscope could be placed on a suction bench with a downwards outlet to avoid inhalation of evaporated preservative.~~

4.3.1.1. Microscopic methods

Identification of *Gyrodactylus* species is based on morphology and morphometry of marginal hooks anchors (hamuli) and bars in the opisthaptor (the attachment organ). Good preparation of specimens is a prerequisite for species identification.

Digestion of the soft tissue, leaving the hard parts only, is recommended when high-resolution morphometrics is required for reliable morphometric diagnosis. The soft tissue can be digested in a solution (approx. 1 µl) of 75 mM Tris, 10 mM EDTA (ethylene diamine tetra-acetic acid), 5% SDS (sodium dodecyl sulphate) and 100 mg ml⁻¹ proteinase K, pH 8.0. After adding the digestion solution, the reaction should be inspected in the microscope until completion and then ended by adding a stop solution (1:1 glycerol and 10% neutral buffered formalin). The procedure for digestion is described in detail in (Harris *et al.*, 1999). Identification of *G. salaris* should be in accordance with references: Cunningham *et al.*, 2001; Malmberg *et al.*, 1957;1970; McHugh *et al.*, 2000; Olstad *et al.*, 2007b; Shinn *et al.*, 2004.

The size of the opisthaptor hard parts in *Gyrodactylus* varies extensively with, for example, temperature, whereas shape is more stable (Mo, 1991a; 1991b; 1991c). The capability of linear measurements to capture morphology might therefore not always be sufficient for reliable diagnosis (Olstad *et al.*, 2007b).

Gyrodactylus salaris is morphologically similar to *G. teuchis* from brown trout, Atlantic salmon, and rainbow trout, and to *G. thymalli* from grayling (Figure 1). The species can be differentiated by trained morphologists on the basis of the shape of the marginal hook sickle. *Gyrodactylus teuchis* has a longer and more constantly curved sickle blade, while *G. thymalli* has a small angle on the shaft of the sickle (Cunningham *et al.*, 2001; McHugh *et al.*, 2000; Shinn *et al.*, 2004).

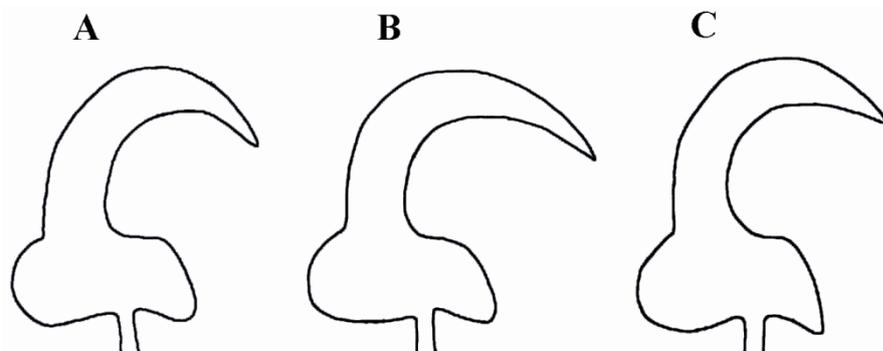


Figure 1. Marginal hooks of (A) *Gyrodactylus salaris*, (B) *G. teuchis* and (C) *G. thymalli*. Drawings are modified after Cunningham *et al.*, 2001.

4.3.1.1.1. Wet mounts

Not applicable.

4.3.1.1.2. Smears

Not applicable.

4.3.1.1.3. Fixed sections

Not applicable.

4.3.1.2. Agent isolation and identification

4.3.1.2.1. Cell culture/artificial media

Not applicable.

4.3.1.2.2. Antibody-based antigen detection methods

Not applicable.

4.3.1.2.3. Molecular techniques

Preparation of samples

Template DNA should be prepared from live/fresh or EtOH-preserved specimens using a suitable DNA preparation protocol. A DNA extraction kit may be used in accordance with the manufacturer's recommendations.

4.3.1.2.3.1. Analysis of the ribosomal RNA gene internal transcribed spacer region

i) Polymerase chain reaction (PCR) amplification of the internal transcribed spacer (ITS)

For amplification of a 1300 base pair product of the ITS-region, primers, such as 5'-TTT-CCG-TAG-GTG-AAC-CT-3' and 5'-TCC-TCC-GCT-TAG-TGA-TA-3', may be used. The cycling conditions for PCR are as follows, initial denaturation at 95°C for 5 minutes; 30 cycles of 94°C for 1 minute, 50°C for 1 minute, 72°C for 2 minutes; final extension at 72°C for 7 minutes (Cunningham, 1997). If partially degraded material is analysed, the ITS1 and ITS2 spacers can be amplified in two separate reactions using primer sets and PCR conditions described in Matejusova *et al.* (2001).

Annex 24 (contd)

ii) ITS sequencing and sequence analysis

Amplified ITS fragments prepared as in Section 4.3.1.2.3.1.i above should be sequenced and the sequences subjected to a BLAST search in GenBank/EMBL to establish identity with known sequences. In addition to the PCR primers, at least two internal primers should be used such as; 5'-ATT-TGC-GTT-CGA-GAG-ACC-G and 5'-TGG-TGG-ATC-ACT-CGG-CTC-A (Ziętara & Lumme, 2003). Several sequences of other species infecting salmonids, e.g. *G. derjavini*, *G. derjavinoides*, *G. truttae*, *G. teuchis* and *G. thymalli* are available in GenBank/EMBL. *Gyrodactylus salaris* and *G. thymalli* cannot be distinguished by this method, but sequences of ITS distinguishes *G. salaris* and *G. thymalli* from all other known species.

~~Note: Several sequences of *G. salaris* and *G. thymalli* are available in GenBank/EMBL, all differing by only a few point mutations, but with no specific mutations that distinguish *G. salaris* from *G. thymalli*.~~

4.3.1.2.3.2. Analysis of the mitochondrial cytochrome oxidase I gene

i) PCR amplification of the mitochondrial cytochrome oxidase 1 (CO1) gene

For amplification of the CO1-gene, the primers 5'-TAA-TCG-GCG-GGT-TCG-GTA-A-3' and 5'-GAA-CCA-TGT-ATC-GTG-TAG-CA-3' (Meinilä *et al.*, 2002) may be used. The cycling conditions for PCR are as follows, initial denaturation at 95°C for 5 minutes; 35 cycles of 95°C for 1 minute, 50°C for 1 minute, 72°C for 2 minutes; final extension at 72°C for 7 minutes. Additional primer sets for amplification of CO1 can be found in references: 4 Meinilä *et al.*, 2002; 2004.

ii) CO1 sequencing and sequence analysis

Amplified CO1 fragments prepared as described above should be sequenced and compared with other sequences using a BLAST search in GenBank/EMBL. In addition to the PCR primers, at least two internal primers can be used, such as 5'-CCA-AAG-AAC-CAA-AAT-AAG-TGT-TG-3'), and 5'-TGT-CYC-TAC-CAG-TGC-TAG-CCG-CTG-G-3' (Hansen *et al.*, 2003).

If the obtained sequence does not have a 100% match in GenBank/EMBL, a phylogenetic analysis should be performed to establish the relationship to other available sequences. Different clades of *G. salaris* and *G. thymalli* can be distinguished with this method.

NOTE: CO1 sequences cannot unambiguously differentiate between *G. salaris* and *G. thymalli* but can be used to assign specimens to a clade. Clades of *G. salaris* and *G. thymalli* generally correspond well to host preferences and/or the geographical distribution of the parasites, with a few exceptions. ~~CO1 cannot be applied as a pathogenicity marker.~~

Note that some researchers have chosen to submit all their sequences from both Atlantic salmon and grayling as *G. salaris*, causing confusion when comparing sequences (both ITS and CO1) with those in GenBank/EMBL in a BLAST search. Host identity of sequences in GenBank/EMBL should thus always be checked.

4.3.1.2.4. Agent purification

Not applicable.

4.3.2. Serological methods

Not applicable.

5. Rating of tests against purpose of use

Not applicable.

6. Test(s) recommended for targeted surveillance to declare freedom from infection with *G. salaris*

Diagnostic/detection methods to declare freedom are the same as those mentioned in for Section 4.3.

7. Corroborative diagnostic criteria

7.1. Definition of suspect case

Observation of *Gyrodactylus* specimen(s) on Atlantic salmon or rainbow trout (or other susceptible hosts) either in skin scrapings examined in a light microscope or on fins or skin examined under a stereomicroscope.

7.2. Definition of confirmed case

~~A molecular identification of *Gyrodactylus* specimen(s) to *G. salaris* (or *G. thymalli*) by sequencing of ITS followed by sequencing and phylogenetic analysis of CO1 to assign the sequence to the nearest known relative is preferred. Trained morphologists can perform morphological identification of *Gyrodactylus* specimen(s) to *G. salaris* based on structures of the attachment organ. However, a morphological diagnosis should be confirmed by molecular tools. A combination of both morphological and molecular methods as described in this chapter is recommended.~~

Infection with *G. salaris* shall be confirmed if the following criteria are met:

i) Morphology consistent with *G. salaris*:

ii) Molecular identification of *Gyrodactylus* specimen(s) to *G. salaris* (or *G. thymalli*) by sequencing of ITS followed by sequencing and phylogenetic analysis of CO1 to assign the sequence to the nearest known relative is preferred.

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* *

NB: There is an OIE Reference Laboratory for infection with *Gyrodactylus salaris* (see Table at the end of this *Aquatic Manual* or consult the OIE web site for the most up-to-date list: <http://www.oie.int/en/our-scientific-expertise/reference-laboratories/list-of-laboratories/>).

Please contact the OIE Reference Laboratories for any further information on infection with *G. salaris*.

NB: FIRST ADOPTED IN 1997 AS GYRODACTYLOSIS OF ATLANTIC SALMON (GYRODACTYLUS SALARIS); MOST RECENT UPDATES ADOPTED IN 2012.

CHAPTER 2.3.5.

INFECTION WITH HPR-DELETED OR HPR0 INFECTIOUS SALMON ANAEMIA VIRUS

1. Scope

For the purpose of this chapter, Infection with infectious salmon anaemia virus (ISAV) means infection with the pathogenic agent highly polymorphic region (HPR)-deleted ISAV or HPR0 ISAV (with a non-deleted HPR) of the Genus *Isavirus* of the and Family Orthomyxoviridae.

Infection with HPR-deleted ISAV may cause ~~infectious salmon anaemia (ISA)~~ infection with ISAV in Atlantic salmon (*Salmo salar*), which is a generalised and lethal condition characterised by severe anaemia, and variable haemorrhages and necrosis in several organs. The disease course is prolonged with low daily mortality (0.05–0.1%) typically only in a few cages. Cumulative mortality may become very high for a period lasting several months if nothing is done to limit disease dissemination (Rimstad *et al.*, 2011).

Detection of HPR0 ISAV has never been associated with clinical signs of infection with ISAV ~~ISA~~ in Atlantic salmon (Christiansen *et al.*, 2011). This virus genotype replicates transiently and has mainly been localised to the gills. A link between non-pathogenic HPR0 ISAV and pathogenic HPR-deleted ISAV, with some outbreaks potentially occurring as a result of the emergence of HPR-deleted ISAV from HPR0 ISAV has been suggested (Christiansen *et al.*, 2017; Cunningham *et al.*, 2002; Mjaaland, *et al.*, 2002).

2. Disease information

2.1. Agent factors

2.1.1. Aetiological agent, agent strains

ISAV is an enveloped virus, 100–130 nm in diameter, with a genome consisting of eight single-stranded RNA segments with negative polarity (Dannevig *et al.*, 1995). The virus has haemagglutinating, receptor-destroying and fusion activity (Falk *et al.*, 1997; Mjaaland *et al.*, 1997; Rimstad *et al.*, 2011).

The morphological, physiochemical and genetic properties of ISAV are consistent with those of the *Orthomyxoviridae*, and ISAV has been classified as the type species of the genus *Isavirus* (Kawaoka *et al.*, 2005) within this virus family. The nucleotide sequences of all eight genome segments, encoding at least ten proteins, have been described (Clouthier *et al.*, 2002; Rimstad *et al.*, 2011), including the 3' and 5' non-coding sequences (Kulshreshtha *et al.*, 2010). Four major structural proteins have been identified, including a 68 kDa nucleoprotein, a 22 kDa matrix protein, a 42 kDa haemagglutinin-esterase (HE) protein responsible for receptor-binding and receptor-destroying activity, and a 50 kDa surface glycoprotein with putative fusion (F) activity, encoded by genome segments 3, 8, 6 and 5, respectively. Segment 1, 2, and 4 encode the viral polymerases PB2, PB1 and PA. The two smallest genomic segments, segments 7 and 8, each contain two open reading frames (ORF). The ORF1 of segment 7 encodes a protein with type I interferon antagonistic properties, while ORF2 has been suggested to encode for a nuclear export protein (NEP). Whether the ORF1 gene product is nonstructural or a structural component of the virion remains to be determined. The smaller ORF1 of segment 8 encodes the matrix protein, while the larger ORF2 encodes an RNA-binding structural protein also with type I interferon antagonistic properties.

Annex 25 (contd)

Sequence analysis of various gene segments has revealed differences between isolates both within and between defined geographical areas. ~~According to sequence differences in the 5' region of the HE gene, ISAV isolates have been divided into two major groups, one European and one North American group. According to sequence differences in all eight genomic segments, two groups are clearly defined: one European and one North American (Gagné & LeBlanc, 2017).~~ In the HE gene, a small HPR near the transmembrane domain has been identified. This region is characterised by the presence of gaps rather than single-nucleotide substitutions (Cunningham *et al.*, 2002; Mjaaland *et al.*, 2002). A full-length gene (HPR0) has been suggested to represent a precursor from which all ISAV HPR-deleted (pathogenic) variants of ISAV originate. The presence of non-pathogenic HPR0 ISAV genome has been reported in both apparently healthy wild and farmed Atlantic salmon, but has not been detected in diseased fish with clinical disease and pathological signs consistent with infection with ISAV ISA (Christiansen *et al.*, 2011; Cunningham *et al.*, 2002; Lyngstad *et al.*, 2012; Markussen *et al.*, 2008; McBeath *et al.*, 2009; Nylund *et al.*, 2007). A mixed infection with ~~of~~ HPR-deleted and HPR0 ISAV variants has been reported (Cardenas *et al.*, 2014; Kibenge *et al.*, 2009). Recent studies show that HPR0 ISAV variants occur frequently in sea-reared Atlantic salmon. The HPR0 ISAV strain seems to be more seasonal and transient in nature and displays a tissue tropism with high prevalence in gills (Christiansen *et al.*, 2011; Lyngstad *et al.*, 2011). To date there has been no direct evidence linking the presence of HPR0 ISAV to a subsequent clinical infection with ISAV ISA outbreak. The risk of emergence of pathogenic HPR-deleted ISAV variants from a reservoir of HPR0 ISAV is considered to be low but not negligible (Christiansen *et al.*, 2011; 2017; EFSA, 2012; Lyngstad *et al.*, 2012).

In addition to the variations seen in the HPR of the HE gene, other gene segments may also be of importance for development of infection with ISAV ISA. A putative virulence marker has been identified in the fusion (F) protein. Here, a single amino acid substitution, or a sequence insertion, near the protein's putative cleavage site has been found to be a prerequisite for virulence (Kibenge *et al.*, 2007; Markussen *et al.*, 2008). Aside from insertion/recombination, ISAV also uses gene segment reassortment in its evolution, with potential links to virulence (Devold *et al.*, 2006; Markussen *et al.*, 2008; Mjaaland *et al.*, 2005).

2.1.2. Survival outside the host

ISAV has been detected by reverse-transcription polymerase chain reaction (RT-PCR) in seawater sampled at farming sites with ISAV-positive Atlantic salmon (Kibenge *et al.*, 2004). It is difficult to estimate exactly how long the virus may remain infectious in the natural environment because of a number of factors, such as the presence of particles or substances that may bind or inactivate the virus. Exposing cell culture-propagated ISAV to 15°C for 10 days or to 4°C for 14 days had no effect on virus infectivity (Falk *et al.*, 1997).

2.1.3. Stability of the agent (effective inactivation methods)

ISAV is sensitive to UV irradiation (UVC) and ozone. A 3-log reduction in infectivity in sterile fresh water and seawater was obtained with a UVC dose of approximately 35 Jm⁻² and 50 Jm⁻², respectively, while the corresponding value for ISAV in wastewater from a fish-processing plant was approximately 72 Jm⁻². Ozonated seawater (4 minutes with 8 mg ml⁻¹, 600–750 mV redox potential) may inactivate ISAV completely. Incubation of tissue homogenate from diseased fish at pH 4 or pH 12 for 24 hours inactivated ISAV infectivity. Incubation in the presence of chlorine (100 mg ml⁻¹) for 15 minutes also inactivated virus (Rimstad *et al.*, 2011). Cell culture-isolated ISAV may survive for weeks at low temperatures, but virus infectivity is lost within 30 minutes of exposure at 56°C (Falk *et al.*, 1997).

2.1.4. Life cycle

The main infection route is most likely through the gills for both HPR0 and HPR-deleted ISAV, but infection via the intestine or skin cannot be excluded. ~~HPR-deleted ISAV has been used in the studies referred to below. Endothelial cells lining blood vessels seem to be the primary target cells for ISAV as demonstrated by electron microscopy immunohistochemistry and *in situ* hybridisation. Virus replication has also been demonstrated in leukocytes, and sinusoidal macrophages in kidney tissue stain positive for ISAV using immunohistochemistry (IHC). As endothelial cells are the target cells (see Section 2.2.4), virus replication may occur in any organ (Aamelfot *et al.*, 2012; Rimstad *et al.*, 2011).~~

The haemagglutinin-esterase (HE) molecule of ISAV, like the haemagglutinin (HA) of other orthomyxoviruses (influenza A, B and C viruses), is essential for binding of the virus to sialic acid residues on the cell surface. In the case of ISAV, the viral particle binds to glycoprotein receptors containing 4-O-acetylated sialic acid residues, which also functions as a substrate for the receptor-destroying enzyme. Further uptake and replication seem to follow the pathway described for influenza A viruses, indicated by demonstration of low pH-dependent fusion, inhibition of replication by actinomycin D and α -amanitin, early accumulation of nucleoprotein followed by matrix protein in the nucleus and budding of progeny virions from the cell surface (Cottet *et al.*, 2011; Rimstad *et al.*, 2011).

The route of shedding of ISAV from infected fish may be through natural excretions/secretions.

The HPR0 variant has not been isolated in cell culture, which hampers *in-vivo* and *in-vitro* studies of characteristics and the life cycle of this virus variant.

2.2. Host factors

2.2.1. Susceptible host species

Natural outbreaks of ISA have only been recorded in farmed Atlantic salmon, and in Coho salmon (*Oncorhynchus kisutch*) in Chile (Kibenge *et al.*, 2001). Subclinically infected feral Atlantic salmon, brown trout and sea trout (*S. trutta*) have been identified by RT-PCR (Kibenge *et al.*, 2004; Plarre *et al.*, 2005). In marine fish, detection of ISAV by RT-PCR has been reported in tissues of pollock (*Pollachius virens*) and cod (*Gadus morhua*), but only in fish collected from cages with Atlantic salmon exhibiting ISA (MacLean SA *et al.*, 2003). Following experimental infection by bath immersion, ISAV has been detected by RT-PCR in rainbow trout (*Oncorhynchus mykiss*) (Biacchesi *et al.*, 2007) and herring (*Clupea harengus*), the latter in a subsequent transmission to Atlantic salmon. Attempts have been made to induce infection or disease in pollock, *Pollachius virens*, but with negative results.

Species that fulfil the criteria for listing as susceptible to infection with ISAV according to Chapter 1.5. of the Aquatic Animal Health Code (Aquatic Code) include: amago trout (*Oncorhynchus masou*), Atlantic salmon (*Salmo salar*), brown trout (*Salmo trutta*) and rainbow trout (*Oncorhynchus mykiss*).

2.2.2. Species with incomplete evidence for susceptibility

Species for which there is incomplete evidence for susceptibility according to Chapter 1.5. of the Aquatic Code include: Atlantic herring (*Clupea harengus*) and amago trout (*Oncorhynchus masou*).

2.2.3. Susceptible stages of the host

In Atlantic salmon, life stages from fingerlings to adults are known to be susceptible. Disease outbreaks are mainly reported in seawater cages, and only a few cases have been reported in the freshwater stage, including one case in yolk sac fry (Rimstad *et al.*, 2011). Infection with ISAV ISA has been experimentally induced in both Atlantic salmon fry and parr kept in freshwater. Genetics may also play an important role in the susceptibility of Atlantic salmon to infection with ISAV+ISA, as differences in susceptibility among different family groups have been observed.

2.2.4. Species or subpopulation predilection (probability of detection)

HPR deleted forms of infection with ISAV ISA is primarily a cause disease of in Atlantic salmon.

2.2.5. Target organs and infected tissue

For fish that have developed infection with ISAV+ISA: endothelial cells in all organs become infected (gills, heart, liver, kidney, spleen and others) (Aamelfot *et al.*, 2012). HPR0 ISAV variants seem primarily to target the gills, but this variant has also been detected in kidney and heart (Christiansen *et al.*, 2011; Lyngstad *et al.*, 2011).

Annex 25 (contd)**2.2.65. Persistent infection with lifelong carriers**

Persistent infection in lifelong carriers has not been documented in Atlantic salmon, but at the farm level, infection may persist in the population by continuous infection of new individuals that do not develop clinical signs of disease. This may include infection with the HPR0 ISAV variants, which seems to be only transient in nature (Christiansen *et al.*, 2011; Lyngstad *et al.*, 2011). Experimental infection of rainbow trout and brown trout with ISAV indicate that persistent infection in these species could be possible (Rimstad *et al.*, 2011).

2.2.76. Vectors

Passive transfer of ISAV by salmon lice (*Lepeophtheirus salmonis* and *Caligus rogercresseyi*; Oelkers *et al.*, 2014) has been demonstrated under experimental conditions. Although natural vectors have not been identified, several different vector groups could be possible vectors under certain defined conditions (reviewed in Rimstad *et al.*, 2011).

2.2.87. Known or suspected wild aquatic animal carriers

Wild Atlantic salmon and brown trout and sea trout may be carriers of ISAV (Rimstad *et al.*, 2011). The importance of wild marine fish (see Section 2.2.1) as virus carriers needs to be clarified. The results from a study from the Faroe Islands point to the potential presence of an unknown marine reservoir for this virus (Christiansen *et al.*, 2011).

2.3. Disease pattern**2.3.1. Transmission mechanisms**

Studies of recurrent epidemics of infection with ISAV in different salmon-producing areas conclude that the virus spreads locally between adjoining-adjacent sites. Proximity to sites with infection with ISAV outbreaks is a risk of primary importance, and the risk for a susceptible farm increases the nearer it is to an infected farm. Sequence analysis of ISAV from infection with ISAV outbreaks in Norway shows a high degree of similarity between viruses isolated from neighbouring ISA affected sites, further supporting ISAV transmission between proximate sites. The risk of transmission of ISAV is dependent on the level of biosecurity measures in place. Suggested pathways for ISAV transmission are through sea water, shipment of live fish, transmission through sea lice, and via infected wild salmonids (Aldrin *et al.*, 2011; Gustafson *et al.*, 2007; Lyngstad *et al.*, 2011; Mardones *et al.*, 2011; Rimstad *et al.*, 2011).

Many ISA-outbreaks of clinical disease caused by infection with ISAV in Norway appear to be isolated in space and time from other outbreaks with unknown sources of infection (Aldrin *et al.*, 2011). A suggested hypothesis for disease emergence is occasional transition of HPR0 ISAV into HPR-deleted ISAV variants causing solitary outbreaks or local epidemics through local transmission (Lyngstad *et al.*, 2011; 2012). The risk of emergence of HPR-deleted ISAV variants from a reservoir of HPR0 ISAV is considered to be low but not negligible (EFSA, 2012). A direct link between HPR0 variants and HPR-deleted ISAV remains to be demonstrated.

As infection with ISAV has also been reported from smolt-producing sites with Atlantic salmon, transmission of ISAV from parent to progeny cannot be excluded. Even though there is no evidence of true vertical transmission, eggs and embryos could be a risk of transmission if ISAV biosecurity measures are not adequate (Mardones *et al.*, 2014; Marshall *et al.*, 2014; Rimstad *et al.*, 2011).

2.3.2. Prevalence

In a-net pens containing diseased fish, the prevalence of HPR-deleted ISAV may vary widely, while in adjacent net pens (without diseased fish) ISAV may be difficult to detect, even by the most sensitive methods. Therefore, for diagnostic investigations it is important to sample from net pens containing diseased fish.

There is increasing evidence that the prevalence of the non-pathogenic HPR0 ISAV genotype may be high in Atlantic salmon production areas. HPR0 variants in Atlantic salmon appear to be a seasonal and transient infection (Christiansen *et al.*, 2011). HPR0 variants of ISAV have also been detected in wild salmonids (reviewed in Rimstad *et al.*, 2011).

2.3.3. Geographical distribution

Initially reported in Norway in the mid-1980s (Thorud & Djupvik, 1988), infection with ISAV ~~ISA~~ in Atlantic salmon has since then been reported in Canada (New Brunswick in 1996; Mullins *et al.*, 1998), the United Kingdom (Scotland in 1998), the Faroe Islands (2000), the USA (Maine in 2001) and in Chile (2007) (Cottet *et al.*, 2011; Rimstad *et al.*, 2011). The presence of the HPR0 ISAV variant has been reported in all countries where infection with HPR-deleted ISAV has occurred.

2.3.4. Mortality and morbidity

During ~~ISA~~ outbreaks of infection with ISAV, morbidity and mortality may vary greatly within and between ~~different~~ net pens in a seawater fish farm, and between ~~different~~ fish farms. Morbidity and mortality within a net pen may start at very low levels. Typically, daily mortality ranges from 0.5 to 1% in affected cages. Without intervention, mortality increases and ~~seems to often~~ peaks in early summer and winter. The range of cumulative mortality during an outbreak is from insignificant to moderate, but in severe cases, cumulative mortality exceeding 90% may be recorded ~~during over~~ several months. Initially, an outbreak of infection with ISAV ~~ISA~~ may be limited to one or two net pens over a long time period. In such cases, if net pens with clinical infection with ISAV ~~ISA~~ are slaughtered immediately, further development of clinical infection with ISAV ~~ISA~~ at the site may be prevented. In outbreaks where smolts have been infected in well boats during transport, simultaneous outbreaks may occur.

HPR0 ISAV has not been associated with ~~ISA~~ clinical disease in Atlantic salmon.

2.3.5. Environmental factors

Generally, outbreaks of infection with ISAV ~~ISA~~ tend to be seasonal with most outbreaks in late spring and late autumn; however outbreaks can occur at any time of the year. Handling of fish (e.g. sorting or treatment, splitting or moving of cages) may initiate disease outbreaks on infected farms, especially if long-term undiagnosed problems have been experienced in advance (Lyngstad *et al.*, 2008).

2.4. Control and prevention

2.4.1. Vaccination

Vaccination against infection with ISAV ~~ISA~~ has been carried out in North America since 1999 and the Faroe Islands since 2005. In Norway vaccination against infection with ISAV was carried out for the first time in 2009 in a region with a high rate of infection with ISAV ~~ISA~~ outbreaks. Chile started vaccinating against infection with ISAV ~~ISA~~ in 2010. However, the currently available vaccines do not seem to offer complete protection in Atlantic salmon.

2.4.2. Chemotherapy

~~Most recently, it has been demonstrated that~~ The broad-spectrum antiviral drug Ribavirin (1- β -D-ribofuranosyl-1,2,4-triazole-3-carboxamide) is effective in inhibiting ISAV replication both *in vitro* and *in vivo* (Rivas-Aravena *et al.*, 2011).

2.4.3. Immunostimulation

Not applicable.

2.4.4. Resistance breeding

Differences in susceptibility among different family groups of Atlantic salmon in fresh water have been observed in challenge experiments and in field tests, indicating the potential for resistance breeding (Gjøen *et al.*, 1997).

2.4.5. Restocking with resistant species

Not applicable.

2.4.6. Blocking agents

Not applicable.

Annex 25 (contd)**2.4.7. Disinfection of eggs and larvae**

Disinfection of eggs according to standard procedures is suggested as an important control measure (chapter 4.4 of the Aquatic Code).

2.4.8. General husbandry practices

The incidence of infection with ISAV ISA may be greatly reduced by implementation of legislative measures or husbandry practices regarding the movement of fish, mandatory health control, transport and slaughterhouse regulations. Specific measures including restrictions on affected, suspected and neighbouring farms, enforced sanitary slaughtering, generation segregation ('all in/all out') as well as disinfection of offal and wastewater from fish slaughterhouses and fish processing plants may also contribute to reducing the incidence of the disease. The experience from the Faroe Islands, where the prevalence of HPR0 is high, demonstrates that the combination of good biosecurity and husbandry reduces the risk of outbreaks of infection with ISAV ISA ~~outbreaks~~ substantially.

3. Sampling**3.1. Selection of individual specimens**

For HPR-deleted ISAV, fish displaying clinical signs or gross pathology should be sampled.

For HPR0 ISAV, randomly selected individuals should be sampled at different time points throughout the production cycle.

~~The following is primarily for verification of suspected cases based on clinical signs and gross pathology or positive RT-PCR for HPR-deleted ISAV.~~

~~For detection of HPR0 ISAV, gill tissue should be sampled in randomly selected individuals at different points of time through the production cycle. Only detection using RT-PCR is possible for this genotype.~~

3.2. Preservation of samples for submission

Haematology:	Heparin or EDTA (ethylene diamine tetra-acetic acid)
Cell culture:	Virus transport medium
Histology and immunohistochemistry:	Fixation in neutral phosphate-buffered 10% formalin
Immunofluorescence (smears):	Either submitted dried, or dried and fixed in 100% acetone
Molecular biology (RT-PCR and sequencing):	Appropriate medium for preservation of RNA

3.3. Pooling of samples

Pooling of samples may be acceptable, however, the impact on sensitivity and design prevalence must be considered.

3.4. Best organs or tissues**3.4.1. Detection of HPR-deleted ISAV**

Blood is preferred for non-lethal sampling. Generally, as infection with ISAV ISA is a generalised infection, internal organs not exposed to the environment should be used for diagnostic testing.

Virological examination (cell culture and PCR): heart (should always be included) and mid-kidney;

Histology (prioritised): mid-kidney, liver, heart, pancreas/intestine, spleen;

Immunofluorescence (smears): mid-kidney;

Immunohistochemistry: mid-kidney, heart (including valves and bulbus arteriosus).

3.4.2. Detection of HPR0 ISAV

Gill tissue ~~Gills~~ should be tested by RT-PCR.

3.5. Samples/tissues that are not suitable

None known.

4. Diagnostic methods

4.1. Field diagnostic methods

4.1.1. Clinical signs

The most prominent external signs of infection with ISAV ISA are pale gills (except in the case of blood stasis in the gills), exophthalmia, distended abdomen, blood in the anterior eye chamber, and sometimes skin haemorrhages especially of the abdomen, as well as scale pocket oedema.

Generally, Atlantic salmon naturally infected with HPR-deleted ISAV appear lethargic and may keep close to the wall of the net pen.

Affected fish are generally in good condition, but diseased fish have no feed in the digestive tract.

4.2. Pathological evaluation

4.2.1. Gross pathology

Fish infected with HPR-deleted ISAV may show a range of pathological changes, from none to severe, depending on factors such as infective dose, virus strain, temperature, age and immune status of the fish. No lesions are pathognomonic to infection with ISAV, but anaemia and circulatory disturbances are always present. The following findings have been described to be consistent with infection with ISAV, though all changes are seldom observed in one single fish.

- Yellowish or blood-tinged fluid in peritoneal and pericardial cavities.
- Oedema of the swim bladder.
- Small haemorrhages of the visceral and parietal peritoneum.
- Focal or diffusely dark red liver (a thin fibrin layer may be present on the surface).
- Swollen, dark red spleen with rounded margins.
- Dark redness of the intestinal wall mucosa in the blind sacs, mid- and hind-gut, without blood in the gut lumen of fresh specimens.
- Swollen, dark red kidney with blood and liquid effusing from cut surfaces.
- Pinpoint haemorrhages of the skeletal muscle.

4.2.2. Clinical chemistry

- Haematocrit <10 in end stages (25–30 often seen in less advanced cases). Haematocrit <10 should always be followed up by investigation for infection with ISAV ISA in sea-water reared Atlantic salmon.
- Blood smears with degenerate and vacuolised erythrocytes and the presence of erythroblasts with irregular nuclear shape. Differential counts show a reduction in the proportion of leucocytes relative to erythrocytes, with the largest reduction being among lymphocytes and thrombocytes.

Liver pathology will lead to increased levels of liver enzymes in the blood.

4.2.3. Microscopic pathology

Histological changes in clinically diseased Atlantic salmon are variable, but can include the following:

- Numerous erythrocytes in the central venous sinus and lamellar capillaries where erythrocyte thrombi also form in the gills.

Annex 25 (contd)

- Multifocal to confluent haemorrhages and/or hepatocyte necrosis at some distance from larger vessels in the liver. Focal accumulations of erythrocytes in dilated hepatic sinusoids.
- Accumulation of erythrocytes in blood vessels of the intestinal lamina propria and eventually haemorrhage into the lamina propria.
- Spleen stroma distended by erythrocyte accumulation.
- Slight multifocal to extensive diffuse interstitial haemorrhage with tubular necrosis in the haemorrhagic areas, erythrocyte accumulation in the glomeruli in the kidney.
- Erythrophagocytosis in the spleen and secondary haemorrhages in liver and kidney.

4.2.4. Wet mounts

Not applicable.

4.2.5. Smears

See Section 4.3.1.1.2.

4.2.6 Fixed sections

See Section 4.3.1.1.3.

4.2.7. Electron microscopy/cytopathology

Virus has been observed in endothelial cells and leukocytes by electron microscopy of tissue preparations, but this method has not been used for diagnostic purposes.

4.2.8. ~~Differential diagnoses~~

~~Other anaemic and haemorrhagic conditions, including erythrocytic inclusion body syndrome, winter ulcer and septicaemias caused by infections with *Moritella viscosa*. Disease cases in Atlantic salmon with haematocrit values below 10 is not a unique finding for ISA, however cases with such low haematocrit values without any obvious explanation should always be tested for the presence of ISAV.~~

4.3. Agent detection and identification methods**4.3.1. Direct detection methods**

With the exception of molecular techniques (see 4.3.1.2.3), these direct detection methods are only recommended for fish with clinical signs of infection with HPR-deleted ISAV.

4.3.1.1. Microscopic methods*4.3.1.1.1. Wet mounts*

Not applicable.

*4.3.1.1.2. Smears**4.3.1.1.2.1 Indirect fluorescent antibody test*

An indirect fluorescent antibody test (IFAT) using validated monoclonal antibodies (MAbs) against ISAV haemagglutinin-esterase (HE) on kidney smears (imprints) or on frozen tissue sections of kidney, heart and liver has given positive reactions in both experimentally and naturally infected Atlantic salmon. Suspected cases (see Section 7.1) may be confirmed with a positive IFAT.

i) Preparations of tissue smears (imprints)

A small piece of the mid-kidney is briefly blotted against absorbent paper to remove excess fluid, and several imprints in a thumbnail-sized area are fixed on poly-L-lysine-coated microscope slides. The imprints are air-dried, fixed in chilled 100% acetone for 10 minutes and stored either at 4°C for a few days or at -80°C until use.

ii) Staining procedure

After blocking with 5% non-fat dry milk in phosphate-buffered saline (PBS) for 30 minutes, the preparations are incubated for 1 hour with an appropriate dilution of anti-ISAV MAb, followed by three washes. For the detection of bound antibodies, the preparations are incubated with fluorescein isothiocyanate (FITC)-conjugated anti-mouse Ig for 1 hour. PBS with 0.1% Tween 20 is used for washing. All incubations are performed at room temperature.

4.3.1.1.3. Fixed sections

4.3.1.1.3.1 Immunohistochemistry (IHC)

Polyclonal antibody against ISAV nucleoprotein is used on paraffin sections from formalin-fixed tissue. This IHC staining has given positive reactions in both experimentally and naturally infected Atlantic salmon. Preferred organs are mid-kidney and heart (transitional area including all three chambers and valves). Suspected cases due to pathological signs are verified with a positive IHC. Histological sections are prepared according to standard methods.

i) Preparation of tissue sections

The tissues are fixed in neutral phosphate-buffered 10% formalin for at least 1 day, dehydrated in graded ethanol, cleared in xylene and embedded in paraffin, according to standard protocols. Approximately 5 µm thick sections (for IHC sampled on poly-L-lysine-coated slides) are heated at 56–58°C (maximum 60°C) for 20 minutes, dewaxed in xylene, rehydrated through graded ethanol, and stained with haematoxylin and eosin for pathomorphology and IHC as described below.

ii) Staining procedure for IHC

All incubations are carried out at room temperature on a rocking platform, unless otherwise stated.

- a) Antigen retrieval is done by boiling sections in 0.1 M citrate buffer pH 6.0 for 2 × 6 minutes followed by blocking with 5% non-fat dry milk and 2% goat serum in 50 mM TBS (TBS; Tris/HCl 50 mM, NaCl 150 mM, pH 7.6) for 20 minutes.
- b) Sections are then incubated overnight with primary antibody (monospecific rabbit antibody against ISAV nucleoprotein) diluted in TBS with 1% non-fat dry milk, followed by three washes in TBS with 0.1% Tween 20.
- c) For detection of bound antibodies, sections are incubated with Alkaline phosphatase-conjugated antibodies to rabbit IgG for 60 minutes. Following a final wash, Fast Red (1 mg ml⁻¹) and Naphthol AS-MX phosphate (0.2 mg ml⁻¹) with 1 mM Levamisole in 0.1 M TBS (pH 8.2) is added to develop for 20 minutes. Sections are then washed in tap water before counterstaining with Harris haematoxylin and mounted in aqueous mounting medium. ISAV positive and ISAV negative tissue sections are included as controls in every setup.

iii) Interpretation

Negative control sections should not have any significant colour reactions. Positive control sections should have clearly visible red-coloured cytoplasmic and intranuclear staining of endothelial cells in blood vessels or heart endocardium. A test sample section should only be regarded as positive if clear, intranuclear red staining of endothelial cells is found. The intranuclear localisation is particular to the orthomyxovirus nucleoprotein during a stage of virus replication. Concurrent cytoplasmic staining is often dominant. Cytoplasmic and other staining patterns without intranuclear localisation must be considered as nonspecific or inconclusive.

The strongest positive staining reactions are usually obtained in endothelial cells of heart and kidney. Endothelial staining reactions within very extensive haemorrhagic lesions can be slight or absent, possibly because of lysis of infected endothelial cells.

Annex 25 (contd)**4.3.1.2. Agent isolation and identification***4.3.1.2.1. Cell culture*

ASK cells (Devold *et al.*, 2000) are recommended for primary ISAV isolation, but other susceptible cell lines, such as SHK-1 (Dannevig *et al.*, 1995), may be used. However, strain variability and the ability to replicate in different cell lines should be taken into consideration. The ASK cells seem to support isolation and growth of the hitherto known virus isolates. A more distinct cytopathic effect (CPE) may appear in ASK cells. Both the SHK-1 and ASK cell lines appear to lose susceptibility for ISAV with increasing passage level.

The SHK-1 and ASK cells are grown at 20°C in Leibovitz's L-15 cell culture medium supplemented with fetal bovine serum (5% or 10%), L-glutamine (4 mM), gentamicin (50 µg ml⁻¹) and 2-mercapto-ethanol (40 µM) (this latter may be omitted).

For virus isolation, cells grown in 25 cm² tissue culture flasks or multi-well cell culture plates, which may be sealed with parafilm or a plate sealer to stabilise the pH of the medium, may be used. Cells grown in 24-well plates may not grow very well into monolayers, but this trait may vary between laboratories and according to the type of cell culture plates used. Serially diluted ISAV-positive controls should be inoculated in parallel with the tissue samples as a test for cell susceptibility to ISAV (this should be performed in a separate location from that of the test samples).

i) Inoculation of cell monolayers

Prepare a 2% suspension of tissue homogenate using L-15 medium without serum or other medium with documented suitability. Remove growth medium from actively growing monolayers (1–3 day old cultures or cultures of 70–80 % confluency) grown in 25 cm² tissue culture flasks or multi-well cell culture plates (see above). Inoculate monolayers (25 cm² tissue culture flasks) with 1.5 ml of the 2% tissue homogenate. Adjust volume to the respective surface area in use. Allow 3–4 hours incubation at 15°C followed by removal of the inoculum, and addition of fresh, L-15 medium supplemented with 2–5% FCS. Alternatively, a 1/1000 dilution and direct inoculation without medium replacement can be used.

When fish samples come from production sites where infectious pancreatic necrosis virus (IPNV) is regarded as endemic, the tissue homogenate supernatant should be incubated (for a minimum of 1 hour at 15°C) with a pool of antisera to the indigenous serotypes of IPNV prior to inoculation.

ii) Monitoring incubation

Inoculated cell cultures (kept at 15°C) are examined at regular intervals (at least every 7 days) for the occurrence of CPE. Typical CPE due to ISAV appears as vacuolated cells that subsequently round up and loosen from the growth surface. If CPE consistent with that described for ISAV or IPNV appears, an aliquot of the medium for virus identification, as described below, must be collected. In the case of an IPNV infection, re-inoculate cells with tissue homogenate supernatant that has been incubated with a lower dilution of IPNV antisera. If no CPE has developed after 14 days, subculture to fresh cell cultures.

iii) Subcultivation procedure

Aliquots of medium (supernatant) from the primary cultures are collected 14 days (or earlier when obvious CPE appears) after inoculation. Supernatants from wells inoculated with different dilutions of identical samples may be pooled for surveillance purposes.

Supernatants are inoculated into fresh cell cultures as described for the primary inoculation: remove growth medium, inoculate monolayers with a small volume of diluted supernatant (1/5 and higher dilutions) for 3–4 hours before addition of fresh medium. Alternatively, add supernatants (final dilutions 1/10 and higher) directly to cell cultures with growth medium.

Inoculated cell cultures are incubated for at least 14 days and examined at regular intervals, as described for the primary inoculation. At the end of the incubation period, or earlier if obvious CPE appears, the medium is collected for virus identification, as described below. Cell cultures with no CPE should always be examined for the presence of ISAV by immunofluorescence (IFAT), haemadsorption or by PCR because virus replication may occur without development of apparent CPE.

The procedure described below has been successful for isolation of HPR-deleted ISAV from fish with clinical signs or from suspected cases. HPR0 has hitherto not been isolated in cell culture.

4.3.1.2.2. Antibody-based antigen detection methods

4.3.1.2.2.1 Virus identification by IFAT

All incubations are carried out at room temperature unless otherwise stated.

- i) Prepare monolayers of cells in appropriate tissue culture plates (e.g. 96-well or 24-well plates), in slide flasks or on cover-slips dependent on the type of microscope available (an inverted microscope equipped with UV light is necessary for monolayers grown on tissue culture plates). SHK-1 cells grow rather poorly on glass cover-slips. The necessary monolayers for negative and positive controls must be included.
- ii) Inoculate the monolayers with the virus suspensions to be identified in tenfold dilutions, two monolayers for each dilution. Add positive virus control in dilutions known to give a good staining reaction. Incubate inoculated cell cultures at 15°C for 7 days or, if CPE appears, for a shorter time.
- iii) Fix in 80% acetone for 20 minutes after removing cell culture medium and rinsing once with 80% acetone. Remove the fixative and air dry for 1 hour. The fixed cell cultures may be stored dry for less than 1 week at 4°C or at –20°C for longer storage.
- iv) Incubate the cell monolayers with anti- ISAV MAb in an appropriate dilution in PBS for 1 hour, and rinse twice with PBS/0.05% Tween 20. If unspecific binding is observed, incubate with PBS containing 0.5% dry skimmed milk.
- v) Incubate with FITC-conjugated goat anti-mouse immunoglobulin for 1 hour (or if antibody raised in rabbits is used as the primary antibody, use FITC-conjugated antibody against rabbit immunoglobulin), according to the instructions of the supplier. To increase the sensitivity, FITC-conjugated goat anti-mouse Ig may be replaced with biotin-labelled anti-mouse Ig and FITC-labelled streptavidin with the described rinsing in between the additional step. Rinse once with PBS/0.05% Tween 20, as described above. The nuclei can be stained with propidium iodide (100 µg ml⁻¹ in sterile distilled water). Add PBS (without Tween 20) and examine under UV light. To avoid fading, the stained plates should be kept in dark until examination. For long periods of storage (more than 2–3 weeks) a solution of 1,4-diazabicyclooctane (DABCO 2.5% in PBS, pH 8.2) or similar reagent may be added as an anti-fade solution.

4.3.1.2.3. Molecular techniques

4.3.1.2.3.1 Reverse-transcription polymerase chain reaction (RT-PCR)

The primers described below for RT-PCR and real-time RT-PCR will detect both European and North-American HPR-deleted ISAV, and also HPR0 ISAV.

RT-PCR may be used for detection of ISAV from total RNA (or total nucleic acid) extracted from recommended organs/tissues (see Section 3.4). The real-time RT-PCR for the detection of ISAV is recommended as it increases the specificity and, probably, also the sensitivity of the test. Though several primer sets for ISAV real-time RT-PCR have been reported, recommended primer sets are presented in the table below. The primer sets derived from genomic segment 8 and segment 7 have been used by several laboratories and have been found suitable for detection of ISAV during disease outbreaks and in apparently healthy carrier fish.

With the widespread occurrence of HPR0 ISAV variants, it is essential to follow up any positive PCR results based on segment 7 or 8 primer sets by sequencing the HPR of segment 6 in order to determine the ISAV HPR variant present (HPR-deleted or HPR0 or both). Adequate primers, designed and validated by the OIE Reference Laboratory are given in the table below. Validation of the HPR primer set for the North American isolates is restricted by the limited sequence data available in the Genbank for the 3' end of ISAV segment 6.

Annex 25 (contd)

The primers for segment 7 and 8 as well as sequencing primers for segment 6 HPR, are listed below and may also be used for conventional RT-PCR if necessary.

Real-time <u>and conventional</u> RT-PCR: Primer and probe sequences	Named	Genomic segment	Product size	Reference
5'-CAG-GGT-TGT-ATC-CAT-GGT-TGA-AAT-G-3' 5'-GTC-CAG-CCC-TAA-GCT-CAA-CTC-3' 5'-6FAM-CTC-TCT-CAT-TGT-GAT-CCC-MGBNFQ-3'	forward primer reverse primer Taqman@probe	7	155 nt	Snow <i>et al.</i> , 2006
5'-CTA-CAC-AGC-AGG-ATG-CAG-ATG-T-3' 5'-CAG-GAT-GCC-GGA-AGT-CGA-T-3' 5'-6FAM-CAT-CGT-CGC-TGC-AGT-TC-MGBNFQ-3'	forward primer reverse primer Taqman@probe	8	104 nt	Snow <i>et al.</i> , 2006
5'-GAC-CAG-ACA-AGC-TTA-GGT-AAC-ACA-GA-3' 5'-GAT-GGT-GGA-ATT-CTA-CCT-CTA-GAC-TTG-TA-3'	forward primer reverse primer	6 (HPR)	304 nt if HPR0	Designed by OIE Ref. Lab.

4.3.1.2.3.2 Real-time RT-PCR

4.3.1.2.3.2.1 Sampling

Target organs are normally the heart, kidney and gills. Under surveillance protocols, pooled organs of three fish are recommended while individual analysis of samples is required for confirmatory purposes as well as for molecular studies. Immediately after organ extraction from fish, 0.5 mm³ slices are independently imbibed in RNAlater (or ethanol) as preservative in Eppendorf tubes properly labelled to be sent in isothermal containers with cooling units to the diagnostic laboratories. The cold chain must be maintained during the delivery process.

4.3.1.2.3.2.2 Processing and analysis of samples via real-time RT-PCR

i) RNA extraction

Samples are removed from the RNA preservative, weighed and the sum of the three target organ slices must be kept in the range from 30 to 40 mg. Samples are then homogenised in lysis buffer (according to the kit used) supplemented with 1.4 mm Zirconium oxide beads in an automated Roche's Magalis device kit followed by RNA extraction using the E.Z.N.A.® Total RNA Kit (TRK) I (Catalog Number R683402CH), under the following conditions:

Amount of tissue	Amount of TRK lysis buffer	2-Mercaptoetanol
30–40 mg	700 µl	14 µl

ii) Real-time RT-PCR reactions

Three parallel reactions are normally run for each sample, the first two target viral coding segment 8, and the third is a cellular housekeeping gene acting as quality control: (1) carried out according to Snow *et al.* (2006); (2) under an optimised mix named GIM⁴; (3) measures the reporter gene ELF-1α as a reference for the integrity of the RNA recovered.

Mixes are distributed either in ELISA plates or tube strips and kept at 4°C until use. Reactions are recorded using the SuperScript™ III Platinum™ One-Step qRT-PCR Kit, (Catalogue Number 11732088). Each mix is prepared for a final volume of 20 µl considering a maximum simple volume of 4 µl according to the following tables.

Master Mix	Forward primer 20 µM	Reverse primer 20 µM	Probe 20 µM	ROX	Enzyme	Water	Sample	Final volume
10 µl	1 µl	1 µl	0.3 µl	0.4 µl	0.4 µl	4 µl	4 µl	20 µl

⁴ GIM: Available from the OIE Reference Laboratory in Chile

Annex 25 (contd)

<u>Assay</u>	<u>Primer/probe</u>	<u>Sequence</u>
<u>Snow et al., 2006</u>	<u>Forward</u>	<u>5'-TGC-TAC-ACA-GCA-GGA-TGC-AG-3'</u>
	<u>Reverse</u>	<u>5'-CAT-CTT-CTC-TGT-CGA-GCA-GGA-3'</u>
	<u>Probe</u>	<u>6FAM-CAT-CGT-CGC-TGC-AGT-TC-MGBNFQ</u>
<u>GIM*</u>	<u>Forward</u>	<u>5'-ATC-AGT-AAA-CTT-CAG-AGG-AAC-ATC-3'</u>
	<u>Reverse</u>	<u>5'-GAA-ATG-AAG-ATG-TTG-CTC-AAC-3'</u>
	<u>Probe</u>	<u>5'-/56-FAM/AGC-GAC-GAT-ZEN-GAC-TCT-CTA-CTG-TGT-GAT-G-/3IABkFQ/-3'</u>
<u>ELF-1α</u> <u>Sepulveda et al., 2012</u>	<u>Forward</u>	<u>5'-GCC-CCT-CCA-GGA-YGT-YTA-CAA-3'</u>
	<u>Reverse</u>	<u>5'-CCA-CAC-GGC-CCA-CRG-GTA-C-3'</u>
	<u>Probe</u>	<u>5'-/56-FAM/ATC-GGY-GGT-AT+T+G+G+A+AC-/3BHQ</u>

*Developed by the OIE Reference Laboratory in Chile.

iii) Sample processing

ELISA plates or strips with reaction mix are taken from 4°C and loaded with adequate volume of samples. Controls are then loaded: a) a positive amplification control (RNA from an ISAV positive reference tissue); b) a negative extraction control (RNA from an ISAV negative reference tissue, extracted along with the testing samples); c) a negative amplification control (free water). Finally, the plates are sealed with parafilm or the tube strips covered and taken to the thermocycler where they are placed before passing by a spin.

iv) Real-time PCR programme

The three reactions (Snow et al., GIM and ELF-1 α) are run in parallel and analysed under a simplex format; temperatures for each were carefully set as follows:

<u>Steps</u>	<u>Temperature</u>	<u>Time</u>	<u>Steps</u>
<u>RT</u>	<u>50°C</u>	<u>15 minutes</u>	<u>1</u>
<u>Initial denaturation</u>	<u>95°C</u>	<u>2 minutes</u>	<u>1</u>
<u>Denaturation, annealing and extension</u>	<u>95°C</u>	<u>10 seconds</u>	<u>45</u>
	<u>60°C</u>	<u>1 minute</u>	

4.3.1.2.3.2.3 Interpretation of the results

Results are read and interpreted using the StepOne software version 2.3, according to the following steps:

- i) Thresholds are set manually by assigning 0.1 values to the Snow et al. and GIM assay and 0.4 to the ELF-1 α assay.
- ii) Controls are checked. If the results are as expected, the reading is continued. If not, the run is aborted.
- iii) Ct values for ELF-1 α should be within established ranges (14–25) together with a reasonably shaped curve.
- iv) Sample results for Snow et al. and GIM should give similar Ct values with delta values ranging from 1 to 2 units and share similar curve shapes.

Annex 25 (contd)

- v) Once this procedure is done, results are recorded in a pre-established form and sent to the OIE Reference Laboratory in Chile no later than 24–48 hours upon sample reception.
- vi) For positive results, a second analysis is required to determine if the putative virus detected is a HPR-deleted variant or a HPRO.

4.3.1.2.4. Agent purification

ISAV propagated in cell culture can be purified by sucrose gradient centrifugation (Falk *et al.*, 1997) or by affinity purification using immunomagnetic beads coated with anti-ISAV MAb.

4.3.2. Serological methods

None published or validated.

~~Both Atlantic salmon and rainbow trout develop a humoral immune response to the ISAV infection. Enzyme-linked immunosorbent assays (ELISAs) with either purified virus or lysates from ISAV-infected cell cultures have been established for detection of ISAV-specific antibodies. ELISA titres can be very high and appear to be quite specific for the nucleoprotein in Western blots (K. Falk, pers. comm.). The test is not standardised for surveillance or diagnostic use, but may be used as a supplement to direct virus detection and pathology in obscure cases. Furthermore, the level and distribution of seroconversion in an ISAV-infected population may give some information about the spread of infection, particularly in cases where vaccination is not practised, and in wild fish.~~

5. Rating of tests against purpose of use

As an example, the methods currently available for targeted surveillance for infection with HPR-deleted ISAV and diagnosis of infection with ISAV ISA are listed in Table 5.1. For surveillance of infection with HPR0 ISAV, real-time RT-PCR followed by conventional RT-PCR and sequencing are the only recommended methods (not included in the table). The designations used in the Table indicate: a = the method is the recommended method for reasons of availability, utility, and diagnostic specificity and sensitivity; b = the method is a standard method with good diagnostic sensitivity and specificity; c = the method has application in some situations, but cost, accuracy, or other factors severely limits its application; and d = the method is presently not recommended for this purpose. These are somewhat subjective as suitability involves issues of reliability, sensitivity, specificity and utility. Although not all of the tests listed as category a or b have undergone formal standardisation and validation, their routine nature and the fact that they have been used widely without dubious results, makes them acceptable.

*Table 5.1. Methods for targeted surveillance and diagnosis**

Method	Targeted surveillance for infection with HPR-deleted ISAV				Presumptive diagnosis	Confirmatory diagnosis
	Fry	Parr	Smolt	Adults		
Gross signs	d	d	d	d	c	b
Histopathology	d	d	d	b	b	b
IFAT on kidney imprints	d	d	d	d	b	a
Immunohistochemistry	d	d	d	d	b	a
Isolation in cell culture with virus identification	a	a	a	a	a	a
RT-PCR or real-time RT-PCR followed by sequencing	<u>a-c</u>	<u>a-c</u>	<u>a-c</u>	<u>a-c</u>	b	<u>a-c</u>
<u>Real-time RT-PCR</u>	<u>a</u>	<u>a</u>	<u>a</u>	<u>a</u>	<u>a</u>	<u>b</u>
<u>Sequencing</u>	<u>d</u>	<u>d</u>	<u>d</u>	<u>d</u>	<u>d</u>	<u>a</u>

*As the diagnosis of infection with ISAV is not based on the results of a single method, the information in this Table should be used with care. See Section 7 for the criteria for infection with ISAV diagnosis.
 PLs = postlarvae; IFAT = indirect fluorescent antibody test; EM = electron microscopy;
 RT-PCR = reverse-transcriptase polymerase chain reaction.

6. Test(s) recommended for targeted surveillance to declare freedom from infection with ISAV infectious salmon anaemia virus

For infection with ISAV, real-time RT-PCR is the recommended test for surveillance. ~~Regular health inspections combined with investigation for ISA when increased mortality is associated with one of the given clinical signs and/or pathological changes consistent with ISA is an efficient way of obtaining data on the occurrence of ISA in farmed populations. In addition to regular health inspections, testing for HPR-deleted ISAV, preferentially by PCR-based methodology, at certain intervals may be carried out. However, due to the expected low prevalence in apparently healthy populations and the uneven spread of infection within a farm, statistically appropriate numbers of samples need to be tested. The significance of positive findings of ISAV by PCR alone for the risk of developing ISA disease is not clear, and therefore any positive findings would have to be followed up by either further testing and/or surveillance of the production site.~~

~~Because of the transient nature of HPR0 ISAV, statistically appropriate sample sizes need to be tested at time points through the production cycle to be able to document freedom of this infection.~~

7. Corroborative diagnostic criteria

Reasonable grounds to suspect fish of being infected with ISAV (HPR-deleted or HPR0) are outlined below. The Competent Authority should ensure that, following the suspicion of fish infected with ISAV on a farm, an official investigation to confirm or rule out the presence of the disease will be carried out as quickly as possible, applying inspection and clinical examination, as well as collection and selection of samples and using the methods for laboratory examination as described in Section 4.

7.1. Definition of suspect case (~~HPR-deleted ISAV~~)

Infection with HPR0 or HPR-deleted ISAV shall be suspected if the following criterion is met:

~~ISA or infection with HPR-deleted ISAV would be suspected if at least one of the following criteria is met:~~

~~i) Positive conventional RT-PCR or real-time RT-PCR result~~

In addition, infection with HPR-deleted ISAV shall be suspected if one of the following criteria is met:

~~ii) Clinical signs consistent with ISA and/or pathological changes consistent with ISA (Section 4.2) whether or not the pathological changes are associated with clinical signs of disease;~~

~~ii) CPE typical of ISAV in cell cultures Isolation and identification of ISAV in cell culture from a single sample (targeted or routine) from any fish on the farm, as described in Section 4.3.1.2.1;~~

~~iii) Evidence for the presence of ISAV from two independent laboratory tests such as RT-PCR (Section 4.3.1.2.3) and/or (Section 4.3.1.1.2.1) or IHC (Section 4.3.1.1.3.1)~~

iii) Positive IFAT on tissue imprints

Annex 25 (contd)**7.2. Definition of a confirmed case (HPR-deleted ISAV)**

The presence of HPR-deleted ISAV is considered to be confirmed if, in addition to the criteria in Section 7.1, one or more of the following criteria are met:

- i) ISAV isolation is carried out in cell culture followed by virus identification by either an antibody-based test (IFAT) and/or conventional PCR followed by sequencing of the amplicon;
- ii) ISAV is detected in histological sections by immunoassay using specific anti-ISAV antibodies;
- iii) Detection of ISAV in tissue preparations by conventional PCR followed by sequencing of the amplicon

7.2.1. Definition of confirmed ISA

~~The following criteria should be met for confirmation of ISA: detection of ISAV in tissue preparations by means of specific antibodies against ISAV (IHC on fixed sections [Section 4.3.1.1.3.1] or IFAT on tissue imprints [Section 4.3.1.1.2] or fixed sections as described in Section 4.3.1.1.3) in addition to either:~~

- ~~i) Isolation and identification of ISAV in cell culture from at least one sample from any fish on the farm, as described in Section 4.3.1.2.1~~

~~or~~

- ~~ii) Detection of ISAV by RT-PCR by the methods described in Section 4.3.1.2.3;~~

7.2.2 Definition of confirmed HPR-deleted ISAV infection

~~The criteria given in i) or ii) should be met for the confirmation of infection with HPR-deleted ISAV.~~

- ~~i) Isolation and identification of ISAV in cell culture from any fish sample on the farm as described in Section 4.3.1.2.1.~~
- ~~ii) Isolation and identification of ISAV in cell culture from at least one sample from any fish on the farm with corroborating evidence of ISAV in tissue preparations using either RT-PCR (Section 4.3.1.2.3) or IFAT/IHC (Sections 4.3.1.1.2 and 4.3.1.1.3).~~

7.3. Definition of a confirmed infection with case(HPR0 ISAV)**7.3.1. Definition of confirmed infection with HPR0 ISAV**

~~The criteria given in i) should be met for the confirmation of HPR0 ISAV infection. The presence of HPR0 ISAV is considered to be confirmed if, in addition to the criteria in Section 7.1, the following criterion is met:~~

- ~~i) Detection of ISAV by RT-PCR followed by independent amplification and sequencing of the HPR region of segment 6 to confirm the presence of HPR0 only.~~

7.3. Definition of confirmed infection with HPR0 ISAV**7.3.1. Definition of confirmed infection with HPR0 ISAV**

The criteria given in i) should be met for the confirmation of HPR0 ISAV infection.

- i) Detection of ISAV by RT-PCR followed by independent amplification and sequencing of the HPR region of segment 6 to confirm the presence of HPR0 only.
- ii) Detection of ISAV by RT-PCR followed by independent amplification and sequencing of the HPR region of segment 6 to confirm the presence of HPR0 only.

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*
* *

NB: There are OIE Reference Laboratories for Infection with infectious salmon anaemia virus (see Table at the end of this *Aquatic Manual* or consult the OIE Web site for the most up-to-date list: <http://www.oie.int/en/our-scientific-expertise/reference-laboratories/list-of-laboratories/>).

Please contact the OIE Reference Laboratory for any further information on Infection with infectious salmon anaemia virus

NB: FIRST ADOPTED IN 1995 AS INFECTIOUS SALMON ANAEMIA; MOST RECENT UPDATES ADOPTED IN 2014.

CHAPTER 2.2.3.

INFECTION WITH INFECTIOUS HYPODERMAL AND HAEMATOPOIETIC NECROSIS VIRUS

[...]

2.2. Host factors

2.2.1. Susceptible host species

Species that fulfil the criteria for listing as susceptible to infection with IHNV according to Chapter 1.5. of the *Aquatic Animal Health Code (Aquatic Code)* include: ~~giant river prawn (*Macrobrachium rosenbergii* [under study])~~, yellowleg shrimp (*Penaeus californiensis*), giant tiger prawn (*Penaeus monodon*), northern white shrimp (*Penaeus setiferus*), blue shrimp (*Penaeus stylirostris*), and white leg 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 a species as susceptible to infection with IHNV according to Chapter 1.5. of the *Aquatic Code* include: northern brown shrimp (*Penaeus aztecus*).

In addition, pathogen-specific positive polymerase chain reaction (PCR) results have been reported in the following organisms, but an active infection has not been demonstrated: giant river prawn (*Macrobrachium rosenbergii*), northern pink shrimp (*Penaeus duorarum*), western white shrimp (*Penaeus occidentalis*), kuruma prawn (*Penaeus japonicus*), green tiger prawn (*Penaeus semisulcatus*), *Hemigrapsus penicillatus*, Argentine stiletto shrimp (*Artemesia longinaris*), Cuata swimcrab (*Callinectes arcuatus*), Mazatlan sole (*Achirus mazatlanus*), yellowfin mojarra (*Gerres cinereus*), tilapias (*Oreochromis* sp.), Pacific piquitinga (*Lile stolifera*) and blackfin snook (*Centropomus medius*).

[...]

ASSESSMENT OF KURUMA SHRIMP (*PENAEUS JAPONICUS*) TO ACUTE HEPATOPANCREATIC NECROSIS DISEASE (AHPND) AGAINST CRITERIA IN CHAPTER 1.5.

The *ad hoc* Group on Susceptibility of crustacean species to infection with OIE listed diseases (the *ad hoc* Group) assessed *Penaeus japonicus* for susceptibility to infection with AHPND causing bacteria based on the reference Tinwongger *et al.* (2016).

Criteria for susceptibility to infection with AHPND causing bacteria are detailed in Table 1 (as per Article 1.5.6. of the *Aquatic Code*). This table includes Replication (A), Viability/Infectivity (B), Pathology/Clinical signs (C) and location (D). Hosts were considered to be infected with AHPND causing bacteria if they fulfilled either criterion A, or at least two of criteria B, C and D (as per point 3 of Article 1.5.7. of the *Aquatic Code*).

Table 1. Criteria for susceptibility to infection with AHPND causing bacteria

A: Replication	B: Viability / Infectivity	C: Pathology / Clinical signs	D: Location
Presence of characteristic histopathology Demonstration of increasing copy number over time with qPCR with confirmatory PCR/sequencing specific for Pir toxin gene Serial passage from individual to SPF individual of the same species*	Single passage bioassay to a SPF (target pathogen) of any susceptible host species and confirmation of pathogen identification**	Clinical signs and mortality can start as early as ten days post-stocking. Clinical signs include a pale-to-white hepatopancreas (HP), significant HP atrophy, soft shells, guts with discontinuous, or no, contents, black spots or streaks visible within the HP (due to melanised tubules). <u>Acute phase:</u> Characterised by a massive and progressive degeneration of the HP tubules from proximal to distal, with significant rounding and sloughing of HP tubule epithelial cells into the HP tubules, HP collecting ducts and posterior stomach in the absence of bacterial cells. <u>Terminal phase:</u> Characterised by marked intra-tubular haemocytic inflammation and development of massive secondary bacterial infections that occur in association with the necrotic and sloughed HP tubule cells.***	Gut-associated tissues and organs, such as hepatopancreas (HP), stomach, the midgut and the hindgut.

Key:

- * To demonstrate replication by this approach requires evidence for multiple passages in confirmed target pathogen-free hosts of the same species as being assessed.
- ** To demonstrate viability or infectivity of the target pathogen within the host being assessed, single passage in any known susceptible SPF host is required.
- *** Demonstration of terminal phase is insufficient evidence for fulfilment of this category when evidence from acute phase histopathology is not available.

Taking into account information in Tinwongger *et al.* (2016) the *ad hoc* Group agreed that the identity of the pathogenic agent had been confirmed in accordance with Article 1.5.5.

Annex 27A (contd)

The *ad hoc* Group assessment for host susceptibility to infection with AHPND causing bacteria is provided in Table 2.

The *ad hoc* Group agreed that *P. japonicus* did not fulfil criteria A, B, C or D (see Table 2). Regarding criterion C (Pathology/Clinical signs; Article 1.5.6.) the *ad hoc* Group noted that although high mortality was reported in Tinwongger *et al.* (2016) there were no other pathological signs specific for AHPND relative to control group. The *ad hoc* Group agreed that although *P. japonicus* is probably susceptible to the effects of the *Photorhabdus* insect-related (Pir) toxins, PirA and PirB, there is insufficient evidence to be conclusive and they therefore allocated a 'No' to criterion C.

Table 2. Outcome of assessment for host susceptibility to AHPND

Genus	Species	Stage 1: Transmission*	Stage 2: Toxin gene identification	Stage 3: Evidence for infection				Outcome**	References
				A Replication	B Viability/ Infectivity	C Pathology/ Clinical signs	D Location		
<i>Penaeus</i>	<i>japonicus</i>	E (immersion)	PCR	No	No (because infectivity has not been proven)	No	No	3	Tinwongger <i>et al.</i> (2016)

Transmission Key*:

N: Natural infection

E: Experimental infection

Outcome Key**:

Outcome 1: Host species proposed to be listed in Article 9.3.2. of the *Aquatic Code*.

Outcome 2: Host species proposed to be listed in Chapter 2.2.1. of the *Aquatic Manual* under the revised Section 2.2.2. '*Species with incomplete evidence for susceptibility*'.

Outcome 3: Host species proposed to be listed in Chapter 2.2.1. of the *Aquatic Manual* under the revised Section 2.2.2. '*Species with incomplete evidence for susceptibility*' where pathogen-specific positive PCR results (but an active infection has not been demonstrated) have been reported.

The *ad hoc* Group agreed that *Penaeus japonicus* did not meet the criteria in Chapter 1.5. for listing in the *Aquatic Code* but agreed it should be included in the *Aquatic Manual* (Chapter 2.2.1., Section 2.2.2. *Species with incomplete evidence for susceptibility*) with the following new text:

'In addition, pathogen-specific positive polymerase chain reaction (PCR) results have been reported in the following organisms, but an active infection has not been demonstrated: Kuruma prawn (*Penaeus japonicus*).'

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CHAPTER 2.2.X.

ACUTE HEPATOPANCREATIC NECROSIS DISEASE

1. Scope

Acute hepatopancreatic necrosis disease (AHPND) means infection with strains of *Vibrio parahaemolyticus* (V_{AHPND}) that contain a ~70-kbp plasmid with genes that encode homologues of the *Photobacterium* insect-related (Pir) toxins, PirA and PirB. Although there are reports of the isolation of other *Vibrio* species from clinical cases of AHPND, only V_{AHPND} has been demonstrated to cause AHPND.

[...]

2.2. Host factors

2.2.1. Susceptible host species

Species that fulfil the criteria for listing as susceptible to AHPND according to Chapter 1.5. of the *Aquatic Code* include: giant tiger prawn (*Penaeus monodon*) 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 a species as susceptible AHPND for susceptibility according to Chapter 1.5. of the *Aquatic Code* include: fleshy prawn (*Penaeus chinensis*).

In addition, pathogen-specific positive polymerase chain reaction (PCR) results have been reported in the following organisms, but an active infection has not been demonstrated: kuruma prawn *Penaeus japonicus*.

Assessment of Tilapia lake virus against the new criteria for listing aquatic animal diseases in accordance with Chapter 1.2. of the *Aquatic Code* (2017 edition)

Overall assessment

The OIE Aquatic Animal Health Standards Commission assessed Tilapia lake virus (TiLV) against the criteria for listing aquatic animal diseases in accordance with Article 1.2.2. of the *Aquatic Code* (2017 edition) (see Table 1 below).

Table 1. Summary of assessment of TiLV

	Listing criteria						Conclusion
	1	2	3	4a	4b	4c	
Tilapia lake virus	+	+	-	NA	+	+	do not list

NA = not applicable.

Background

A novel orthomyxo-like virus, named as Tilapia lake virus (TiLV), has been identified as the cause of mass die-offs of tilapia (Eyngor *et al.*, 2014) in both farms and the wild environment. The host range is not well known but a number of tilapines are known to be susceptible (Eyngor *et al.*, 2014). Tilapia is the second most important group of farmed fish after carps. Global production of tilapia, predominantly *Oreochromis niloticus*, is estimated at 4.5 million metric tonnes (FAO data). Farming occurs primarily in tropical and subtropical countries though some production in recirculation systems has started in other regions. *O. niloticus* was first introduced to developing countries to support subsistence farming. However, larger scale commercial production is now significant and tilapia products are traded globally.

Assessment for TiLV in accordance with Article 1.2.2. of the *Aquatic Code* (2017 edition)

Criterion	Criterion text	Assessment
1	International spread of the <i>pathogenic agent</i> (via <i>aquatic animals, aquatic animal products, vectors or fomites</i>) is likely.	<p>TiLV has been reported in Chinese Taipei, Colombia, Ecuador and Israel (Bacharach <i>et al.</i>, 2016; Ferguson <i>et al.</i>, 2014; Tsofack <i>et al.</i>, 2016), and most recently, Egypt (Fathi <i>et al.</i>, 2017) and Thailand (Dong <i>et al.</i>, 2017). Despite geographic separation strains were highly homologous, suggesting an epidemiological link and international spread. Historically, tilapia has been traded internationally to establish populations for production in new regions, and there is still extensive trade in tilapia. The current driver for international trade is the dissemination of improved genetic strains (though current pattern and volume of trade has not been determined for this assessment). Tilapia products are traded internationally and while a risk of transmission with some product types should be expected, specific risks have not been considered in this assessment.</p> <p>Given the evidence of spread and the broad distribution of tilapia (Asia, Africa and South America), international spread is likely.</p> <p>Criterion met</p>

Annex 28 (contd)

AND			
2		<p>At least one country may demonstrate country or zone freedom from the <i>disease</i> in susceptible <i>aquatic animals</i>, based on provisions of Chapter 1.4.</p>	<p>Tilapia are farmed in many countries (Asia, Africa and South America) and in a range of production systems. While TiLV has been reported in Chinese Taipei, Colombia, Ecuador and Israel (Bacharach <i>et al.</i>, 2016; Ferguson <i>et al.</i>, 2014; Tsofack <i>et al.</i>, 2016), and most recently, Egypt (Fathi <i>et al.</i>, 2017) and Thailand (Dong <i>et al.</i>, 2017). However, a lack of thorough investigation of all mortality incidents and possible associated international trade in tilapia means that the geographic distribution of TiLV may be wider than currently reported. For example, reports of mortality in tilapia in Ghana and Zambia in 2016 have not been attributed to TiLV but the available information does not indicate that the presence of the virus has been investigated. There is significant production of tilapia in other countries with no report of unexplained mortality; therefore, it is considered likely that some countries remain free.</p> <p>Criterion met</p>
AND			
3		<p>A precise <i>case definition</i> is available and a reliable means of detection and <i>diagnosis</i> exist.</p>	<p>A virus has been cultured from affected fish and the genome has been characterised and classified as a novel orthomyxovirus (Eyngor <i>et al.</i>, 2014). In-situ hybridisation indicates presence of the agent in association with lesions (Eyngor <i>et al.</i>, 2014). Cohabitation of infected and naïve fish demonstrated waterborne transmission between fish with the latter developing a lethal disease (mortality was similar to levels achieved by lethal injection) (Eyngor <i>et al.</i>, 2014).</p> <p>TiLV can be cultured in primary tilapia brain cells or in an E-11 cell line, inducing a cytopathic effect at 5-10 days (Eyngor <i>et al.</i>, 2014). A PCR primer set has been designed. However, it is not known whether these primers will detect all strains of the virus (Eyngor <i>et al.</i>, 2014).</p> <p>A more highly sensitive, nested RT-PCR has been published and is suitable for the detection of TiLV in clinical cases (Tsofack <i>et al.</i>, 2016). Most recently a semi-nest RT-PCR with improved detection sensitivity (7.5 viral copies per reaction) over the nested RT-PCR, has been published (Dong <i>et al.</i>, 2017). However, additional information on the validation of these assays is required to determine whether this criterion has been met.</p> <p>Criterion not met</p>

Annex 28 (contd)

AND			
4	a)	Natural transmission to humans has been proven, and human infection is associated with severe consequences.	Not applicable.
	OR		
4	b)	The <i>disease</i> has been shown to affect the health of cultured <i>aquatic animals</i> at the level of a country or a <i>zone</i> resulting in significant consequences e.g. production losses, morbidity or mortality at a <i>zone</i> or country level.	Very high levels of mortality (>80%) have been observed in affected populations (both farmed and wild) (Bacharach <i>et al.</i> , 2016; Ferguson <i>et al.</i> , 2014; Gophen <i>et al.</i> , 2015). Decreases of catch of tilapines, specifically <i>Sarotherodon (Tilapia) galilaeus</i> , from the Sea of Galilee have been observed since 2007. Dong <i>et al.</i> (2017) reported approximately 90% mortality in red tilapia fingerlings within one months of stocking into cages. Since 2009 episodic losses of tilapia (<i>Oreochromis niloticus</i>) were recorded in fish farms all over Israel (Eyngor <i>et al.</i> , 2014). Mortality in farmed <i>O. niloticus</i> in Ecuador have also been attributed to TiLV (Ferguson <i>et al.</i> , 2014). Losses are significant regionally and at a national level. Criterion met
	OR		
4	c)	The <i>disease</i> has been shown to, or scientific evidence indicates that it would, affect the health of wild <i>aquatic animals</i> resulting in significant consequences e.g. morbidity or mortality at a population level, reduced productivity or ecological impacts.	Very high levels of mortality (>80%) have been observed in affected populations (both farmed and wild) (Bacharach <i>et al.</i> , 2016; Ferguson <i>et al.</i> , 2014; Gophen <i>et al.</i> , 2015). Decreases of catch of tilapines, specifically <i>Sarotherodon (Tilapia) galilaeus</i> , from the Sea of Galilee have been observed since 2007. Since 2009 episodic losses of tilapia (<i>Oreochromis niloticus</i>) were recorded in fish farms all over Israel (Eyngor <i>et al.</i> , 2014). Losses are significant regionally and at a national level. Criterion met

Conclusion

TiLV is considered to meet criterion 1, 2, 4 b and c.

The virus can be cultured but antibody-based tests or nucleic acid-based tests to confirm the identification have not yet been validated sufficiently. A primer set is available but it is not clear whether all strains of the virus can be detected hence confirmation requires genetic sequencing. As a result, it cannot be concluded that a repeatable and robust means of diagnosis, criterion 3, has been met.

The disease does not meet the criteria for inclusion in the OIE list of diseases in Chapter 1.2.

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BATRACHOCHYTRIUM SALAMANDRIVORANS

INFORMATION ON THE PATHOGENIC AGENT

1. CAUSATIVE AGENT

1.1. Pathogenic agent type

Fungus.

1.2. Disease name and synonyms

Infection with *Batrachochytrium salamandrivorans*.

1.3. The pathogen agent's common names and synonyms

B. salamandrivorans, Bsal

1.4. Taxonomic affiliation

Batrachochytrium salamandrivorans (Kingdom Fungi, Phylum Chytridiomycota, Order Rhizophydiales, Genus *Batrachochytrium*).

1.5. Authority (first scientific description, reference)

B. salamandrivorans was first identified in 2013 following dramatic declines among populations of European fire salamanders (*Salamandra salamandra*) in the Netherlands (Martel *et al.*, 2013).

1.6. Pathogenic agent environment (fresh, brackish, marine waters)

Fresh water.

2. MODES OF TRANSMISSION

2.1. Routes of transmission (horizontal, vertical, indirect)

The pathogenic agent transmits horizontally. It can persist in the environment as non-motile or motile spores (Stegen *et al.*, 2017). It is not known whether direct animal to animal contact is epidemiologically important.

2.2. Reservoir

Based on characteristics of *B. dendrobatidis*, it is expected that resistant non-motile spores can survive in water and soil (Johnson & Speare, 2003). Individuals surviving disease can be persistently infected (Martel *et al.*, 2013; Martel *et al.*, 2014).

2.3. Risk factors (temperature, salinity, etc.)

B. salamandrivorans thrives best at temperatures around 15°C (Martel *et al.*, 2013). Salamanders have been shown experimentally not to be colonised by *B. salamandrivorans* at temperatures above 25°C (Bloom *et al.*, 2015a).

The zoospores actively swim in water, the fungus is dependent on water, and desiccation is fatal to all life stages (EFSA, 2017).

3. HOST RANGE

3.1. Susceptible species

B. salamandrivorans has a wide and as yet not fully characterised host range. It has been found to be susceptible in 14 amphibian species (EFSA, 2017).

Infection with *B. salamandrivorans* has also been reported in several species of wild, captive or museum specimens (Martel *et al.*, 2014; Spitzen van der Sluijs *et al.*, 2016).

3.2. Affected life stage

No published work has investigated susceptibility of different life stages; however there are no reports of resistant life stages.

4. GEOGRAPHICAL DISTRIBUTION

B. salamandrivorans was first detected in the Netherlands in 2013, and has later been found on several locations in neighbouring regions of Belgium in 2013 and 2014 (Martel *et al.*, 2014). The fungus has also been identified in captive populations of salamanders and newts in Germany (Spitzen van der Sluijs *et al.*, 2016) and in the United Kingdom (Cunningham *et al.*, 2015).

The origin of *B. salamandrivorans* is thought to lie in south-east Asia and it has been identified in Japan, Thailand and Vietnam (Laking *et al.*, 2017; Martel *et al.*, 2014).

Outside of Western Europe, *B. salamandrivorans* is known to infect a number of species of newts native to Asia, but does not appear to cause significant disease or mortality in those species. Current evidence strongly suggests that *B. salamandrivorans* is endemic to Asia and that species within this region may act as a disease reservoir (Laking *et al.*, 2017).

5. CLINICAL SIGNS AND CASE DESCRIPTION

5.1. Host tissues and infected organs

The main organ where pathology is observed is the skin (Martel *et al.*, 2013; Gray *et al.*, 2015).

5.2. Gross observations and macroscopic lesions

B. salamandrivorans parasitises epidermal cells of salamanders, causing skin ulcerations with significant degradation of the epidermis, impairment of vital skin functions, and subsequent death of susceptible species within two to three weeks (Martel *et al.*, 2013; Gray *et al.*, 2015; Laking *et al.*, 2017).

Annex 29 (contd)

Clinical signs of disease caused by fungi of the Genus *Batrachochytrium* are in general variable and not pathognomonic, although the lesions linked to *B. salamandrivorans* are characterised by marked skin ulcerations, in contrast to those caused by *B. dendrobatidis*, which typically induces epidermal hyperplasia and hyperkeratosis (Martel *et al.*, 2013 and 2014). As a consequence, clinical signs alone are not a suitable means for diagnosis.

5.3. Microscopic lesions and tissue abnormality

Histopathological lesions consist of focal epidermal ulcerations with high numbers of colonial thalli of *B. salamandrivorans* (Martel *et al.*, 2013).

5.4. OIE status

Infection with *B. salamandrivorans* was listed by the OIE in 2017 (see Chapter 1.3. of the *Aquatic Animal Health Code*) (OIE, 2017).

6. SOCIAL, ECONOMIC AND ENVIRONMENTAL SIGNIFICANCE

Amphibians are the most commonly traded animal across many regions of the world (Smith *et al.*, 2009; Nijman, 2010), though much of this trade has been reported to remain unregulated and unrecorded (Rowley *et al.*, 2016).

Investigations by Martel *et al.* (2013) provide very solid evidence that *B. salamandrivorans* is causing disease in fire salamanders in the Netherlands. Field observations and experimental studies indicate that case fatality approaches 100%. Between 2010 and 2013 the fire salamander in affected populations in the Netherlands was reduced by 96%.

The disease has the potential to negatively impact many amphibian populations. Yap *et al.* (2015) modelled the likely impact of *B. salamandrivorans* in North America and concluded that it is a serious threat to biodiversity there if introduced.

B. salamandrivorans could cause significant negative population level effects (including extinction) in many amphibian species (Yap *et al.*, 2015).

7. ZOONOTIC IMPORTANCE

None

8. DIAGNOSTIC METHODS**8.1. Definition of suspicion**

High levels of mortality in amphibian populations with or without erosive skin disease.

8.2. Presumptive test methods

A presumptive diagnosis can be made on the basis of histology and the identification of characteristic skin lesions and colonial thalli (Martel *et al.*, 2013). Thus this method is only suitable for diagnosis in clinical cases.

8.3. Confirmatory test methods

Diagnosis can be confirmed by PCR or culture.

Currently the most reliable and widely used method for *B. salamandrivorans* detection is quantitative PCR (qPCR) (Bloo *et al.*, 2013).

Bloo *et al.* (2013) also described a PCR protocol (Duplex Real-Time PCR) which allows for the simultaneous detection and quantification of both *B. dendrobatidis* and *B. salamandrivorans* in amphibian samples.

Isolation by culture methods has a low sensitivity (Martel *et al.*, 2014).

9. CONTROL METHODS

A protocol for treating infected salamanders has been developed (Bloo *et al.*, 2015a; Bloo *et al.*, 2015b). Infected salamanders exposed to temperatures of 25°C for 10 days were demonstrated to be cleared of infection. However, the margin between the temperature required to eliminate *B. salamandrivorans* and the upper thermal limit tolerated by most urodelans is narrow (Bloo *et al.*, 2015a). Alternatively, combinations of antibiotics have been shown to be similarly effective, but only kept or captured animals can be treated.

Trade in salamanders is considered to be the most likely pathway for entry of *B. salamandrivorans* into new geographical regions (Yap *et al.*, 2015; Grant *et al.*, 2016). Movement restrictions to limit pathogen introduction and early detection through surveillance of high-risk areas should be implemented to control pathogen invasion.

10. TRANSMISSION RISK

As *B. salamandrivorans* has been horizontally transmitted through cohabitation, disease transmission is likely with movement of aquatic animals.

The principal route for the global spread in *B. salamandrivorans* is considered to be the international trade in salamanders and newts (Martel *et al.*, 2014; Stephen *et al.*, 2015; Cooper, 2016; RAVON Reptielen Amfibieën Onderzoek Nederland 2016; U.S. Fish & Wildlife Service 2016). In Europe hobbyists are known to transport salamanders across borders to attend shows. This presents a potential risk for the spread of the pathogenic agent that is difficult to control.

B. salamandrivorans is also more likely to spread within crowded conditions, which create stress and weaken individuals' natural immune systems (Rachowicz *et al.*, 2005; Rowley *et al.*, 2007; Rollins-Smith *et al.*, 2011 in: U.S. Fish & Wildlife Service, 2016). *B. salamandrivorans* may also be introduced to the natural environment through improper disposal of contaminated water or material to transport salamanders (Stephen *et al.*, 2015; U.S. Fish & Wildlife Service, 2016). The intentional release of non-native salamanders (often as fishing baits) or unintentional escapes from enclosures may also result in introduction and establishment of *B. salamandrivorans* in wild populations (Picco and Collins, 2008; Krysko *et al.*, 2011 in: U.S. Fish & Wildlife Service, 2016).

The fact that salamanders can originate either from wildlife or from captivity (farms, hobby breeders, pet traders, etc.) is an aspect that should be considered when assessing the feasibility of a trade restriction.

For *B. dendrobatidis* all water and transport materials (*e.g.*, moist soil, packaging) used to import amphibians should be treated as contaminated for quarantine purposes (Johnson & Speare, 2003). It must be assumed that this also applies to *B. salamandrivorans*.

Annex 29 (contd)

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Annex 29 (contd)

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Original: English
August 2017

REPORT OF THE MEETING OF THE OIE AD HOC GROUP ON SUSCEPTIBILITY OF FISH SPECIES TO INFECTION WITH OIE LISTED DISEASES

Paris, 25–27 April 2017

The OIE *ad hoc* Group on susceptibility of fish species to infection with OIE listed diseases (the *ad hoc* Group) met for their second meeting at OIE Headquarters from 25–27 April 2017. (Please note, the report of the first *ad hoc* Group meeting held from 17–19 January 2017 was not published).

The list of participants and the Terms of Reference are presented in [Annex I](#) and [Annex II](#), respectively.

Dr Gillian Mylrea, Deputy Head Standards Department, welcomed members to this meeting, the second for this *ad hoc* Group and thanked them for their ongoing work on this important topic.

The Chair of the *ad hoc* Group, Dr Mark Crane, clarified that the purpose of this meeting was to finalise the assessments started at their previous meeting for infection with epizootic haematopoietic necrosis virus (EHNV), infection with *Gyrodactylus salaris* (*G. salaris*) and infection with koi herpesvirus (KHV); and to start work on the assessment for infection with infectious salmon anaemia (ISAV). He explained that the *ad hoc* Group would progressively apply the criteria to the OIE listed fish diseases, noting that it would take several meetings to complete this task. During this meeting the *ad hoc* Group finalised assessments for EHNV, ISAV and *G. salaris*.

The *ad hoc* Group applied the three-stage approach, outlined in Article 1.5.3. of the *Aquatic Code*, to assess susceptibility of a species, described as shown below:

1. criteria to determine whether the route of transmission is consistent with natural pathways for the infection (as described in Article 1.5.4.);
2. criteria to determine whether the pathogenic agent has been adequately identified (as described in Article 1.5.5.);
3. criteria to determine whether the evidence indicates that presence of the pathogenic agent constitutes an infection (as described in Article 1.5.6.).

Hosts that were classified as susceptible species (as described in Article 1.5.7.) were proposed for inclusion in Article 10.X.2. of the relevant *disease-specific* chapter of the *Aquatic Code*.

Hosts that were classified as species for which there is incomplete evidence for susceptibility (as described in Article 1.5.8.) were proposed for inclusion in a new Section 2.2.2. *Species with incomplete evidence for susceptibility* of the relevant chapter of the *Aquatic Manual*.

Annex 30 (contd)

The detailed assessments for each specific pathogenic agent assessed by the *ad hoc* Group are provided in Annexes III to V.

Disease	Annex Number
Infection with epizootic haematopoietic necrosis virus	Annex III
Infection with infectious salmon anaemia virus	Annex IV
Infection with <i>Gyrodactylus salaris</i>	Annex V

The *ad hoc* Group wished to note the following:

1. In many of the older publications accurate pathogen identification was not carried out because molecular typing techniques were not available at the time. Therefore, for many of these cases, a weight of evidence approach using combined data from relevant studies was used to assess susceptibility.
2. The *ad hoc* Group worked on the assumption that authors had correctly identified the host species on which they were reporting.
3. References that reported invasive experimental procedures as the route of transmission were not progressed past Stage 1 (i.e. Article 1.5.4.). In these cases the criteria A-D were noted as not applicable and the outcome inconclusive.
4. The *ad hoc* Group used the following outcome key when assessing the susceptibility of the species:
 - 1: The species meets the criteria for susceptibility and is proposed for inclusion in Article X.X.2. of the Aquatic Code;*
 - 2: The species meets some but not all of the criteria and is proposed for inclusion in Section 2.2.2. 'Species with incomplete evidence for susceptibility' of the Aquatic Manual;*
 - 3: The species does not meet the criteria (e.g. PCR- positive on gills or intestines and no other evidence; studies with questionable methodology or inconsistent results) and is not proposed for inclusion in either the Aquatic Code or Aquatic Manual;*
 - 4: There is evidence of non-susceptibility and the species is not proposed for inclusion in either the Aquatic Code or Aquatic Manual.*
5. Where there was conflicting evidence in the scientific literature for the same host species, or assessments differed (e.g. assessments ranging between '1' and '3'), the *ad hoc* Group provided some explanatory text in the relevant Annex as to their rationale for the final outcome.
6. For assessments that were inconsistent with known pathogen epidemiology (e.g. when a virus previously presumed as highly species-specific is shown to occur in a distant taxonomic group), the *ad hoc* Group required two or more independent studies to justify a new host species to be considered as susceptible.
7. The *ad hoc* Group separately identified hosts for which there was only evidence for criteria in Article 1.5.4. ('natural pathways for infection') and 1.5.5. ('pathogenic agent has been adequately identified'), but not 1.5.6. ('presence of the pathogenic agent constitutes an infection'), e.g. shown to be PCR positive without virus isolation, i.e. Outcome '3'.

The *ad hoc* Group recommended that these organisms not be included in Section 2.2.2. (Species with incomplete evidence for susceptibility) of the relevant chapter of the *Aquatic Manual*, as has been done in the revised crustacean disease chapters of the *Aquatic Manual* because finfish viruses can be cultured and therefore virus isolation from internal organs was required to be considered conclusive evidence of the presence of infectious virus. (Note: this differs from crustacean viruses where there are no *in-vitro* methods for virus isolation.)

Annex 30 (contd)

The *ad hoc* Group made the following recommendations:

- The *ad hoc* Group agreed to commence work electronically on KHV, Spring viraemia of carp and infection with Salmonid alphavirus.
- The *ad hoc* Group requested that another physical meeting be held in 2017 to finalise these assessments and to start applying the criteria to the remaining OIE listed fish diseases.

.../Annexes

Annex 30 (contd)Annex I**MEETING OF THE AD HOC GROUP ON SUSCEPTIBILITY OF FISH SPECIES TO INFECTION
WITH OIE LISTED DISEASES****Paris, 25–27 April 2017**

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Annex 30 (contd)Annex II

MEETING OF THE AD HOC GROUP ON SUSCEPTIBILITY OF FISH SPECIES TO INFECTION WITH OIE LISTED DISEASES

Paris, 25–27 April 2017

Terms of reference

Background

A new Chapter 1.5. 'Criteria for listing species as susceptible to infection with a specific pathogen' was introduced in the 2014 edition of the *Aquatic Code*. The purpose of this chapter is to provide criteria for determining which host species are listed as susceptible in Article X.X.2. of each disease specific chapter in the *Aquatic Code*. The criteria are to be applied progressively to each disease-specific chapter in the *Aquatic Code*.

These assessments will be undertaken by *ad hoc* Groups and the assessments will be provided to Member Countries' for comment prior to any change in the list of susceptible species in Article X.X.2. of the disease specific chapters in the *Aquatic Code*.

For species where there is some evidence of susceptibility but insufficient evidence to demonstrate susceptibility through the approach described in Article 1.5.3, information will be included in the relevant disease-specific chapter in the *Aquatic Manual*.

Purpose

The *ad hoc* Group on susceptibility of fish species to infection with OIE listed diseases will undertake assessments for the ten OIE listed fish diseases.

Terms of Reference

1. Consider evidence required to satisfy the criteria in Chapter 1.5.
2. Review relevant literature documenting susceptibility of species for OIE listed fish diseases.
3. Propose susceptible species for OIE listed diseases for fish based on Article 1.5.7.
4. Propose susceptible species for OIE listed diseases for fish based on Article 1.5.8.

Expected outputs of the *ad hoc* Group

1. Develop a list of susceptible species for inclusion in the relevant Article X.X.2. of fish disease-specific chapters in the *Aquatic Code*.
 2. Develop a list of species with incomplete evidence for susceptibility for inclusion in Section which 2.2.2. of the *Aquatic Manual*.
 3. Draft a report for consideration by the Aquatic Animals Commission at their September 2017 meeting.
-

ASSESSMENT OF HOST SUSCEPTIBILITY TO INFECTION WITH EPIZOOTIC HAEMATOPOIETIC NECROSIS VIRUS (EHNV)

Criteria for susceptibility to infection with EHNV are detailed in Table 1 (as per Article 1.5.6. of the *Aquatic Code*). This table includes Replication (A), Viability/Infectivity (B), Pathology/Clinical Signs (C) and Location (D). Hosts were considered to be infected with EHNV if they fulfilled either criterion A, or at least two of criteria B, C and D (as per point 3 of Article 1.5.7. of the *Aquatic Code*).

Table 1. Criteria for susceptibility to infection with EHNV

A: Replication	B: Viability/Infectivity	C: Pathology/Clinical signs	D: Location
Sequential virus titration showing increase in viral titres OR Demonstration of increasing copy number over time by qPCR with confirmatory PCR/sequencing OR TEM showing virions in host cells OR Products (e.g. antigens) of virus replication detected	Isolation by cell culture OR Cohabitation with passage to a susceptible host with confirmed infection in the sentinel species by PCR and demonstrating at least one of the following: i. clinical signs, with or without associated mortality, ii. Histopathology, iii. Re-isolation of virus in cell culture.*	Tropism for vascular endothelium and haematopoietic necrosis. Perivascular mononuclear inflammatory response in liver.	Gills, cardiovascular system, kidney, liver **

Key:

* To demonstrate viability or infectivity of the target pathogen within the host being assessed, single passage in any known susceptible SPF host is required.

** It is noted that target organs may be differ from those described for existing susceptible species. Where gills are used surface contamination should be ruled out.

Annex 30 (contd)

Annex III (contd)

ASSESSMENT FOR HOST SUSCEPTIBILITY

The assessment for host susceptibility to infection with EHNV is provided in Table 2.

Table 2. Outcome of assessment for host susceptibility to infection with EHNV

Genus	Species	Common name	Stage 1: Transmission*	Stage 2: Pathogen identification	Stage 3: Evidence for infection				Outcome**	References
					A	B	C	D		
<i>Oncorhynchus</i>	<i>mykiss</i>	rainbow trout	N/E	PCR/IFAT/ELISA	Y	Y	Y	Y	1	4, 3, 10, 11
<i>Perca</i>	<i>fluviatilis</i>	European perch	N/E	PCR/IFAT	Y	Y	Y	Y	1	2, 4, 9, 11
<i>Macquaria</i>	<i>australasica</i>	macquarie perch	E	PCR	Y	Y	Y	Y	1	2, 11
<i>Bidyanus</i>	<i>bidyanus</i>	silver perch	E	PCR	Y	Y	Y	Y	1	2, 11
<i>Galaxias</i>	<i>olidus</i>	mountain galaxias	E	Incomplete	Y	Y	Y	Y	1	11 (virus later characterized by 2)
<i>Gambusia</i>	<i>affinis</i>	mosquito fish	E	Incomplete	Y	Y	Y	Y	1	11 (virus later characterized by 2)
<i>Ameiurus</i>	<i>melas</i>	black bullhead	E	IFAT	N	Y	Y	Y	1	5
<i>Esox</i>	<i>lucius</i>	northern pike	E	IHC	Y	Y	Y	Y	1	6
<i>Sander</i>	<i>luciperca</i>	pike-perch	E	PCR/sequencing	N	Y	Y	Y	1	7
<i>Melanotaenia</i>	<i>fluviatilis</i>	crimson spotted rainbow fish	E	PCR	N	Y	Y	Y	1	2
<i>Gambusia</i>	<i>holbrooki</i>	eastern mosquito fish	E	PCR	N	Y	Y	Y	1	2
<i>Macquaria</i>	<i>ambigua</i>	golden perch	E	PCR	N	N	N	N	3	2

Annex 30 (contd)

Annex III (contd)

Genus	Species	Common name	Stage 1: Transmission*	Stage 2: Pathogen identification	Stage 3: Evidence for infection				Outcome**	References
					A	B	C	D		
<i>Tandanus</i>	<i>tandanus</i>	freshwater catfish	EI	PCR	NA	NA	NA	NA	3	2
<i>Mogurnda</i>	<i>adpersa</i>	purple spotted gudgeon	E	PCR	N	Y	N	N	3	2
<i>Salmo</i>	<i>salar</i>	atlantic salmon	EI	First report	NA	NA	NA	NA	3	10
<i>Maccullochella</i>	<i>peelii</i>	murray cod	E/EI	PCR	N	N	N	N	3/4	2, 11
<i>Nannoperca</i>	<i>australis</i>	southern pigmy perch	E	PCR	N	N	N	N	4	2
<i>Maccullochella</i>	<i>macquariensis</i>	trout cod	E	PCR	N	N	N	N	4	2
<i>Hypseleotris</i>	<i>species</i>		E	PCR	N	N	N	N	4	2
<i>Craterocephalus</i>	<i>stercusmuscarum fulvus</i>	unspecked Hardyhead	E	PCR	N	N	N	N	4	2
<i>Cyprinus</i>	<i>carpio</i>	common carp	E	PCR/sequencing	N	N	N	N	4	6
<i>Carassius</i>	<i>auratus</i>	goldfish	E	PCR/sequencing	N	N	N	N	4	6, 9

Route of infection Key**N: Natural infection**E: Experimental (non-invasive)**EI: Experimental (invasive)**NA: Not applicable (e.g. PCR negative, no other data)*

Criterion A alone is sufficient to determine infection. Otherwise at least two of criteria B/C/D.

Outcome Key***1: Meets the criteria for susceptibility.**2: Some but not all of the criteria have been met.**3: Criteria have not been met (e.g., PCR- positive on gills or intestines and no other evidence; studies with questionable methodology or inconsistent results).**4: Evidence of non-susceptibility.*

Annex 30 (contd)Annex III (contd)**Additional information relevant to assessments for EHN**Macquarie perch (*Macquaria australasica*)

The *ad hoc* Group assessed two papers resulting in outcome assessments of a '1' and '3' and agreed to include this species as susceptible in the *Aquatic Code*. In Becker *et al.* (2013), the only indication of infection by bath challenge was positive histopathology in one fish (of one sufficiently tested), the evidence was considered inconclusive, and the *ad hoc* Group assessed it as a '3'. However, the *ad hoc* Group considered the Langdon *et al.* (1989) paper to be a strong study and the outcome status of '1' is based on this paper in addition to the strain being characterized (PCR sequencing) by Becker *et al.* (2013).

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Annex 30 (contd)

Annex III (contd)

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Annex 30 (contd)

Annex IV

ASSESSMENT OF HOST SUSCEPTIBILITY TO INFECTION WITH SALMON INFECTIOUS ANAEMIA VIRUS (ISAV)

Criteria for susceptibility to infection with ISAV are detailed in Table 1 (as per Article 1.5.6. of the *Aquatic Code*). This table includes Replication (A), Viability/Infectivity (B), Pathology/Clinical Signs (C) and Location (D). Hosts were considered to be infected with ISAV if they fulfilled either criterion A, or at least two of criteria B, C and D (as per point 3 of Article 1.5.7. of the *Aquatic Code*).

Table 1. Criteria for susceptibility to infection with ISAV

A: Replication	B: Viability / Infectivity	C: Pathology / Clinical signs	D: Location
Sequential virus titration showing increase in viral titres; OR Demonstration of increasing copy number over time by qPCR with confirmatory PCR/sequencing; OR TEM showing virions in host cells; OR Products of virus replication detected e.g. demonstration of viral antigen by specific immunoassay of tissue imprints or fixed tissue sections;	Isolation by cell culture. This needs to be from an internal organ. OR Cohabitation with passage to a susceptible host with confirmed infection in the sentinel species by PCR and demonstrating at least one of the following: i. clinical signs with or without associated mortality, ii. Histopathology, iii. Re-isolation of virus in cell culture.	Yellowish or blood-tinged fluid in peritoneal and pericardial cavities. Oedema of the swim bladder. Small haemorrhages of the visceral and parietal peritoneum. Focal or diffusely dark red liver. A thin fibrin layer may be present on the surface. Swollen, dark red spleen with rounded margins. Dark redness of the intestinal wall mucosa in the blind sacs, mid- and hind-gut, without blood in the gut lumen of fresh specimens. Swollen, dark red kidney with blood and liquid effusing from cut surfaces. Pinpoint haemorrhages of the skeletal muscle. Low hematocrit (severe anemia).	Gill, heart, mid-kidney, spleen, liver, pancreas/intestine*

* It is noted that target organs may differ from those described for existing susceptible species. Where gills and pancreas/intestines are used surface contamination should be ruled out.

Annex 30 (contd)

Annex IV (contd)

ASSESSMENT FOR HOST SUSCEPTIBILITY

The assessment for host susceptibility to infection with salmon anaemia virus is provided in Table 2.

Table 2. Outcome of assessment for host susceptibility to infection with ISAV

Genus	Species	Common name	Stage 1: Transmission	Stage 2 : Pathogen identification	Stage 3:				Outcome**	References
					A	B	C	D		
<i>Oncorhynchus</i>	<i>mykiss</i>	Rainbow trout	E and EI	RT-PCR and cell culture	N	Y	Y	Y	1	2, 22
<i>Salmo</i>	<i>salar</i>	Atlantic salmon	E	RT-PCR	Y	N	Y	Y	1	11
<i>Salmo</i>	<i>trutta</i>	Brown trout = sea trout	N	RT-PCR	N	Y	N	Y	1	17
<i>Oncorhynchus</i>	<i>masou</i>	Amago trout	I and E	RT-PCR	N	N	Y	Y	2	3
<i>Clupea</i>	<i>harengus</i>	Atlantic herring	E	RT-PCR and culture -ve	N	Y	N	N	2	14
<i>Oncorhynchus</i>	<i>kisutch</i>	Coho salmon	N	RT-PCR	N	Y	N	N	3	5, 6, 7, 8, 9, 24
<i>Gadus</i>	<i>morhua</i>	Cod	I and N	Cell culture and RT-PCR	N	N	N	N	4	10, 21
<i>Pollachius</i>	<i>virens</i>	Saithe	I and E	- veRT-PCR	N	N	N	N	4	23
<i>Mytilus</i>	<i>edulis</i>	Blue mussel	N	RT-PCR	N	N	N	N	4	13, 19
<i>Oncorhynchus</i>	<i>tshawytscha</i>	Chinook salmon	I	Cell culture	N	N	N	N	4	18
<i>Cyprinus</i>	<i>carpio</i>	Common carp	I	RT-PCR	N	N	N	N	4	4
<i>Carassius</i>	<i>auratus</i>	Goldfish	I	RT-PCR	N	N	N	N	4	4

Annex 30 (contd)Annex IV (contd)

Genus	Species	Common name	Stage 1: Transmission	Stage 2 : Pathogen identification	Stage 3:				Outcome**	References
					A	B	C	D		
<i>Hippoglossus</i>	<i>hippoglossus</i>	Atlantic halibut	I	RT-PCR	N	N	N	N	4	21
<i>Caligus</i>	<i>rogercresseyi</i>	Sea lice	N	RT-PCR and cell culture	N	N	N	N	4	15
<i>Pollachius</i>	<i>virens</i>	Pollock	N	RT-PCR	N	N	N	Y	4	10, 12
<i>Cyclopterus</i>	<i>lumpus L.</i>	Lumpfish	N	RT-PCR and cell culture	N	N	N	N	4	10
<i>Salvelinus</i>	<i>alpinus</i>	Arctic charr	I	RT-PCR	N	N	N	N	N/A	22
<i>Oncorhynchus</i>	<i>keta</i>	Chum salmon	I	CELL CULTURE	N	Y	N	N	N/A	18
<i>Oncorhynchus</i>	<i>nerka</i>	Sockeye salmon	I	RT-PCR	N	N	N	N	NA	4
<i>Salvelinus</i>	<i>leucomaenis</i>	Japanese Char	I	RT-PCR	N	Y	N	N	NA	4
<i>Plecoglossus</i>	<i>altivelis</i>	Ayu sweetfish	I	RT-PCR	N	N	N	N	NA	4
<i>Gnathopogon</i>	<i>elongatus caerulescens</i>	Biwa gudgeon	I	RT-PCR	N	N	N	N	NA	4
<i>Anguilla</i>	<i>anguilla</i>	European eel							Not assessed	
<i>Alosa</i>	<i>pseudoharengus</i>	Alewife							Not assessed	

Route of infection Key*N: Natural infection**E: Experimental (non-invasive)**EI: Experimental (invasive)**NA: Not applicable; (e.g. PCR negative, no other data)*

Criterion A alone is sufficient to determine infection. Otherwise at least two of criteria B/C/D.

Outcome Key*1: Meets the criteria for susceptibility.**2: Some but not all of the criteria have been met.**3: Criteria have not been met (e.g., PCR- positive on gills or intestines and no other evidence; studies with questionable methodology or inconsistent results).**4: Evidence of non-susceptibility.*

Annex 30 (contd)Annex IV (contd)**Additional information relevant to ISAV assessments**

In this assessment the *ad hoc* Group assumed that the susceptibility to HPR-deleted was the same as for HPR0 (EFSA Journal 2012; 10 (11):2971. [22 pp.]). Below is more detailed explanatory text for some of the assessments.

Amago trout (*Oncorhynchus masou*)

The *ad hoc* Group assessed *Oncorhynchus masou* as having an outcome score of a '2' because while 6 out of 20 fish were positive via PCR and one fish died with clinical signs, the virus was not transmitted to Atlantic salmon. Based on the limited evidence the *ad hoc* Group considered that there was incomplete evidence for inclusion in the *Aquatic Code* as a susceptible host because this was the first and only study on the species and the results were based on only one fish. Therefore, there is a need for corroborating evidence before indicating it to be a susceptible species.

Coho salmon (*Oncorhynchus kisutch*)

A natural outbreak of ISA in Coho salmon is reported in Chapter 2.3.5. of the *Aquatic Manual* based on a study published by Kibenge *et al.* (2001) where ISAV was detected by RT-PCR in tissue homogenates from animals undergoing a mortality event. Since that study, substantial evidence on the susceptibility of Coho salmon to this virus has been published indicating that this species is not a viable host for ISAV.

Given the new information from surveillance data and from other researchers, and that the original findings (Kibenge *et al.*, 2001) may have been a laboratory contamination, the *ad hoc* Group proposed to include Coho salmon (*Oncorhynchus kisutch*) in the Section 2.2.2. 'Species with incomplete evidence for susceptibility' of the *Aquatic Manual* until more conclusive information is made available.

The following is a compilation of the information supporting the assessment that Coho salmon is not susceptible to ISAV (Original study by Kibenge *et al.* [2001]):

- The isolate detected in Chilean Coho salmon in 1999 was a perfect match for an isolate in Canada that was routinely used in the laboratory that identified the virus in Coho salmon as a positive control and in exposure studies (Kibenge *et al.*, 2002; Kibenge *et al.*, 2006).
 - Since this time, genetic analyses of field isolates from farms in close proximity are seldom a perfect match (Kibenge *et al.*, 2009; Lyngstad *et al.*, 2011) suggesting it is unlikely that we would find a perfect match on isolates from Canada and Chile.
- There were several outbreaks of ISA in Canada at the time that the original Coho salmon were tested so the laboratory had opportunity to cross-contaminate both with research and field specimens.
 - The laboratory was later inspected and deemed to have insufficient separation of samples for laboratory GOP (OIE inspection at UPEI, 2012).
- The culture of the virus in this study could only be done on one tissue homogenate sample and only on TO cell lines with trypsin. Given the opportunities for cross-contamination and the lack of replicability of the results it is likely that the finding is a false positive.

Annex 30 (contd)

Annex IV (contd)

Other evidence suggesting Coho salmon is not a susceptible host for ISAV.

- There is now a well-described infectious condition in Coho salmon in Chile called “jaundice disease”, which resembles what Kibenge *et al.* (2001) described. Although data suggest Coho salmon jaundice disease is an infectious condition (Smith *et al.*, 2006), no pathogen has been isolated from affected fish, including ISAV, despite extensive investigation of this disease (Alba *et al.*, submitted for publication).
- No outbreaks of ISA occurred in Chile in 1999 when the Coho salmon were reported by Kibenge *et al.* to be positive for the virus despite millions of susceptible Atlantic salmon hosts in the area and the fact that the strain of virus detected in the Coho salmon was known to be pathogenic to Atlantic salmon. It was not until eight years later that the first case of ISA occurred in Chile.
- The Chilean ISAV isolate in 2007 associated with clinical ISA was more closely related to the Norwegian ISAV isolates than the North American isolates.
- A study by Kibenge in 2006 found that even by intraperitoneal (IP) infection of high virus titres, ISAV could not induce disease in Coho salmon, and although not reported in the paper, the virus presumably could not be detected by RT-PCR at the end of the study. The actual findings of the PCR testing were not presented in the paper, but they were presumed negative given the authors did not discuss them in the manuscript and they would have aided in the conclusion that Coho salmon are asymptomatic carriers of the ISA virus.
- Another study, in which injected Coho salmon IP with high concentrations of ISAV, was able to re-isolate the virus from 1 of 5 fish sampled 13 days post-injection, but the other 10 fish sampled later in the study were not positive. In a second trial in the same study, none of the Coho salmon injected with ISAV (n=15) were positive for virus on cell culture despite the successful infection of the Atlantic salmon in the study.
- Lastly, the Chilean government has been testing Coho salmon as part of their ISAV surveillance programme using Taqman RT-PCR as described in Snow *et al.* (2006). Between 2008 and 2012, while known cases of ISA were occurring in Chile, Sernapesca evaluated 39,214 pools of Coho salmon representing 118,864 fish samples and none were positive for the virus. During the same time period they sampled 144,472 pools of Atlantic salmon representing 414,583 fish and detected 3105 positive pools. The government of Chile also tested several pools of fish from farms rearing multiple species including Coho salmon together (n=28,873), and reported 19 positive samples. All of these positive pools were determined, based on individual fish analysis, to be from the Atlantic salmon in the pools (personal comm. M. Lara Sernapesca). The latter suggests that even on farms with positive Atlantic salmon the Coho salmon do not test positive by RT-PCR.
 - The government data were also analysed statistically to determine the probability of freedom from disease in farmed Coho salmon in Chile (Alba *et al.*, submitted for publication). The authors concluded with high certainty based on their models that Coho salmon in Chile were free of ISAV.

Annex 30 (contd)Annex IV (contd)

Given the new information from surveillance data and from other researchers, and that the original findings (Kibenge *et al.*, 2001) may have been a laboratory contamination, the *ad hoc* Group proposed to include Coho salmon (*Oncorhynchus kisutch*) in the Section 2.2.2. 'Species with incomplete evidence for susceptibility' of the *Aquatic Manual* until more conclusive information is made available.

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Annex 30 (contd)

Annex IV (contd)

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Annex 30 (contd)Annex V

ASSESSMENT OF HOST SUSCEPTIBILITY TO INFECTION WITH *GYRODACTYLUS SALARIS*

The *ad hoc* Group noted that for *G. salaris* the only criterion used to determine Stage 3 (as per Article 1.5.6. of the *Aquatic Code*) was (A) ‘Evidence of replication’ because attachment of the parasite occurs transiently on many species and therefore clinical signs and location of infection alone do not constitute a true infection. Therefore, Viability/Infectivity (B), Pathology/Clinical Signs (C) and Location (D) were not applicable.

Criteria for replication aimed to differentiate between replication versus maturation of existing parasites. Because *G. salaris* is hyperviviparous, adult parasites likely contain embryos when transferred to test species. Thus a limited increase in parasite numbers upon transfer may reflect maturation of existing embryos rather than new reproduction/replication. Consequently, the *ad hoc* Group defined replication as a doubling, or more, in parasite numbers that is maintained beyond the lifespan expected for *G. salaris* on a susceptible host at the given water temperature. Jensen and Bakke (1999) provide average lifespans and reproductive rates for *G. salaris* on *Salmo salar* (their preferred host) held at different water temperatures.

Table 1. Criteria for susceptibility to infection with *G. salaris*

A: Replication	B: Viability/Infectivity	C: Pathology/Clinical signs	D: Location
Sequential examination showing at least a two-fold increase in parasite numbers beyond the expected lifespan for the given conditions.	N/A	N/A	N/A

Annex 30 (contd)

Annex V (contd)

ASSESSMENT FOR HOST SUSCEPTIBILITY

The assessment for host susceptibility to infection with *G. salaris* is provided in Table 2.

Table 2. Outcome of assessment for host susceptibility to infection with *G. salaris*

Genus	Species	Common name	Stage 1: Transmission*	Stage 2: Pathogen identification	Stage 3: Evidence for infection				Outcome	References
					A	B	C	D		
<i>Salmo</i>	<i>salar</i>	Atlantic salmon	N/E	PCR/genotyping	Y	NA	NA	NA	1	7, 9, 11, 12
<i>Oncorhynchus</i>	<i>mykiss</i>	Rainbow trout	N/E	PCR/genotyping	Y	NA	NA	NA	1	10
<i>Salvelinus</i>	<i>alpinus</i>	Arctic char	N/E	PCR/genotyping	Y	NA	NA	NA	1	9, 12, 16
<i>Salvelinus</i>	<i>fontinalis</i>	North American brook trout	N	PCR/genotyping	Y	NA	NA	NA	1	4, , 15
<i>Thymallus</i>	<i>thymallus</i>	Grayling	E	PCR/genotyping	Y	NA	NA	NA	1	11
<i>Salmo</i>	<i>trutta</i>	Brown trout	N/E	PCR/genotyping	Y	NA	NA	NA	1	11
<i>Coregonus</i>	<i>lavaretus</i>	Whitefish	E	Morphology	N	NA	NA	NA	3	14
<i>Anguilla</i>	<i>anguilla</i>	European eel	E	Morphology	N	NA	NA	NA	3	3
<i>Salvelinus</i>	<i>namaycush</i>	North American lake trout	E	Morphology	N	NA	NA	NA	3	5, 6
<i>Gasterosteus</i>	<i>aculeatus</i>	3 spine stickleback	E	Morphology	N	NA	NA	NA	3	13
<i>Pungitius</i>	<i>pungitius</i>	9 spine stickleback	E	Morphology	N	NA	NA	NA	3	13
<i>Platichthys</i>	<i>flesus</i>	Flounder	E	Morphology	N	NA	NA	NA	3	13
<i>Coregonus</i>	<i>lavaretus</i>	Whitefish	E	Morphology	N	NA	NA	NA	3	14
<i>Lampetra</i>	<i>planeri</i>	Lamprey	E	Morphology	N	NA	NA	NA	3	2
<i>Rutilus</i>	<i>rutilus</i>	Roach	E	Morphology	N	NA	NA	NA	3	2
<i>Phoxinus</i>	<i>phoxinus</i>	Minnows	E	Morphology	N	NA	NA	NA	3	1
<i>Perca</i>	<i>fluviatilis</i>	Perch	E	Morphology	N	NA	NA	NA	3	2

Annex 30 (contd)

Annex V (contd)

Route of infection Key*

N: Natural infection

E: Experimental (non-invasive)

EI: Experimental (invasive)

NA: Not applicable; (e.g. PCR negative, no other data)

Outcome Key**

1: Meets the criteria for susceptibility

2: Some but not all of the criteria have been met.

3: Criteria have not been met (e.g. PCR- positive on gills or intestines and no other evidence; studies with questionable methodology or inconsistent results).

4: Evidence of non-susceptibility.

Additional information relevant to *G. salaris*

The *ad hoc* Group accepted pathological identification based on morphology when assessed by a recognised expert (i.e. did not require molecular confirmation).

The *ad hoc* Group noted that many species can sustain viable populations for short durations and could thus act as temporary vectors for spread for the parasite, even though the species do not fulfil the criterion used to determine Stage 3 because there is no supporting evidence of replication as defined by the *ad hoc* Group.

Annex 30 (contd)Annex V (contd)**References**

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Annex 30 (contd)

Annex V (contd)

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AQUATIC ANIMAL HEALTH STANDARDS COMMISSION WORK PLAN 2017–2018

AQUATIC CODE		
Task	September 2017	February 2018
User's Guide	Reviewed and updated in line with changes in the <i>Terrestrial Code</i> adopted in 2016 and amended as relevant and provided for comment.	Review Member Country comments
Glossary	Amended several definitions and provided for comment.	Review Member Country comments
Assessment of Tilapia lake virus against listing criteria	Assessed Tilapia lake virus against the new criteria for listing in Chapter 1.2. and provided for comment.	Review Member Country comments
Diseases listed by the OIE (Chapter 1.3.)	Amended names of the listed fish diseases in line with 'Infection with' approach.	Review Member Country comments
Criteria for listing species as susceptible (Chapter 1.5.)	Reviewed Member Country comments and provided for comment.	Review Member Country comments
OIE procedures relevant to the Agreement on the application of sanitary and phytosanitary measures of the World Trade Organization (Chapter 5.3.)	Reviewed relevant changes to the <i>Terrestrial Code</i> adopted in 2017 and amended, as relevant and provided for comment.	Review Member Country comments
Criteria to assess the safety of aquatic animal commodities (Chapter 5.4.)	Reviewed comments and provided for comment.	Review Member Country comments
New chapter for infection with <i>Batrachochytrium salamandrivorans</i> (Chapter 8.X.)	Developed a new draft chapter including horizontal changes and provided for comment.	Review Member Country comments
Infection with <i>Batrachochytrium dendrobatidis</i> (Chapter 8.1.) and Infection with ranavirus (Chapter 8.2.)	Applied horizontal changes applied to Chapter 8.X. and fish disease-specific chapters to ensure that all three amphibian chapters are aligned. Provided for comment.	Review Member Country comments
Acute hepatopancreatic necrosis disease (Chapter 9.1.)	Reviewed scope in light of new literature and agreed no changes were justified	
Infection with infectious hypodermal and haematopoietic necrosis (Chapter 9.4.)	Reviewed the <i>ad hoc</i> Group assessment of <i>M. rosenbergii</i> and deleted from Article 9.4.2.	Review Member Country comments
Epizootic haematopoietic necrosis (Chapter 10.1.)	Reviewed list of susceptible species in Article 10.1.2. after consideration of the work of the <i>ad hoc</i> Group; also applied horizontal changes and provided for comment.	Review Member Country comments

Annex 31 (contd)

AQUATIC CODE (CONTD)		
Task	September 2017	February 2018
Infection with <i>Gyrodactylus salaris</i> (Chapter 10.3.)	Reviewed list of susceptible species in Article 10.3.2. after consideration of the work of the <i>ad hoc</i> Group; applied horizontal changes and provided for comment.	Review Member Country comments
Infection with infectious salmon anaemia virus (Chapter 10.4.)	Reviewed list of susceptible species in Article 10.4.2. after consideration of the work of the <i>ad hoc</i> Group; applied horizontal changes and provided for comment.	Review Member Country comments
Model Articles X.X.8., X.X.9., X.X.10. and X.X.11.	Developed model articles showing all horizontal changes and provided for comment.	Review Member Country comments
Tilapia lake virus – technical disease card	Reviewed technical disease card and considered it did not need amending	Review disease card and update if necessary
<i>Batrachochytrium salamandrivorans</i> – technical disease card	Developed a technical disease card and uploaded onto the OIE website.	Review disease card and update if necessary
Guidance document for applying criteria for listing	Reviewed a draft document developed by the OIE Headquarters	Finalise document
Develop principles for determining surveillance periods in disease-specific chapters	Requested <i>ad hoc</i> Group on Disease freedom meet electronically prior to February 2018 to develop principles for determining surveillance periods in disease-specific chapters	Review eAHG report
New chapter on Biosecurity (Chapter 4.X.)	Requested a second meeting to finalise the draft chapter prior to the February 2018	Review <i>ad hoc</i> Group report
AQUATIC MANUAL		
Task	September 2017	February 2018
Epizootic haematopoietic necrosis (Chapter 2.3.1.)	Amended Section 2.2.2. Species with incomplete evidence for susceptibility after consideration of the work of the <i>ad hoc</i> Group; reviewed the chapter proposed further amendments and provided for comment.	Review Member Country comments
Infection with <i>Gyrodactylus salaris</i> (Chapter 2.3.3)	Amended Section 2.2.2. Species with incomplete evidence for susceptibility after consideration of the work of the <i>ad hoc</i> Group; reviewed the chapter proposed further amendments and provided for comment.	Review Member Country comments
Infection with infectious salmon anaemia virus (Chapter 2.3.5.)	Amended Section 2.2.2. Species with incomplete evidence for susceptibility after consideration of the work of the <i>ad hoc</i> Group; reviewed the chapter proposed further amendments and provided for comment.	Review Member Country comments

Annex 31 (contd)

AQUATIC MANUAL (CONTD)		
Task	September 2017	February 2018
Infection with infectious hypodermal and haematopoietic necrosis (IHHN) (Chapter 5.5.4., Sections 2.2.1. and 2.2.2.)	Deleted <i>Macrobrachium rosenbergii</i> as a susceptible species and presented for comment.	Review Member Country comments
Assessment of kuruma shrimp (<i>Penaeus japonicas</i>) as susceptible species for acute hepatopancreatic necrosis disease Chapter (2.2.1.).	Agreed to include kuruma shrimp (<i>Penaeus japonicas</i>) in Section 2.2.2. of the <i>Aquatic Manual</i> . Presented for comment.	Review Member Country comments
New draft chapter for Infection with <i>Batrachochytrium salamandrivorans</i> (Chapter 2.1.X.)	In the absence of a Reference Laboratory for <i>Batrachochytrium salamandrivorans</i> the Commission proposed to request an expert to <i>prepare</i> a draft chapter.	
Review of the <i>Aquatic Manual</i> disease chapter template proposed by the <i>ad hoc</i> Group	Reviewed the disease chapter template that had been <i>further</i> amended by the <i>ad hoc</i> Group following feedback from the Commission at the February 2017 meeting. The <i>ad hoc</i> Group will further amend the <i>template</i> taking into account feedback provided to them by the Commission.	Review the finalised template and the three example chapters, and provide these to Members for information

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