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Organisation Mondiale de la Santé Animale

World Organisation for Animal Health

Organización Mundial de Sanidad Animal

Annex 2

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

Paris, 7–14 February 2019

EU comment
The EU would like to commend the OIE Aquatic Animal Health Standards Commission for its work and for having taken into consideration EU comments on the Aquatic Code and Manual submitted previously.
The EU notes the reply from the Aquatic Animals Commission to some of the previous EU comments and the justification why some of those comments have not been taken into consideration.
The EU only has few specific comments on this report of the February 2019 meeting of the Aquatic Animals Commission (inserted in the text below) and in general supports the proposed changes to chapters and articles presented therein.
Please note that the EU positions regarding Annexes 3 to 16 are appended to this document, while the EU comments on Annexes 17 to 22 will be provided to the OIE separately by 7 August 2019.
The EU would like to stress again its continued commitment to participate in the work of the OIE and to offer all technical support needed by the Aquatic Animals Commission and its ad hoc groups for future work on the Aquatic Code and Manual.

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OIE Aquatic Animal Health Standards Commission (hereinafter referred to as the Aquatic Animals Commission) met at OIE Headquarters in Paris from 7 to 14 February 2019. The list of participants is attached as **Annex 1**.

The Aquatic Animals Commission thanked the following Member Countries for providing written comments on draft texts for the OIE *Aquatic Animal Health Code* (hereinafter referred to as the *Aquatic Code*) and OIE *Manual of Diagnostic Tests for Aquatic Animals* (hereinafter referred to as the *Aquatic Manual*) circulated after the Commission's September 2018 meeting: Australia, Canada, China (People's Rep. of), Chinese Taipei, Japan, Malaysia, Mexico, New Caledonia, New Zealand, Norway, Switzerland, Thailand, Vietnam, the United States of America (USA), 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 *Aquatic Code* and the *Aquatic Manual* where appropriate. The amendments are shown in the usual manner by 'double underline' and '~~strikethrough~~' and are presented in the Annexes to this report. For Annexes that have been circulated previously, 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 relevant ad hoc Groups, which include important information, and encourages Member Countries to review these reports together with the report of the Commission. These reports are available on the OIE website.

The table below lists the texts as presented in the Annexes. Member Countries should note that texts in **Annexes 3 to 16** are proposed for adoption at the 87th General Session in May 2019; **Annexes 17 to 22** are presented for Member Country comment; and **Annexes 23 and 24** are presented for information.

Comments on **Annexes 17 to 22** of this report must reach OIE Headquarters by the **7 August 2019** to be considered at the September 2019 meeting of the Aquatic Animals Commission. Comments received after the due date will not be submitted to the 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 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 '~~strikethrough~~' 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 Country submissions into the Aquatic Animals Commission's working documents.

Item	Texts proposed for adoption	Annex number	Page number
	AQUATIC CODE		
1.2.	Glossary	3	27
1.3.	Criteria for listing species as susceptible (Chapter 1.5.); in track changes (A) and clean text (B)	4 (A&B)	29
1.4.1.	Article 10.5.2. Infection with salmonid alphavirus (Chapter 10.5.)	5	37
1.4.2.	Article 10.7.2. Infection with koi herpesvirus (Chapter 10.7.)	6	39
1.4.3.	Article 10.9.2. Infection with spring viraemia of carp virus (Chapter 10.9.)	7	41
1.5.	Infection with Ranavirus species (Chapter 8.3.)	8	43
1.6.	Acute hepatopancreatic necrosis disease (Chapter 9.1.)	9	51
1.7.	Articles 10.2.1. and 10.2.2. of Infection with *Aphanomyces invadans* (Chapter 10.2.)	10	57
1.8.	Infection with infectious haematopoietic necrosis virus (Chapter 10.6.)	11	59
1.9.	Article X.X.8.	12	67
	AQUATIC MANUAL		

UNOFFICIAL VERSION

5.1.	Scope and Sections 2.1 and 2.2 of Infection with yellow head virus genotype 1 (Chapter 2.2.9.)	13	69
5.2.	Infectious haematopoietic necrosis (Chapter 2.3.4.)	14	71
5.3.	Infection with salmonid alphavirus (Chapter 2.3.6.)	15	87
5.4.	Scope and Sections 2.1 and 2.2 of Infection with koi herpesvirus (Chapter 2.3.7.)	16	101
	Texts for Member Country comments	**Annex number**	**Page number**
	AQUATIC CODE		
2.1.	New draft chapter on Biosecurity for Aquaculture Establishments (Chapter 4.X.)	17	103
3.1.1.	Infection with shrimp haemocyte iridescent virus (SHIV); assessment for SIHV against the criteria for listing an aquatic animal disease in accordance with Chapter 1.2. Criteria for listing aquatic animal diseases (A) and revised Article 1.3.3. of Chapter 1.3 Diseases listed by the OIE (B)	18 (A&B)	113
3.2.	Model Article for 10.X.13.	19	119
1.8.	Article 10.6.13. Infection with infectious haematopoietic necrosis virus (Chapter 10.6.)	20	123
	AQUATIC MANUAL		
6.1.1.	Infection with spring viraemia of carp virus (Chapter 2.3.9.); in track changes (A) and clean text (B)	21 (A&B)	125
6.1.2.	New draft Chapter 2.1.X. Infection with *Batrachochytrium salamandrivorans*	22	171
Item	**Annexes for Member Country information:**	**Annex number**	**Page number**
3.2.	Report of the electronic *ad hoc* Group on Tilapia lake virus	23	185
J	Work plan	24	187

A. INTRODUCTIONS AND THE PERFORMANCE MANAGEMENT FRAMEWORK

Matthew Stone, Deputy Director General, International Standards and Science, presented the new Performance Management Framework to the Aquatic Animals Commission. He explained that the objective of this framework is continuous improvement of the work all of the Specialist Commissions and the OIE Secretariat in order to improve their work for the benefit of the OIE Members. He noted that this process includes regular meetings between Commission members and himself, and between all Commission Presidents and the Director General and a brief review at the end of each meeting.

Ingo Ernst, President of the Aquatic Animals Commission, encouraged all Commission members to approach the Performance Management Framework in a positive way. He noted that transparency is a key aspect of any performance management framework requiring assessments to be made in an open way and clearly communicated. He commented that the initiative could assist the Commission in undertaking its work in a productive manner where expectations of roles and responsibilities are clearly understood.

B. ADOPTION OF THE AGENDA

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The draft agenda circulated prior to the meeting was discussed, updated and agreed. The adopted agenda of the meeting is attached in **Annex 2.**

C. COOPERATION WITH OTHER SPECIALIST COMMISSIONS

1. The Aquatic Animals Commission and the Biological Standards Commission

The Aquatic Animals Commission and the Biological Standards Commission held a joint meeting on 13 February 2019 to share information and explore areas of common interest and how to strengthen ways of working together. Topics addressed included: each Commission's approach to working on the *Aquatic Manual* and the OIE *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals* (hereinafter referred to as the *Terrestrial Manual*); Reference Centre activities, specifically how to collaborate on the development of procedures and decision making.

All agreed that this meeting assisted in strengthening the collaboration between the two Commissions.

Given it is unlikely that the two Commissions will meet at the same time for future meetings, they agreed to hold teleconference calls between meetings to progress relevant items, e.g. guidance for Reference Centres networking.

D. OIE *AQUATIC ANIMAL HEALTH CODE*

1. Texts to be proposed for adoption at the 2019 General Session (that were circulated for Member Country comments in September 2018)

1.1. General comments

In response to a comment to develop new chapters for the *Aquatic Code* to ensure safe trade in broodstock and genetic material, the Aquatic Animals Commission added this topic to its work plan.

1.2. Glossary

Comments were received from Canada, China (People's Rep. of), Mexico, Vietnam, the EU and AU-IBAR.

Basic biosecurity conditions

The Aquatic Animals Commission reviewed the comments and noted that most were either in support of the proposed changes or of an editorial nature. In response to a Member Country suggestion to include additional text providing the option of disease notification to the OIE or the Competent Authority, the Commission did not agree as the definition of basic biosecurity conditions addresses biosecurity at the national level. The Commission highlighted that notification obligations to the OIE are addressed in Chapter 1.1.

The Aquatic Animals Commission agreed not to make any amendments to the text as they considered it to be clear as currently proposed.

The revised definition for 'basic biosecurity conditions' is presented in Annex 3 and will be proposed for adoption at the 87th General Session in May 2019.

EU position

The EU supports the adoption of this modified Glossary.

1.3. Criteria for listing species as susceptible (Chapter 1.5.)

Comments were received from Australia, Canada, China (People's Rep. of), Chinese Taipei, Japan, Malaysia, Mexico, Thailand, USA, Vietnam, the EU and AU-IBAR.

The Aquatic Animals Commission reviewed the comments and amended the chapter, where relevant.

Article 1.5.2.

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The Aquatic Animals Commission did not agree with a comment to delete the text after the word 'infection', specifying the definition of infection to include presence of multiplying, developing pathogenic agent, as they considered it useful to be explicit about the meaning of infection at the start of the chapter.

In response to a comment, the Aquatic Animals Commission clarified that the definition for infection in the Glossary should continue to include latency and therefore does not need to be reviewed. The Commission reiterated that in terms of demonstrating susceptibility the criteria do not need to include demonstration of true latency as this offers no advantage in the identification of susceptible species over demonstration of other forms of infection.

Article 1.5.4.

The Aquatic Animals Commission did not agree with a comment to delete point 3 in Article 1.5.4. The Commission noted that this article describes stage 1 which is only for classification of the evidence. It considered that to support transparency and defensibility all relevant and available evidence should be classified before determining which evidence is used to assess species susceptibility. The Commission also noted that some experimental evidence could be used to demonstrate that some species are refractory to infection, which is useful for Article 1.5.9.

The Aquatic Animals Commission agreed to change 'inoculation' to 'injection' to clarify that this is not vaccination. It also agreed, for clarity, to change 'infectivity' to 'infective' in the last paragraph of Article 1.5.4.

In response to a comment requesting a definition or guidance for 'high loads' the Aquatic Animals Commission agreed to reinsert the word 'unnaturally' to indicate that high loads are meant to be a level greater than would be experienced under natural conditions.

Article 1.5.6.

The Aquatic Animals Commission did not agree with a comment to change 'naïve' to 'apparently healthy' as naive was the appropriate term and apparently healthy animals could in fact be infected or immune to infection.

Article 1.5.8.

The Aquatic Animals Commission agreed with a comment to change the cross-reference of Article 1.5.3. to Article 1.5.7. noting that this amendment clarified the intention of this text which is to identify species having incomplete evidence for susceptibility. A similar edit was made in point 1 of Article 1.5.9. to ensure consistency.

The Aquatic Animals Commission agreed with a comment to change 'risk analysis' to 'risk assessment' because in this context risk management is not included.

Article 1.5.9.

A Member Country requested the scientific rationale for setting the threshold for low host species specificity, namely at least one susceptible species in each of three or more taxa at the ranking of Family. The Aquatic Animals Commission explained that the threshold has been established at a level considered appropriate to restrict application of this article to pathogenic agents that have a broad host range, and where gaps in the scientific knowledge mean that new species would be likely to be judged as susceptible, if they were exposed to the pathogenic agent. For example, for infection with *Aphanomyces astacii,* all species of fresh water crayfish in the Families of Cambaridae, Astacidae and Parastacidae tested to date have proven to be susceptible. However, the susceptibility of many species within these families has not been investigated.

In response to a comment to provide examples to facilitate the understanding of this article, the Aquatic Animals Commission referred Members to page 7 of its February 2018 report, where it provided a table of examples of how the threshold for application of Article 1.5.9. might apply.

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http://www.oie.int/fileadmin/Home/eng/Internationa_Standard_Setting/docs/pdf/A_AAC_Feb_2018.pdf

The Aquatic Animals Commission agreed with comments to edit the numbering of points in this article to improve readability.

The Aquatic Animals Commission did not agree with a comment to replace 'appropriately designed experimental procedures' with 'invasive experimental procedures'. The Commission reiterated that it had replaced the words 'controlled challenges' as this was considered to be too limited, and that all relevant procedures, invasive or not, should be considered.

The Aquatic Animals Commission did not agree with a comment to amend point 2(a) as it considered it clear as written.

The revised Chapter 1.5. Criteria for listing species as susceptible to infection with a specific pathogen is presented in track changes (A) and clean text (B) in **Annex 4** and will be proposed for adoption at the 87th General Session in May 2019.

EU position

The EU thanks the OIE and supports the adoption of this modified chapter.

1.4. Amendments to fish disease-specific chapters regarding susceptible species

1.4.1. Article 10.5.2. Infection with salmonid alphavirus (Chapter 10.5.)

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

Article 10.5.2.

A Member proposed that brown trout (*Salmo trutta*) be removed from Article 10.5.2. as it did not consider that this species met the criteria for susceptibility. The Aquatic Animals Commission requested the *ad hoc* Group on Susceptibility of fish species to infection with OIE listed diseases review its assessment of brown trout (*Salmo trutta*) to infection with salmonid alphavirus.

The *ad hoc* Group reviewed its assessment and agreed that brown trout (*Salmo trutta*) did not meet the criteria and should therefore not be included in Article 10.5.3. of the *Aquatic Code*. But it did meet the criteria to be included in Section 2.2.2. *Species with incomplete evidence for susceptibility* of the *Aquatic Manual*. The rationale for this change was because the study of Boucher *et al*., 1995, only included pathology and no virus isolation/detection for an invasive experimental trial, which is not sufficient to meet the listing criteria.

The Aquatic Animals Commission became aware of a new publication reporting susceptibility of ballan wrasse (*Labrus bergylta)* to infection with salmonid alphavirus (Ruane *et al.,* 2018) and requested that the *ad hoc* Group undertake an assessment of this species against the criteria in Chapter 1.5. in light of this study.

The *ad hoc* Group reviewed its assessment and noted that the results of the new study were based on a single positive result from a single location/survey and that supplementary evidence is needed to prove susceptibility. The Commission agreed that based on this information, ballan wrasse (*Labrus bergylta*) should not be included in Article 10.5.2. of the *Aquatic Code* before there is corroborating evidence. It would, however, be proposed for inclusion in Section 2.2.2. *Species with incomplete evidence for susceptibility* of the *Aquatic Manual* (refer to Item 5.2).

References

Boucher, P., Raynard, R. S., Houghton, G., & Laurencin, F. B. (1995). Comparative experimental transmission of pancreas disease in Atlantic salmon, rainbow trout and brown trout. Diseases of Aquatic Organisms, **22 (1)**, 19–24.

UNOFFICIAL VERSION

Ruane, N. M., Swords, D., Morrissey, T., Geary, M., Hickey, G., Collins, E. M., Geoghegan, F., Swords, F. (2018). Isolation of salmonid alphavirus subtype 6 from wild- caught ballan wrasse, *Labrus bergylta* (Ascanius). *Journal Fish Diseases* 41 (11), 1643-1651).

The revised Article 10.5.2. of Chapter 10.5. Infection with salmonid alphavirus (is presented in **Annex 5** and will be proposed for adoption at the 87th General Session in May 2019.

EU position

The EU thanks the OIE and supports the adoption of this modified chapter.

1.4.2. Article 10.7.2. Infection with koi herpesvirus (Chapter 10.7.)

Comments were received from Australia and the EU.

The Aquatic Animals Commission reviewed a comment and agreed not to make any additional amendments as they considered the text clear as written.

The revised Article 10.7.2. of Chapter 10.7. Infection with koi herpesvirus is presented in Annex 6 and will be proposed for adoption at the 87th General Session in May 2019.

EU position

The EU supports the adoption of this modified this chapter.

1.4.3. Article 10.9.2. Infection with spring viraemia of carp virus (Chapter 10.9.)

Comments were received from Australia, Canada, New Zealand and the EU.

A Member requested the rationale for delisting species such as tench (*Tinca tinca*) that have been recognised and controlled in some Member Countries as susceptible to infection with spring viraemia of carp (SVCV). The Aquatic Animals Commission noted that the *ad hoc* Group on Susceptibility of fish species to infection with OIE listed diseases had undertaken assessments of the susceptibility of all relevant species to infection with SVCV against the criteria in Chapter 1.5. The Commission noted that tench and several of the other species currently considered as susceptible in some Member Countries were found not to meet the criteria for listing when applying the criteria in Chapter 1.5.

The Commission reminded Members that all reports of the *ad hoc* Group are available at the OIE website: http://www.oie.int/standard-setting/specialists-commissions-working-ad-hoc-groups/ad-hoc-groups-reports/

In response to a comment that raised concerns about the inclusion of zebrafish (*Danio rerio*) in Article 10.9.2., the Aquatic Animals Commission noted that the species had been assessed by the *ad hoc* Group and was found to meet the criteria for susceptibility. The Aquatic Animals Commission noted that the assessment for zebrafish (*Danio rerio*) had been based on one available study. The Commission agreed that based on this information, zebrafish (*Danio rerio*) should not be included in Article 10.9.2. of the *Aquatic Code* until there is corroborating evidence to support the assessment of susceptibility, even though the study provided strong evidence of susceptibility. The species would, however, be proposed for inclusion in Section 2.2.2. *Species with incomplete evidence for susceptibility* of the *Aquatic Manual* (see Item 6.1.1.).

The revised Article 10.9.2. of Chapter 10.9. Infection with spring viraemia of carp virus is presented in **Annex 7** and will be proposed for adoption at the 87th General Session in May 2019.

EU position

The EU supports the adoption of this modified chapter.

1.5. Infection with Ranavirus species (Chapter 8.3.)

Comments were received from Australia, Canada, and the EU.

UNOFFICIAL VERSION

The Aquatic Animals Commission reviewed comments and noted that they were either in support of the proposed changes or of an editorial nature.

The Aquatic Animals Commission did not agree with a comment to include the word 'infection' before 'status' in the following phrase, 'infection with *Ranavirus* species status of the exporting country, zone or compartment' that is used throughout this and other disease-specific chapters. The Commission reiterated that the it had agreed some time ago to move away from referring to the disease name but rather to 'infection with pathogenic agent'. The Commission noted this approach has now been applied in all disease-specific chapters with the exception of acute hepatopancreatic necrosis disease because of the aetiology of the disease.

Article 8.3.1.

The Aquatic Animals Commission agreed with a comment to remove 'member virus' as it was unnecessary. The Commission highlighted that, unlike other listed diseases, the pathogenic agent in this chapter is listed at the Genus level not at the species level, and so the wording differs for this chapter.

Article 8.3.8.

The Aquatic Animals Commission amended text to ensure alignment with amendments made to the model Article X.X.8. (see Item 1.8.).

The revised Chapter 8.3. Infection with Ranavirus is presented in **Annex 8** and will be proposed for adoption at the 87th General Session in May 2019.

EU position

The EU supports the adoption of this modified chapter.

1.6. Acute hepatopancreatic necrosis disease (Chapter 9.1.)

Comments were received from Australia, Malaysia, Mexica, New Caledonia and the EU.

The Aquatic Animals Commission reviewed comments and noted that they were either in support of the proposed changes or of an editorial nature.

Article 9.1.8.

The Aquatic Animals Commission amended text to ensure alignment with amendments made in the model Article X.X.8. (see Item 1.8.).

The revised Chapter 9.1. Acute hepatopancreatic necrosis disease is presented in **Annex 9** and will be proposed for adoption at the 87th General Session in May 2019.

EU position

The EU supports the adoption of this modified chapter.

1.7. Articles 10.2.1. and 10.2.2. of Infection with *Aphanomyces invadans* (Chapter 10.2.)

The Aquatic Animals Commission reminded Members that it had amended Article 10.2.1. at its September 2018 meeting to ensure consistency with other amended fish disease-specific chapters and it had also amended some Family names in Article 10.2.2. to remove the use of italics as Family names for fish should not appear in italics. The Commission considered these amendments to be of an editorial nature. The Commission noted that during the review of these articles it corrected misspelling of the names of torpedo-shaped catfishes (*Clarias* spp.) and terapon (*Terapon* sp.).

The revised Articles 10.2.1. and 10.2.2. of Chapter 10.2. Infection with *Aphanomyces invadans* is presented in **Annex 10** will be proposed for adoption at the 87th General Session in May 2019.

EU position

UNOFFICIAL VERSION

The EU supports the adoption of this modified chapter.

1.8. Infection with infectious haematopoietic necrosis virus (Chapter 10.6.)

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

Article 10.6.1.

In response to comments to revert from 'Salmonid novirhabdovirus' back to 'infectious haematopoietic necrosis virus' as some susceptible species are non-salmonid, the Aquatic Animals Commission noted that it would use the official ICTV designation but that the previously used virus name, 'infectious haematopoietic necrosis virus', should also be referred to in Article 10.6.1. The Commission made minor edits consistent with the approach in other Aquatic Code chapters.

Article 10.6.2.

The Aquatic Animals Commission agreed with a comment to correct a misspelling of masu salmon (*Oncorhynchus masou*).

A Member Country recommended pike (*Esox lucius*), grayling (*Thymallus thymallus*) and eel (*Anguilla anguilla*) be included in Article 10.6.2. of the *Aquatic Code* as susceptible to infection with infectious haematopoietic necrosis virus (IHNV) based on information in two scientific studies: Reschova *et al.*, 2008 and Dorson *et al.,* 1987. The Aquatic Animals Commission requested that the *ad hoc* Group on Susceptibility of fish species to infection with OIE listed diseases review its previous assessments of these species against the criteria in Chapter 1.5.

The *ad hoc* Group reviewed its previous assessments and agreed that the Northern pike met the criteria and should be included in Article 10.6.2. The Aquatic Animals Commission therefore proposed the inclusion of pike in Article 10.6.2 of the *Aquatic Code*

The Aquatic Animals Commission agreed that it was not possible to include grayling (*Thymallus thymallus*) and eel (*Anguilla anguilla*) in Article 10.6.2. because there was insufficient available scientific evidence to assess the species against the criteria in Chapter 1.5.

References:

Dorson, M., Chevassus, B., & Torhy, C. (1987). Susceptibility of pike (*Esox lucius*) to different salmonid viruses (IPN, VHS, IHN) and to the perch rhabdovirus. *Bulletin français de la pêche et de la protection des milieux aquatiques*, 307, 91-101.

Reschova, S., Pokorova, D., Hulova, J., Kulich, P., & Vesely, T. (2008). Surveillance of viral fish diseases in the Czech Republic over the period January 1999 - December 2006. *Veterinarni Medicina*, 53(2), 86–92.

Article 10.6.8.

The Aquatic Animals Commission amended text to ensure alignment with amendments made to the model Article X.X.8. (see Item 1.8.).

Article 10.6.13.

In response to a comment the Aquatic Animals Commission amended point 1 to improve clarity regarding assessment of the disease risks associated with imported disinfected eggs. The Commission proposed to circulate this amendment for comment.

The revised Chapter 10.6. Infection with infectious haematopoietic necrosis virus is presented in **Annex 11** and will be proposed for adoption at the 87th General Session in May 2019.

UNOFFICIAL VERSION

EU position

The EU thanks the OIE and in general supports the adoption of this modified chapter. We have one comment included in Annex 11.

The revised Article 10.6.13 of Chapter 10.6. Infection with infectious haematopoietic necrosis virus is presented in **Annex 20** for Member Country comments.

EU comment

[*Will be provided separately by 7 August 2019*]

1.9. Article X.X.8.

Comments were received from Australia, Canada, China (People's rep of), Chinese Taipei, Thailand, Vietnam and the EU.

In response to a comment seeking clarification for the meaning of 'high health status' in point 2(a)(ii) of Article X.X.8. the Aquatic Animals Commission clarified that 'high health status' means the highest feasible disease status for a source population from a country not declared free from 'infection with pathogen X' and is based on information from testing and surveillance of the source population. The Commission emphasised that this article addresses the importation of aquatic animals for aquaculture from a country, zone or compartment not declared free from 'infection with pathogenic agent X'.

The Aquatic Animals Commission agreed to edit point 1(a) to clarify that aquatic animals could be killed and processed either in the original quarantine facility or following biosecure transport to another quarantine facility. This edit will be applied to Article X.X.8. of all disease-specific chapters of the *Aquatic Code* when they are being amended.

The Aquatic Animals Commission amended text in point 2(b)(iv) to clarify the period required for quarantine, in response to a comment.

The revised model Article X.X.8. is presented in **Annex 12** and will be proposed for adoption at the 87th General Session in May 2019.

EU position

The EU supports the adoption of this modified chapter.

2. Texts circulated for Member Country comments

2.1. New draft chapter on Aquatic Animal Biosecurity for Aquaculture Establishments (Chapter 4.X.)

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

The Aquatic Animals Commission considered all comments and amended the text to improve readability and clarity, where relevant.

General comments

In response to some comments, the Aquatic Animals Commission reminded Members that the purpose of this chapter is primarily to mitigate the risk of the introduction of specific pathogenic agents into aquaculture establishments.

A Member Country requested that the Aquatic Animals Commission edit the chapter to provide more context about the biosecurity framework provided by governance and regulation, in which biosecurity at the level of the aquaculture establishment operates. The Commission highlighted that

UNOFFICIAL VERSION

this chapter is intended to focus on biosecurity at the aquaculture establishment level but Article 4.X.3. recognises that biosecurity can also be applied at the level of country, zone or compartment. The Commission acknowledged the importance of broader biosecurity frameworks but agreed that the scope of the chapter could not be extended to include this content without making it less accessible and applicable for aquaculture establishments. The Commission plans to revise other relevant chapters in Section 4 of the *Aquatic Code* to include the application of biosecurity for zoning and compartmentalisation.

In response to a comment, the Aquatic Animals Commission highlighted that they would ensure the inclusion of cross references to this new chapter in other *Aquatic Code* chapters and vice versa, as relevant, when this chapter is adopted.

Article 4.X.5.

In response to a comment that all input and output risk factors should be used to define three systems (open, semi-closed and closed), the Aquatic Animals Commission commented that this approach does not provide a model that allows categorisation of individual establishments. A suggestion that categories of semi-open and semi-closed systems should be merged was not accepted as the Commission considered that there are important differences between these systems that need to be distinguished.

The Aquatic Animals Commission did not agree with a comment to include open systems in the scope of the chapter, as they are not 'aquaculture establishments', which is the subject of this chapter. The Commission noted that the production of aquatic animals for stocking into open systems takes place within aquaculture establishments and therefore, associated biosecurity matters are addressed by articles in this chapter. However, the Commission accepted the comment that the health status of aquatic animals stocked into open systems should be subject to disease mitigation measures and amended the text accordingly.

The Aquatic Animals Commission agreed with a comment to include 'mollusc aquaculture' as an example of a semi-open aquaculture production system.

In response to a comment that not all recirculating production systems discharge water that is effectively treated to inactivate pathogens and therefore they cannot be considered a closed production system, the Aquatic Animals Commission explained that for these systems to be regarded as closed, all incoming and outgoing water should be subjected to an effective treatment.

Article 4.X.6.

In response to a comment the Aquatic Animals Commission agreed to amend the title of this article to 'Transmission pathways, associated risks and mitigation measures' to better reflect its content.

The Aquatic Animals Commission also agreed to add some text prior to listing the risk mitigation measures in each point in this article specifying that the list of mitigation measures is not exhaustive, but rather is aimed at addressing the most important measures. Other pathways may be identified in some systems that also need to be mitigated.

Comments to include detailed information that can already be found in other chapters in the *Aquatic Code*, e.g. Chapter 4.3. *Disinfection of aquaculture establishments and equipment* and 4.8. *Control of pathogenic agents in aquatic animal feed,* were not accepted in order to avoid duplication.

<u>Point 1. Aquatic animals</u>

In response to a comment the Aquatic Animals Commission amended point b) to emphasise the defined term for 'quarantine' is being used as the glossary definition describes in detail the purpose of maintaining aquatic animals in isolation.

The Aquatic Animals Commission did not agree to include vectors in point h), noting that vectors are addressed in point 6.

UNOFFICIAL VERSION

Point 2. Aquatic animal products and aquatic animal waste

The Aquatic Animals Commission agreed to amend point 2 (aquatic animal products and waste) to be consistent with the rest of the text regarding waste moved out of establishments.

Point 5. Fomites

The Aquatic Animals Commission agreed to include ‘footwear’ after ‘clothing’ as a potential fomite.

Point 6. Vectors

The Aquatic Animals Commission agreed with a comment to provide more guidance on mitigation measures associated with vectors. Accordingly, the text was revised to clarify that the mitigation measures described in point 1 for aquatic animals can also be applied to mitigate risks associated with vectors. Mitigation measures for other types of vectors were also added.

Article X.X.7.

In response to a comment the Aquatic Animals Commission clarified that this article on risk analysis is consistent with Chapter 2.1. *Import Risk Analysis* of the *Aquatic Code* but it agreed to add a new sentence noting that this article elaborates the principles in Chapter 2.1. and applies them for the development of biosecurity for aquaculture establishments.

Step 1 – Hazard Identification

The Aquatic Animals Commission revised text to clarify that many hazards will share the same pathway and that information and pathways of introduction need to be combined to identify the most effective mitigation measures. The Commission emphasised that the current text clearly describes a hazard as a specific pathogenic agent or as a group of pathogenic agents.

Step 2 – Risk Assessment

In response to a suggestion to include a visual representation of the pathway of all physical and biological events required for a hazard to occur, the Aquatic Animals Commission noted that it would explore this option and report back at its September 2019 meeting.

Table 2. Qualitative descriptors of consequences

The Aquatic Animals Commission agreed with a comment to amend text in Table 2 to provide clearer explanations of the impacts.

Table 4. Interpretation of risk estimates

The Aquatic Animals Commission agreed with a comment to amend text in Table 4 to provide clearer explanations of the management responses.

Step 3 – Risk Management

The Aquatic Animals Commission did not agree with a comment to address environmental factors, as they considered this to be outside the scope of the chapter.

Article 4.X.8.

The Aquatic Animals Commission did not agree with a comment to include risk communication in the title as they saw no need to highlight risk communication over and above other elements of the biosecurity plan.

The Aquatic Animals Commission agreed to expand section 1(b) to include epidemiologic units and separation measures.

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The Aquatic Animals Commission found it necessary to reorganise the text in this article because it considered it important to highlight the key components of the biosecurity plan.

1. Development of a biosecurity plan

The Aquatic Animals Commission agreed with a comment to include additional examples in points b) and d) to provide more guidance to Member Countries.

2. Key components of a biosecurity plan

In response to comments the Aquatic Animals Commission agreed to add more examples of documentation required in point b) *Documentation and record keeping,* to strengthen biosecurity requirements and to have more robust evidence of the effectiveness of the biosecurity protocols. The Commission also decided to provide examples of who, e.g. producer, aquatic animal health professional or veterinarian, might carry out routine monitoring of stock for important health and production parameters in point d) *Health monitoring*.

The revised new draft Chapter 4.X. Biosecurity for aquaculture establishments is presented in **Annex 17** for Member Country comments.

EU comment

[*Will be provided separately by 7 August 2019*]

2.2. Discussion paper on Approaches for determining periods required to demonstrate disease freedom

Comments were received from Australia, Canada, China (People's Rep. of), Chinese Taipei, Japan, Malaysia, Mexico, New Caledonia, New Zealand, Norway, Thailand, Vietnam, the EU and AU-IBAR.

The Aquatic Animals Commission acknowledged the extensive number of comments submitted from Member Countries and appreciated the quality of comments and the high level of engagement on this topic. The Commission indicated that it will develop a revised paper at its next meeting in September 2019, taking into account Member Country comments, sound science and with a view to achieving consensus, in particular focusing on refining the recommended approaches. The revised paper will be sent for Member Country comments in the September 2019 report of the Commission.

3. Other *Aquatic Code* topics

3.1. Disease listed by the OIE (Chapter 1.3.)

3.1.1. Infection with shrimp haemocyte iridescent virus (SHIV)

The Aquatic Animals Commission noted the identification of a novel virus which had been named shrimp haemocyte iridescent virus (SHIV) and undertook an assessment of SHIV against the criteria for listing an aquatic animal disease in accordance with Chapter 1.2. The Commission concluded that infection with SHIV meets the criteria for listing in Article 1.2.2. and should be proposed for listing under Article 1.3. *Diseases listed by the OIE.*

The Aquatic Animals Commission recognised the potential significance of infection with SHIV to many countries given the worldwide importance of crustacean farming and trade. The Commission reminded Member Countries that infection with SHIV meets the definition of an 'emerging disease' and, as such, should be reported in accordance with Article 1.1.4. of the *Aquatic Code*.

The Aquatic Animals Commission also encouraged Member Countries to investigate mortality and morbidity events in crustaceans, emphasising that an understanding of the geographic distribution of SHIV is essential for efforts to control its possible spread.

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The Aquatic Animals Commission suggested that Member Countries wishing for more information or advice on diagnostic testing for SHIV could contact the Reference Laboratories experts for Infection with white spot syndrome virus (see link below).

http://www.oie.int/en/scientific-expertise/reference-laboratories/list-of-laboratories/

The Aquatic Animals Commission agreed to develop a Technical Disease Card for SHIV to provide information for Member Countries on available detection methods and transmission risks for this virus. The Technical Disease Card will be made available on the OIE website once completed.

The revised Article 1.3.3. of Chapter 1.3 Diseases listed by the OIE (A) and the assessment for SHIV against the listing criteria (B) are presented at **Annex 18** for Member Country comments.

EU comment
[*Will be provided separately by 7 August 2019*]

3.2. Model Article for 10.X.13.

In response to a comment, the Aquatic Animals Commission acknowledged that the current text in point 1 of Article 10.X.13. was not very clear as written. Therefore, the Commission proposed some amendments to clarify the intended purpose of this text. The Commission agreed to present this change in a model article that, once adopted, would be applied to relevant disease-specific chapters when they are being amended (see also Item 1.8.).

The model Article 10.X.13. is presented in **Annex 19** for Member Country comments.

EU comment
[*Will be provided separately by 7 August 2019*]

3.3. Chapter 10.3. Infection with *Gyrodactylus salaris*

The Aquatic Animals Commission continued discussions on the taxonomy of *Gyrodactylus salaris* and reviewed advice provided by the OIE Reference Laboratory expert for *G. salaris*. The Commission agreed to retain the position set out in the report of the September 2018 meeting that it does not support synonymisation of *G. salaris* and *G. thymalli,* given the clear phenotypic differences between the two parasites, notably host predilection and pathogenicity in different host species (see also item 6.2.).

3.4. Chapter 11.4. Infection with *Marteilia refringens*

The Aquatic Animals Commission received no comments on the question, raised in its September 2018 meeting report, of splitting *Marteilia refringens* into two species (*M. refringens* and *M. pararefringens*) based on evidence presented in a paper by Kerr *et al.,* (2018). The Commission agreed it will revise Chapter 11.4. Infection with *Marteilia refringens* and in addition, consider whether *M. pararefringens* needs to be assessed against criteria for listing diseases (see also Item 6.3.).

E. OIE *AD HOC* GROUPS

4.1. *Ad hoc* Group on Susceptibility of fish species to infection with OIE listed diseases

The *ad hoc* Group on Susceptibility of fish species to infection with OIE listed diseases met from 13 to 15 November 2018. The *ad hoc* Group continued its work in undertaking assessments of susceptible species to infection with viral haemorrhagic septicaemia virus (VHSV) using the 'Criteria for listing species as susceptible to infection with a specific pathogen' (Chapter 1.5. of the *Aquatic Code*). Due to the large number of potential susceptible species the *ad hoc* Group did not complete all the assessments.

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The Aquatic Animals Commission requested that the *ad hoc* Group continue its work on VHSV and also undertake assessments to review the list of susceptible species for Infection with Aphanomyces invadans (Epizootic ulcerative syndrome) and Infection with red sea bream iridovirus.

4.2. Electronic *ad hoc* Group on tilapia lake virus

The Aquatic Animals Commission reviewed the report of the *ad hoc* Group on Tilapia lake virus (TiLV) which worked electronically from October 2018 to January 2019 on the assessment of TiLV diagnostics and their validation.

The Aquatic Animals Commission noted that the *ad hoc* Group will undertake test validation studies in April 2019. The Commission will review the results at its September 2019 meeting and will, depending on the progress made with test validation, review its assessment for TiLV against the listing criteria in Chapter 1.2.

The *ad hoc* Group was requested to continue its work and report back to the next meeting of the Aquatic Animals Commission in September 2019.

The report of the OIE *ad hoc* Group on tilapia lake virus is presented at **Annex 23** for Member Country information.

F. OIE *MANUAL OF DIAGNOSTIC TESTS FOR AQUATIC ANIMALS*

5. Texts to be proposed for adoption at the 2019 General Session (that were circulated for Member Country comments in September 2018)

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

5.1. Scope and Sections 2.1 and 2.2. of Infection with yellow head virus genotype 1 (Chapter 2.2.9.)

One comment was received regarding consistency of grammar and content in the standard sentence in the scope: that the scope refers to Genus and Family and not Order of the pathogenic agent. The Aquatic Animals Commission agreed with the comment and would check and amend the scope of chapters in the *Aquatic Code* and *Aquatic Manual* accordingly.

The revised scope and Sections 2.1 and 2.2. of Chapter 2.2.9. Infection with yellow head virus genotype 1 is attached as **Annex 13** and is proposed for adoption at the 87th General Session in May 2019.

EU position

The EU supports the adoption of this modified chapter.

5.2. Infectious haematopoietic necrosis (Chapter 2.3.4.)

Section 1. Scope

A Member Country disagreed with the proposed amendment to the name of the pathogenic agent: Salmonid novirhabdovirus as some susceptible species are non-Salmonids. The Aquatic Animals Commission reiterated that the amendment was in accordance with the classification in the database of the International Committee of Taxonomy of Viruses (ICTV) (https://talk.ictvonline.org/taxonomy/p/taxonomy-history?taxnode_id=20171739).

Section 2.1.1. Aetiological agent, agent strains

In response to a comment to include 'with a mean period of occurrence of a single haplotype with a maximum of one calendar year, the genetic diversity of European IHNV is very high (Cieslak *et al.*,

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2017)' at the end of the last paragraph, the Aquatic Animals Commission determined that the issue would be addressed when this chapter is reformatted using the new chapter template.

Section 2.2.1. Susceptible host species

The list of susceptible species was amended following the recommendations of the *ad hoc* Group on Susceptibility of fish species to infection with OIE listed diseases (see item 1.7.).

A Member Country asked for the rationale for not including Atlantic cod (*Gadus morhua*) and Japanese charr (*Salvelinus leucomaenis*) in the report of the *ad hoc* Group. The Aquatic Animals Commission clarified that the two species were not included in the report as they had not been scored in the assessments because of insufficient scientific evidence.

Section 2.2.6. Persistent infection with lifelong carriers

The Aquatic Animals Commission agreed to delete the words 'at normal temperatures' from the last sentence in this paragraph as they are inaccurate.

Section 2.4.3. Immunostimulation, Section 4. Diagnostic methods and Section 6. Test(s) recommended for targeted surveillance to declare freedom from infectious haematopoietic necrosis

In response to comments on immunostimulation, 'gold standard' diagnostic methods, and technical issues relating to the PCR protocol and recommendations for targeted surveillance, the Aquatic Animals Commission determined that these issues would be provided to the OIE Reference Laboratory expert who is currently updating and reformatting the chapter using the new chapter template.

The revised Chapter 2.3.4. Infection with Infectious haematopoietic necrosis is attached as **Annex 14** and is proposed for adoption at the 87th General Session in May 2019.

EU position

The EU thanks the OIE and in general supports the adoption of this modified chapter. We have a few comments included in Annex 14.

5.3. Infection with salmonid alphavirus (Chapter 2.3.6.)

The Aquatic Animals Commission reviewed the comments and amended text, where relevant.

Section 2.1.1. Aetiological agent, agent strains

The Aquatic Animals Commission agreed to delete the column on geographical distribution from Table 2.*1. SAV genotypes by host, environment and geographic distribution* as it would rapidly be out-dated. As the remaining information is important, the Commission agreed to retain it as Table 2.1. *SAV genotypes by susceptible species and environment*.

Section 2.1.2. Survival outside the host

A Member Country proposed deletion of text referring to long-distance spread of fat droplets from dead fish from which SAV can be detected. The Aquatic Animals Commission disagreed with the request as this is important information that is supported by a scientific publication.

Section 2.2.1. Susceptible host species

A Member Country has proposed adding ballan wrasse (*Labrus bergylta*) to Section 2.2.1. *Susceptible host species* for this disease. The *ad hoc* Group on Susceptibility of fish species to infection with OIE listed diseases advised the Aquatic Animals Commission that to date there has only been one published study on this species and that until a corroborative study is published, the species should be added to Section 2.2.2. *Species with incomplete evidence for susceptibility.*

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Section 7.1. Definition of suspect case and Section 7.2. Definition of confirmed case

The Aquatic Animals Commission agreed that the definitions of suspect and confirmed cases needed to be thoroughly reviewed. Rather than proposing amendments in a piecemeal manner, the Commission agreed that this revision would be undertaken systematically when the chapters are reformatted using the new chapter template.

4.3.1.1.2. Reverse-transcription polymerase chain reaction (RT-PCR), real-time RT-PCR, and genotyping by sequencing

The Aquatic Animals Commission agreed with a comment that for genotyping of different SAV-isolates, sequencing of the E2 gene is sufficient. Sequencing of the nsP3 gene may add information regarding deletions between different isolates but is not necessary and the Commission proposed deleting the reference to nsP3 of the third paragraph of the Section. Consequently, it also proposed deleting the nsP3-primerset in table Table 3.1. Characteristic of primers and probe sequences.

The revised Chapter 2.3.6. Infection with salmonid alphavirus is attached as **Annex 15** and is proposed for adoption at the 87th General Session in May 2019.

EU position

The EU thanks the OIE and in general supports the adoption of this modified chapter. We have suggested a few comments included in Annex 15.

5.4. Scope and Sectiosn 2.1. and 2.2. of Infection with koi herpesvirus (Chapter 2.3.7.)

Section 2.2.1. Susceptible host species

A Member Country reiterated a comment regarding the common name of one of the susceptible fish species given in this Section. The Aquatic Animals Commission stated that it uses the common names included in the FAOTERM database. If there is any confusion, Member Countries should rely on the Latin names, which are always given.

The revised Scope and Sections 2.1 and 2.2. of Chapter 2.3.7. Infection with koi herpesvirus are attached as **Annex 16** and are proposed for adoption at the 87th General Session in May 2019.

EU position

The EU supports the adoption of this modified chapter.

6. Other *Aquatic Manual* chapters

6.1. Revision of disease-specific chapters using the new chapter template

6.1.1. Infection with spring viraemia of carp virus (Chapter 2.3.9.)

The Aquatic Animals Commission reviewed Chapter 2.3.9. *Infection with spring viraemia of carp virus*, which had been updated and reformatted using the new disease chapter template. In accordance with OIE protocol, all new text is double underlined and deleted text is struck through; the existing text to be retained has been left unmarked. Amendments to the structure of the chapter has resulted in some changes to the order of the sub-heading titles. Minor changes were made to the previous circulated version of the new *Aquatic Manual* template. For information, the revised template is attached as Annex XX.

The revised Chapter 2.3.9. is presented in track changes (A) and clean text (B) at **Annex 21** for Member Country comments.

EU comment

[*Will be provided separately by 7 August 2019*]

UNOFFICIAL VERSION

6.1.2. Infection with *Batrachochytrium salamandrivorans* (Chapter 2.1.X.)

The Aquatic Animals Commission reviewed a new draft Chapter 2.1.X. *Infection with Batrachochytrium salamandrivorans*, which had been developed by experts using the new disease chapter template.

The new Chapter 2.1.X. **Infection with *Batrachochytrium salamandrivorans*** is presented at **Annex 22** for Member Country comments.

EU comment

[*Will be provided separately by 7 August 2019*]

6.2. Infection with *Gyrodactylus salaris* (Chapter 2.3.3.)

The decision by NCBI GenBank to reclassify gene sequences submitted as *G. thymalli* to *G. salaris* necessitates a revision to the guidance in the *Aquatic Manual*. The Aquatic Animals Commission recommended that the current approach to distinguishing *G. salaris* from *G. thymalli*, by comparison of the sequenced amplified CO1 fragments to reference sequences, continues. Given that the GenBank/EMBL resource is no longer suitable for this purpose, the information would need to be retained by the OIE Reference Laboratory expert for *G. salaris*. In addition, it was recommended to Member Countries that they seek guidance from the Reference Laboratory expert if they need to distinguish *G. salaris* from *G. thymalli*. In the longer term it is hoped that further molecular analysis of *G. salaris* and *G. thymalli* isolates will identify differences that can be used for the development of improved diagnostic assays (see Item 3.3.).

6.3. Infection with *Marteilia refringens* (Chapter 2.4.4.)

The Aquatic Animals Commission requested that the OIE Reference Laboratory expert for *M. refringens* revises the *Aquatic Manual* chapter to take into account the scientific information presented by Kerr *et al*., (2018) and to highlight to the Commission any diagnostic challenges that may arise (see Item 3.4.).

6.4. Chapters that are being updated and reformatted using the new chapter template

The Aquatic Animals Commission selected a number of chapters for reformating by applying the new *Aquatic Manual* chapter template and revisions, where relevant. The Commission will review these revised chapters in a progressive manner at future meetings. The first chapters to be reformatted are:

Chapter 2.3.2. Infection with *Aphanomyces invadans* (epizootic ulcerative syndrome)

Chapter 2.3.3. Infection with *Gyrodactylus salaris*

Chapter 2.3.4. Infection with infectious haematopoietic necrosis

Chapter 2.3.6. Infection with salmonid alphavirus

Chapter 2.3.7. Infection with koi herpesvirus disease

Chapter 2.3.8. Infection with red sea bream iridoviral disease

Chapter 2.3.10. Infection with viral haemorrhagic septicaemia.

G. OIE REFERENCE CENTRES

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

No applications were received.

7.2. Review of annual reports of Reference Centre activities in 2018

UNOFFICIAL VERSION

Annual reports had been received from all but one OIE Reference Laboratory for diseases of aquatic animals and both Collaborating Centres for aquatic animal issues. In accordance with the adopted *Procedures for designation of OIE Reference Laboratories* (the SOPs) (http://www.oie.int/en/scientific-expertise/reference-laboratories/sops/) and the *Procedures for designation of OIE Collaborating Centres* http://www.oie.int/en/scientific-expertise/collaborating-centres/sops/, the Aquatic Animals Commission reviewed all the reports, noting in particular the performance of each Reference Centre with regard to fulfilling the Terms of Reference (ToR) to the benefit of OIE Member Countries. The Commission expressed its on-going appreciation for the enthusiastic support and expert advice given to the OIE by the Reference Centres. A small number of Reference Laboratories did not provide evidence of communication with other Reference Laboratories. They will be reminded that one of the ToRs is to 'establish and maintain a network with other OIE Reference Laboratories designated for the same pathogen or disease'.

7.4. Twinning

As of February 2019, six aquatic animal health twinning projects have been completed: Canada and Chile for infection with infectious salmon anaemia virus; Denmark and Republic of Korea for infection with viral haemorrhagic septicaemia virus; Japan and Indonesia for infection with koi herpesvirus; Norway and Brazil for infection with infectious salmon anaemia virus; USA and China (People's Rep. of) for infection with infectious haematopoietic necrosis virus; USA and Indonesia for crustacean diseases. Two other twinning projects are underway: Italy and Tunisia for viral encephalopathy and retinopathy; USA and Saudi Arabia for shrimp diseases.

The Aquatic Animals Commission reviewed one twinning project proposal between China (People's Rep. of) and Indonesia on infection with white spot syndrome virus and infection with infectious haematopoietic necrosis virus. The Commission provide comments concerning the objectives and workplan of the project.

H. OTHER ISSUES

8.1. Technical disease cards

8.1.1. Tilapia lake virus

The Aquatic Animals Commission reviewed the technical disease card for tilapia lake virus and amended the sections on geographical distribution and confirmatory test methods in line with recent publications.

The technical disease card is available on the OIE website at: http://www.oie.int/en/international-standard-setting/specialists-commissions-groups/aquatic-animal-commission-reports/disease-information-cards/

8.1.2. Infection with *Batrachochytrium salamandrivorans*

The Aquatic Animals Commission reviewed the technical disease card for Infection with *Batrachochytrium salamandrivorans* and concluded that no amendments were necessary.

The technical disease card is available on the OIE website at: http://www.oie.int/en/international-standard-setting/specialists-commissions-groups/aquatic-animal-commission-reports/disease-information-cards/

I. OIE GLOBAL CONFERENCE ON AQUATIC ANIMAL HEALTH

The Aquatic Animals Commission finalised the programme for the OIE Global Conference on Aquatic Animal Health: Collaboration, sustainability: our future to be held from 2 to 4 April, 2019 in Santiago Chile, ensuring that the Global Conference would be engaging and relevant to all Member Countries.

J. WORK PLAN OF THE AQUATIC ANIMAL HEALTH STANDARDS COMMISSION FOR 2018/2019

The Aquatic Animals Commission reviewed and updated its work programme. The programme will be comprehensively reviewed at the next Commission meeting.

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The revised 2018/2019 work programme is presented at **Annex 24** for Member Country information.

K. NEXT MEETING

The next meeting of the Aquatic Animals Commission is scheduled for 25 September to 2 October 2019.

/Annexes

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MEETING OF THE OIE
AQUATIC ANIMAL HEALTH STANDARDS COMMISSION

Paris, 7–14 February 2019

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UNOFFICIAL VERSION

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Annex II

MEETING OF THE OIE AQUATIC ANIMAL HEALTH STANDARDS COMMISSION

Paris, 7–14 February 2019

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Agenda

A. INTRODUCTIONS AND THE PERFORMANCE MANAGEMENT FRAMEWORK

B. ADOPTION OF THE AGENDA

C. COOPERATION WITH OTHER SPECIALIST COMMISSIONS

a) Joint meeting of the Biological Standards Commission and the Aquatic Animals Commission

D. OIE *AQUATIC ANIMAL HEALTH CODE*

1. Texts proposed for adoption at the General Session in May 2019

1.1. General comments

1.2. Glossary

1.3. Criteria for listing species as susceptible (Chapter 1.5.)

1.4. Amendments to fish disease-specific chapters

1.4.1. Article 10.5.2. Infection with salmonid alphavirus (Chapter 10.5.)

1.4.2. Article 10.7.2. Infection with koi herpesvirus (Chapter 10.7.)

1.4.3. Article 10.9.2. Infection with spring viraemia of carp virus (Chapter 10.9.)

1.5. Infection with *Ranavirus* species (Chapter 8.3.)

1.6. Acute hepatopancreatic necrosis disease (Chapter 9.1.)

1.7. Articles 10.2.1. and 10.2.2. of Infection with *Aphanomyces invadans* (Chapter 10.2.)

1.8. Infection with infectious haematopoietic necrosis virus (Chapter 10.6.)

1.9. Article X.X.8.

2. Texts for Member Country comments

2.1. New draft chapter on Aquatic Animal Biosecurity for Aquaculture Establishments (Chapter 4.X.)

2.2. Discussion paper on Approaches for determining periods required to demonstrate disease freedom

3. Other *Aquatic Code* topics

3.1. Disease listed by the OIE (Chapter 1.3.)

3.1.1. Infection with shrimp haemocyte iridescent virus (SIHV

UNOFFICIAL VERSION

3.2. Model Article 10.X.13.

3.2.1. Model Article 10.X.3.

3.2.2. Article 10.6.13. of Infection with infectious haematopoietic necrosis virus (Chapter 10.6.)

3.3. Chapter 10.3. Infection with *Gyrodactylus salaris*

3.4. Chapter 11.4. Infection with *Marteilia refringens*

E. *AD HOC GROUPS*

4.1. A*d hoc* Group on Susceptibility of fish species to infection with OIE listed diseases

4.2. Electronic *ad hoc* Group on tilapia lake virus

F. *MANUAL OF DIAGNOSTIC TESTS FOR AQUATIC ANIMALS*

5. Text proposed for adoption at the General Session in May 2019

5.1. Sections 1, 2.2.21 and 2.2.2. Infection with yellow head virus genotype 1 (Chapter 2.2.9.)

5.2. Infectious haematopoietic necrosis (Chapter 2.3.4.)

5.3. Infection with salmonid alphavirus (Chapter 2.3.6.)

5.4. Koi herpesvirus disease (Chapter 2.3.7.)

6. Other *Aquatic Manual* topics

6.1. Revision of disease-specific chapters using the new template

6.1.1. Infection with spring viraemia of carp virus (Chapter 2.3.9.)

6.1.2. Infection with *Batrachochytrium salamandrivorans* (Chapter 2.1.X)

6.2. Infection with *Gyrodactylus salaris* (Chapter 2.3.3.)

6.3. Infection with *Marteilia refringens* (Chapter 2.4.4)

6.4. Chapters that are being updated and reformatted using the new chapter template

G. OIE REFERENCE CENTRES OR CHANGE OF EXPERTS

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

7.2. Review of annual reports of Reference Centre activities in 2018

7.3. Twinning

UNOFFICIAL VERSION

H. OTHER ISSUES

8.1. Technical disease cards

8.1.1. Tilapia lake virus

8.1.2. Infection with *Batrachochytrium salamandrivorans*

I. OIE GLOBAL CONFERENCE ON AQUATIC ANIMAL HEALTH

J. WORK PLAN OF THE AQUATIC ANIMAL HEALTH STANDARDS COMMISSION FOR

K. NEXT MEETING

UNOFFICIAL VERSION

G L O S S A R Y

EU position
The EU supports the adoption of this modified Glossary.

BASIC BIOSECURITY CONDITIONS

means a minimum set of conditions required to ensure *biosecurity* applying to for a particular *disease,* and a particular *zone* or in a country, *zone* or *compartment* that should include required to ensure adequate disease security, such as:

a) compulsory notification of the *disease*, including or suspicion of the *disease*, is compulsorily notifiable to the *Competent Authority*; and

b) an *early detection system* is in place within the *zone* or country; and

c) import requirements to prevent the introduction of the *pathogenic agent* *disease* into the a *free* country *country*, or *zone* or *compartment*, or the spread within or from *infected zones* and *protection zones*, in accordance with the relevant disease-specific chapter as outlined in the *Aquatic Code*, are in place.

UNOFFICIAL VERSION

CHAPTER 1.5.

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

EU position

The EU thanks the OIE and supports the adoption of this modified chapter.

Article 1.5.1.

Purpose

In each disease-specific chapter, Article X.X.2. lists the *aquatic animal* species that have been found to be susceptible to *infection* with the relevant *pathogenic agent*. The recommendations of each disease-specific chapter apply only to the species listed in Article X.X.2.

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~~.

Species of *aquatic animal*s are considered susceptible to *infection* with a *pathogenic agent* when the presence of a multiplying~~,~~ or developing ~~or latent~~ *pathogenic agent* has been demonstrated by the occurrence of natural *cases* or by experimental exposure that mimics natural transmission pathways. Susceptibility includes clinical or non-clinical *infection.*

The decision to list an individual ~~a~~ species as susceptible in a disease-specific chapter~~s~~ 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.~~ A taxonomic ranking higher than species is listed when the criteria in Article 1.5.9. are met.

~~However,~~ ~~possible~~ Possible susceptibility of a species is also important information and, in accordance with Article 1.5.8., these species are ~~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 Article1.5.6.).

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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 procedures; includes injection, exposure to unnaturally ~~unnaturally~~ high loads of ~~pathogen~~ *pathogenic agent*, or exposure to stressors (e.g. temperature) not encountered in the host's natural or culture environment.

Consideration needs to be given to whether experimental procedures (e.g. ~~inoculation~~ injection, ~~infectivity~~ infective 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.;

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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, after application of Article 1.5.7., if 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 undertake a *risk assessment analysis* for 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

Some *pathogenic agents* have low host species specificity and can infect numerous species across multiple taxa. These *pathogenic agents* are eligible for assessment using this article if they have at least one *susceptible species* in each of three or more taxa at the ranking of Family. The outcome of applying this article may be that *susceptible species* are listed in Article X.X.2. of each disease-specific chapter at a ranking of Genus or higher. 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.

1) For *pathogenic agents* that have a broad host range low host species specificity, 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

BAa) after application of Article 1.5.7., 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

CBb) no species within the taxonomic group ranking has been found to be refractory non-susceptible to *infection*;

AND

Cc) The the taxa taxonomic ranking is at chosen should be the lowest level supported by this evidence of points A a) and b)B.

22) Evidence that a of non-susceptibility of a species is refractory to *infection* may include includes:

a)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 co-located *susceptible species*;

OR

b)B. absence of *infection* in a species exposed to the *pathogenic agent* through a controlled challenges appropriately designed experimental procedures.

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CHAPTER 1.5.

CRITERIA FOR LISTING SPECIES AS SUSCEPTIBLE TO INFECTION WITH A SPECIFIC PATHOGENIC AGENT

Article 1.5.1.

Purpose

In each disease-specific chapter, Article X.X.2. lists the *aquatic animal* species that have been found to be susceptible to *infection* with the relevant *pathogenic agent*. The recommendations of each disease-specific chapter apply only to the species listed in Article X.X.2.

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

Article 1.5.2.

Scope

Species of *aquatic animal*s are considered susceptible to *infection* with a *pathogenic agent* when the presence of a multiplying or developing *pathogenic agent* has been demonstrated by the occurrence of natural *cases* or by experimental exposure that mimics natural transmission pathways. Susceptibility includes clinical or non-clinical *infection*.

The decision to list an individual species as susceptible in a disease-specific chapter should be based on a finding that the evidence is definite in accordance with Article 1.5.3. A taxonomic ranking higher than species is listed when the criteria in Article 1.5.9. are met.

Possible susceptibility of a species is also important information and, in accordance with Article 1.5.8., these species are included in Section *2.2.2. Species with incomplete evidence for susceptibility* of the relevant disease-specific chapter of the *Aquatic Manual*.

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.).

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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 procedures: includes injection, exposure to unnaturally high loads of *pathogenic agent*, or exposure to stressors (e.g. temperature) not encountered in the host's natural or culture environment.

Consideration needs to be given to whether experimental procedures (e.g. injection, infective 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 *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 4 (diagnostic methods) 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 *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.;

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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, after application of Article 1.5.7., if there is incomplete evidence to demonstrate susceptibility of a species but partial information is available, these species 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 incomplete evidence to demonstrate susceptibility of a species, the *Competent Authority* should, prior to the implementation of any import health measures for the species, undertake a *risk assessment* for the *pathogenic agent* under consideration, in accordance with the recommendations in Chapter 2.1.

Article 1.5.9.

Listing susceptible species at a taxonomic ranking of Genus or higher

Some *pathogenic agents* have low host species specificity and can infect numerous species across multiple taxa. These *pathogenic agents* are eligible for assessment using this article if they have at least one *susceptible species* in each of three or more taxa at the ranking of Family. The outcome of applying this article may be that *susceptible species* are listed in Article X.X.2. of each disease-specific chapter at a ranking of Genus or higher.

1) For *pathogenic agents* that have a low host species specificity, a decision to conclude susceptibility of species at a taxonomic ranking of Genus or higher should only be made where:

   a) after application of Article 1.5.7., more than one species within the taxonomic ranking has been found to be susceptible;

   AND

   b) no species within the taxonomic ranking has been found to be non-susceptible to *infection*;

   AND

   c) the taxonomic ranking is at the lowest level supported by evidence of points a) and b).

2) Evidence of non-susceptibility of a species to *infection* includes:

   a) absence of *infection* in a species exposed to the *pathogenic agent* in natural settings where the *pathogenic agent* is known to be present and has caused *infection* in co-located *susceptible species*;

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OR

b) absence of *infection* in a species exposed to the *pathogenic agent* through appropriately designed experimental procedures.

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Annex 5

CHAPTER 10.5.

INFECTION WITH SALMONID ALPHAVIRUS

EU position

The EU thanks the OIE and supports the adoption of this modified chapter.

Article 10.5.1.

General provisions

For the purposes of the *Aquatic Code*, infection with salmonid alphavirus means *infection* with any ~~subtype~~ genotype of the *pathogenic agent* salmonid alphavirus (SAV), of the Genus *Alphavirus* and 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 the following species that meet the criteria for listing as susceptible in accordance with Chapter 1.5.: Arctic charr *(Salvelinus alpinus)*, Atlantic salmon *(Salmo salar)*, ~~brown trout *(Salmo trutta)*~~, common dab *(Limanda limanda)* and rainbow trout *(Onchorynchus mykiss)*. ~~These recommendations also apply to any other *susceptible species* referred to in the *Aquatic Manual* when traded internationally~~.

[…]

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CHAPTER 10.7.

INFECTION WITH KOI HERPESVIRUS

EU position

The EU supports the adoption of this modified this chapter.

[…]

Article 10.7.2.

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.: All varieties and subspecies of common carp (*Cyprinus carpio* ~~*carpio*~~)~~,~~ and common carp hybrids (e.g. *Cyprinus carpio* x *Carassius auratus*)~~, ghost carp *(Cyprinus carpio goi)*~~, ~~and 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.~~

[…]

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Annex 7

CHAPTER 10.9.

INFECTION WITH SPRING VIRAEMIA OF CARP VIRUS

EU position
The EU supports the adoption of this modified chapter.

[…]

Article 10.9.2.

Scope

The recommendations in this chapter apply to the following species that meet the criteria for listing as susceptible in accordance with Chapter 1.5.: all varieties and subspecies of common carp *(Cyprinus carpio carpio)* bighead carp *(Aristichthys nobilis)*, bream *(Abramis brama)*, Caspian white fish *(Rutilus frisiikutum)*, common carp *(Cyprinus carpio carpio)*, fathead minnow *(Pimephales promelas)*, golden shiner *(Notemigonus crysoleucas)*, goldfish *(Carassius auratus)*, grass carp (white amur)*(Ctenopharyngodon idellaidellus)*, and koi carp *(Cyprinus carpio koi)*, crucian carp *(Carassius carassius)*, roach *(Rutilus rutilus)*, and sheatfish (also known as European or wels catfishor wels) *(Silurus glanis)*, and silver carp *(Hypophthalmichthys molitrix)*, bighead carp *(Aristichthys nobilis)*, grass carp (white amur) *(Ctenopharyngodon idellus)*, goldfish *(Carassius auratus)*, orfe *(Leuciscus idus)* and zebrafish *(Sander vitreus)* *(Danio rerio)* 'tench *(Tinca tinca)*. These recommendations also apply to any other *susceptible species* referred to in the *Aquatic Manual* when traded internationally.

[…]

CHAPTER 8.3.

INFECTION WITH *RANAVIRUS* SPECIES

EU position

The EU supports the adoption of this modified chapter.

Article 8.3.1.

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

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

Article 8.3.2.

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

Article 8.3.3.

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

1) *Competent Authorities* should not require any conditions related to *Ranavirus* species, regardless of the infection with ~~ranavirus~~ *Ranavirus* species status of the *exporting country*, *zone* or *compartment*, when authorising the importation or transit of the following *aquatic animal products* derived from a species referred to in Article 8.3.2. that are intended for any purpose and comply with Article 5.4.1.:

   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* species ~~[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 has been demonstrated to inactivate ~~all virus species of the genus~~ *Ranavirus* species~~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 has been demonstrated to inactivate ~~all virus species of the genus~~ *Ranavirus* species ~~[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 has been demonstrated to inactivate~~all virus species of the genus~~ *Ranavirus* species ~~[with the exception of epizootic haematopoietic necrosis virus and European catfish virus]~~).

2) When authorising the importation or transit of *aquatic animal products* derived from a species referred to in Article 8.3.2., other than those referred to in point 1 of Article 8.3.3., *Competent Authorities* should

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require the conditions prescribed in Articles 8.3.7. to 8.3.12. relevant to the infection with ~~ranavirus~~ *Ranavirus* species status of the *exporting country*, *zone* or *compartment*.

3) When considering the importation or transit of *aquatic animal products* derived from a species not referred to in Article 8.3.2. but which could reasonably be expected to pose a *risk* of transmission of ~~ranavirus~~ *Ranavirus* species, the *Competent Authority* should conduct a *risk analysis* in accordance with the recommendations in Chapter 2.1. The *Competent Authority* of the *exporting country* should be informed of the outcome of this analysis.

Article 8.3.4.

Country free from infection with ~~ranavirus~~ *Ranavirus* species

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~~ *Ranavirus* species if all the areas covered by the shared water bodies are declared countries or *zones* free from infection with ~~ranavirus~~ *Ranavirus* species (see Article 8.3.5.).

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

1) none of the *susceptible species* referred to in Article 8.3.2. 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 8.3.2. are present and the following conditions have been met:

   a) there has been no occurrence of infection with ~~ranavirus~~ *Ranavirus* species 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~~ *Ranavirus* species 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 *Ranavirus* species;

OR

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

   a) on detection of *Ranavirus* species, 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 *Ranavirus* species, 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~~ *Ranavirus* species; and

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

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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 8.3.5.

Article 8.3.5.

Zone or compartment free from infection with ~~ranavirus~~ *Ranavirus* species

If a *zone* or *compartment* extends over more than one country, it can only be declared a *zone* or *compartment* free from infection with ~~ranavirus~~ *Ranavirus* species 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~~ *Ranavirus* species may be declared free by the *Competent Authority* of the country concerned if:

1) none of the *susceptible species* referred to in Article 8.3.2. 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 8.3.2. are present in the *zone* or *compartment* and the following conditions have been met:

   a) there has been no occurrence of infection with ~~ranavirus~~ *Ranavirus* species 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~~ *Ranavirus* species 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 *Ranavirus* species;

OR

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

   a) on detection of *Ranavirus* species, 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 *Ranavirus* species, 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~~ *Ranavirus* species; and

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

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Article 8.3.6.

Maintenance of free status

A country, *zone* or *compartment* that is declared free from infection with ~~ranavirus~~ *Ranavirus* species following the provisions of points 1 or 2 of Articles 8.3.4. or 8.3.5. (as relevant) may maintain its status as free from infection with ~~ranavirus~~ *Ranavirus* species provided that *basic biosecurity conditions* are continuously maintained.

A country, *zone* or *compartment* that is declared free from infection with ~~ranavirus~~ *Ranavirus* species following the provisions of point 3 of Articles 8.3.4. or 8.3.5. (as relevant) may discontinue *targeted surveillance* and maintain its free status provided that conditions that are conducive to clinical expression of infection with ~~ranavirus~~ *Ranavirus* species, 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 ~~ranavirus~~ *Ranavirus* species, *targeted surveillance* should be continued at a level determined by the *Aquatic Animal Health Service* on the basis of the likelihood of *infection*.

Article 8.3.7.

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

When importing *aquatic animals* of a species referred to in Article 8.3.2., or *aquatic animal products* derived thereof, from a country, *zone* or *compartment* declared free from infection with ~~ranavirus~~ *Ranavirus* species, 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 8.3.4. or 8.3.5. (as applicable) and 8.3.6., the place of production of the *aquatic animals* or *aquatic animal products* is a country, *zone* or *compartment* declared free from infection with ~~ranavirus~~ *Ranavirus* species.

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 8.3.3.

Article 8.3.8.

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

When importing, for *aquaculture*, *aquatic animals* of a species referred to in Article 8.3.2. from a country, *zone* or *compartment* not declared free from infection with ~~ranavirus~~ *Ranavirus* species, 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) before leaving the *quarantine* ~~facility~~ (either in the original facility or following biosecure transport to another *quarantine* facility) the *aquatic animals* are killed and processed into one or more of the *aquatic animal products* referred to in point 1~~)~~ of Article 8.3.3. or other products authorised by the *Competent Authority*; and

   c~~cb~~) the treatment of all transport water, equipment, effluent and waste materials to inactivate *Ranavirus* species in accordance with Chapters 4.3., 4.7. and 5.5.

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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~~ *Ranavirus* species;

   b) in the *importing country*:

      i) import the F-0 population into a *quarantine* facility;

      ii) test the F-0 population for *Ranavirus* species 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~~ *Ranavirus* species, and sample and test for *Ranavirus* species in accordance with Chapter 1.4. of the *Aquatic Code* and Chapter 2.1.2. of the *Aquatic Manual*;

      v) if *Ranavirus* species ~~is~~ are not detected in the F-1 population, it may be defined as free from infection with ~~ranavirus~~ *Ranavirus* species and may be released from *quarantine*;

      vi) if *Ranavirus* species are detected in the F-1 population, those animals should not be released from *quarantine* and should be killed and disposed of in a biosecure manner in accordance with Chapter 4.7.

Article 8.3.9.

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 ~~ranavirus~~ *Ranavirus* species

When importing, for processing for human consumption, *aquatic animals* of a species referred to in Article 8.3.2., or *aquatic animal products* derived thereof, from a country, *zone* or *compartment* not declared free from infection with ~~ranavirus~~ *Ranavirus* species, 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 8.3.3. or in point 1 of Article 8.3.12., 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 *Ranavirus* species 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 *Ranavirus* species 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.

UNOFFICIAL VERSION

Annex 8 (contd)

Article 8.3.10.

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 ~~ranavirus~~ *Ranavirus* species

When importing *aquatic animals* of a species referred to in Article 8.3.2., 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 ~~ranavirus~~ *Ranavirus* species, 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 8.3.3. 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 *R~~r~~anavirus* species 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 *R~~r~~anavirus* species or disposed of in a biosecure manner in accordance with Chapters 4.3. and 4.7.

Article 8.3.11.

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

When importing, for use in laboratories or zoos, *aquatic animals* of a species referred to in Article 8.3.2. from a country, *zone* or *compartment* not declared free from infection with ~~ranavirus~~ *Ranavirus* species, 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 *R~~r~~anavirus* species 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 *R~~r~~anavirus* species 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 8.3.12.

Importation (or transit) of aquatic animal products for retail trade for human consumption regardless of the infection with ~~ranavirus~~ *Ranavirus* species status of the exporting country, zone or compartment

1) *Competent Authorities* should not require any conditions related to *R~~r~~anavirus* species, regardless of the infection with ~~ranavirus~~*Ranavirus* species status of the *exporting country*, *zone* or *compartment*, when authorising the importation (or transit) of the following *aquatic animal products* that have been prepared and packaged for retail trade and comply with Article 5.4.2.:

   – no *aquatic animal products* listed.

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2) When importing *aquatic animal products*, other than those referred to in point 1 above, derived from a species referred to in Article 8.3.2. from a country, *zone* or *compartment* not declared free from infection with ~~ranavirus~~*Ranavirus* species, the *Competent Authority* of the *importing country* should assess the *risk* and apply appropriate *risk* mitigation measures.

UNOFFICIAL VERSION

CHAPTER 9.1.

ACUTE HEPATOPANCREATIC NECROSIS DISEASE

EU position

The EU supports the adoption of this modified chapter.

Article 9.1.1.

For the purposes of the *Aquatic Code*, acute hepatopancreatic necrosis disease (AHPND) means *infection* with strains of *Vibrio parahaemolyticus* (Vp_{AHPND}), of the Family Vibrionaceae, that contain a ~70-kbp plasmid with genes that encode homologues of the *Photorhabdus* insect-related (Pir) toxins, PirA and PirB.

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

Article 9.1.2.

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.: giant tiger prawn *(Penaeus monodon)* and whiteleg shrimp *(Penaeus vannamei)*.

Article 9.1.3.

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

1) *Competent Authorities* should not require any conditions related to AHPND, regardless of the AHPND status of the *exporting country*, *zone* or *compartment*, when authorising the importation or transit of the following *aquatic animal products* derived from a species referred to in Article 9.1.2., which are intended for any purpose and comply with Article 5.4.1.:

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

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

    c) crustacean oil;

    d) crustacean *meal*;

    e) chemically extracted chitin.

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

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

UNOFFICIAL VERSION

Annex 9 (contd)

Article 9.1.4.

Country free from AHPND

If a country shares a *zone* with one or more other countries, it can only make a *self-declaration of freedom* from AHPND if all the areas covered by the shared water bodies are declared countries or *zones* free from AHPND (see Article 9.1.5.).

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

1) none of the *susceptible species* referred to in Article 9.1.2. 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 9.1.2. are present and the following conditions have been met:

   a) there has been no occurrence of AHPND 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 two years;

OR

3) the AHPND 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 Vp_{AHPND} ~~AHPND~~;

OR

4) it previously made a *self-declaration of freedom* from AHPND and subsequently lost its free status due to the detection of Vp_{AHPND} ~~AHPND~~ but the following conditions have been met:

   a) on detection of Vp_{AHPND} ~~AHPND~~, 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 Vp_{AHPND} ~~AHPND~~, 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 AHPND; and

   d) *targeted surveillance*, as described in Chapter 1.4., has been in place for at least the last two years without detection of Vp_{AHPND} ~~AHPND~~.

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 9.1.5.

Article 9.1.5.

Zone or compartment free from AHPND

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

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

1) none of the *susceptible species* referred to in Article 9.1.2. 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 9.1.2. are present in the *zone* or *compartment* and the following conditions have been met:

   a) there has not been any occurrence of AHPND 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 two years;

OR

3) the AHPND 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 Vp_{AHPND} ~~AHPND~~;

OR

4) it previously made a *self-declaration of freedom* for a *zone* from AHPND and subsequently lost its free status due to the detection of Vp_{AHPND} ~~AHPND~~ in the zone but the following conditions have been met:

   a) on detection of Vp_{AHPND} ~~AHPND~~, 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 Vp_{AHPND} ~~AHPND~~, 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 AHPND; and

   d) *targeted surveillance*, as described in Chapter 1.4., has been in place for at least the last two years without detection of Vp_{AHPND} ~~AHPND~~.

Article 9.1.6.

Maintenance of free status

A country, *zone* or *compartment* that is declared free from AHPND following the provisions of points 1 or 2 of Articles 9.1.4. or 9.1.5. (as relevant) may maintain its status as free from AHPND provided that *basic biosecurity conditions* are continuously maintained.

A country, *zone* or *compartment* that is declared free from AHPND following the provisions of point 3 of Articles 9.1.4. or 9.1.5. (as relevant) may discontinue *targeted surveillance* and maintain its free status provided that conditions are conducive to clinical expression of AHPND, 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 AHPND, *targeted surveillance* should be continued at a level determined by the *Aquatic Animal Health Service* on the basis of the likelihood of *infection*.

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Annex 9 (contd)

Article 9.1.7.

Importation of aquatic animals or aquatic animal products from a country, zone or compartment declared free from AHPND

When importing *aquatic animals* of a species referred to in Article 9.1.2., or *aquatic animal products* derived thereof, from a country, *zone* or *compartment* declared free from AHPND, 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, on the basis of the procedures described in Articles 9.1.4. or 9.1.5. (as applicable) and 9.1.6., the place of production of the *aquatic animals* or *aquatic animal products* is a country, *zone* or *compartment* declared free from AHPND.

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 9.1.3.

Article 9.1.8.

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

When importing, for *aquaculture*, *aquatic animals* of a species referred to in Article 9.1.2. from a country, *zone* or *compartment* not declared free from AHPND, 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) before leaving the *quarantine* ~~facility~~ (either in the original facility or following biosecure transport to another *quarantine* facility) the *aquatic animals* are killed and processed into one or more of the *aquatic animal products* referred to in point 1~~)~~ of Article 9.1.3. or other products authorised by the *Competent Authority*; and

   ~~*b)*~~*c)* the treatment of transport water, equipment, effluent and waste materials to inactivate Vp_{AHPND} 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 AHPND.

   b) In the importing country:

      i) import the F-0 population into a *quarantine* facility;

      ii) test the F-0 population for Vp_{AHPND} in accordance with Chapter 1.4. to determine their suitability as broodstock;

      iii) produce a first generation (F-1) population in *quarantine*;

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iv) culture F-1 population in *quarantine* under conditions that are conducive to the clinical expression of AHPND (as described in Chapter 2.2.1. of the *Aquatic Manual*) and test for Vp_{AHPND} in accordance with Chapter 1.4.;

v) if Vp_{AHPND} is not detected in the F-1 population, it may be defined as free from AHPND and may be released from *quarantine*;

vi) if Vp_{AHPND} is detected in the F-1 population, those animals should not be released from *quarantine* and should be killed and disposed of in a biosecure manner in accordance with Chapter 4.7.

Article 9.1.9.

Importation of aquatic animals or aquatic animal products for processing for human consumption from a country, zone or compartment not declared free from AHPND

When importing, for processing for human consumption, *aquatic animals* of a species referred to in Article 9.1.2., or *aquatic animal products* derived thereof, from a country, *zone* or *compartment* not declared free from AHPND, 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 9.1.3. or in point 1 of Article 9.1.11., or other products authorised by the *Competent Authority*; and

2) all containers and water used in transport are treated to ensure inactivation of Vp_{AHPND} or disposed of in a biosecure manner in accordance with Chapters 4.3., 4.7. and 5.5.; and

3) all processing effluent and waste materials are treated to ensure inactivation of Vp_{AHPND} 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 animals* or *aquatic animal products* being used for any purpose other than for human consumption.

Article 9.1.10.

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

When importing, for use in animal feed or for agricultural, industrial, research or pharmaceutical use, *aquatic animals* of a species referred to in Article 9.1.2., or *aquatic animal products* derived thereof, from a country, *zone* or *compartment* not declared free from AHPND, 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 9.1.3. or other products authorised by the *Competent Authority*; and

2) all containers and water used in transport are treated to ensure inactivation of Vp_{AHPND} or disposed of in a biosecure manner in accordance with Chapters 4.3., 4.7. and 5.5.; and

3) all processing effluent and waste materials are treated to ensure inactivation of Vp_{AHPND} or disposed of in a biosecure manner in accordance with Chapters 4.3. and 4.7.

Article 9.1.11.

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

When importing, for use in laboratories or zoos, *aquatic animals* of a species referred to in Article 9.1.2. from a country, *zone* or *compartment* not declared free from AHPND, the *Competent Authority* of the *importing country* should ensure:

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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 Vp_{AHPND} 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 Vp_{AHPND} 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 9.1.12.

Importation (or transit) of aquatic animal products for retail trade for human consumption regardless of the AHPND status of the exporting country, zone or compartment

1) *Competent Authorities* should not require any conditions related to Vp_{AHPND} ~~AHPND~~, regardless of the AHPND status of the *exporting country*, *zone* or *compartment*, when authorising the importation (or transit) of frozen peeled shrimp (shell off, head off) that have 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 9.1.2. from a country, *zone* or *compartment* not declared free from AHPND, the *Competent Authority* of the *importing country* should assess the *risk* and apply appropriate *risk* mitigation measures.

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Annex 10

CHAPTER 10.2.

INFECTION WITH *APHANOMYCES INVADANS* (EPIZOOTIC ULCERATIVE SYNDROME)

EU position

The EU supports the adoption of this modified chapter.

Article 10.2.1.

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

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*~~ Anguillidae), bagrid catfishes (~~*Bagridae*~~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 (*Clarias* ~~*Clarius*~~ spp.), halfbeaks flying fishes (~~*Exocoetidae*~~ Exocoetidae), tank goby *(Glossogobius giuris)*, marble goby *(Oxyeleotris marmoratus)*, gobies (~~*Gobiidae*~~ *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*~~ Siluridae spp.), 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)*, ~~therapon~~ terapon (*Terapon* sp.) and three-spot gouram~~y~~i *(Trichogaster trichopterus)*. These recommendations also apply to any other *susceptible species* referred to in the *Aquatic Manual* when traded internationally.

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CHAPTER 10.6.

INFECTION WITH INFECTIOUS HAEMATOPOIETIC NECROSIS VIRUS

EU position

The EU thanks the OIE and in general supports the proposed changes to this chapter. One comment is inserted in the text below.

Article 10.6.1.

For the purposes of the *Aquatic Code*, infection with infectious haematopoietic necrosis virus means *infection* with the *pathogenic agent* S~~s~~almonid ~~*Novirhabdovirus*~~ novirhabdovirus (~~also~~ commonly known as infectious haematopoietic necrosis virus ~~(~~[IHNV]) of the Genus *Novirhabdovirus* and Family *Rhabdoviridae*.

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

Article 10.6.2.

Scope

The recommendations in this chapter apply to the following species that meet the criteria for listing as susceptible in accordance with Chapter 1.5.: Arctic charr *(Salvelinus alpinus)*, Atlantic salmon *(Salmo salar)*, brook trout *(Salvelinus fontinalis)*, brown trout *(Salmo trutta)*, chinook salmon *(Oncorhynchus tshawytscha)*, chum salmon *(Oncorhynchus keta)*, coho salmon *(Oncorhynchus kisutch)*, cutthroat trout *(Onchorynchus clarkii)*, lake trout *(Salvelinus namaycush)*, mas~~e~~ou salmon *(Oncorhynchus masou)*, marble trout *(Salmo marmoratus)*, pike (*Esox lucius*), 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 sockeye salmon (*Oncorhynchus nerka*) ~~Atlantic salmon *(Salmo salar)*. These recommendations also apply to any other *susceptible species* referred to in the *Aquatic Manual* when traded internationally.~~

EU comment

The name 'Pike' used here for *Esox Lucius* is different to the name given in the Manual 'Northern Pike'.

We have no objections to add 'Pike' as a susceptible species but it would be preferable to use the same name in both the Code and the Manual.

Article 10.6.3.

Importation or transit of aquatic animal products for any purpose regardless of the infection with IHNV 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 species referred to in Article 10.6.2. that are intended for any purpose and 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 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

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time/temperature equivalent 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 animal products* derived from 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 10.6.13. relevant to the infection with IHNV status of the *exporting country*, *zone* or *compartment*.

3) When considering the importation or transit of *aquatic animal products* derived from a species not referred to in Article 10.6.2. but which could reasonably be expected to pose a *risk* of transmission 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.

Article 10.6.4.

Country free from infection with IHNV

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 occurrence of infection with IHNV 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 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 free status due to the detection of IHNV but the following conditions have been met:

a) on detection of IHNV, 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 IHNV, and the appropriate *disinfection* procedures (as described in Chapter 4.3.) have been completed; and

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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; 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 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

If a *zone* or *compartment* extends over more than one country, it can only be declared a *zone* or *compartment* free from infection with IHNV 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* of the country 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 occurrence of infection with IHNV 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 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 free status due to the detection of IHNV in the *zone* but the following conditions have been met:

   a) on detection of IHNV, 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 IHNV, 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; 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.

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Annex 11 (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 provided that conditions that are conducive to clinical expression of infection with IHNV, 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 IHNV, *targeted surveillance* 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 or aquatic animal products from a country, zone or compartment declared free from infection with IHNV

When importing *aquatic animals* of a species referred to in Article 10.6.2., or *aquatic animal products* 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*. The *international aquatic animal health certificate* should state 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 *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 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

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) before leaving the *quarantine* ~~facility~~ (either in the original facility or following biosecure transport to another *quarantine* facility) the *aquatic animals* are killed and processed into one or more of the *aquatic animal products* referred to in point 1~~)~~ of Article 10.6.3. or other products authorised by the *Competent Authority*; and

   ~~b~~c) the treatment of all transport water, equipment, effluent and waste materials to inactive IHNV in accordance with Chapters 4.3., 4.7. and 5.5.

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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 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* for a duration sufficient for, and under conditions that are conducive to, the clinical expression of infection with IHNV, 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 in accordance with Chapter 4.7.

Article 10.6.9.

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 IHNV

When importing, for processing for human consumption, *aquatic animals* 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 in point 1 of Article 10.6.12., 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 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 holding of the *aquatic animals* 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* 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.

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Annex 11 (contd)

Article 10.6.10.

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 IHNV

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 animal *feed* and agricultural, industrial, research or pharmaceutical use, from a country, *zone* or *compartment* not declared free from infection with IHNV, 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 10.6.3. 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 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 are treated to ensure inactivation of IHNV or disposed of in a biosecure manner in accordance with Chapters 4.3. and 4.7.

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 IHNV

When importing, for use in laboratories or 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 IHNV, 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 IHNV 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 animal products for retail trade for human consumption regardless of the infection with IHNV 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 fish fillets or steaks (chilled) that have 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.

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For these *aquatic animal products* Member Countries may wish to consider introducing internal measures to address the *risks* associated with the *aquatic animal product* 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 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* and apply appropriate *risk* mitigation measures.

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Model Article X.X.8. for all disease-specific chapters (or Article 10.4.12. for infection with infectious salmon anaemia virus)

EU position

The EU supports the adoption of this modified chapter.

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

When importing for *aquaculture*, *aquatic animals* of species referred to in Article X.X.2. from a country, *zone* or *compartment* not declared free from 'infection with pathogen X'/'*disease X*', 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) before leaving ~~the~~ *quarantine* ~~facility~~ (either in the original facility or following biosecure transport to another *quarantine* facility) the *aquatic animals* are killed and processed into one or more of the *aquatic animal products* referred to in point 1~~)~~ of Article X.X.3. or other products authorised by the *Competent Authority*; and

   ~~b)~~c) the treatment of all transport water, equipment, effluent and waste materials to inactive 'pathogen X' 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 pathogen X'/ 'disease X'.

   b) In the *importing country*:

      i) import the F-0 population into a *quarantine* facility;

      ii) test the F-0 population for 'pathogen X' in accordance with Chapter 1.4. to determine their suitability as broodstock;

      iii) produce a first generation (F-1) population in *quarantine*;

      iv) culture the F-1 population in *quarantine* for a duration sufficient for, and under conditions that are conducive to~~,~~ the clinical expression of 'infection with pathogen X'/'disease X' (as described in Chapter X.X.X. of the *Aquatic Manual*) and test for 'pathogen X' in accordance with Chapter 1.4.;

      v) if 'pathogen X' is not detected in the F-1 population, it may be defined as free from 'infection with pathogen X'/'disease X' and may be released from *quarantine*;

      vi) if 'pathogen X' is detected in the F-1 population, those animals should not be released from *quarantine* and should be killed and disposed of in a biosecure manner in accordance with Chapter 4.7.

CHAPTER 2.2.9.

INFECTION WITH YELLOW HEAD VIRUS GENOTYPE 1

EU position

The EU supports the adoption of this modified chapter.

1. Scope

Infection with yellow head virus genotype 1 means infection with the pathogenic agent yellow head virus genotype 1 (YHV1) of the Ggenus *Okavirus*, and Family *Roniviridae* and Order *Nidovirales*.

[…]

2.2. Host factors

2.2.1. Susceptible host species

Species that fulfil the criteria for listing a species as susceptible to infection with YHV1 according to Chapter 1.5 of *Aquatic Animal Health Code* (*Aquatic Code*) include: Blue shrimp (*Penaeus stylirostris*), dagger blade grass shrimp (*Palaemonetes pugio*), giant tiger prawn (*Penaeus monodon*), grass shrimp (*Palaemonetes pugio*), jinga shrimp (*Metapenaeus affinis*) and whiteleg shrimp (*Penaeus vannamei*). giant tiger prawn (*P. monodon*), white leg shrimp (*P. vannamei*), blue shrimp (*P. stylirostris*), daggerblade grass shrimp (*Palaemonetes pugio*), and Jinga shrimp (*Metapenaeus affinis*).

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 for susceptibility susceptible to infection with YHV1 according to Chapter 1.5 of the *Aquatic Code* include: Banana prawn (*Penaeus merguiensis*), Carpenter prawn (*Palaemon serrifer*), kuruma prawn (*Penaeus japonicus*), northern brown shrimp (*Penaeus aztecus*), northern pink shrimp (*Penaeus duorarum*), northern white shrimp (*Penaeus setiferus*), Pacific blue prawn (*Palaemon styliferus*), red claw crayfish (*Cherax quadricarinatus*), Sunda river prawn (*Macrobrachium sintangense*) and yellow shrimp (*Metapenaeus brevicornis*). Sunda river prawn (*Macrobrachium sintangense*), yellow shrimp (*Metapenaeus brevicornis*), Carpenter prawn (*Palaemon serrifer*), Pacific blue prawn (*Palaemon styliferus*), northern brown shrimp (*Penaeus aztecus*), northern pink shrimp (*Penaeus duorarum*), kuruma prawn (*Penaeus japonicus*), banana prawn (*Penaeus merguiensis*), northern white shrimp (*Penaeus setiferus*) and red claw crayfish (*Cherax quadricarinatus*). Evidence is lacking for these species to either confirm that the identity of the pathogenic agent is YHV1, transmission mimics natural pathways of infection, or presence of the pathogenic agent constitutes an infection.

In addition, pathogen-specific positive polymerase chain reaction (PCR) results have been reported in the following species, but an active infection has not been demonstrated: Acorn barnacle (*Chelonibia patula*), blue crab (*Callinectes sapidus*), cyclopoid copepod (*Ergasilus manicatus*), gooseneck barnacle (*Octolasmis muelleri*), Gulf killifish (*Fundulus grandis*) and paste shrimp (*Acetes* sp.).

[…]

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Annex 14

CHAPTER 2.3.4

INFECTION WITH INFECTIOUS HAEMATOPOIETIC NECROSIS VIRUS

EU position

The EU thanks the OIE and in genral supports the adoption of this modified chapter.

Additional comments have been included in the text below.

1. Scope

~~Infectious haematopoietic necrosis (IHN)~~ Infection with infectious haematopoietic necrosis virus means infection with the *pathogenic agent* ~~S~~salmonid ~~n~~N*ovirhabdovirus* (also known as infectious haematopoietic necrosis virus [IHNV]) of the Genus *Novirhabdovirus* and Family *Rhabdoviridae*. ~~is a viral disease affecting most species of salmonid fish reared in fresh water or sea water. Caused by the rhabdovirus, infectious haematopoietic necrosis virus (IHNV), the principal clinical and economic consequences of IHN occur on farms rearing rainbow trout where acute outbreaks can result in very high mortality. However, both Pacific and Atlantic salmon can be severely affected. For the purpose of this chapter, IHN is considered to be infection with IHNV~~.

2. Disease information

~~For detailed reviews of the disease, see Bootland & Leong (1999) or Wolf (1988).~~

2.1. Agent factors

2.1.1. Aetiological agent, agent strains

The fish rhabdovirus, IHNV, has a bullet-shaped virion containing a non-segmented, negative-sense, single-stranded RNA genome of approximately 11,000 nucleotides that encodes six proteins in the following order: a nucleoprotein (N), a phosphoprotein (P), a matrix protein (M), a glycoprotein (G), a non-virion protein (NV), and a polymerase (L). The presence of the unique NV gene and sequence similarity with certain other fish rhabdoviruses, such as viral haemorrhagic septicaemia virus, has resulted in the creation of the *Novirhabdovirus* genus of the family *Rhabdoviridae*, with IHNV as the type species. The type strain of IHNV is the Western Regional Aquaculture Center (WRAC) strain available from the American Type Culture Collection (ATCC VR-1392). The GenBank accession number of the genomic sequence of the WRAC strain is L40883 (Morzunov *et al*., 1995; Winton & Einer-Jensen, 2002).

Sequence analysis has been used to compare IHNV isolates from North America, Europe and Asia (Emmenegger *et al*., 2000; Enzmann *et al*., 2005; Enzmann *et al*., 2010; Johansson *et al*., 2009; Kim *et al*., 2007; Kolodziejek *et al*., 2008; Kurath *et al*., 2003; Nishizawa *et al*., 2006; Troyer & Kurath, 2003). Within the historical natural range of the virus in western North America, most isolates of IHNV from Pacific salmon form two genogroups that are related to geographical location and not to year of isolation or host species. The isolates within these two genogroups show a relatively low level of nucleotide diversity, suggesting evolutionary stasis or an older host-pathogen relationship. Conversely, isolates of IHNV from farmed rainbow trout in the USA form a third genogroup with more genetic diversity and an evolutionary pattern indicative of ongoing adaptation to a new host or rearing conditions. Isolates from farmed rainbow trout in Europe and Asia appear to have originated from North America, but show further, independent, divergence within their new geographical range (Enzmann *et al*., 2010; Kim *et al*., 2007; Nishizawa *et al*., 2006).

On the basis of antigenic studies using neutralising polyclonal rabbit antisera, IHNV isolates form a single serogroup (Engelking *et al.,* 1991), while mouse monoclonal antibodies have revealed a number of neutralising epitopes on the glycoprotein (Huang *et al.,* 1994; Ristow & Arnzen De Avila, 1991; Winton *et al.,* 1988), as well as the existence of a non-neutralising group epitope borne by the nucleoprotein (Ristow & Arnzen, 1989). However, there appears to be little or no correlation between genotypes and serotypes (Johansson *et al.,* 2009). Variations in the virulence and host preference of IHNV strains have been recorded during both natural cases of disease and in experimental infections (Garver *et al.,* 2006; LaPatra *et al.,* 1993a).

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2.1.2. Survival outside the host

IHNV is heat, acid and ether labile. The virus will survive in fresh water for at least 1 month at cooler temperatures, especially if organic material is present.

2.1.3. Stability of the agent (effective inactivation methods)

IHNV is readily inactivated by common disinfectants and drying (Wolf, 1988).

2.1.4. Life cycle

Reservoirs of IHNV are clinically infected fish and covert carriers among cultured, feral or wild fish. Virus is shed via urine, sexual fluids and from external mucus, whereas kidney, spleen and other internal organs are the sites in which virus is most abundant during the course of overt infection (Bootland & Leong, 1999; Wolf, 1988).

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: The principal hosts for IHNV are members of the family *Salmonidae*. Species reported to be naturally infected with IHNV include Arctic charr (*Salvelinus alpinus*), Atlantic salmon (*Salmo salar*), brook trout (*Salvelinus fontinalis*), brown trout (*Salmo trutta*), chinook salmon (*Oncorhynchus tshawytscha*), chum salmon (*Oncorhynchus keta*), coho salmon (*Oncorhynchus kisutch*), cutthroat trout (*Onchorynchus clarkii*), lake trout (*Salvelinus namaycush*), maseu salmon (*Oncorhynchus masou*), marble trout (*Salmo marmoratus*), Northern pike (*Esox lucius*), rainbow trout or steelhead (*Oncorhynchus mykiss*) Chinook (*O. tshawytscha*), sockeye (*O. nerka*), chum (*O. keta*), amago (*O. rhodurus*), masou (*O. masou*), coho (*O. kisutch*), and sockeye salmon (*Oncorhynchus nerka*) Atlantic salmon (*Salmo salar*). Other salmonids including brown trout (*S. trutta*) and cutthroat trout (*O. clarki*), some chars (*Salvelinus namaycush*, *S. alpinus*, *S. fontinalis*, and *S. leucomaenis*), ayu (*Plecoglossus altivelis*) and non-salmonids including European eel (*Anguilla anguilla*), herring (*Clupea pallasi*), cod (*Gadus morhua*), sturgeon (*Acipenser transmontanus*), pike (*Esox lucius*), shiner perch (*Cymatogaster aggregata*) and tube-snout (*Aulorhychus flavidus*) have occasionally been found to be infected in the wild or shown to be susceptible by a natural route of infection (Bootland & Leong, 1999; EFSA, 2008; Wolf, 1988).

EU comment

The name 'Northern Pike' used here for *Esox Lucius* is different to the name given in the Code 'Pike'.

We have no objections to add 'Pike' as a susceptible species but it would be preferable to use the same name in both the Code and the Manual.

2.2.2. Species with incomplete evidence for susceptibility

Species for which there is incomplete evidence of susceptibility to fulfil the criteria for listing as susceptible to infection with IHNV according to Chapter 1.5. of the *Aquatic Code* include: Northern pike (*Esox lucius*), Pacific herring (*Clupea pallasii*), shiner perch (*Cymatogaster aggregate*), tube-snout (*Aulorhynchus flavidus*), burbot (*Lota* lota) and white sturgeon (*Acipenser transmontanus*).

In addition, pathogen-specific positive polymerase chain reaction (PCR) results have been reported in the following species, but an active infection has not been demonstrated: all varieties and subspecies of common carp (*Cyprinus carpio*) *and* American yellow perch (*Perca flavescens*).

2.2.23. Susceptible stages of the host

Infection with IHNV occurs among several species of salmonids with fry being the most highly susceptible stage. Older fish are typically more resistant to clinical disease, but among individuals, there is a high degree of variation in susceptibility to infection with IHNV. As with viral haemorrhagic septicaemia virus, good fish health condition seems to decrease susceptibility to overt infection with IHNV, while co-infections with bacterial diseases (e.g. bacterial coldwater disease), handling and other stressors can cause subclinical infections to become overt. Fish become increasingly resistant to infection with age until spawning, when they once again become highly susceptible and may shed large amounts of virus in sexual products. Survivors of infection with IHNV demonstrate a strong protective immunity with the synthesis of circulating antibodies to the virus (LaPatra *et al.*, 1993b).

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2.2.~~3~~4. Species or subpopulation predilection (probability of detection)

IHNV shows a strong phylogeographic signature (Enzmann *et al*., 2010; Kurath *et al*., 2003; Nishizawa *et al*., 2006) that reflects the host species from which the virus is most commonly isolated in various geographical areas (e.g. sockeye salmon in the Northeast Pacific – U genogroup; Chinook salmon in California, USA – L genogroup; and rainbow trout in Europe, Asia and Idaho, USA – E, J and M genogroups, respectively).

2.2.~~4~~5. Target organs and infected tissue

Virus entry is thought to occur through the gills and at bases of fins while kidney, spleen and other internal organs are the sites in which virus is most abundant during the course of overt infection (Bootland & Leong, 1999; Wolf, 1988).

2.2.~~5~~6. Persistent infection with lifelong carriers

Historically, the geographic range of infection with IHNV was limited to western North America, but the disease has spread to Europe and Asia via the importation of infected fish and eggs. Once IHNV is introduced into a farmed stock, the disease may become established among susceptible species of wild fish in the watershed. The length that individual fish are infected with IHNV varies with temperature; however, unlike infectious pancreatic necrosis virus (IPNV) or channel catfish virus (CCV), a true, life-long carrier state with IHNV appears to be a rare event ~~at normal temperatures~~.

2.2.~~6~~7. Vectors

Horizontal transmission of IHNV is typically by direct exposure, but invertebrate vectors have been proposed to play a role in some cases (Bootland & Leong, 1999).

Mayfly (*Callibaetis* sp.) (Shors & Winston, 1988) and salmon lice (*Lepeophtheirus salmonis*) (Jakob *et al*., 2011) are potential vectors for IHNV.

2.2.~~7~~8. Known or suspected wild aquatic animal carriers

IHNV is endemic among many populations of free-ranging salmonids. A marine reservoir has been proposed, but not confirmed.

2.3. Disease pattern

Infection with IHNV often leads to mortality due to the impairment of osmotic balance and occurs within a clinical context of oedema and haemorrhage. Virus multiplication in endothelial cells of blood capillaries, haematopoietic tissues, and cells of the kidney underlies the clinical signs.

2.3.1. Transmission mechanisms

The transmission of IHNV between fish is primarily horizontal and high levels of virus are shed from infected juvenile fish, however, cases of vertical or egg-associated transmission have been recorded. Although egg-associated transmission is significantly reduced by the now common practice of surface disinfection of eggs with an iodophor solution, it is the only mechanism accounting for the occurrence of infection with IHNV in new geographical locations among alevins originating from eggs that were incubated and hatched in virus-free water (Winton, 1991).

2.3.2. Prevalence

Infection with IHNV is endemic and widely prevalent among populations of free-ranging salmonids throughout much of its historical range along the west coast of North America. The virus has also become established with a high prevalence of infection in major trout growing regions of North America, Europe and Asia where IHNV was introduced through the movement of infected fish or eggs.

2.3.3. Geographical distribution

Infection with IHNV has been detected in North America, Asia and Europe, but not in the Southern Hemisphere. Countries reporting confirmed or suspect cases of infection with IHNV to the OIE include: Austria, Belgium, Canada, China (People's Rep. of), Croatia, Czech Republic, France, Germany, Iran, Italy, Japan, Korea (Rep. of), Netherlands, Poland, Russia, Slovenia, Spain, Switzerland and United

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States of America. Infections and overt disease have been reported among fish reared in both fresh and sea water.

EU comment

There is a risk that very detailed geographical distribution could soon become outdated. It may be more appropriate to refer to WAHIS which contains official information and is constantly updated, or use a broader geographical distribution rather than individual countries (e.g. by continent).

2.3.4. Mortality and morbidity

Depending on the species of fish, rearing conditions, temperature, and, to some extent, the virus strain, outbreaks of infection with IHNV may range from explosive to chronic. Losses in acute outbreaks will exceed several per cent of the population per day and cumulative mortality may reach 90–95% or more (Bootland & Leong, 1999). In chronic cases, losses are protracted and fish in various stages of disease can be observed in the pond.

2.3.5. Environmental factors

The most important environmental factor affecting the progress of infection with IHNV is water temperature. Experimental trials have demonstrated infection with IHNV can produce mortality from 3°C to 18°C (Bootland & Leong, 1999); however, clinical disease typically occurs between 8°C and 15°C under natural conditions.

2.4. Control and prevention

Control methods for infection with IHNV currently rely on avoidance of exposure to the virus through the implementation of strict control policies and sound hygiene practices (Winton, 1991). The thorough disinfection of fertilised eggs, the use of virus-free water supplies for incubation and rearing, and the operation of facilities under established biosecurity measures are all critical for preventing infection with IHNV at a fish production site.

2.4.1. Vaccination

Experimental vaccines to protect salmonids against infection with IHNV have been the subject of research for more than 40 years with some showing promise in both laboratory and field trials when delivered by immersion or injection (Kurath, 2008; Winton, 1991; Winton, 1997). Both autogenous, killed vaccines and a DNA vaccine have been licensed for commercial use in Atlantic salmon net-pen aquaculture on the west coast of North America where such vaccines can be delivered economically by injection. However, vaccines against infection with IHNV have not yet been licensed in other countries where the application of vaccines to millions of smaller fish will require additional research on novel mass delivery methods.

2.4.2. Chemotherapy

Although chemotherapeutic approaches for control of infection with IHNV have been studied, they have not found commercial use in aquaculture against ~~IHNV~~ the disease (Winton, 1991).

2.4.3. Immunostimulation

Immunostimulants are an active area of research, but have not found commercial use in aquaculture against infection with IHNV.

2.4.4. Resistance breeding

Experimental trials of triploid or inter-species hybrids have shown promise (Barroso *et al*., 2008; Winton, 1991) and the genetic basis of resistance to IHNV has been an active area of recent research (Miller *et al*., 2004; Purcell *et al*., 2010).

2.4.5. Restocking with resistant species

Within endemic areas, the use of less susceptible species has been used to reduce the impact of infection with IHNV in aquaculture.

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2.4.6. Blocking agents

Natural compounds have been identified from aquatic microbes that have antiviral activity; however, these have not found commercial use in aquaculture against infection with IHNV (Winton, 1991).

2.4.7. Disinfection of eggs and larvae

Disinfection of eggs is a highly effective method to block egg-associated transmission of IHNV in aquaculture settings (Bovo *et al.*, 2005). The method is widely practiced in areas where the virus is endemic.

2.4.8. General husbandry practices

In addition to disinfection of eggs, use of a virus-free water supply has been shown to be a critical factor in the management of infection with IHNV within endemic areas. Several approaches include use of wells or springs that are free of fish or other sources of IHNV and disinfection of surface water sources using UV light or ozone (Winton, 1991).

3. Sampling

3.1. Selection of individual specimens

Clinical inspections are best carried out during a period whenever the water temperature is below 14°C. All production units (ponds, tanks, net-cages, etc.) must be inspected for the presence of dead, weak or abnormally behaving fish. Particular attention must be paid to the water outlet area where weak fish tend to accumulate.

In farms with salmonids, if rainbow trout are present, only fish of that species are selected for sampling. If rainbow trout are not present, the sample has to be obtained from fish of all other infection with IHNV susceptible species present, as listed in Section 2.2.1. Susceptible species should be sampled proportionally, or following risk-based criteria for targeted selection of lots or populations with a history of abnormal mortality or potential exposure events (e.g. via untreated surface water, wild harvest or replacement with stocks of unknown risk status).

If more than one water source is used for fish production, fish from all water sources must be included in the sample. If weak, abnormally behaving or freshly dead (not decomposed) fish are present, such fish are selected. If such fish are not present, the fish selected must include normal appearing, healthy fish collected in such a way that all parts of the farm as well as all year classes are proportionally represented in the sample.

3.2. Preservation of samples for submission

Before shipment or transfer to the laboratory, parts of the organs to be examined must be removed from the fish with sterile dissection instruments and transferred to sterile plastic tubes containing transport medium, i.e. cell culture medium with 10% foetal calf serum (FCS) and antibiotics. Addition of 200 International Units (IU) penicillin, 200 µg streptomycin, and 200 µg kanamycin per ml are recommended, although other antibiotics of proven efficiency may also be used.

3.3. Pooling of samples

Ovarian fluid or organ pieces from a maximum of ten fish may be collected in one sterile tube containing at least 4 ml transport medium and this represents one pooled sample. The tissue in each sample should weigh a minimum of 0.5 g. The tubes should be placed in insulated containers (for instance, thick-walled polystyrene boxes) together with sufficient ice or 'freezer blocks' to ensure chilling of the samples during transportation to the laboratory. Freezing must be avoided. The temperature of a sample during transit.

should never exceed 10°C and ice should still be present in the transport box at receipt or one or more freeze blocks must still be partly or completely frozen. Virological examination must be started as soon as possible and not later than 48 hours after collection of the samples. In exceptional cases, the virological examination may be started at the latest within 72 hours after collection of the material, provided that the material to be examined is protected by transport medium and that the temperature requirements during transportation are fulfilled.

Whole fish may be sent to the laboratory if the temperature requirements during transportation can be fulfilled. Whole fish may be wrapped in paper with absorptive capacity and must be shipped in a plastic bag, chilled as mentioned above. Live fish can also be shipped. All packaging and labelling must be performed in accordance with present national and international transport regulations, as appropriate.

3.4. Best organs or tissues

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The optimal tissue material to be examined is spleen, anterior kidney, and either heart or encephalon. In some cases, ovarian fluid and milt must be examined.

In case of small fry, whole fish less than 4 cm long can be minced with sterile scissors or a scalpel after removal of the body behind the gut opening. If a sample consists of whole fish with a body length between 4 cm and 6 cm, the viscera including kidney should be collected. If a sample consisted of whole fish less than 4 cm long, these should be minced with sterile scissors or a scalpel, after removal of the body behind the gut opening. If a sample consisted of whole fish with a body length between 4 cm and 6 cm, the viscera, including kidney, should be collected. If a sample consisted of whole fish more than 6 cm long, tissue specimens should be collected as described above. The tissue specimens should be minced with sterile scissors or a scalpel, homogenised and suspended in transport medium.

3.5. Samples/tissues that are not suitable

IHNV is very sensitive to degradation, therefore sampling tissues with high enzymatic activities or large numbers of contaminating bacteria such as the intestine or skin should be avoided when possible. Muscle tissue is also less useful as it typically contains a lower virus load.

4. Diagnostic methods

The “Gold Standard” for detection of IHNV is the isolation of the virus in cell culture followed by its immunological or molecular identification. While the other diagnostic methods listed below can be used for confirmation of the identity of virus isolated in cell culture or for confirmation of overt infections in fish, they are not approved for use as primary surveillance methods for obtaining or maintaining approved infection with IHNV-free status.

Due to substantial variation in the strength and duration of the serological responses of fish to virus infections, the detection of fish antibodies to viruses has not thus far been accepted as a routine diagnostic method for assessing the viral status of fish populations. In the future, validation of serological techniques for diagnosis of fish virus infections could render the use of fish serology more widely acceptable for diagnostic purposes. However, when present, a positive serological response is considered presumptive evidence of past exposure to infection with IHNV (Jorgensen *et al.*, 1991).

4.1. Field diagnostic methods

4.1.1. Clinical signs

The disease is typically characterised by gross signs that include lethargy interspersed with bouts of frenzied, abnormal activity, darkening of the skin, pale gills, ascites, distended abdomen, exophthalmia, and petechial haemorrhages internally and externally.

4.1.2. Behavioural changes

During outbreaks, fish are typically lethargic with bouts of frenzied, abnormal activity, such as spiral swimming and flashing. A trailing faecal cast is observed in some species. Spinal deformities are present among some of the surviving fish (Bootland & Leong, 1999).

4.2. Clinical methods

4.2.1. Gross pathology

Affected fish exhibit darkening of the skin, pale gills, ascites, distended abdomen, exophthalmia, and petechial haemorrhages internally and externally. Internally, fish appear anaemic and lack food in the gut. The liver, kidney and spleen are pale. Ascitic fluid is present and petechiae are observed in the organs of the body cavity.

4.2.2. Clinical chemistry

The blood of affected fry shows reduced haematocrit, leukopenia, degeneration of leucocytes and thrombocytes, and large amounts of cellular debris. As with other haemorrhagic viraemias of fish, blood chemistry is altered in severe cases (Bootland & Leong, 1999).

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4.2.3. Microscopic pathology

Histopathological findings reveal degenerative necrosis in haematopoietic tissues, kidney, spleen, liver, pancreas, and digestive tract. Necrosis of eosinophilic granular cells in the intestinal wall is pathognomonic of infection with IHNV ~~infection~~ (Bootland & Leong, 1999).

4.2.4. Wet mounts

Wet mounts have limited diagnostic value.

4.2.5. Tissue imprints and smears

Necrobiotic bodies and foamy macrophages, indicative of a clinical manifestation of infection with IHNV, can be best observed using tissue imprints obtained from the kidney and spleen rather than smears.

4.2.6. Electron microscopy/cytopathology

Electron microscopy of virus-infected cells reveals bullet-shaped virions of approximately 150–190 nm in length and 65–75 nm in width (Wolf, 1988). The virions are visible at the cell surface or within vacuoles or intracellular spaces after budding through cellular membranes. The virion possesses an outer envelope containing host lipids and the viral glycoprotein spikes that react with immunogold staining to decorate the virion surface.

4.3. Agent detection and identification methods

The traditional procedure for detection of IHNV is based on virus isolation in cell culture. Confirmatory identification may be achieved by use of immunological (neutralisation, indirect fluorescent antibody test or enzyme-linked immunosorbent assay), or molecular (polymerase chain reaction, DNA probe or sequencing) methods (Arakawa *et al*., 1990; Arnzen *et al*., 1991; Deering *et al*., 1991; Dixon & Hill, 1984; Jorgensen *et al*., 1991; LaPatra *et al*., 1989; Purcell *et al*., 2006; Winton & Einer-Jensen, 2002).

4.3.1. Direct detection methods

4.3.1.1. Microscopic methods

4.3.1.1.1. Wet mounts

Wet mounts are not appropriate for detection or identification of IHNV.

4.3.1.1.2. Smears

Smears are not appropriate for detection or identification of IHNV.

4.3.1.1.3. Fixed sections

Immunohistochemistry and *in-situ* hybridisation (ISH) methods have been used in research applications, but are not appropriate for detection or identification of IHNV in a diagnostic setting.

4.3.1.2. Agent isolation and identification

4.3.1.2.1. Cell culture/artificial media

Cell lines to be used: EPC or FHM.

Detection of virus through the development of viral cytopathic effect (CPE) in cell culture would be followed by virus identification through either antibody-based tests or nucleic acid-based tests. Any antibody-based tests would require the use of antibodies validated for their sensitivity and specificity.

4.3.1.2.1.1. Virus extraction

In the laboratory the tissue in the tubes must be completely homogenised (either by stomacher, blender mortar and pestle with sterile sand or any other suitable and validated homogeniser) and subsequently suspended in the original transport medium. The final ratio between tissue material and transport medium must be adjusted in the laboratory to 1:10.

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The homogenate is centrifuged in a refrigerated centrifuge at 2°C–5°C at 2000–4000 ***g*** for 15 minutes and the supernatant collected and treated for either four hours at 15°C or overnight at 4°C with antibiotics (e.g. 1 mg ml^{-1} gentamicin may be useful at this stage). If shipment of the sample has been made in a transport medium (i.e. with exposure to antibiotics) the treatment of the supernatant with antibiotics may be omitted. The antibiotic treatment aims at controlling bacterial contamination in the samples and makes filtration through membrane filters unnecessary.

Where practical difficulties arise (e.g. incubator breakdown, problems with cell cultures, etc.), which make it impossible to inoculate cells within 48 hours after the collection of the tissue samples, it is acceptable to freeze the supernatant at –80°C and carry out virological examination within 14 days. If the collected supernatant is stored at –80°C within 48 hours after the sampling it may be reused only once for virological examination.

Optional treatment of homogenate to inactivate competing virus: treatment of inocula with antiserum to IPNV (which in some parts of the world occurs in 50% of fish samples) aims at preventing CPE due to IPNV from confounding the ability to detect IHNV in cell culture. When samples come from production units, which are considered free from IPN, treatment of inocula with antiserum to IPNV should be omitted. Prior to the inoculation of the cells, the supernatant is mixed with equal parts of a suitably diluted pool of antisera to the indigenous serotypes of IPNV and incubated with this for a minimum of one hour at 15°C or a maximum of 18 hours at 4°C. The titre of the antiserum must be at least 1/2000 in a 50% plaque neutralisation test.

4.3.1.2.1.2. Inoculation of cell monolayers

EPC or FHM cells are grown at ~~20–30–~~25°C in suitable medium, e.g. Eagle's MEM (or modifications thereof) with a supplement of 10% fetal bovine serum (FBS) and antibiotics in standard concentrations. When the cells are cultivated in closed vials, it is recommended to buffer the medium with bicarbonate. The medium used for cultivation of cells in open units may be buffered with Tris/HCl (23 mM) and Na-bicarbonate (6 mM). The pH must be 7.6 ± 0.2. Cell cultures to be used for inoculation with tissue material should be young (4-48 hours old) and actively growing (not confluent) at inoculation.

Antibiotic-treated organ suspension is inoculated into cell cultures in at least two dilutions, i.e. the primary dilution and, in addition, a 1:10 dilution thereof, resulting in final dilutions of tissue material in cell culture medium of 1:100 and 1:1000, respectively, (in order to prevent homologous interference). The ratio between inoculum size and volume of cell culture medium should be about 1:10. For each dilution and each cell line, a minimum of about 2 cm^2 cell area, corresponding to one well in a 24-well cell culture tray, has to be used. Use of cell culture trays is recommended, but other units of similar or with larger growth area are acceptable as well.

4.3.1.2.1.3. Incubation of cell cultures

Inoculated cell cultures are incubated at 15°C for 7–10 days. If the colour of the cell culture medium changes from red to yellow, indicating medium acidification, pH adjustment with sterile bicarbonate solution or equivalent substances has to be performed to maintain cell susceptibility to virus infection.

At least every six months or if decreased cell susceptibility is suspected, titration of frozen stocks of IHNV is performed to verify the susceptibility of the cell cultures to infection.

4.3.1.2.1.4. Microscopy

Inoculated cell cultures must be inspected regularly (at least three times a week) for the occurrence of CPE at 40–150 × magnification. The use of a phase-contrast microscope is recommended. If obvious CPE is observed, virus identification procedures have to be initiated immediately.

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4.3.1.2.1.5. Subcultivation

If no CPE has developed after the primary incubation for 7–10 days, subcultivation is performed to fresh cell cultures utilising a cell area similar to that of the primary culture.

Aliquots of medium (supernatant) from all cultures/wells constituting the primary culture are pooled according to the cell line 7–10 days after inoculation. The pools are then inoculated into homologous cell cultures undiluted and diluted 1:10 (resulting in final dilutions of 1:10 and 1:100, respectively, of the supernatant) as described in Section 4.3.1.2.1.2 above.

Alternatively, aliquots of 10% of the medium constituting the primary culture are inoculated directly into a well with fresh cell culture (well-to-well subcultivation). In case of salmonid samples, the inoculation may be preceded by preincubation of the dilutions with the antiserum to IPNV at an appropriate dilution as described above.

The inoculated cultures are then incubated for 7–10 days at 15°C with observation as in Section 4.3.1.2.1.4. If toxic CPE occurs within the first three days of incubation, subcultivation may be performed at that stage, but the cells must then be incubated for seven days and subcultivated again with a further seven days incubation. When toxic CPE develops after three days, the cells may be passed once and incubated to achieve the total of 14 days from the primary inoculation. There should be no evidence of toxicity in the final seven days of incubation.

If bacterial contamination occurs, despite treatment with antibiotics, subcultivation must be preceded by centrifugation at 2000–4000 ***g*** for 15–30 minutes at 2–5°C, and/or filtration of the supernatant through a 0.45 µm filter (low protein-binding membrane). In addition to this, subcultivation procedures are the same as for toxic CPE.

If no CPE occurs the test may be declared negative.

4.3.1.2.2. Antibody-based antigen detection methods

4.3.1.2.2.1. Neutralisation test (identification in cell culture)

i) Collect the culture medium of the cell monolayers exhibiting CPE and centrifuge an aliquot at 2000 ***g*** for 15 minutes at 4°C, or filter through a 0.45 µm (or 450 nm) pore membrane to remove cell debris.

ii) Dilute virus-containing medium from 10^2–10^4.

iii) Mix aliquots (for example 200 µl) of each dilution with equal volumes of an IHNV antibody solution.

The neutralising antibody (Nab) solution must have a 50% plaque reduction titre of at least 2000. Likewise, treat a set of aliquots of each virus dilution with cell culture medium to provide a non-neutralised control.

iv) In parallel, a neutralisation test must be performed against a homologous IHNV strain (positive neutralisation test) to confirm the reactivity of the antiserum.

v) Incubate all the mixtures at 15°C for 1 hour.

vi) Transfer aliquots of each of the above mixtures on to 24-hour-old monolayers overlaid with cell culture medium containing 10% FBS (inoculate two wells per dilution) and incubate at 15°C; 24- or 12-well cell culture plates are suitable for this purpose, using a 50 µl inoculum.

vii) Check the cell cultures for the onset of CPE and read the results for each suspect IHNV sample as soon as it CPE occurs in non-neutralised controls. Results are recorded either after a simple microscopic examination (phase contrast preferable) or after discarding the cell culture medium and staining cell monolayers with a solution of 1% crystal violet in 20% ethanol.

viii) The tested virus is identified as IHNV when CPE is prevented or noticeably delayed in the cell cultures that received the virus suspension treated with the IHNV-specific antibody, whereas CPE is evident in all other cell cultures.

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Other neutralisation tests of proven efficiency may be used alternatively.

4.3.1.2.2.2. Indirect fluorescent antibody test (IFAT)

Antibody-based antigen detection methods such as IFAT, ELISA and various immunohistochemical procedures for the detection of IHNV have been developed over the years. These techniques can provide detection and identification relatively quickly compared with virus isolation in cell culture. However, various parameters such as antibody sensitivity and specificity and sample preparation can influence the results; a negative result should be viewed with caution. These techniques should not be used in attempts to detect carrier fish.

4.3.1.2.2.2.1. Indirect fluorescent antibody test in cell cultures

i) Prepare monolayers of cells in 2 cm^2 wells of cell culture plastic plates or on cover slips in order to reach around 80% confluency, which is usually achieved within 24 hours of incubation at 22°C (seed six cell monolayers per virus isolate to be identified, plus two for positive and two for negative controls). The FBS content of the cell culture medium can be reduced to 2–4%. If numerous virus isolates have to be identified, the use of black 96-well plates for immunofluorescence is recommended.

ii) When the cell monolayers are ready for infection (i.e. on the same day or on the day after seeding) inoculate the virus suspensions to be identified by making tenfold dilution steps directly in the cell culture wells or flasks.

iii) Dilute the control virus suspension of IHNV in a similar way, in order to obtain a virus titre of about 5,000–10,000 plaque-forming units (PFU) per ml in the cell culture medium.

iv) Incubate at 15°C for 24 hours.

v) Remove the cell culture medium, rinse once with 0.01 M phosphate buffered saline (PBS), pH 7.2, then three times briefly with a cold mixture of acetone 30%/ethanol 70% (v/v) (stored at –20°C).

vi) Let the fixative act for 15 minutes. A volume of 0.5 ml is adequate for 2 cm^2 of cell monolayer.

vii) Allow the cell monolayers to air-dry for at least 30 minutes and process immediately or freeze at –20°C.

viii) Prepare a solution of purified IHNV antibody or serum in 0.01 M PBS, pH 7.2, containing 0.05% Tween-80 (PBST), at the appropriate dilution (which has been established previously or is given by the reagent supplier).

ix) Rehydrate the dried cell monolayers by four rinsing steps with the PBST solution, and remove this buffer completely after the last rinsing.

x) Treat the cell monolayers with the antibody solution for 1 hour at 37°C in a humid chamber and do not allow evaporation to occur (e.g. by adding a piece of wet cotton to the humid chamber). The volume of solution to be used is 0.25 ml 2 cm^{-2} well.

xi) Rinse four times with PBST as above.

xii) Treat the cell monolayers for 1 hour at 37°C with a solution of FITC- or tetramethylrhodamine-5-(and-6-) isothiocyanate (TRITC)-conjugated antibody to the immunoglobulin used in the first layer and prepared according to the instructions of the supplier. These conjugated antibodies are most often rabbit or goat antibodies.

xiii) Rinse four times with PBST.

xiv) Examine the treated cell monolayers on plastic plates immediately, or mount the cover slips using, for example, glycerol saline, pH 8.5 prior to microscopic observation.

xv) Examine under incident UV light using a microscope with × 10 eye pieces and × 20–40 objective lens having numerical aperture >0.65 and >1.3, respectively. Positive and negative controls must be found to give the expected results prior to any other observation.

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4.3.1.2.2.2.2. Indirect fluorescent antibody test on imprints

i) Bleed the fish thoroughly.

ii) Make kidney imprints on cleaned glass slides or at the bottom of the wells of a plastic cell culture plate.

iii) Store the kidney pieces together with the other organs required for virus isolation in case this becomes necessary later.

iv) Allow the imprint to air-dry for 20 minutes.

v) Fix with acetone or ethanol/acetone and dry.

vi) Rehydrate the above preparations and block with 5% skim milk or 1% bovine serum albumin, in PBST for 30 minutes at 37°C. vii) Rinse four times with PBST.

viii) Treat the imprints with the solution of antibody to IHNV and rinse.

ix) Block and rinse.

x) Reveal the reaction with suitable fluorescein isothiocyanate (FITC)-conjugated specific antibody, rinse and observe.

xi) If the test is negative, process the organ samples stored at 4°C for virus isolation in cell culture, as described above.

Other IFAT or immunocytochemical (alkaline phosphatase or peroxidase) techniques of proven efficiency may be used alternatively.

4.3.1.2.2.3. Enzyme-linked immunosorbent assay (ELISA)

i) Coat the wells of microplates designed for ELISAs with appropriate dilutions of purified immunoglobulins (Ig) or serum specific for IHNV, in 0.01 M PBS, pH 7.2 (200 µl/well).

ii) Incubate overnight at 4°C.

iii) Rinse four times with 0.01 M PBS containing 0.05% Tween-20 (PBST).

iv) Block with skim milk (5% in PBST) or other blocking solution for 1 hour at 37°C (200 µl/well).

v) Rinse four times with PBST.

vi) Add 2% Triton X-100 to the virus suspension to be identified.

vii) Dispense 100 µl/well of two- or four-step dilutions of the virus to be identified and of IHNV control virus, and a heterologous virus control (e.g. viral haemorrhagic septicaemia virus). Allow the samples to react with the coated antibody to IHNV for 1 hour at 20°C.

viii) Rinse four times with PBST.

ix) Add to the wells either biotinylated polyclonal IHNV antiserum or MAb to N protein specific for a domain different from the one of the coating MAb and previously conjugated with biotin.

x) Incubate for 1 hour at 37°C.

xi) Rinse four times with PBST.

xii) Add streptavidin-conjugated horseradish peroxidase to those wells that have received the biotin-conjugated antibody, and incubate for 1 hour at 20°C.

xiii) Rinse four times with PBST. Add the substrate and chromogen. Stop the course of the test when positive controls react, and read the results.

xiv) Interpretations of the results is according to the optical absorbencies achieved by negative and positive controls and must follow the guidelines for each test, e.g. absorbency at 450 nm of positive control must be minimum 5–10 × A450 of negative control.

The above biotin-avidin-based ELISA version is given as an example. Other ELISA versions of proven efficiency may be used instead.

4.3.1.2.3. Molecular techniques

EU comment

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We suggest updating this section including a reference to real time RT-PCR methods. Currently, there are real time RT-PCR protocols fully validated and widely used; for example the methods described by Purcell *et al*. 2013 has been incorporated into the EU Commission Implementing Decision (EU) 2015/1554 of 11 September 2015 laying down rules for the application of Directive 2006/88/EC as regards requirements for surveillance and diagnostic methods. If this suggestion is accepted Table 5.1. (Methods for targeted surveillance and diagnosis) should aslo be updated.

4.3.1.2.3.1. Polymerase chain reaction

4.3.1.2.3.1.1. Viral RNA preparation

Total RNA from infected cells is extracted using a phase-separation method (e.g. phenol-chloroform or Trizol) or by use of a commercially-available RNA isolation kit used according to the manufacturer's instructions. While all of these methods work well for drained cell monolayers or cell pellets, RNA binding to affinity columns can be affected by salts present in tissue culture media and phase-separation methods should be used for extraction of RNA from cell culture fluids.

4.3.1.2.3.1.2. Reverse-transcription (RT) and standard PCR protocol

i) Prepare a master mix for the number of samples to be analysed. Work under a hood and wear gloves.

ii) The master mix for one 50 µl reverse-transcription PCR is prepared as follows: 23.75 µl ribonuclease-free (DEPC-treated) or molecular biology grade water; 5 µl 10 × buffer; 5 µl 25 mM $MgCl_2$; 5 µl 2 mM dNTP; 2.5 µl (20 pmoles µl^{-1}) Upstream Primer

   5'-AGA-GAT-CCC-TAC-ACC-AGA-GAC-3'; 2.5 µl (20 pmoles µl^{-1}) Downstream Primer

   5'-GGT-GGT-GTT-GTT-TCC-GTG-CAA-3'; 0.5 µl *Taq* polymerase (5 U µl^{-1}); 0.5 µl AMV reverse transcriptase (9 U µl^{-1}); 0.25 µl RNasin (39 U µl^{-1}).

iii) Centrifuge the tubes briefly (10 seconds) to make sure the contents are at the bottom.

iv) Place the tubes in the thermal cycler and start the following cycles – 1 cycle: 50°C for 30 minutes; 1 cycle: 95°C for 2 minutes; 30 cycles: 95°C for 30 seconds, 50°C for 30 seconds, 72°C for 60 seconds; 1 cycle: 72°C for 7 minutes and soak at 4°C.

v) Visualise the 693 bp PCR amplicon by electrophoresis of the product in 1.5% agarose gel with ethidium bromide and observe using UV transillumination.

NOTE: These PCR primers target a central region of the IHNV G gene (Emmenegger *et al*., 2000). While other primer sets can be used for amplification of portions of the N or G genes of IHNV (Winton & Einer-Jensen, 2002), the primer sequences listed above have been shown to be conserved among a broad range of IHNV isolates and are not present in the G gene of the related fish rhabdoviruses, viral haemorrhagic septicaemia virus or hirame rhabdovirus. Additionally, the new primers produce an amplicon that can be used as a template for sequence analysis of the 'mid-G' region of the IHNV genome for epidemiological purposes (Emmenegger *et al*., 2000; Kurath *et al*., 2003).

4.3.1.2.3.2. Other amplification-based assays

Other methods to detect IHNV based on amplification of target sequences of genomic or messenger RNA have been developed that use a loop-mediated isothermal amplification (LAMP) method (Gunimaladevi *et al*., 2005) or a highly sensitive quantitative reverse-transcriptase PCR assay (Overturf *et al*., 2001). However, these assays have not yet undergone sufficient laboratory validation using a panel of isolates representing the various IHNV genotypes to make them suitable for listing as a confirmatory method.

4.3.1.2.3.3. Sequencing

Sequence analysis of PCR amplicons has become much more rapid and less costly in recent years and is a good method for confirmation of IHNV (Winton & Einer-Jensen, 2002). In addition, sequence analysis provides one of the best approaches for identification of genetic strains and for epidemiological tracing of virus movement (Emmenegger *et al*., 2000; Kim *et al*., 2007; Kurath *et al*., 2003; Nishizawa *et al*., 2006).

5. Rating of tests against purpose of use

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The methods currently available for surveillance, detection, and diagnosis of infection with IHNV 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
	Gametes	Fry	Juveniles	Adults		
Gross signs	d	c	c	d	b	d
Virus isolation	a	a	a	a	a	c
Direct LM	d	c	d	d	b	c
Histopathology	d	c	d	d	b	c
Transmission EM	d	d	d	d	b	c
Antibody-based assays	d	c	c	c	a	b
PCR assays	c	c	c	c	a	a
Sequencing	d	d	d	d	c	a

LM = light microscopy; EM = electron microscopy; PCR = polymerase chain reaction.

EU comment

This Table 5.1. (Methods for targeted surveillance and diagnosis) should be updated considering our suggestion to include a reference to real time RT-PCR methods in section 4.3.1.2.3 – Molecular techniques.

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6. Test(s) recommended for targeted surveillance to declare freedom from infectious haematopoietic necrosis

The method for targeted surveillance to declare freedom from infection with IHNV is isolation of virus in cell culture. For this purpose, the most susceptible stages of the most susceptible species should be examined. Reproductive fluids and tissues collected from adult fish of a susceptible species at spawning should be included in at least one of the sampling periods each year.

7. Corroborative diagnostic criteria

7.1. Definition of suspect case

A suspect case is defined as the presence of typical, gross clinical signs of the disease in a population of susceptible fish, OR a typical internal histopathological presentation among susceptible species, OR detection of antibodies against IHNV in a susceptible species, OR typical cytopathic effect in cell culture without identification of the agent, OR a single positive result from one of the diagnostic assays ranked as ‘a’ or ‘b’ in Table 5.1.

7.2. Definition of confirmed case

A confirmed case is defined as a suspect case that has EITHER: 1) produced typical cytopathic effect in cell culture with subsequent identification of the agent by one of the antibody-based or molecular tests listed in Table 5.1., OR: 2) a second positive result from a different diagnostic assay ranked as 'a' or 'b' in the last column of Table 5.1.

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* *

NB: There are OIE Reference Laboratories for infection with infectious haematopoietic necrosis 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/scientific-expertise/reference-laboratories/list-of-laboratories/).
Please contact the OIE Reference Laboratories for any further information on infection with infectious haematopoietic necrosis virus.

NB: FIRST ADOPTED IN 1995 AS INFECTIOUS HAEMATOPOIETIC NECROSIS; MOST RECENT UPDATES ADOPTED IN 2012.

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CHAPTER 2.3.6.

INFECTION WITH SALMONID ALPHAVIRUS

EU position

The EU thanks the OIE and in general supports the adoption of this modified chapter. We have suggested a few comments and a change of style for a table below.

1. Scope

~~For the purpose of this chapter, i~~Infection with salmonid alphavirus (SAV) means infection with any ~~subtype~~ genotype of the pathogenic agent SAV, ~~of the~~ Genus *Alphavirus*, and Family *Togaviridae.*

~~Infection with SAV may cause pancreas disease (PD) or sleeping disease (SD) in Atlantic salmon (*Salmo salar* L.), rainbow trout (*Oncorhynchus mykiss*) and brown trout (*Salmo trutta* L.) (Boucher *et al.*, 1995; McLoughlin & Graham, 2007). The virus is horizontally transmitted, and the main reservoirs of SAV are clinically diseased or covertly infected fish (Viljugrein *et al.*, 2009). The disease is a systemic disease characterised microscopically by necrosis and loss of exocrine pancreatic tissue, and heart and skeletal muscle changes. The mortality varies significantly, from negligible to over 50% in severe cases, and up to 15% of surviving fish will develop into long, slender fish ('runts') (McLoughlin & Graham, 2007).~~

2. Disease information

2.1. Agent factors

2.1.1. Aetiological agent, agent strains

SAV is an enveloped, spherical, single-stranded, positive-sense RNA virus, approximately 60-70 nm in diameter, with a genome of ~12 kb. The genome codes for eight proteins: four capsid glycoproteins (E1, E2, E3 and 6K) and four nonstructural proteins (nsP1–4). Glycoprotein E2 is considered to be the site of most neutralising epitopes, while E1 contains more conserved, cross-reactive epitopes (McLoughlin & Graham, 2007). SAV is considered to belong to the Genus *Alphavirus* of the Family *Togaviridae*. This is based on nucleotide sequence studies of SAV isolates, and is also supported by biological properties of the virus, including cross-infection and neutralisation trials. In addition, four conserved nucleotide sequence elements (CSEs) and a conserved motif (GDD), characteristic of alphaviruses, are present in the SAV genome (McLoughlin & Graham, 2007).

SAV has been divided into six genotypes (SAV1–SAV6) based solely on nucleic acid sequences for the proteins E2 and nsP3 (Fringuelli *et al.,* 2008). The level of antigenic variation among genotypes is considered low as monoclonal antibodies (MAbs) raised against a specific SAV genotype are likely to cross react with other SAV isolates (Graham *et al.,* 2014; Jewhurst *et al.,* 2004).

Infection with SAV may cause pancreas disease (PD) or sleeping disease (SD) in Atlantic salmon (*Salmo salar* L.), common dab (*Limanda limanda*), ~~and~~ rainbow trout (*Oncorhynchus mykiss*) McLoughlin & Graham, 2007) and Arctic charr (*Salvelinus alpinus*) (Lewisch *et al.*, 2018). The disease is a systemic disease characterised microscopically by necrosis and loss of exocrine pancreatic tissue, and heart and skeletal muscle changes.

The genotype groups by susceptible species and environment ~~and their geographical distributions~~ are presented in the table below ~~(abbreviations: SW = sea water, FW = fresh water, PD = pancreas disease, SD = sleeping disease)~~:

Table 2.1. SAV genotypes by susceptible species and environment ~~host, environment and geographic distribution~~

SAV ~~subtype~~ genotype	~~Host and environment~~ Freshwater	Sea ~~W~~water	~~Country~~
SAV 1 ~~(PD)~~	~~Atlantic salmon (SW)~~ Rainbow trout ~~(FW)~~	Atlantic salmon ~~(SW)~~	~~Ireland, UK (Northern Ireland, Scotland)~~

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SAV subtype genotype	Host and environment Freshwater	Sea Wwater	Country
SAV 2 FW (SD)	Rainbow trout (FW) Atlantic salmon (SW) Atlantic salmon (FW) Arctic charr (FW)	Atlantic salmon (SW)	France, Germany, Italy, Spain, Switzerland, Poland, UK (England, Scotland) Scotland Austria
SAV 2 Marine (PD)	Atlantic salmon (SW)	Atlantic salmon (SW)	Norway, UK (Scotland)
SAV 3 (PD)	Rainbow trout (SW) Atlantic salmon (SW)	Rainbow trout (SW) Atlantic salmon (SW)	Norway
SAV 4 (PD)	Atlantic salmon (SW)	Atlantic salmon (SW)	Ireland, UK (Northern Ireland, Scotland)
SAV 5 (PD)	Atlantic salmon (SW) Common dab (SW)	Atlantic salmon (SW) Common dab (SW)	UK (Scotland) UK (Scotland), Ireland
SAV 6 (PD)	Atlantic salmon (SW)	Atlantic salmon (SW)	Ireland

2.1.2. Survival outside the host

Laboratory tests suggest that SAV would survive for extended periods in the aquatic environment. In these tests, virus survival was inversely related to temperature. In the presence of organic matter, marked longer survival times were observed in sea water compared with fresh water (Graham *et al.,* 2007c). SAV has been detected in fat leaking from dead fish, indicating that this may be a route for transmission. Fat droplets may accumulate at the sea water surface, contributing to long distance spread of the virus (Stene *et al.,* submitted2013).

The half-life of SAV in serum has been found to be inversely related to temperature, emphasising the need for rapid shipment of samples at 4°C to laboratories for virus isolation. For long-term conservation of SAV-positive samples and cultured virus, storage at –80°C is recommended (Graham *et al.,* 2007c).

2.1.3. Stability of the agent

SAV is rapidly inactivated in the presence of high levels of organic matter at 60°C, at pH 7.2, and at pH 4 and pH 12 at 4°C, suggesting that composting, ensiling and alkaline hydrolysis would all be effective at inactivating virus in fish waste (Graham *et al.,* 2007a).

2.1.4. Life cycle

Probable infection routes are through the gills or via the intestine. In the acute stages of the disease, large amounts of SAV can be detected and live virus can be isolated from the heart, kidney, blood and several other organs, but the actual target cells for the virus has not yet been identified.

Viraemia precedes both the onset of histological changes and clinical signs (McLoughlin & Graham, 2007). The route of shedding may be through natural excretions/secretions, supported by the detection of SAV by reverse-transcriptase polymerase chain reaction (RT-PCR) in the faeces and mucous of experimentally infected Atlantic salmon. These matrices may therefore play a role in the horizontal transmission of SAV through water (Graham *et al.,* 2012). Virus has been detected in water 4–13 days after post-infection, indicating that virus shedding coincides with the viraemic stage (Andersen *et al.,* 2010). An incubation period of 7–10 days at sea water temperatures of 12–15°C has been estimated based on analysis of antibody production in intraperitoneally infected fish and cohabitants in an experimental trial (McLoughlin & Graham, 2007). Several studies have shown that SAV RNA can be detected in fish for an extended period post-infection (Jansen *et al.,* 2010a; McLoughlin & Graham, 2007). Subclinical infection has been reported, suggesting that the severity of an outbreak may be influenced by several environmental factors (McLoughlin & Graham, 2007), and recent data show that seasonal increases in water temperature may trigger disease outbreaks in SAV-infected farms (Stene *et al.,* 2014).

2.2. Host factors

2.2.1. Susceptible host species

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~~Disease outbreaks and infection experiments have shown that Atlantic salmon, rainbow trout and brown trout are susceptible (Boucher *et al.,* 1995; McLoughlin & Graham, 2007).~~

Species that fulfil the criteria for listing ~~a species~~ as susceptible to infection with SAV according to Chapter 1.5. of the Aquatic Animal Health Code (Aquatic Code) include: Arctic charr (*Salvelinus* alpinus), Atlantic salmon (*Salmo salar*), common dab (*Limanda limanda*) 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: long rough dab (*Hippoglossoides platessoides*) and plaice (*Pleuronectes platessa*).

In addition, pathogen-specific positive PCR results have been reported in the following ~~organisms~~ species, but an active infection has not been demonstrated: Argentine hake (*Merluccius hubbsi*), ~~b~~Ballan wrasse (*Labrus bergylta*), brown trout (*Salmo trutta*), cod (*Gadus morhua*), European flounder (*Platichthys flesus*), haddock (*Melanogrammus aeglefinus*), herring (*Glupea harengus*), Norway pout (*Trisopterus esmarkii)*, saithe (*Pollachius virens*), ~~salmon louse (*Lepeophtheirus salmonis*),~~ sculpin sp. (*Myoxocephalus octodecemspinosus*) and whiting (*Merlangius merlangus*).

EU comment

We suggest that Ballan wrasse (*Labrus bergylta*) should be included in the first paragraph of section 2.2.2 as a species for which there is incomplete evidence for susceptibility. Currently, Ballan wrasse are included in paragraph 2 indicating that pathogen-specific positive PCR results only have been recorded for this species. This is incorrect, the paper by Ruane *et. al.* 2018 reports that the SAV was isolated on cell culture and then confirmed by PCR. The current text conveys the impression that SAV has only been detected in ballan wrasse by PCR.

Ruane, N. M., Swords, D., Morrissey, T., Geary, M., Hickey, G., Collins, E. M., Geoghegan, F., Swords, F. (2018). Isolation of salmonid alphavirus subtype 6 from wild - caught ballan wrasse, Labrus bergylta (Ascanius). Journal Fish Diseases 41 (11), 1643-1651).

2.2.~~2~~3. Susceptible stages of the host

All life stages should be considered as susceptible to infection with SAV.

Farmed rainbow trout in fresh water are affected at all stages of production (Kerbart Boscher *et al.,* 2006). Experience from Norway shows that farmed rainbow trout and Atlantic salmon are susceptible at all stages in sea water, probably reflecting a sea water reservoir of SAV. Experimental infection by injection indicates susceptibility of Atlantic salmon parr in fresh water (McVicar, 1990).

2.2.~~3~~4. Species or subpopulation predilection (probability of detection)

There is no known species or subpopulation predilection.

2.2.~~4~~5. Target organs and infected tissue

Infection with SAV is a systemic disease with an early viraemic phase. After infection, SAV has been detected in all organs that have been examined: brain, gill, pseudobranch, heart, pancreas, kidney and skeletal muscle (Andersen *et al.,* 2007; McLoughlin & Graham, 2007) as well as in mucous and faeces (Graham *et al.,* 2012).

2.2.~~5~~6. Persistent infection with lifelong carriers

SAV has been detected in surviving fish 6 months after experimental infection (Andersen *et al.,* 2007). At the farm level, an infected population will harbour SAV until slaughter (Jansen *et al.,* 2010a; 2010b). On an individual level, however, lifelong persistent infection has not been documented.

2.2.~~6~~7. Vectors

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SAV has been detected by RT-PCR in salmon lice (*Lepeophtheirus salmonis*) collected during acute disease outbreaks in Atlantic salmon, but transfer to susceptible fish species has not been studied (Petterson *et al.,* 2009). Vectors are not needed for transmission of SAV.

2.2.~~7~~8. Known or ~~S~~suspected wild aquatic animal carriers

In surveys of wild marine fish, SAV RNA has been detected in the flatfish species common dab (*Limanda limanda*), long rough dab (*Hippoglossoides platessoides*) and plaice (*Pleuronectes platessa*) (McCleary *et al.,* 2014; Snow *et al.,* 2010). The importance of wild marine or fresh water species as ~~virus~~ carriers needs to be determined ~~clarified~~.

EU comment

On the basis of *Ruane et al.* 2018 mentioned above, we suggest that Ballan wrasse (*Labrus bergylta*) should also be included in section 2.2.8.

2.3. Disease pattern

2.3.1. Transmission mechanisms

Transmission of SAV occurs horizontally. This is supported by phylogenetic studies, successful transmission among fish in cohabitant studies, proven transmission between farming sites, studies on survival of SAV in sea water and the spread via water currents (Graham *et al.,* 2007c; 2011; Jansen *et al.,* 2010a; Kristoffersen *et al.,* 2009; Viljugrein *et al.,* 2009).

Long-distance transmission and thus introduction of SAV in a previously uninfected area is most likely assigned to movement of infected live fish (Kristoffersen *et al.,* 2009; Rodger & Mitchell, 2007). Once SAV has been introduced into an area, ~~shared ownership and close site~~ farm proximity and water currents are factors involved in local transmission (Aldrin *et al.,* 2010; Kristoffersen *et al.,* 2009; Viljugrein *et al.,* 2009). Risk factors for outbreaks on a farming site include a previous history of infection with SAV, high feeding rate, high sea lice burden, the use of autumn smolts and previous outbreaks of infectious pancreatic necrosis (IPN) (Bang Jensen *et al.,* 2012; Kristoffersen *et al.,* 2009; Rodger & Mitchell, 2007).

Vertical transmission of SAV has been suggested (Bratland & Nylund, 2009), but the evidence is not convincing (Kongtorp *et al.,* 2010; McLoughlin & Graham, 2007). The Norwegian Scientific Committee for Food Safety, 2010 ~~has recently~~ carried out a risk assessment ~~on brood fish surveillance~~ and ~~vertical transmission of infection,~~ concluded that the risk of vertical transmission of SAV is negligible.

2.3.2. Prevalence

The prevalence of ~~infected fish within an~~ infection with SAV~~-infected fish farm~~ may vary. During disease outbreaks, the prevalence is usually high; prevalences of 70–100% have been reported in Atlantic salmon farming sites (Graham *et al.,* 2010). If moribund or thin fish or runts are sampled, the probability of detecting SAV~~-infected fish~~ is higher than if randomly selected, apparently healthy fish are sampled (Jansen *et al.,* 2010b). Prevalence estimates will also vary with the diagnostic method used.

Prevalence in wild fish is largely unknown. SAV RNA has been detected in some flatfish species in sea water in Scotland (Snow *et al.,* 2010). A serological survey of wild salmonids in fresh water river systems in Northern Ireland did not detect virus neutralisation antibodies against SAV in any of 188 sera tested, whereas the majority of sera from farmed salmon in sea water in the same area tested positive (Graham *et al.,* 2003).

EU comment

This comment refers to the second paragraph of section 2.3.2. on prevalence in wild fish. We suggest that this paragragh should be updated to reflect the detection of SAV RNA in flatfish species in sea water in Ireland as well Scotland.

The reference is: *McCleary S.J., Giltrap M., Henshilwood K. & Ruane N.M. (2014). Detection of salmonid alphavirus RNA in Celtic and Irish Sea flatfish. Dis. Aquat. Org. 109: 1–7, 2014 doi: 10.3354/dao02719*

2.3.3. Geographical distribution

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Infection with SAV is known to be present in farmed salmonid fish in Croatia, France, Germany, Ireland, Italy, Norway, Poland, Spain, Switzerland and the United Kingdom (England, Scotland and Northern Ireland).

2.3.4. Mortality and morbidity

Mortality rates due to infection with SAV may vary with genotype ~~subtype~~, season, year, use of biosecurity measures and species of fish (Bang Jensen *et al.,* 2012; Graham *et al.,* 2011; Rodger & Mitchell, 2007; Stormoen *et al.,* 2013). The cumulative mortality at the farm level ranges from negligible to over 50% in severe cases (Bang Jensen *et al.,* 2012; Graham *et al.,* 2003; Rodger & Mitchell, 2007; Ruane *et al.,* 2008; Stene *et al.,* 2014).

Duration of disease outbreaks, defined as the period with increased mortality, varies from 1 to 32 weeks (Jansen *et al.* 2010a; 2014; Ruane *et al.,* 2008).

2.3.5. Environmental factors

Clinical outbreaks and mortality are influenced by water temperature and season (McLoughlin & Graham, 2007; Rodger & Mitchell, 2007; Stene *et al.,* 2014; Stormoen *et al.,* 2013). Stressing the fish by movement, crowding or treatment may initiate disease outbreaks on infected farms.

2.4. Control and prevention

2.4.1. Vaccination

DNA-based and virus-inactivated vaccines against SAV are both commercially available. ~~At present, one vaccine is commercially available. This vaccine was introduced in 2007 and is widely used in Atlantic salmon farms in endemic areas in Norway, Ireland and Scotland. This vaccine is based on inactivated SAV~~ genotype ~~subtype 1, and claims a reduction in mortality of at least 50% in comparisons of vaccinated fish against unvaccinated fish at the same farm. The vaccine does not seem to offer complete protection, but a field evaluation carried out in Norway demonstrated that the mortality in farms with vaccinated fish is comparable with mortality in farms without infection with SAV. Furthermore, a small reduction in the number of outbreaks was seen (Bang Jensen *et al.,* 2012).~~

~~A vaccine based on inactivated SAV of another~~ genotype ~~subtype is under development. Furthermore, a DNA-based vaccine is showing promising results. To date, only Canada has allowed the use of DNA-based vaccines for control of fish diseases; it is not certain whether this vaccine will be licensed for use in other markets.~~

2.4.2. Chemotherapy

No chemotherapy is available.

2.4.3. Immunostimulation

No immunostimulation is available.

2.4.4. Resistance breeding

Differences in susceptibility among different family groups of Atlantic salmon have been observed in challenge experiments and in the field, indicating the potential for resistance breeding. Both in Ireland and Norway, efforts are being made to breed fish that are more resistant to infection with SAV (McLoughlin & Graham, 2007). ~~Selection of brood fish by using gene markers for resistance is in an early phase.~~

2.4.5. Restocking with resistant species

Not relevant.

2.4.6. Blocking agents

Not relevant.

2.4.7. Disinfection of eggs and larvae

Disinfection procedures were evaluated in fertilised ova from SAV genotype 3 positive broodstock (Kongtorp *et al.,* 2010). Nevertheless, further investigation is needed. ~~(See Graham *et al.,* 2007b; Kongtorp *et al.,* 2010.)~~

2.4.8. General husbandry practices

To avoid infection with SAV, general good hygiene practices should be applied: use of appropriate sites for farming, segregation of generations, stocking with good quality fish, removal of dead fish, regular cleaning of tanks and pens, controlling parasites and other pathogens as well as careful

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handling of fish. Once a site has been infected, mortality may be reduced by imposing a general stop on handling of the fish as well as a general stop on feeding the fish.

3. Sampling

3.1. Selection of individual specimens

All production units (ponds, tanks, net-cages, etc.) should be inspected for the presence of dead, weak or abnormally behaving fish. Extremely weak ('sleeping') fish may be found at the bottom of a tank or in the net-cages. If the number of clinically diseased fish is low, samples from long, thin fish ('runts') may be added (Jansen *et al.,* 2010b).

3.2. Preservation of samples for submission

Table 3.1. *Preservative used for each method*

Method	**Preservative**
Histology and immunohistochemistry	Fixation in neutral phosphate-buffered 10% formalin
Molecular biology (RT-PCR and sequencing)	Appropriate medium for preservation of RNA
Cell culture	Virus transport medium
Serology	Blood plasma or serum

3.3. Pooling of samples

~~For diagnostic purposes, pooling of samples from different individuals is not considered necessary or recommended as detection of SAV and characteristic histopathological changes in the same individual will strengthen the connection between the virus and the observed disease. For surveillance purposes, pooling of samples for virological examination (PCR or cell culture) may be accepted, but may decrease the sensitivity of the tests.~~

Pooling of samples may be acceptable, however, the impact on sensitivity and design prevalence must be considered.

3.4. Best organs or tissues

Heart and mid-kidney are the recommended organs for detection of SAV either by molecular biological methods or by cell culture. During the course of the disease, the heart usually contains more SAV than other tissues and should always be sampled. After disease outbreaks, gills and heart (Graham *et al.,* 2010) and pools of heart and mid-kidney (Jansen *et al.,* 2010a; 2010b) remained RT-PCR positive for months after initial detection.

During the initial viraemic phase, serum samples are also suitable for detection of SAV either by molecular biological methods or by cell culture. Serum sampling may therefore be used for early warning screening tests (Graham *et al*., 2010). From approximately 3 weeks after SAV infection, blood serum or plasma is suitable for a virus neutralisation test that identifies neutralising antibodies against SAV in fish exposed to SAV (Graham *et al.,* 2003).

Tissues for histological examinations should include gill, heart, pyloric caeca with attached pancreatic tissue, liver, kidney, spleen and skeletal muscle containing both red (aerobe) and white (anaerobe) muscle. Skin with associated skeletal muscle sample should be taken at the lateral line level and deep enough to include both red and white muscle.

4. Diagnostic methods

4.1. Field diagnostic methods

4.1.1. Clinical signs

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A sudden drop in appetite may be observed 1–2 weeks before the detection of ~~enhanced~~ elevated mortality. Clinically diseased fish may be observed swimming slowly at the water surface. In some cases, extremely weak (“sleeping”) fish can be found at the bottom of tanks or in net-cages. An increased number of faecal casts may also be observed ~~in the water~~. However, it is important to ~~notice~~ note that clinical signs are not pathognomonic. ~~and that careful observation and examinations~~ Careful investigation of any dead, ~~weak~~ moribund or abnormally behaving fish is necessary to determine involvement of SAV and rule out other *pathogenic agents*.

Initially, nutritional status is usually normal, but in the months after an outbreak or in the later stages of disease, long slender fish (‘runts’) with ~~low~~ poor body condition are typically observed. The ~~development~~ presentation of long, slender fish can be caused by factors other than SAV.

4.2. Clinical methods

4.2.1. Gross pathology

Yellow mucoid gut contents are a usual post-mortem finding, as is typically seen in fish that are not eating. Occasionally signs of circulatory disturbances, such as petechial haemorrhages, small ascites or reddening of the pancreatic region between the pyloric caeca, may be seen. Some diseased fish may show pale hearts or heart ruptures. It is important to note that post-mortem findings are not pathognomonic.

4.2.2. Clinical chemistry

Not documented for diagnostic use.

4.2.3. Microscopic pathology

The changes most commonly found in clinically diseased fish are severe loss of exocrine pancreatic tissue, cardiomyocytic necrosis and inflammation, red (aerobe) skeletal muscle inflammation and white (anaerobe) skeletal muscle degeneration or inflammation. A less frequent but supporting finding is the detection of cells with many cytoplasmic eosinophilic granules along kidney sinusoids.

As the disease progresses, the development of these changes is not simultaneous in all organs: In a very short, early phase, the only lesion present can be necrosis of exocrine pancreatic tissue and a variable inflammatory reaction in the peripancreatic fat. Shortly thereafter, heart muscle cell degeneration and necrosis develop~~s~~ before the inflammation response in the heart becomes more pronounced. The pancreatic necrotic debris will seemingly disappear and the typical picture of severe loss of exocrine pancreatic tissue will soon appear simultaneously with the increasing inflammation in the heart. Somewhat later, skeletal muscle degeneration, inflammation and fibrosis develop. In a proportion of fish, severe fibrosis of the peri-acinar tissue may occur, and in this case the pancreas does not recover (runts) (Christie *et al.,* 2007; Kerbart Boscher *et al.,* 2006; McLoughlin & Graham, 2007; Taksdal *et al.,* 2007).

4.2.4. Wet mounts

Not relevant.

4.2.5. Smears

Not relevant.

4.2.6. Fixed sections, immunohistochemistry

~~The single~~ Immunohistochemical ~~method published~~ testing (Taksdal *et al.,* 2007) is only recommended for samples from fish with acute necrosis of exocrine pancreatic tissue.

4.2.6.1. 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 3 µm thick sections (for immunohistochemistry sampled on poly-L-lysine-coated slides) are heated at 56–58°C (maximum 60°C) for 20 minutes, dewaxed in xylene, rehydrated

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through graded ethanol, and stained with haematoxylin and eosin for histopathology and immunohistochemistry as described below.

4.2.6.2. Staining procedure for immunohistochemistry

All incubations are carried out at room temperature and all washing steps are done with Tris-buffered saline (TBS).

i) Nonspecific antibody binding sites are first blocked in 5% bovine serum albumin (BSA) in TBS for 20 minutes. The solution is then poured off without washing.

ii) Sections are incubated with primary antibody (monoclonal mouse antibody 4H1 against E1 SAV glycoprotein [Todd *et al.,* 2001]), diluted 1/3000 in 2.5% BSA in TBS and then incubated overnight, followed by two wash out baths lasting a minimum of 5 minutes.

iii) Sections are incubated with secondary antibody (biotinylated rabbit anti-mouse Ig) diluted 1/300 for 30 minutes, followed by wash out baths as in step ii above.

iv) Sections are incubated with streptavidin with alkaline phosphatase 1/500 for 30 minutes followed by wash out baths as in step ii above.

v) For detection of bound antibodies, sections are incubated with Fast Red[1] (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) and allowed to develop for 20 minutes followed by one wash in tap water before counterstaining with Mayer's haematoxylin and mounting in aqueous mounting medium.

SAV-positive and SAV-negative tissue sections are included as controls in every setup (Taksdal *et al.,* 2007).

4.2.7. Electron microscopy/cytopathology

Not relevant for diagnostic use.

4.2.8. Differential diagnoses

4.2.8.1. Differential diagnoses relevant for microscopic pathology (Section 4.2.3)

Tissues that are changed by infection with SAV are also changed by heart and skeletal muscle inflammation (HSMI), cardiomyopathy syndrome (CMS) and IPN. However, if all the main organs are examined by histopathology, the pattern of affected organs will usually appear different.

Table 4.1. Tissue changes associated with infection with SAV, HSMI, CMS and IPN

	Infection with SAV	HSMI	CMS	IPN
Heart*	+	+	+	–
Pancreas	+	–	–	+
Skeletal muscle	+	+	–	–

*Heart changes in CMS affects mainly the inner spongy layer of the ventricle and the atrium, whereas in Infection with SAV and HSMI, the compact layer of the ventricle is more severely affected. Although these three diseases induce epicarditis, HSMI causes the most severely inflamed epicardium.

In a very short, early acute stage of infection, when only necrosis of exocrine pancreas has developed, infection with SAV might be mistaken for IPN caused by infection with IPN virus (IPNV). In such cases, virological examination will clarify the causal agent.

[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.*

Virological and serological examinations combined with histopathological examination of 5–10 clinically diseased fish will usually clarify the situation. HSMI and CMS have only been detected in Atlantic salmon.

4.3. Agent detection and identification methods

4.3.1. Direct detection methods

4.3.1.1. Agent isolation and identification

4.3.1.1.1. Cell culture

Isolation of field isolates of SAV in cell culture may be challenging (Christie, 1998; Graham, 2007c; Petterson *et al.,* 2013). CHSE-214 are commonly used for primary SAV isolation, but susceptible cell lines such as BF-2, FHM, SHK-1, EPC, CHH-1 or others, may be used. Variation in cell line susceptibility among different SAV field isolates has been reported (Graham *et al.,* 2008; Herath *et al.,* 2009), and it is therefore recommended that several cell lines are tested for initial cell culture isolation of SAV in a new laboratory or for a new virus strain.

The CHSE-214 cells are grown at 20°C in Eagle's minimal essential medium (EMEM) with non-essential amino acids and 0.01 M HEPES (N-2-hydroxyethyl-piperazine-N-2-ethanesulfonic acid) buffer, or Leibovitz's L-15 cell culture medium, both supplemented with fetal bovine serum (FBS) (5% or 10%) and L-glutamine (4 mM).

For virus isolation, cells are grown in tissue culture flasks or multi-well cell culture plates. SAV-positive controls may be inoculated in parallel with the tissue samples as a test for cell susceptibility to SAV. When positive controls are included, measures must be taken to avoid contamination.

i) Inoculation of cell monolayers

   Prepare a 2% suspension of tissue homogenate or a 10% suspension of serum using L-15 medium or EMEM without serum or other medium with documented suitability. Remove growth medium from actively growing monolayers (1- to 2-day-old cultures or cultures of 70–80% confluency) grown in tissue culture flasks or multi-well cell culture plates (see above). Inoculate monolayers with a low volume of the 2% tissue homogenate or 10% serum dilution (for 25 cm^2 flasks: 1.5 ml). Adjust volume to the respective surface area in use. Allow 2–3 hours of incubation at 15°C followed by removal of the inoculum, and addition of fresh L-15 or EMEM medium supplemented with 2–5% fetal bovine serum (for 25 cm^2 flasks: 5 ml).

   When fish samples come from production sites where 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 cytopathic effect (CPE). Typical CPE due to SAV appears as plaques of pyknotic, vacuolated cells. However, Norwegian SAV field isolates (both SAV3 and marine SAV2) usually do not produce CPE in low passages, and this is also reported for other SAV ~~subtypes~~ genotypes (Graham *et al*., 2008; Petterson *et al*., 2013). If no CPE has developed after 14 days, subculture to fresh cell cultures.

iii) Subcultivation procedure

   14 days (or earlier when obvious CPE appears) after inoculation, the cultures are freeze–thawed at –80°C (the procedure can be repeated 1–2 times) to release virus from the infected cells.

   Following centrifugation at 3000 ***g*** for 5 minutes, the 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 2–3 hours before addition of fresh 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 should always be examined for the presence of SAV by immunofluorescence (indirect fluorescent antibody test [IFAT]), as virus replication may occur without development of apparent CPE.

iv) Antibody-based verification of SAV growth in cell culture

   All incubations below are carried out at room temperature unless otherwise stated.

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a) Prepare monolayers of cells in appropriate tissue culture plates (e.g. 96-well plates), or on cover-slips, depending on the type of microscope available (an inverted microscope equipped with UV light is necessary for monolayers grown on tissue culture plates). The necessary monolayers for negative and positive controls must be included.

b) 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 9–11 days.

c) 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. If necessary, the fixed cell cultures may be stored dry for 14 days at 4°C until staining.

d) Incubate the cell monolayers with anti-SAV MAb in an appropriate dilution in phosphate-buffered saline (PBS) for 1 hour and rinse three times with PBS with 0.05% Tween 20.

e) Incubate with fluorescein isothiocyanate (FITC)-conjugated anti-mouse immunoglobulin for 1 hour (or if the primary Ab is polyclonal from rabbits, use FITC-conjugated antibody against rabbit immunoglobulin), according to the instructions of the supplier. To increase the sensitivity of the test, FITC-conjugated anti-mouse Ig may be replaced with biotin-labelled anti-mouse Ig and FITC-labelled streptavidin with rinsing as in step d in between the steps. 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 the 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.

4.3.1.1.2. Reverse-transcription polymerase chain reaction (RT-PCR), real-time RT-PCR, and genotyping by sequencing

The primers described below for real-time RT-PCR and RT-PCR with sequencing will detect all known ~~subtypes~~ genotypes of SAV.

RT-PCR may be used for detection of SAV from total RNA (or total nucleic acids) extracted from recommended organs or tissues (see Section 3.4). Real-time RT-PCR for the detection of SAV is recommended as it increases the specificity and also the sensitivity of the test.

For genotyping, RT-PCR with subsequent sequencing of fragments from the E2 ~~and nsP3~~ genes is recommended.

The primers and probe sequences for real-time RT-PCR from the nsP1 gene, as well as primers for genotyping, are listed in table 3.1. ~~below~~. The E2-primers may also be used for conventional RT-PCR detection of SAV, if necessary. A variety of kits designed for RNA extraction/RT-PCR and qPCR machines can be used. The PCR programme depends on the kit and real-time PCR equipment used in the laboratory. The conditions for performing the real-time RT-PCR in the OIE Reference Laboratory is as follows: 50°C for 10 minutes, 95°C for 3 minutes, and 40 cycles of (95°C for 10 seconds, 60°C for 20 seconds). For the conventional RT-PCRs (sequencing), the following programme is used: 50°C for 30 minutes, 95°C for 15 minutes, and 45 cycles of (94°C for 60 seconds, 55°C for 45 seconds, 72°C for 60 seconds).

Table 3.1. ~~Characteristic of p~~Primers and probe sequences for RT-PCR and real time RT-PCR

~~RT-PCR:~~ Primer and probe sequences	~~Named~~	Genomic segment	Product size	Reference
QnsP1F: 5'-CCG-GCC-CTG-AAC-CAG-TT-3' QnsP1R: 5'-GTA-GCC-AAG-TGG-GAG-AAA-GCT-3' QnsP1probe: 5'FAM-CTG-GCC-ACC-ACT-TCG-A-MGB3' (Taqman®probe)	~~forward primer~~ ~~reverse primer~~ ~~Taqman®probe~~	QnsP1	107 nt	Hodneland *et al.*, 2006
E2F: 5'-CCG-TTG-CGG-CCA-CAC-TGG-ATG-3' E2R: 5'-CCT-CAT-AGG-TGA-TCG-ACG-GCA-G-3'	~~forward primer~~ ~~reverse primer~~	E2	516 nt	Fringuelli *et al.*, 2008
~~nsP3F: 5'-CGC-AGT-CCA-GCG-TCA-CCT-CAT-C-3'~~ ~~nsP3R: 5'-TCA-CGT-TGC-CCT-CTG-CGC-CG-3'~~	~~forward primer~~ ~~reverse primer~~	~~nsP3~~	~~490 nt~~	~~Fringuelli *et al.*, 2008~~

4.3.2. Serological methods

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4.3.2.1 Immunoperoxidase-based serum neutralisation assay (Graham *et al.,* 2003)

Experimental studies have shown that neutralising antibodies can first be detected 10–16 days post-infection (Graham *et al.,* 2003), and serum neutralisation (SN) assays can be used as a diagnostic tool for the detection of SAV antibodies. SN assays are based on the presence or absence of detectable virus growth in cultured cells following incubation with serum that may contain neutralising antibodies. In addition, the assay allows detection of virus in serum or plasma, if present.

CHSE-214 cells are grown as described in Section 4.3.1.1.1 Cell culture. A suspension of trypsinised cells, diluted 1/3 in growth medium (10% FBS) is prepared for the SN assay.

i) 1/20 and 1/40 dilutions of each test serum are prepared in maintenance medium (2% FBS), and transferred to two duplicate wells (15 µl per well) on a flat-bottomed tissue culture grade microtitre plate. An equal volume of virus (100 $TCID_{50}$ [median tissue culture infective dose]) is added and the plate is incubated for 2 hours at room temperature.

ii) 70 µl of maintenance medium, and 50 µl of the CHSE-214 cell suspension is added to each well, and the plates are incubated for 3 days at 15°C.

iii) The cell monolayer is then fixed and stained as described in Section 4.3.1.1.1, step iv *Antibody-based verification of SAV growth in cell culture*, or using the following procedure: monolayers of CHSE-214 cells are fixed for 30 minutes at room temperature in 10% neutral buffered formalin. Following two washes with 0.01 M PBS, a MAb against SAV is added to the monolayers in an appropriate dilution. Bound MAb is visualised using a labelled streptavidin–biotin system according to the manufacturer's instructions.

iv) SN titres (ND_{50}) are then calculated according to the method of Karber (1931), with titres ≥ 1:20 being considered positive. Both serum controls (without virus added) and a virus control (without serum added) must always be included in the assay, to ensure valid results.

5. Rating of tests against purpose of use

As an example, the methods currently available for targeted surveillance and diagnosis of infection with SAV 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
	Fry	Juveniles	Adults		
Gross signs	d	d	d	c	d
Histopathology	c	c	c	~~a~~ b	~~a~~ d
Immunohistochemistry	d	d	d	b	b
Isolation in cell culture	d	d	d	c	c
Serum neutralisation assay	d	c	b	a	b
Real-time RT-PCR	b	b	b	b	b
RT-PCR with sequencing	d	b	b	b	a

RT-PCR = Reverse-transcriptase polymerase chain reaction.

EU comment

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This comment is about the style of the above table. It does not seem to be in line with the style used for other tables in the Manual, for example for Table 4.1 in Annex 21(a). For consistency, you may wish to redraft table 5.1.

6. Test(s) recommended for targeted surveillance to declare freedom from infection with SAV

The recommended test to be used in surveillance of susceptible fish populations for declaration of freedom from SAV is RT-PCR as described in Section 4.3.1.1.2 in this chapter.

7. Corroborative diagnostic criteria

7.1. Definition of suspect case

A suspected case of infection with SAV is defined as:

i) Clinical signs consistent with infection with SAV (Section 4.1.1)

or

ii) Gross and microscopic~~ally~~ pathology consistent with the disease (Sections 4.2.1 and 4.2.3)

or

iii) Detection of antibodies against SAV (Section 4.3.2.1) or detection of SAV (Section 4.3.1.1.)

or

iv) If epidemiological information of infectious contact with suspected or confirmed case(s) appears.

7.2. Definition of confirmed case

Evidence for the presence of SAV from two independent laboratory tests as microscopic pathology (Section 4.2.3), cell culture (Section 4.3.1.1.1), RT-PCR (Section 4.3.1.1.2) or serology (Section 4.3.2).

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NB: There is an OIE Reference Laboratory for infection with salmonid alphavirus
(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/scientific-expertise/reference-laboratories/list-of-laboratories/).
Please contact the OIE Reference Laboratories for any further information on infection with salmonid alphavirus

NB: FIRST ADOPTED IN 2014.

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C H A P T E R 2 . 3 . 7 .

INFECTION WITH KOI HERPESVIRUS ~~DISEASE~~

EU position
The EU supports the adoption of this modified chapter.

1. Scope

Infection with koi herpesvirus ~~disease (KHVD)~~ means infection with the pathogenic agent koi herpesvirus (KHV) of the Genus *Cyprinivirus* and Family *Alloherpesviridae* ~~a herpesvirus infection (Hedrick *et al*., 2000) capable of inducing a contagious and acute viraemia in common carp (*Cyprinus carpio*) and varieties such as koi carp and ghost carp (Haenen *et al.,* 2004)~~.

[…]

2.2. Host factors

2.2.1. Susceptible host species

~~Naturally occurring KHV infections have only been recorded from common carp (Cyprinus carpio) and varieties of this species (e.g. koi carp). Goldfish × common carp hybrids, produced by hybridising male goldfish with female carp, have been reported to show some susceptibility to KHV infections. Although mortality rate was low (5%), approximately 50% of these hybrids examined 25 days after intraperitoneal injection with a high dose of KHV possessed viral genomic DNA, as detected by polymerase chain reaction (PCR) (Hedrick et al., 2006). In a more recent study, infection by bath immersion with different KHV strains caused mortality of 35–42% in goldfish × koi carp hybrids and 91–100% in crucian carp × koi carp hybrids. The most marked clinical signs were large skin ulcers, excess mucus production and haemorrhages in the fins with the most extensive signs noted in the crucian carp × koi carp hybrids. Viral DNA was detected in all of the hybrid mortalities by PCR assay (Bergmann et al., 2010b).~~

Species that fulfil the criteria for listing ~~a species~~ as susceptible to infection with KHV according to Chapter 1.5. of the *Aquatic Animal Health Code* (*Aquatic Code*) include: All varieties and subspecies of common carp (*Cyprinus carpio* ~~*carpio*~~), and common carp hybrids (e.g. *Cyprinus carpio* × *Carassius auratus*).

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: Goldfish (*Carassius auratus*), grass carp (*Ctenopharyngodon idella*) and Syberian crucian carp (*Carassius auratus*).

In addition, pathogen-specific positive polymerase chain reaction (PCR) results have been reported in the following ~~organisms~~ species, but an ~~active~~ infection has not been demonstrated: Atlantic sturgeon (*Acipenser oxyrinchus* ~~*gueldenstaedtii*~~), blue back ide (*Leuciscus idus*), common roach (*Rutilus rutilus*), Euraseas ruffe (*Gymnocephalus cernuus*), European perch (*Perca fluviatilis*), hybrid sterlet × beluga (*Acipenser ruthenus* × *Huso huso*), rainbow trout (*Oncorhynchus mykiss*), Russian sturgeon (*Acipencer gueldenstaedtii* ~~*oxyrinchus*~~), scud (crustacean) (*Gammarus pulex*), silver carp (*Hypophthalmichthys militrix*), stone loach (*Barbatula barbatula*), swan mussel (*Anodonta cygnea*) and tench (*Tinca tinca*).

[…]

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