Organisation Mondiale de la Santé Animale | World Organisation for Animal Health | Organización Mundial de Sanidad Animal

Original: English

October 2015

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

Paris, 5–9 October 2015

The OIE Aquatic Animal Health Standards Commission (the Aquatic Animals Commission) met at OIE Headquarters in Paris from 5 October to 9 October 2015.

The list of participants and the adopted agenda are presented at Annex 1 and Annex 2.

The Aquatic Animals Commission thanked the following Member Countries for providing written comments on draft texts circulated after the Commission's March 2015 meeting: Australia, Belgium, Canada, Chinese Taipei, New Zealand, Norway, Saudi Arabia, Switzerland, Thailand, the United States of America (USA), the Member States of the European Union (EU), 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 Countries' comments and amended texts in the OIE *Aquatic Animal Health Code* (the *Aquatic Code*) and OIE *Manual of Diagnostic Tests for Aquatic Animals* (the *Aquatic Manual*) where appropriate. The amendments are shown in the usual manner by 'double underline' and '~~strikethrough~~' and may be found in the Annexes to the report. In Annex 10, amendments made at this meeting are highlighted with a coloured background in order to distinguish them from those made previously. The Aquatic Animals Commission considered all Member Countries' comments and documented its responses. However, the Commission was not able to draft a detailed explanation of the reasons for accepting or not each of the comments received.

Member Countries are reminded that many comments submitted without a rationale are difficult to evaluate and respond to. Similarly if comments are resubmitted without modification or new justification, the Aquatic Animals Commission will usually not repeat previous explanations for decisions. The Commission encourages Member Countries to refer to previous reports when preparing comments on longstanding issues.

Member Countries should note that the table below summarises the texts presented in the Annexes. Annexes 3 to 12 are presented for Member Countries' comments; Annexes 13 and 14 are presented for Member Countries' information.

The Aquatic Animals Commission encourages Member Countries to participate in the development of the OIE's intergovernmental standards by submitting comments on this report, and future reports, in preparation for the process of adoption at the General Session. Comments should be submitted as specific proposed text changes, supported by an explanatory rationale. 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 Countries' submissions into the Commission's working documents.

Comments on this report must reach OIE Headquarters by **15 January 2016** to be considered at the February 2016 meeting of the Aquatic Animals Commission. All comments should be sent to the OIE International Trade Department at: trade.dept@oie.int.

Texts for Member Countries' comments	**Annex number**
***Aquatic Code*:**	
Glossary	Annex 3
Proposed revisions to Articles 1.5.2. and 4.2.3. as a consequence of the proposed definition of 'vector'	Annex 4
Criteria for the inclusion of diseases in the OIE list (Chapter 1.2.)	Annex 5A ('track changes') and Annex 5B ('clean' version)
Diseases listed by the OIE (Chapter 1.3.)	Annex 6
Disinfection of aquaculture establishments and equipment (revised Chapter 4.3.)	Annex 7
Proposed restructure of Section 4: Disease prevention and control	Annex 8
General obligations related to certification (Chapter 5.1.)	Annex 9
Acute Hepatopancreatic Necrosis Disease (new Chapter 9.X.)	Annex 10
Infection with yellow head virus (Chapter 9.2.)	Annex 11
***Aquatic Manual*:**	
Infection with yellow head virus (Chapter 2.2.8.)	Annex 12
Texts for Member Countries' information	
Aquatic Animals Commission Work Plan for 2015/2016	Annex 13
Ad hoc Group on Disinfection of aquaculture establishments and equipment	Annex 14

A. MEETING WITH THE DIRECTOR GENERAL AND DEPUTY DIRECTOR GENERAL

The Aquatic Animals Commission met Dr Bernard Vallat, Director General, and Dr Brian Evans, Deputy Director General (Animal Health, Veterinary Public Health, International Standards), on the 6th of October 2015.

Dr Vallat congratulated the Commission members on their election and on behalf of the Member Countries wished them a successful 3-year term. He highlighted the importance of good communication and flexibility of approach among the Specialist Commissions to ensure alignment between the *Codes* and the *Manuals*, and between the *Aquatic* and *Terrestrial Animal Health Codes,* except when differences are necessary. Dr Vallat noted that the Aquatic Animals Commission was a unique Commission in that it was responsible for standards in the *Aquatic Code* and *Aquatic Manual* as well as nominations for Reference Centre experts and the quality of these Centres.

Dr Vallat noted that to put the Sixth Strategic Plan into effect and to safeguard the credibility of the Organisation (for example when we are accountable to the World Trade Organisation) we must strengthen our excellence by increasing our reliance on science and improving the transparency of our work.

Dr Evans highlighted the resolution adopted at the 83rd General Session to establish a performance evaluation framework for the Specialist Commissions, which will provide feedback to Member Countries on the performance of each of the Specialist Commissions via the Council. He also recalled the strong request from Delegates for congruence, coherence and effective sequencing of work between the Specialist Commissions which should be taken into account in the scheduling of meetings, Specialist Commission representation on *ad hoc* groups and reviews and improvements to Specialist Commission procedures.

Dr Evans highlighted Delegates commitment to maintaining the 2-year cycle of standard development, and that requests for standard development or amendment in 1 year should be considered in exceptional circumstances only or for minor updates.

Dr Ingo Ernst, President of the Aquatic Animals Commission, advised that the Commission would develop a work plan for its 3-year term, taking into consideration ongoing work and new priorities. The work plan would include the items to be achieved during this 3-year term of the Commission and would also provide a road map for future work.

Finally Dr Vallat and Dr Evans thanked the Commission Members for their commitment, promised their support, and wished the Commission every success throughout their newly elected term of office.

B. ADOPTION OF THE AGENDA

The draft agenda circulated prior to the meeting was discussed, and several new agenda items were added. The adopted agenda of the meeting is attached as Annex 2.

C. INFORMATION FOR NEW AQUATIC ANIMALS COMMISSION MEMBERS

The Aquatic Animals Commission reviewed and discussed a document developed by OIE Headquarters that compiled relevant information for new Aquatic Animals Commission Members. The Commission Members agreed this was a helpful introductory document, and that there would be value in updating it as and when necessary to provide an on-going single source reference on the role of the Commission, and how it operates.

D. MEETING WITH THE PAST-PRESIDENT OF THE AQUATIC ANIMALS COMMISSION

The Past-President of the Aquatic Animals Commission joined the meeting of the Aquatic Animals Commission (7th October) to discuss the future work plan for the Aquatic Animals Commission.

E. MEETING WITH THE PRESIDENT OF THE TERRESTRIAL ANIMAL HEALTH STANDARDS COMMISSION

The President of the Terrestrial Animal Health Standards Commission (Code Commission) joined the meeting of the Aquatic Animals Commission to discuss issues of mutual interest, notably, the Code Commission work programme, horizontal chapters in common and conventions for naming of diseases.

F. EXAMINATION OF MEMBER COUNTRY COMMENTS AND WORK OF RELEVANT *AD HOC* GROUPS

Item 1 General comments of Member Countries

The Aquatic Animals Commission did not agree with a Member Country comment to amend the title of the *Aquatic Code* Chapter 10.4. and *Aquatic Manual* Chapter 2.3.5. to Infection with infectious salmon anaemia virus. The Commission noted that the chapter covers all known genotypes of the virus as described in Article 10.4.1. The Commission reminded Member Countries that the listed disease, 'Infection with HPR-deleted or HPR0 infectious salmon anaemia virus', has been named this way to ensure the requirement to report all strains of ISAV is explicit.

Item 2 Glossary

Comments were received from the EU.

The Aquatic Animals Commission considered Member Countries' comments and amendments being proposed by the Code Commission to relevant definitions in the Glossary in the *Terrestrial Code*.

The Aquatic Animals Commission did not agree with a Member Country comment to include a reference to parasitic agents and zoonoses in the definition for disinfection, and reiterated that these are covered in the definition for pathogenic agents in the glossary.

The Aquatic Animals Commission agreed to propose the following new definitions for inclusion in the glossary:

OIE Standard and *OIE Guidelines*

In consultation with the Director General, the Code Commission, the Scientific Commission and the Biological Standards Commission, at their February 2015 meetings, agreed on new definitions for *OIE Standards* and *OIE Guidelines.* The Aquatic Animals Commission agreed to propose these new definitions for inclusion in the glossary and noted that once these definitions are adopted the use of these terms throughout the *Code* will be reviewed and aligned with the adopted definitions.

Vector

Given the use of the term 'vector' in the revised draft Chapter 4.3. *Disinfection in aquaculture establishments and equipment* and elsewhere in the *Code*, the Aquatic Animals Commission proposed a new definition for 'vector', taking into consideration the definition of vector used in the *Terrestrial Code*.

The Aquatic Animals Commission also noted that the term 'vector' has been used inconsistently in the *Aquatic Code*. The Commission proposed some minor consequential amendments to Articles 1.5.2. and 4.2.3. to ensure that the use of 'vector' would be consistent with the proposed new definition of 'vector' throughout the *Aquatic Code*.

The new Glossary definitions are attached as Annex 3 for Member Countries' comments.

Proposed revisions to Articles 1.5.2. and 4.2.3. are attached as Annex 4 for Member Countries' comments.

Item 3 Notification of diseases and provision of epidemiological information (Chapter 1.1.)

Comments were received from AU-IBAR, Australia, Canada, EU, New Zealand, Norway, and USA.

The Aquatic Animals Commission considered Member Countries' comments and amendments being proposed by the Code Commission to the corresponding chapter in the *Terrestrial Code.*

Given the importance of standardisation of this chapter with the corresponding chapter in the *Terrestrial Code,* the Aquatic Animals Commission made relevant amendments to the chapter and requested that OIE Headquarters provide these proposed amendments to the February 2016 meeting of the Code Commission for their consideration when reviewing comments on this chapter.

Member Countries are encouraged to review and comment on the corresponding *Terrestrial Code* chapter in the September 2015 report of the Code Commission. The Aquatic Animals Commission plan to meet at the same time as the Code Commission in February 2016 and will discuss both chapters to ensure standardisation, as far as possible, between the two *Code* chapters, and amend Chapter 1.1. of the *Aquatic Code* accordingly.

Item 4 Criteria for listing aquatic animal diseases (Chapter 1.2.)

Comments were received from AU-IBAR, Australia, Belgium, Canada, EU, New Zealand, Norway, Thailand and USA.

The Aquatic Animals Commission considered Member Countries' comments and amendments being proposed by the Code Commission to the corresponding chapter in the *Terrestrial Code*, and made relevant amendments.

The Aquatic Animals Commission appreciated the extensive comments provided by Member Countries on this chapter and noted the divergence of opinion on many of the proposed amendments. In considering Member Countries' comments and possible amendments to the text, the Aquatic Animals Commission noted that Chapter 1.2. of the *Aquatic Code* states *'the objective of listing is to support Member Countries' efforts to prevent the transboundary spread of important diseases of aquatic animals through transparent and consistent reporting*'.

The Aquatic Animals Commission noted that some Member Countries requested revisions that were inconsistent with that objective. Therefore the Aquatic Animals Commission amended the text to improve clarity and to achieve the objective for listing diseases.

The Aquatic Animals Commission proposed some amendments that differ from those proposed in the corresponding chapter in the *Terrestrial Code*; and noted that these differences are justified because of differences in applying the criteria for disease listing in the aquatic context.

The Aquatic Animals Commission noted that to achieve the objective of listing, the criteria must be responsive and flexible for the dynamic circumstances of aquatic animal diseases, such as the rapid growth and expansion of aquaculture, high volumes of trade, diversity of species, the frequent emergence of aquatic animal diseases and the difficulty in achieving eradication. During the last twenty years, 19 new aquatic animal diseases have been added to the OIE list. In addition 16 aquatic animal diseases have been de-listed since 2005.

The Aquatic Animals Commission considered Member Countries' comments on the usefulness of the Explanatory Notes. The Aquatic Commission agreed that these notes should be removed from the chapter but will be used in the development of a document to guide *ad hoc* groups in the application of the listing criteria. The Aquatic Animals Commission agreed to work with the Code Commission in the development of this document.

The Aquatic Animals Commission proposed the following amendments:

Article 1.2.1:

The Commission agreed with a Member Country comment to amend the last sentence of Article 1.2.1. that refers to the *Aquatic Manual* Chapter 1.1.2. *Principles and methods of validation of diagnostic assays for infectious diseases* to accurately reflect the title of the chapter.

Article 1.2.2. – Criterion 1:

The Commission replaced 'has been proven' with 'is likely' and noted that the objective of listing is to '*prevent the transboundary spread of important diseases of aquatic animals through transparent and consistent reporting*'. The Commission emphasised that it would be contrary to the objective of listing to wait for the 'international spread of an agent' to be proven when scientific evidence and international trade patterns indicate that it is very likely. This is important for aquatic animal diseases given the factors described above and in particular the challenge of achieving successful eradication of aquatic animal diseases.

Article 1.2.2. – Criterion 2:

The Commission noted that in the revised criterion the reference to '*a country with a zone*' had been removed and proposed that it be retained because this is consistent with the objective of listing and mechanisms in the *Aquatic Code* to establish zone freedom.

In response to several Member Countries' comments, the Commission agreed to delete '*or impending freedom*' because the only process for declaring freedom from OIE listed aquatic animal diseases in the *Aquatic Cod*e is self-declaration. The Commission recognised that it is difficult to envision how Member Countries would use the process of self-declaration to demonstrate impeding freedom.

The Commission agreed to change '*has*' to '*may*' regarding the demonstration of country freedom because Member Countries are required to have at least 2 years of basic biosecurity in place subsequent to a disease being listed and prior to making a self-declaration of freedom.

Article 1.2.2. – Criterion 3:

The Commission did not agree with a suggestion to replace the word '*reliable'* given that reliability is an appropriate descriptor for the validation of a diagnostic assay as described in Chapter 1.1.2. of the *Aquatic Manual*.

The Commission noted that a reliable means of detection and diagnosis is a fundamental part of every case definition and proposed a reorganisation of the structure of the criterion. The Commission deleted '*to clearly identify cases and allow them to be distinguished from other diseases'* because this meaning is included in the glossary definition of case definition.

Article 1.2.2. – Criterion 4.a:

The Commission made no amendments to this criterion.

Article 1.2.2. – Criterion 4.b:

In response to several Member Countries' comments, the Commission clarified that the definition of aquatic animal refers to both cultured and wild aquatic animals but acknowledged that a revision to improve clarity of the definition should be considered in future work. The Commission did not agree with a Member Country comment to merge points b. and c. in Article 1.2.2. but added the term '*cultured'* to clarify that this criterion specifically applies to cultured aquatic animals while the next criteria refers to wild aquatic animals.

The Commission clarified that this criterion considers both the impact and consequences of a disease in cultured aquatic animals. The Commission revised the criterion considering that the impact of a disease is the effect on the health of aquatic animals which then results in significant consequences at the country or zone level. The Commission also discussed the need to ensure flexibility in this criterion to account for the wide range of possible impacts on cultured aquatic animals resulting from disease occurrence.

The Commission deleted '*taking into account the occurrence and severity of the clinical signs'* because these are not always useful indicators of the consequences of disease in aquatic animals.

Article 1.2.2. – Criterion 4.c.

The Commission clarified that this criterion considers both the impact and consequences of a disease in wild aquatic animals. The Commission revised the criterion considering that the impact of a disease is the effect on the health of aquatic animals which then results in significant consequences at the country or zone level. The Commission also discussed the need to ensure flexibility in this criterion to account for the wide range of possible consequences for wild aquatic animal populations' resulting from disease occurrence.

The Commission considered a Member Country comment regarding the words '*ecological threats*' and proposed that '*ecological impacts*' more suitably reflects the potential consequences of disease in wild aquatic animals.

The Commission deleted '*taking into account the occurrence and severity of the clinical signs'* because these are not always useful indicators of the consequences of disease in wild aquatic animals.

The Commission has provided proposed amendments as 'clean' text and reminds Member Countries that previous versions were presented in Annex 22 of the March 2015 report of the Aquatic Animals Commission.

The revised Chapter 1.2. is attached as Annex 5A (with track changes) and Annex 5B (as clean text) for Member Countries' comments.

Item 5 Diseases listed by the OIE (Chapter 1.3.)

Comments were received from the EU.

The Aquatic Animals Commission amended the name of 'infection with yellow head virus' to 'infection with yellow head virus genotype 1' to align with the name of this disease as per Chapter 9.2.

The Aquatic Animals Commission considered the EU comment requesting that the Commission proceed with work on pathogen differentiation for viral haemorrhagic septicaemia virus (VHSV) genotypes. The Commission agreed that VHSV should be the next pathogen to be considered for strain differentiation and would consider this in the context of its work plan priorities.

The Aquatic Animals Commission discussed the situation regarding new diseases for consideration on the OIE list and in light of recent publications agreed to undertake assessments against the criteria for listing (Chapter 1.2.) for *Batrachochytrium salamandrivorans* and *Marteilia cochillia.* The Commission will review these assessments at their February 2016 meeting.

The revised Chapter 1.3. is attached as Annex 6 for Member Countries' comments.

Item 6 General recommendations on Disinfection (Chapter 4.3.)

The Aquatic Animals Commission reviewed the report of the *ad hoc* Group on Disinfection of aquaculture establishments and equipment and the revised draft Chapter 4.3. *Disinfection of aquaculture establishments and equipment*, and commended the *ad hoc* Group for their substantial work.

The Aquatic Animals Commission revised the draft text to improve clarity and readability.

The Aquatic Animals Commission noted that in point 4b) of Article X.X.4. of all disease-specific chapters in the *Aquatic Code* the reference to disinfection procedures in the *Aquatic Manual* should be amended to the *Aquatic Code*. The Commission requested OIE Headquarters to amend this point in the next edition of the *Aquatic Code.*

The report of the meeting of the *ad hoc* Group on Disinfection of aquaculture establishments and equipment is attached as Annex 14 for Member Countries' information.

The revised draft Chapter 4.3. is attached as Annex 7 for Member Countries' comments.

Item 7 Recommendations for surface disinfection of salmonid eggs (Chapter 4.4.)

Comments were received from Norway.

The Aquatic Animals Commission considered a Member Country comment and agreed that the suggestions were not critical to the understanding of the chapter. Given the recent adoption of this chapter, the Commission agreed to consider these comments when the chapter is next revised.

Item 8 Section 4

The Aquatic Animals Commission reviewed previous work undertaken by the Commission regarding revision of Section 4. *Disease prevention and control*. The Commission proposes substantial changes to this section including the addition of new chapters, and reorganisation and revision of existing chapters.

The Aquatic Animals Commission has developed a proposed restructure of Section 4 and welcomes Member Countries' comments on the new structure and prioritisation of this work. The Commission will consider Member Countries' comments at their February 2016 meeting and develop an approach to prioritise and complete this work.

The proposed restructure for Section 4 is attached as Annex 8 for Member Countries' comments.

Item 9 General obligations related to certification (Chapter 5.1.)

Comments were received from the EU.

The Aquatic Animals Commission agreed with Member Countries' comments and proposed that point 2 of Article 5.1.4. be deleted as this is repeated in the recently revised text of point 3. The Commission explained that point 2 should have been deleted when the revised text of point 3 was adopted in 2014.

The revised Article 5.1.4. is attached as Annex 9 for Member Countries' comments.

Item 10 OIE procedures relevant to the Agreement on the Application of Sanitary and Phytosanitary Measures on the World Trade Organization (Chapter 5.3.)

In consultation with the Director General, the Code Commission revised Chapter 5.3. in the *Terrestrial Code* to take into account comments of recent World Trade Organization Dispute Settlement Body panels, to remove unnecessarily discursive text, and to align with established *Code* format. The Aquatic Animals Commission reviewed the proposed amendments in Chapter 5.3. of the *Terrestrial Code.* The Commission agreed that given text in the two *Code* chapters is almost identical and the importance of standardisation between these two chapters, they will make relevant amendments to the *Aquatic Code* Chapter 5.3. once proposed amendments have been adopted in the *Terrestrial Code* chapter, to avoid divergence between the two chapters.

Item 11 Acute Hepatopancreatic Necrosis Disease (AHPND)

Given the inclusion of acute hepatopancreatic necrosis disease (AHPND) in Chapter 1.3. *Diseases listed by the OIE*, at the May 2015 OIE General Session, the Aquatic Animals Commission developed a new draft chapter on AHPND for inclusion in the *Aquatic Code*.

The Aquatic Animals Commission noted that the lists of commodities in Articles 10.X.3. and 10.X.11. have been placed 'under study' and requested that experts conduct assessments on a range of commodities commonly traded internationally against the criteria provided in Chapter 5.4. The Commission requested that they receive these assessments prior to their February 2016 meeting so that relevant articles can be updated.

The new Chapter 9.X. is attached as Annex 10 for Member Countries' comments.

Item 12 Infection with yellow head virus (Chapter 9.2.)

Comments were received from Australia and the EU.

The Aquatic Animals Commission noted that several Member Countries' comments supported the proposed amendment to the title of the chapter.

The Aquatic Animals Commission made relevant amendments to Article 9.2.2. to improve clarity and replaced the term YHD with infection with YHV1 throughout the chapter, as necessary.

The revised Chapter 9.2. is attached as Annex 11 for Member Countries' comments.

Item 13 Infection with ranavirus (Chapter 8.2.)

In response to a Member Country's comment, advice had been requested from an OIE expert on the listing of infection with Ranavirus at the genus level. The Aquatic Animals Commission reviewed the OIE's expert's advice. Currently research on the taxonomy of Ranavirus is underway and the International Committee on Taxonmomy of Viruses (ICTV) will, in 2016, review the classification. Therefore, the Commission proposed to reconsider the listing of Ranaviruses once the ICTV position is clear.

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

Item 14. OIE *Manual of Diagnostic Tests for Aquatic Animals*

In response to requests from Member Countries and recent OIE Global and Regional Conferences, the Aquatic Animals Commission considered the need to improve critical areas of the *Aquatic Manual* including test validation, case definitions and Table 5.1. *Methods for surveillance, detection and diagnosis.* The Commission requested that an *ad hoc* Group be convened, and work in collaboration with experts from the OIE Reference Laboratories to address these issues.

Item 15 Draft chapter on acute hepatopancreatic necrosis disease

Comments were received from Australia, Japan, Switzerland and Thailand.

A large number of Member Countries' comments were received on the draft chapter on acute hepatopancreatic necrosis disease (AHPND). The Aquatic Animals Commission agreed that comments should be considered by the *ad hoc* Group and the draft chapter amended as necessary. The Commission requested that this work be completed prior to its February 2016 meeting.

Item 16 Infection with yellow head virus (Chapter 2.2.8.)

The Aquatic Animals Commission noted that Article 9.2.1. of the *Aquatic Code* was recently revised so that infection with yellow head virus now means 'infection with yellow head virus genotype 1 (YHV1)'. To ensure consistency, the Commission, in consultation with the OIE Reference Laboratory expert for yellow head disease, updated and amended the corresponding *Aquatic Manual* Chapter 2.2.8.

The Aquatic Animals Commission added a new section 2.2.2. '*Species with incomplete evidence for susceptibility'* to be consistent both with the corresponding *Aquatic Code* Chapter 9.2. and the February 2015 report of the OIE *ad hoc* Group on susceptibility of crustacean species to infection with OIE listed diseases (Annex 27 of the March 2015 report of the Aquatic Animals Commission).

The revised chapter 2.2.8. is attached as Annex 12 for Member Countries' comments.

Item 17 OIE Reference Centres

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

The following proposals for changes to nominated experts at two OIE Reference Laboratories, supported by the Delegates of the Member Countries concerned, were considered.

Yellow head disease

Dr Nick Moody to replace Dr Peter Walker at the Australian Animal Health Laboratory, CSIRO Livestock Industries, Geelong, Victoria, Australia.

Infectious hypodermal and haematopoietic necrosis, Taura syndrome, infectious myonecrosis and *White spot disease*

Dr Kathy Tang-Nelson to replace Dr Donald Lightner at the Aquaculture Pathology Laboratory, Department of Veterinary Science and Microbiology, University of Arizona, Tucson, United States of America.

The Aquatic Animals Commission recommended that these proposed changes to nominated experts be approved.

17.2. Follow up of annual reports of Reference Centre activities in 2014

The Aquatic Animals Commission was informed that annual reports for activities in 2014 had been received from all but one OIE Reference Laboratory. A request for an explanation will be sent to the expert at the Reference Laboratory that has not provided a report.

H. OTHER ISSUES

Item 18 Aquatic Animal Commission's work programme 2015/2016

The Aquatic Animals Commission reviewed and updated its work programme, taking account of Member Countries' and Headquarters' comments, recommendations from recent OIE Global Conferences, and the work completed.

The revised work programme is attached as Annex 13 for Member Countries' information.

Item 19 Aquatic Animal Commission Activities

The Aquatic Animals Commission agreed that it is important to inform Member Countries of activities that Commission members undertake in their role as Commission members.

Since May 2015, Commission members have participated in the following activities:

Dr Ingo Ernst:

(1) Prepared and presented a Technical Item with questionnaire '*The role of Veterinary Services in managing emerging aquatic animal diseases: what are the factors needed for success?*' at the 29th Conference of the OIE Regional Commission for Asia, the Far East and Oceania, held in Ulaanbaatar (Mongolia) from 14 to 18 September 2015.

(2) Represented the Aquatic Animals Commission at a meeting of the *ad hoc* Group on Disinfection of aquaculture establishments and equipment, Paris, 19–21 May 2015.

Item 20 Food and Agricultural Organisation Update

Food and Agricultural Organisation representatives were not able to attend the Aquatic Animals Commission meeting due to other work commitments so a tele-conference call was held prior to the Commission meeting. Rohana Subasinghe and Melba Reantaso from FAO were joined by Ingo Ernst and Gillian Mylrea. FAO representatives provided updates on relevant FAO Technical Cooperation Programmes underway, in particular those focused on acute hepatopancreatic necrosis disease in Asia and Latin America and epizootic ulcerative syndrome in Africa. Dr Ernst provided an update on relevant activities of the Aquatic Animals Commission.

During the Aquatic Animals Commission meeting Dr Ernst presented a summary of information shared. Commission members welcomed this agenda item and noted the importance of the relationship with FAO.

Item 21 Proposed dates for next meetings

The 2016 Aquatic Animals Commission meetings are scheduled for February 15–19, and September 12–16 inclusive.

…Annexes

Annex 1

MEETING OF THE OIE
AQUATIC ANIMAL HEALTH STANDARDS COMMISSION

Paris, 5–9 October 2015

_____

List of participants

MEMBERS OF THE COMMISSION

Dr Ingo Ernst (President)
Director Aquatic Pest and Health Policy
Animal Division
Department of Agriculture and Water Resources
18 Marcus Clarke Street
Canberra ACT 2601
AUSTRALIA
Tel.: +61 2 6272 5615
Ingo.Ernst@agriculture.gov.au

Dr Alicia Gallardo Lagno
Subdirectora nacional de acuicultura
Servicio Nacional de Pesca y Acuicultura
Calle Victoria 2832
CHILE
Tel.: + 56 32 281 9282
agallardol@sernapesca.cl

Dr Maxwell Barson
Senior lecturer
(Parasitology & histopathology)
University of Zimbabwe
Department of Biological Sciences
Box MP 167 Mt. Pleasant
ZIMBABWE
Tel.: + 263 4 303 211
barson001@yahoo.co.uk
banson@science.uz.ac.zw

Dr Edmund Peeler
Group Manager Aquatic Pest & Pathogens
CEFAS
Barrack Road, Weymouth
Dorset, DT4 8UB UK
UNITED KINGDOM
Tel.: + 44 (0)1305 206746
ed.peeler@cefas.co.uk

Dr Joanne Constantine
National Manager
Animal Health Import/Export, Aquatics Section
Canadian Food Inspection Avenue
Floor 3 E, Room 116
59 Camelot Drive
Ottawa ON K1A 0Y9
CANADA
Tel.: + 1-613-773-7426
joanne.constantine@inspection.gc.ca
jonjohn@rogers.com

Prof. Mohamed Shariff Bin Mohamed Din
Faculty of Veterinary Medicine
Universiti Putra Malaysia
43400 Serdang, Selangor
MALAYSIA
Tel.: + 6012 2839 845
shariff@upm.edu.my
pshariff@gmail.com

OTHER PARTICIPANTS

Dr Franck Berthe
Former President – Aquatic Animal Health Standards Commission
European Food Safety Authority - EFSA
Head of Animal Health Plant Health
Via Carlo Magno 1, Parma
ITALY
Tel.: + 39 052 1 036 870
Fax: + 39 052 1 036 0870
Franck.Berthe@efsa.europa.eu

Dr Etienne Bonbon
President – Terrestrial Animal Health Standards Commission
Scientific Counsellor
EU Delegation to the International Organisations in Paris
12, avenue d'Eylau
75116 Paris
FRANCE
Tel.: +33 1 44 05 31 68
etienne.bonbon@eeas.europa.eu
e.bonbon@oie.int

Annex 1 (contd)

OIE HEADQUARTERS

Dr Bernard Vallat
Director General
12, rue de Prony
75017 Paris
FRANCE
Tel.: 33 - (0)1 44 15 18 88
Fax: 33 - (0)1 42 67 09 87
oie@oie.int

Dr Derek Belton
Head
International Trade Department
OIE
d.belton@oie.int

Dr Brian Evans
Deputy Director General
Head
Scientific and Technical Department
d.evans@oie.int

Ms Sara Linnane
Scientific editor
Scientific and Technical Department
OIE
s.linnane@oie.int

Dr Gillian Mylrea
Deputy Head
International Trade Department
OIE
g.mylrea@oie.int

Annex 2

MEETING OF THE OIE
AQUATIC ANIMAL HEALTH STANDARDS COMMISSION

Paris, 5–9 October 2015

———

Adopted agenda

A. MEETING WITH THE DIRECTOR GENERAL AND DEPUTY DIRECTOR GENERAL

B. ADOPTION OF THE AGENDA

C. INFORMATION FOR NEW AQUATIC ANIMALS COMMISSION MEMBERS

D. MEETING WITH THE PAST-PRESIDENT OF THE AQUATIC ANIMALS COMMISSION

E. MEETING WITH THE PRESIDENT OF THE TERRESTRIAL ANIMAL HEALTH STANDARDS COMMISSION

F. EXAMINATION OF MEMBER COUNTRY COMMENTS AND WORK OF RELEVANT *AD HOC* GROUPS

Item 1 General comments

Item 2 Glossary

Item 3 Notification of diseases and provision of epidemiological information (Chapter 1.1.)

Item 4 Criteria for listing aquatic animal diseases (Chapter 1.2.)

Item 5 Diseases listed by the OIE (Chapter 1.3.)

Item 6 Acute hepatopancreatic necrosis disease (AHPND)

Item 7 General recommendations on disinfection (Chapter 4.3.)

Item 8 Recommendations for disinfection of salmonid eggs (Chapter 4.4.)

Item 9 Section 4. Disease Prevention and Control

Item 10 General obligations related to certification (Chapter 5.1.)

Item 11 OIE procedures relevant to the Agreement on the Application of Sanitary and Phytosanitary Measures on the World Trade Organization (Chapter 5.3.)

Item 12 Infection with yellow head virus (Chapter 9.2.)

Item 13 Infection with ranavirus (Chapter 8.2)

Annex 2 (contd)

G. *MANUAL OF DIAGNOSTIC TESTS FOR AQUATIC ANIMALS*

Item 14 *Manual of Diagnostic Tests for Aquatic Animals*

Item 15 Acute hepatopancreatic necrosis disease (AHPND)

Item 16 Infection with yellow head virus (Chapter 2.2.8.)

Item 17 OIE Reference Centres

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

17.2. Follow up of annual reports of Reference Centre activities in 2014

H. OTHER ISSUES

Item 18 Aquatic Animals Commission's work programme 2015/2016

Item 19 Aquatic Animals Commission Activities

Item 20 FAO Update

Item 21 Proposed dates for next meetings

Annex 3

GLOSSARY

For the purpose of the *Aquatic Code*:

OIE STANDARDS

means a text that has been formally adopted by the OIE World Assembly of Delegates, published by the OIE, and that provides requirements, recommendations, specifications and characteristics that should be used consistently to ensure the improvement of animal health, veterinary public health and animal welfare worldwide.

OIE GUIDELINES

means an OIE publication that provides advice to improve animal health, veterinary public health and animal welfare worldwide that has been endorsed by an OIE Specialist Commission or the OIE Council, but has not been formally adopted by the OIE World Assembly of Delegates.

VECTOR

means any living organism that transports an infectious agent to a susceptible individual or its food or immediate surroundings. The organism may or may not pass through a development cycle within the vector.

Annex 4

Proposed revisions to Articles 1.5.2. and 4.2.3. as a consequence of the proposed new definition of 'vector'

CHAPTER 1.5.

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

[…]

Article 1.5.2.

Scope

Susceptibility may include clinical or non-clinical *infection* but does not include ~~mechanical vectors (i.e.~~ species that may carry the *pathogenic agent* without replication~~)~~.

The decision to list a species as susceptible should be based on a finding that the evidence is definite. However, possible susceptibility of a species is also important information and this should also be included in Section 2.2.1. entitled «Susceptible host species» of the relevant *disease*-specific chapter of the *Aquatic Manual*.

- - - - - - - - - - - - - -

— Text deleted.

CHAPTER 4.2.

APPLICATION OF COMPARTMENTALISATION

[…]

Article 4.2.3.

Separation of a compartment from potential sources of infection

[…]

2. Infrastructural factors

Structural aspects of an *establishment* or *establishments* within a *compartment* contribute to the effectiveness of its *biosecurity*. Consideration should be given to:

a) water supply;

b) effective means of physical separation;

c) facilities for people entry including access control;

Annex 4 (contd)

d) *vehicle* and vessel access including washing and *disinfection* procedures;

e) unloading and loading facilities;

f) isolation facilities for introduced *aquatic animals*;

g) facilities for the introduction of material and equipment;

h) infrastructure to store *feed* and veterinary products;

i) disposal of *aquatic animal* waste;

j) measures to prevent exposure to fomites~~, mechanical~~ or ~~biological~~ vectors;

k) *feed* supply/source.

- - - - - - - - - - - - - -

— Text deleted.

Annex 5A

CHAPTER 1.2.

CRITERIA FOR LISTING AQUATIC ANIMAL THE INCLUSION OF DISEASES IN THE OIE LIST

Article 1.2.1.

Introduction

This chapter describes the criteria for listing *diseases* in Chapter 1.3.

The objective of listing is to support Member Countries~~'~~ by providing information needed to take appropriate action ~~efforts~~ to prevent the transboundary spread of important *diseases* of *aquatic animals*. This is achieved by ~~through~~ transparent, timely and consistent ~~reporting~~ *notification*.

For the *diseases* listed in accordance with Article 1.2.2., the corresponding *disease*-specific chapters ~~in the *Aquatic Code*~~ assist Member Countries in the harmonisation of *disease* detection, prevention and control and provide standards for safe *international trade* in *aquatic animals* and *aquatic animal* ~~their~~ *products*.

The requirements for *notification* of *listed diseases* are detailed in Chapter 1.1.

Principles and methods of~~or~~ validation ~~selection~~ of diagnostic tests are provided ~~described~~ in Chapter 1.1.2. of the *Aquatic Manual*.

Article 1.2.2.

The c~~C~~riteria for the inclusion of a ~~listing an aquatic animal~~ *disease* in the OIE list are as follows:

~~*Diseases* proposed for listing should meet the relevant criteria as set out in A. Consequences, B. Spread and C. Diagnosis. Therefore, to be listed, a *disease* should have the following characteristics: 1 or 2 or 3; and 4 or 5; and 6; and 7; and 8. Such proposals should be accompanied by a *case definition* for the *disease* under consideration.~~

~~No.~~		~~Criteria for listing~~	~~Explanatory notes~~
		~~A. Consequences~~	
~~1.~~OR	b.	The *disease* has been shown to affect ~~cause a significant production losses at a national or multinational (zonal or regional) level impact on~~ the health of cultured *aquatic animals* at the level of a country or a *zone* ~~taking into account the occurrence and severity of the clinical signs,~~ resulting in significant consequences ~~impacts~~, e.g. production losses, morbidity and mortality at a *zone* or country level. ~~including direct production losses and mortality.~~	~~There is a general pattern that the disease will lead to losses in susceptible species, and that morbidity or mortality are related primarily to the infectious agent and not management or environmental factors. (Morbidity includes, for example, loss of production due to spawning failure.) The direct economic impact of the disease is linked to its morbidity, mortality and effect on product quality.~~

Annex 5A (contd)

2.OR	c.Or	The *disease* has been shown to, or scientific evidence indicates that it is likely to would, affect cause a significant impact on the health of morbidity or mortality in *wild aquatic animals* resulting in significant consequences, e.g. morbidity and mortality at a population level, and ecological impacts. populations taking into account the occurrence and severity of the clinical signs, including direct production losses and mortality, and ecological threats.	Wild aquatic animal populations can be populations that are commercially harvested (wild fisheries) and hence are an economic asset. However, the asset could be ecological or environmental in nature, for example, if the population consists of an endangered species of aquatic animal or an aquatic animal potentially endangered by the disease.
AND			
3.4.	a.Or	The agent is of public health concern. Natural transmission to humans has been proven, and human infection is associated with severe consequences.	
And B. Spread			
4.	-	Infectious aetiology of the disease is proven.	-
5.	Or	An infectious agent is strongly associated with the disease, but the aetiology is not yet known.	Infectious diseases of unknown aetiology can have equally high risk implications as those diseases where the infectious aetiology is proven. Whilst disease occurrence data are gathered, research should be conducted to elucidate the aetiology of the disease and the results be made available within a reasonable period of time.

No.		**Criteria for listing**	**Explanatory notes**
And B. Spread			
6.1.	And	Likelihood of iInternational spread, of the agent including (via live *aquatic animals*, their *aquatic animal products* or fomites) is likely has been proven.	International trade in aquatic animal species susceptible to the disease exists or is likely to develop and, under international trading practices, the entry and establishment of the disease is likely.
AND			

~~7.~~2.	~~And~~	At least one ~~Several~~ country~~ies~~ or a country with a *zone* may ~~or countries with zones~~ ~~has~~ demonstrate~~d~~ freedom ~~or impending freedom~~ from the *disease* in ~~populations of~~ susceptible *aquatic animals*, ~~may be declared free of the disease~~ based on the ~~general~~ ~~surveillance~~ provisions ~~principles~~ ~~outlined~~ ~~in~~ of Chapter~~s~~ 1.4. ~~and 1.5.~~	~~Free countries/zones could still be protected. Listing of diseases that are ubiquitous or extremely widespread would render notification unfeasible. However, individual countries that run a control programme on such a disease can propose its listing provided they have undertaken a scientific evaluation to support their request. Examples may be the protection of broodstock from widespread diseases, or the protection of the last remaining free zones from a widespread disease.~~
~~And C. Diagnosis~~			
AND			
~~8.~~3.		~~A repeatable and robust~~ A precise *case* definition is available and a r~~R~~eliable means of detection~~/~~ and diagnosis exist~~s~~ ~~and a precise case definition is available to clearly identify *cases* and allow them to be distinguished from other *diseases*.~~	~~A diagnostic test should be widely available and preferably has undergone a formal standardisation and validation process using routine field samples (See *Aquatic Manual*.) or a robust case definition is available to clearly identify cases and allow them to be distinguished from other pathologies.~~

- - - - - - - - - - - - - -

— Text deleted.

Annex 5

CLEAN VERSION

CHAPTER 1.2.

CRITERIA FOR THE INCLUSION OF DISEASES IN THE OIE LIST

Article 1.2.1.

Introduction

This chapter describes the criteria for listing *diseases* in Chapter 1.3.

The objective of listing is to support Member Countries by providing information needed to take appropriate action to prevent the transboundary spread of important *diseases* of *aquatic animals.* This is achieved by transparent, timely and consistent *notification*.

For the *diseases* listed in accordance with Article 1.2.2., the corresponding *disease*-specific chapters assist Member Countries in the harmonisation of *disease* detection, prevention and control, and provide standards for safe *international trade* in *aquatic animals* and *aquatic animal products*.

The requirements for *notification* of *listed diseases* are detailed in Chapter 1.1.

Principles and methods of validation of diagnostic tests are provided in Chapter 1.1.2 of the *Aquatic Manual*.

Article 1.2.2.

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

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

AND

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

AND

3) A precise *case definition* is available and a reliable means of detection and *diagnosis* exist.

AND

4)

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

OR

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

OR

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

Annex 6

CHAPTER 1.3.

DISEASES LISTED BY THE OIE

Preamble: The following *diseases* are listed by the OIE according to the criteria for listing an *aquatic animal disease* (see Article 1.2.2.).

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

Article 1.3.1.

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

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

Article 1.3.2.

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

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

Article 1.3.3.

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

– Acute hepatopancreatic necrosis disease
– Crayfish plague (*Aphanomyces astaci*)
– Infection with yellow head virus genotype 1
– Infectious hypodermal and haematopoietic necrosis
– Infectious myonecrosis
– Necrotising hepatopancreatitis

Annex 6 (contd)

– Taura syndrome
– White spot disease
– White tail disease.

Article 1.3.4.

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

– Infection with *Batrachochytrium dendrobatidis*
– Infection with ranavirus.

Annex 7

CHAPTER 4.3

DISINFECTION OF AQUACULTURE ESTABLISHMENTS AND EQUIPMENT

Article 4.3.1.

Purpose

To provide recommendations on planning and implementation of *disinfection* procedures to prevent the spread of *pathogenic agents*.

Article 4.3.2.

Scope

This chapter describes recommendations on *disinfection* procedures for *aquaculture establishments* and equipment used during routine biosecurity activities and emergency response. Guidance is provided on general principles, planning and implementation of *disinfection* activities.

For specific methods of pathogen inactivation refer to the *disease*-specific chapters in the *Aquatic Manual.*

Article 4.3.3.

Introduction

Disinfection is commonly employed as a *disease* management tool in *aquaculture establishments* as part of a *biosecurity plan*. *Disinfection* is used to prevent entry or exit of target *pathogenic agents* from an *aquaculture establishment* or *compartment*, as well as the spread of *pathogenic agents* within *aquaculture establishments*. *Disinfection* may be used during emergency disease response to support the maintenance of *disease* control *zones* and for *disease* eradication (stamping-out procedures) from affected *aquaculture establishments*. The specific objective of *disinfection* will determine the strategy used and how it is applied.

Where possible, the spread of *pathogenic agents* should be prevented by avoiding *risk* pathways rather than attempting to manage them through *disinfection*. For example, high *risk* and difficult to disinfect items (e.g. gloves, dive and harvest equipment, ropes and nets) should be site specific rather than disinfecting and moving these items between production units and *aquaculture establishments*.

Article 4.3.4.

General principles

Disinfection is a structured process that uses physical and chemical procedures to inactivate target *pathogenic agents*. The process should include planning and implementation stages that take into account potential options, efficacy and *risks*.

Annex 7 (contd)

The *disinfection* process may vary depending on whether the overall objective is *disease* eradication or *disease* control. Procedures addressing eradication will generally involve destocking of all *aquatic animals* as well as *disinfection* of *aquaculture establishments* and equipment, whereas *disease* control aims at limiting the spread of *disease* between or within *aquaculture establishments*. Although different approaches may be used to achieve the identified objective, the general principles described below should be applied in all cases.

1) The *disinfection* process should include the following phases:

a) Cleaning and washing

Cleaning and washing of surfaces and equipment should always precede the application of *disinfectants*. It is necessary to remove solid waste, organic matter and chemical residues as these may reduce the effectiveness of *disinfectants*. The detergent used should be compatible with the *disinfectant* and the surface being treated. Waste produced during this phase should be disposed of in a safe manner because it may contain viable *pathogenic agents* that have the potential to spread *infection* if not controlled. After cleaning procedures, any excess water should be drained before application of *disinfectants*.

Where treatment of water is required, the presence of suspended solids may also reduce the capacity of some *disinfectants*. Removal of suspended solids through various processes such as filtration, sedimentation, coagulation or flocculation should be performed.

Biofilms, often referred to as slime, are a thin film of microorganisms and extracellular polymeric substances that adhere to surfaces. Biofilms physically protect embedded microorganisms against *disinfectants*. In order to achieve effective *disinfection*, biofilms should be removed during the cleaning and washing stage prior to the application of *disinfectants*.

b) Application of disinfectants

This phase involves the application of chemical compounds or physical processes that are appropriate to inactivate the target *pathogenic agent*.

The application of *disinfectants* should take into account the type of material requiring *disinfection* and how *disinfectants* should be applied. Hard non-permeable materials such as polished metal surfaces, plastics and painted concrete can be cleaned thoroughly and allow contact with the *disinfectant* because there is little opportunity for infective material to lodge in crevices. *Disinfection* efficacy will decrease if the surface is corroded, pitted or paint is flaking, so proper maintenance of equipment is essential. For permeable surfaces and materials (e.g. woven material, nets and soil), a higher *disinfectant* concentration and a longer contact time is required because the surface area is greater, chemicals cannot penetrate easily and residual organic matter may be present.

The choice of the application method should ensure all surfaces come into contact with the agent for the required period of time. The application of *disinfectants* should be undertaken methodically (e.g. using a grid pattern) to ensure that complete coverage and adequate contact times are achieved. Each phase should start from the highest point and proceed downwards, commencing from the least contaminated areas. However for some equipment, rinsing of surfaces with the *disinfectant* may be sufficient. When *disinfectants* are applied to vertical surfaces, care should be taken to ensure that the required contact time is maintained before the *disinfectant* drains away. Vertical surfaces may need retreatment or the addition of compatible foaming agents to prolong adherence to surfaces.

For pipes and biofilters, complete filling with the *disinfectant* solution should be done to ensure contact with all surfaces. Difficult to access and complex areas may require fumigation or use of misting equipment.

c) Removal or inactivation of the disinfectant

Removal or inactivation of chemical residues is important to avoid toxicity to *aquatic animals*, corrosion of equipment and environmental impacts. Processes that may be employed for the removal or inactivation of chemical residues may include: rinsing of surfaces, dilution to acceptable levels, treatment to inactivate chemical agents or, time to allow deactivation or dissipation of the active compound. These processes may be used in isolation or in combination.

2) *Disinfectants* should be used in accordance with relevant legislation. *Disinfectants* may present *risks* to the health of users, *aquatic animals* and the environment. Chemical *disinfectants* should be stored, used and disposed of in accordance with regulations and manufacturer's instructions.

3) *Disinfection* should be monitored to ensure appropriate dose of *disinfectant* and *disinfection* efficacy. Depending on the application process and the pathogen of concern, this may be done in different ways. Examples include measurement of the active agent (e.g. residual chlorine levels), indirect measurement of the active agent by an indicator process (e.g. monitoring oxygen reduction potential), and measuring efficacy using indicator bacteria (e.g. heterotrophic bacteria plate counts).

In facilities that have undergone destocking and *disinfection*, the use of a sentinel population prior to restocking may be considered. The sentinel population should be susceptible to the pathogen of concern and exposed to conditions that would be conducive to the expression of clinical *disease* should viable pathogen remain.

4) *Aquaculture establishments* should keep records of the *disinfection* processes applied. The records should be sufficient to allow evaluation of the *disinfection* plan.

Article 4.3.5.

Planning

A *disinfection* plan should be developed that incorporates an assessment of the risk pathways, the type of material to be disinfected, the *pathogenic agents* to be inactivated and the environment in which the process is to be undertaken. The *disinfection* plan should be regularly reviewed and include a mechanism for determining efficacy. Any changes to the *disinfection* plan should also be documented.

The planning process should assess the critical control points where *disinfection* will be most effective. *Disinfection* priorities should be developed by considering potential pathways for spread of *pathogenic agents* and the relative *risk* of contamination. For effective *disinfection* of facilities containing vectors (e.g. ponds) the vectors should be excluded, removed or destroyed as part of the *disinfection* process.

An inventory of all items requiring *disinfection* should be developed and include an assessment of materials used in construction, surface porosity, access to areas and resistance to chemical damage. Then, the appropriate *disinfection* method should be decided for each item.

The level of cleaning required prior to *disinfection* should be assessed for each type of equipment. If heavy soiling with solids and particulate matter is present, specific attention should be given to the cleaning process and the resources required. The physical or chemical cleaning process should be compatible with the *disinfectant* chosen.

Personnel, equipment and materials to be *disinfected* should be assessed taking into account the type and number of items to be treated and how waste material will be managed.

The ability to control water flow and water volumes should be considered at the planning stage and will depend on the enterprise type (recirculation, flow-through and open systems). Water may be disinfected using a variety of methods as described in Article 4.3.11.

Annex 7 (contd)

Article 4.3.6.

Disinfection in an emergency response

Disinfection is essential part of any emergency response to support *disease* control activities such as *quarantine* of affected *aquaculture establishments* and stamping-out procedures. The conditions associated with an emergency response require different approaches for *disinfection* to those used in routine biosecurity. These conditions include a high level of *disease risk* (due to the significance of the *disease*), high pathogen loading, potential high volumes of infected *aquatic animals* and waste, large areas requiring *disinfection* and large volumes of contaminated water. Planning should consider these circumstances, incorporate an evaluation of *risks* and include methods for monitoring efficacy.

In an emergency response it may be preferable to avoid disease *risk* pathways rather than relying on *disinfection*. Equipment should not be moved from an infected premise unless effective *disinfection* has been achieved. In some circumstances, destruction of high *risk* equipment or material in a way that inactivates the pathogen (e.g. incineration) will be required.

Article 4.3.7.

Types of disinfectants

Types of *disinfectants* commonly used in *aquaculture* include the following:

1. Oxidising agents

   The majority of oxidising agents are relatively fast acting and are effective *disinfectants* for a large range of micro-organisms. These compounds are inactivated by organic matter and therefore should be used following an effective cleaning stage. Organic matter consumes oxidising agents and the initial concentration (loading dose) may drop rapidly, making effective dosing levels (residual dose) difficult to predict. Therefore, residual dose levels should always be monitored to ensure that they remain above the minimum effective concentration for the required time period.

   Oxidising agents may be toxic to *aquatic animals* and therefore should be removed or inactivated.

   Common oxidising agents include chlorine compounds, chloramine-T, iodophores, peroxygen compounds, chlorine dioxide and ozone.

2. pH modifiers (alkalis and acids)

   pH modifiers consist of either alkalis or acid compounds used to modify ambient pH. They have the advantage that they are not inactivated by organic matter and therefore can be used in areas where an effective cleaning phase is not possible such as in pipes and biofilters.

3. Aldehydes

   Aldehydes act by denaturing protein. Two aldehyde compounds that may be used during decontamination of *aquaculture establishments* are formaldehyde and glutaraldehyde. They are highly effective against a wide range of organisms but require long exposure times. Aldehydes maintain their activity in the presence of organic matter and are only mildly corrosive. Formalin can also be used to produce formaldehyde gas for fumigation.

4. Biguanides

   Of the many biguanides available, chlorhexidine is the most commonly used. However they are not effective in hard or alkaline water and are less effective against many *pathogenic agents* compared to other groups of *disinfectants*. These compounds are comparatively non-corrosive and relatively safe, thus they are commonly used in the *disinfection* of people and delicate equipment.

5. Quaternary ammonium compounds (QACs)
The biocidal efficacy of QACs is variable and selective. They are effective against some vegetative bacteria and some fungi, but not all viruses. QACs are most active against gram-positive bacteria; action against gram-negative bacteria is slow, with some strains showing resistance. These compounds are not effective against spores. The advantages of QACs are that they are noncorrosive and have wetting properties that enhance contact with surfaces. QACs may be toxic to *aquatic animals* and should be removed from surfaces following *disinfection* procedures.

6. Ultraviolet (UV) irradiation

UV irradiation is a viable option for the treatment of water entering or leaving *aquaculture establishments* where there is some control of water flows in recirculation or flow-through systems. UV irradiation should be used following effective filtration because suspended solids reduce UV transmission and the effectiveness of this method.

7. Heat treatment

The effectiveness of heat treatment is dependent on the combination of temperature and exposure time. Susceptibility of *pathogenic agents* to heat treatment varies significantly, therefore, the characteristics of the target *pathogenic agent* should be taken into consideration. Under most conditions, moist heat is more effective than dry heat.

8. Desiccation

Desiccation may be an effective *disinfectant* for susceptible *pathogen agents* and may be used in circumstances where other *disinfection* methods are impractical or as an ancillary method to other *disinfection* methods.

Desiccation can be considered to be a *disinfection* method if complete drying of the item is achieved because the absence of water will kill many *pathogen agents*. However, moisture content may be difficult to monitor in some circumstances. The effectiveness will vary depending on environmental conditions such as temperature and humidity.

9. Combined disinfection methods

Combined *disinfection* methods should be considered wherever they are synergistic and provide a higher assurance of effective *pathogenic agent* inactivation. Some examples include:

a) direct sunlight and drying as a combined *disinfection* method provides three potential *disinfection* actions, i.e. UV irradiation, heating and desiccation. It has no operational cost and may be used subsequent to other methods;

b) ozone and UV irradiation are often combined in series as they provide back-up systems and different modes of action. UV irradiation also has the advantage of removing ozone residues from treated water.

Antagonistic effects may occur when chemical agents or detergents are combined.

Article 4.3.8.

Selection of a disinfectant

The *disinfectant* should be selected considering the following:

– effectiveness against the *pathogenic agents*;
– effective concentration and exposure time;
– ability to measure efficacy;

Annex 7 (contd)

- nature of the items to be disinfected;
- compatibility with the available water type (e.g. fresh water, hard water or seawater);
- availability of the *disinfectant* and equipment;
- ease of application;
- cost;
- impacts of residues on aquatic animals and the environmental; and
- user safety.

Article 4.3.9.

Types of aquaculture establishments and equipment

Aquaculture establishments and equipment differ widely in their characteristics. This section presents some considerations for effective *disinfection* of different types of *aquaculture establishments* and equipment.

1. Ponds

   Ponds are generally large and may be earthen based or be fitted with plastic liners. These characteristics together with the large volumes of water make cleaning prior to decontamination difficult and high organic loads may affect many chemical *disinfectants*. Ponds should be drained of water and have as much organic matter removed prior to *disinfection*. Earthen ponds should be dried thoroughly and lime compounds applied to raise pH and aid the inactivation of *pathogenic agents*. Cultivation of the base of unlined ponds will also aid in incorporation of liming compounds and drying.

2. Tanks

   Tank construction material (e.g. fibreglass, concrete or plastic) will determine the type of *disinfection* method used. Bare concrete tanks are susceptible to corrosion by acids and potential damage by high pressure sprayers. They are also porous and therefore require longer application of chemicals to ensure *disinfection*. Plastic, painted and fibreglass tanks are more easily disinfected because they have smooth, non-porous surfaces that facilitate thorough cleaning and are resistant to most chemicals.

   Prior to *disinfection*, water should be drained from tanks. Tank equipment should be removed for separate cleaning and *disinfection*, and all organic waste and debris removed. Tank surfaces should be washed using high-pressure sprayers or mechanical scrubbing with detergent to remove fouling such as algae and biofilms. Heated water may be used to enhance the cleaning process. Any excess water should be drained before application of *disinfectants*.

   When *disinfectants* are applied to vertical surfaces, care should be taken to ensure that adequate contact time is maintained before the *disinfectant* is drained. Following *disinfection*, tanks should be rinsed to remove all residues and allowed to dry completely.

3. Pipes

   Disinfection of pipes may be difficult due to lack of access. Pipe construction material should be taken into consideration when selecting the *disinfection* method.

   Pipes can be cleaned effectively through the use of alkaline or acid solutions, or foam projectile pipe cleaning systems. Effective *disinfection* in pipes requires the removal of biofilms, followed by flushing of the resulting particulate matter and thorough rinsing.

   Once pipes are cleaned, chemical *disinfectants* or circulation of heated water can be used. All steps require pipes to be fully filled so that internal surfaces are treated.

4. Cage nets and other fibrous materials

Nets used in cage culture are often large, difficult to handle, have significant levels of biofouling and are usually made from fibrous materials that trap organic matter and moisture. Due to the difficulty associated with disinfecting large nets and their close contact with fish populations they are considered to be high *risk* items that should be dedicated to a single *aquaculture establishment* or area.

Once the net has been removed from the water, it should be transferred directly to the net washing site. Nets should be thoroughly cleaned prior to *disinfection* to remove organic matter and aid in the penetration of chemical *disinfectants*. Cleaning of nets is best achieved by first removing gross biofouling and then washing with a detergent solution.

Following cleaning, nets may be disinfected by complete immersion in chemical *disinfectants* or heated water. Treatment duration should be sufficient to allow penetration into net material. Following *disinfection*, nets should be dried before storage. If rolled nets are not completely dry they will retain moisture which may enhance survival of the *pathogenic agent*.

Other fibrous materials such as wood, ropes and dip nets have characteristics similar to cage nets and they require special consideration. Wherever possible, it is recommended that equipment is site specific if it includes fibrous material.

5. Vehicles

The *risk* associated with *vehicles* will be determined by their use, e.g. transportation of mortalities, live *aquatic animals*, harvested *aquatic animals*. All potentially contaminated internal and external surfaces should be disinfected. *S*pecial consideration should be given to high *risk* areas such as the internal surface of *containers*, pipes, transportation water and waste. Corrosive *disinfectants* should be avoided or corrosive residues removed following *disinfection* by thorough rinsing. Oxidative compounds such as chlorines are the most commonly used *disinfectants* for vehicles.

6. Buildings

Aquaculture establishments include buildings for culture, harvesting and processing of *aquatic animals*, and other buildings associated with storage of feed and equipment.

The approach to *disinfection* may vary depending on the structure of the building and degree of contact with contaminated material and equipment.

Buildings should be designed to allow effective cleaning and thorough application of *disinfectants* to all internal surfaces. Some buildings will contain complex piping, machinery and tank systems that may be difficult to disinfect. Wherever possible, buildings should be cleared of debris and emptied of equipment, prior to *disinfection*.

Misting or foaming agents are options for *disinfection* of complex areas and vertical surfaces. Fumigation can be considered for large or difficult to access areas if buildings can be adequately sealed.

7. Containers

Containers range from simple plastic bins used to transport harvested *aquatic animal products* or dead *aquatic animals* through to complex tank systems used for the transport of live *aquatic animals*.

Containers are generally manufactured using smooth non-porous material (i.e. plastic, steel) which can be easily disinfected. They should be considered high *risk* items because they are in close contact with *aquatic animals* or their *products* (e.g. blood, diseased *aquatic animals*). In addition the need to move them between locations makes them potential fomites for the spread of *pathogenic agents*. In the case of transport of live *aquatic animals*, *containers* may also have pipes and pumping systems and confined spaces that should also be disinfected.

Annex 7 (contd)

All water should be drained from the *container* and any *aquatic animals*, faecal matter and other organic material removed by flushing with clean water. All pipes and associated pumps should also be inspected and flushed. *Containers* should then be washed using appropriate chemical detergents combined with high-pressure water cleaners or mechanical scrubbing.

All internal and external surfaces of *containers* should be treated using an appropriate *disinfection* method. They should then be rinsed and inspected to ensure there are no organic residues and stored in a manner that allows them to drain and dry quickly.

8. Boats

All boats should undergo routine *disinfection* to ensure that they do not transfer *pathogenic agents*. The level of contamination of boats will be determined by their use. Boats used to harvest or to remove dead *aquatic animals* from *aquaculture* sites should be considered as high *risk*. Organic material should be regularly removed from decks and work areas.

As part of the *disinfection* planning process, an assessment should be made to identify high *risk* areas such as in and around machinery, holding tanks, bilges and pipes. All loose equipment should be removed prior to *disinfection*. Additional procedures should be developed for well-boats because of their potential to transfer *pathogenic agents* through the discharge of contaminated water. Where there is a *risk* of *pathogenic agent*, effluent water from well-boats should be disinfected prior to discharge (refer to Article 4.3.10.).

Where possible, boats should be placed on land for *disinfection* in order to limit waste water entering the aquatic environment and to allow access to hull areas. Biofouling organisms that may act as mechanical carriers or intermediate hosts should be removed.

Where boats cannot be removed to land, a *disinfection* method should be chosen that minimises the discharge of toxic chemicals into the aquatic environment. Divers should be used to inspect and clean hulls. Where appropriate, mechanical methods such as high-pressure sprayers or steam cleaners should be considered as an alternative to chemical *disinfection* for cleaning above and below the water-line. Fumigation may also be considered for large areas if they can be adequately sealed.

9. Biofilters

Biofilters associated with closed or semi-closed production systems are an important control point for *disease*. Biofilters are designed to maintain a colony of beneficial bacteria used to enhance water quality. The conditions that support these bacteria also enhance survival of some *pathogenic agents* should they be present. It is normally not possible to disinfect biofilters without also destroying beneficial bacteria. Therefore potential water quality issues should be taken into account when planning strategies for *disinfection* of biofilters.

When disinfecting biofilters the system should be drained, organic residues removed and surfaces cleaned. All filters should be removed for separate *disinfection*.

Disinfection of biofilter systems can be undertaken by modifying water pH levels (using either acid or alkaline solutions). Where this is undertaken, the pH levels must be sufficient to inactivate the target pathogen, but should not be corrosive to pumps and equipment within the biofilter system. Alternatively, the biofilter can be completely dismantled, including removal of biofiter substrate, and the components cleaned and *disinfectants* applied separately. In the case of emergency *disease* response, the latter procedure is recommended. The biofilter substrate should be replaced if it cannot be effectively disinfected. Biofilter systems should be thoroughly rinsed before re-stocking.

10. Husbandry equipment

Aquaculture establishments will normally have a range of husbandry equipment items that come into close contact with *aquatic animals* and have potential to act as fomites. Examples include graders, automatic vaccinators and fish pumps.

The general principles described in Article 4.3.4. should be applied to *disinfection* of husbandry equipment. Each item should be examined to identify areas that come into close contact with *aquatic animals* and where organic material accumulates. If required, equipment should be dismantled to allow adequate cleaning and application of *disinfectants.*

Article 4.3.10.

Personal equipment

Disinfection of personal equipment should consider the level of *risks* associated with previous use. Where possible, personal equipment should be site specific to avoid the need for regular *disinfection*.

Equipment should be chosen which is non-absorbent and easy to clean. All staff entering a production area should use protective clothing that is clean and uncontaminated. On entry and exit of production areas boots should be cleaned and disinfected. When footbaths are used they should incorporate a cleaning procedure to remove accumulations of mud, be sufficiently deep to cover boots, use a *disinfectant* solution that is resistant to organic matter and be regularly refreshed with a new solution.

High *risk* equipment such as dive equipment requires special attention because it may be exposed to very high levels of contaminated material and is often susceptible to chemical corrosion. Frequent rinsing of equipment will assist in reducing build-up of organic matter and make *disinfection* more efficient. Equipment should be allowed to dry thoroughly to ensure that moist microenvironments that may harbour pathogens are minimised.

Article 4.3.11.

Disinfection of water

Aquaculture establishments may need to disinfect water, as a general biosecurity measure for intake water, to exclude entry of target *pathogenic agents*, or to eliminate pathogens in effluent water. The most appropriate *disinfection* method will differ depending on the *disinfection* objective and the characteristics of the water to be disinfected.

Exclusion of *aquatic animals* and removal of suspended solids from the water to be treated are essential prior to the application of *disinfectants*. Pathogens are known to adhere to organic and inorganic matter and removal of suspended solids can significantly reduce loading of *pathogenic agents* in water. Removal of suspended solids can be achieved by filtration or settlement of suspended material. The most suitable filtration system will depend on the initial quality of water, volumes to be filtered, capital and operating costs and reliability.

Physical (e.g. UV irradiation) and chemical (e.g. ozone, chlorine and chlorine dioxide) *disinfectants* are commonly used to disinfect water. Suspended solids should be removed prior to the application of these *disinfectants* because organic matter may inhibit oxidative *disinfection* processes and suspended solids inhibit UV transmission and reduce efficacy of UV irradiation by shielding pathogenic agents. A combination of methods may be beneficial where they are synergistic or where a level of redundancy is required.

It is essential to monitor the efficacy of water *disinfection*. This can be achieved by direct testing for pathogens of concern, indirect monitoring of indicator organisms or monitoring of residual levels of *disinfectants*.

Management of chemical residues is important to avoid toxic effects on *aquatic animals*. For example, residuals formed between ozone and seawater such as bromide compounds are toxic to early life stages of *aquatic animals* and may be removed using charcoal filtration. Residual chlorine should be removed from water by chemical deactivation or off gassing.

Annex 8

Proposed Restructure of Section 4 'Disease prevention and control' of the *Aquatic Code*

CURRENT CHAPTER	COMMENT	PROPOSED NEW CHAPTER
Section 4 – Disease prevention and control		
	Introduction to the chapters in this section.	*4.1. Introduction to disease prevention and control*
4.1. Zoning and compartmentalisation	Requires revision to improve readability and clarity on the general principles for establishing zones and compartments.	*4.2. Zoning and compartmentalisation* (revised Chapter 4.1.)
	Develop a new chapter specific to the application of zoning to provide clearer guidance on establishing zones for trade and disease control purposes. Would integrate with other chapters.	*4.3. Application of zoning* (new chapter)
4.2. Application of compartmentalisation	Requires revision to improve readability and clarity and to improve guidance for establishing compartments for trade purposes. Would integrate with other chapters e.g. biosecurity, disinfection.	*4.4. Application of compartmenta-lisation* (revised Chapter 4.2.)
	Develop a new chapter on principles of aquaculture biosecurity. Would cover key approaches to biosecurity planning such as risk analysis and identification of transmission pathways. Would integrate with other chapters e.g. disinfection, compartmentalisation.	*4.5. Aquaculture biosecurity* (new chapter)
4.3. General recommendations on disinfection	Currently under revision to provide more detailed recommendations on the principles of disinfection.	*4.6. Disinfection of aquaculture establishments and equipment* (revised Chapter 4.3. under development)
4.4. Recommendations for surface disinfection of salmonid eggs	New chapter adopted in 2015. If amended in the future consider changes suggested at Oct 2015 AAC meeting.	*4.7. Recommendations for surface disinfection of salmonid eggs*
4.5. Contingency planning	Requires substantial revision to provide adequate guidance on the principles of contingency planning and emergency response. Required to support articles in each disease-specific chapter on returning to freedom following an outbreak. Would integrate with other chapters e.g. biosecurity, disinfection.	*4.10. Emergency disease preparedness* (new chapter)
4.6. Fallowing	Delete this chapter and integrate relevant information in the proposed new chapter on biosecurity.	To be included in proposed new *Chapter 4.4. Aquaculture biosecurity*
4.7. Handling, disposal and treatment of aquatic animal waste	May require some revision to integrate with other new and revised chapters in this section (e.g. emergency disease preparedness, disinfection) and ensure recommendations are sound.	*4.8. Handling, disposal and treatment of aquatic animal waste*
4.8. Control of pathogenic agents in aquatic animal feed	Recently revised and adopted (2015). Would integrate with other chapters e.g. biosecurity.	*4.9. Control of pathogenic agents in aquatic animal feed*

Annex 9

CHAPTER 5.1.

GENERAL OBLIGATIONS RELATED TO CERTIFICATION

[...]

Article 5.1.4.

Responsibilities in case of an incident related to importation

1) *International trade* involves a continuing ethical responsibility. Therefore, if within a reasonable period subsequent to an export taking place, the *Competent Authority* becomes aware of the appearance or reappearance of a *disease* that has been specifically included in the *international aquatic animal health certificate* or other *disease* of potential epidemiological importance to the *importing country* there is an obligation for the *Competent Authority* to notify the *importing country*, so that the imported *commodities* may be inspected or tested and appropriate action be taken to limit the spread of the *disease* should it have been inadvertently introduced.

~~2) If a *disease* condition appears in imported *aquatic animals* within a reasonable period after importation, the *Competent Authority* of the *exporting country* should be informed so as to enable an investigation to be made, because this may be the first available information on the occurrence of the *disease* in a previously free *aquatic animal* population. The *Competent Authority* of the *importing country* should be informed of the result of the investigation because the source of *infection* may not be in the *exporting country*.~~

2~~3~~) If a *disease* appears in *aquatic animals* in an *importing country* and is associated with importation of *commodities*, the *Competent Authority* of the *exporting country* should be informed. This will enable the *exporting country* to investigate as this may be the first available information on the occurrence of the *disease* in a previously free *aquatic animal* population.

3~~4~~) In case of suspicion, on reasonable grounds, that an *international aquatic animal health certificate* may be fraudulent, the *Competent Authority* of the *importing country* and *exporting country* should conduct an investigation. Consideration should also be given to notifying any third country(ies) that may have been implicated. All associated consignments should be kept under official control, pending the outcome of the investigation. *Competent Authorities* of all countries involved should fully cooperate with the investigation. If the *international aquatic animal health certificate* is found to be fraudulent, every effort should be made to identify those responsible so that appropriate action can be taken in accordance with the relevant legislation.

[...]

— Text deleted.

Annex 10

CHAPTER 9.X.

ACUTE HEPATOPANCREATIC NECROSIS DISEASE

Article 9.X.1.

For the purposes of the *Aquatic Code*, acute hepatopancreatic necrosis disease (AHPND) means *infection* with strains of the bacteria *Vibrio parahaemolyticus* carrying one or more extrachromosal plasmid(s) that encode for a toxin (Pir^{vp}) that induces AHPND histopathological changes in the hepatopancreas (Vp_{AHPND}). *V. parahaemolyticus* is classified as a member of the *V. harveyi* clade.

[Information on methods for diagnosis are provided in the *Aquatic Manual*.]

Article 9.X.2.

Scope

The recommendations in this chapter apply to the following *susceptible species* which meet the criteria for listing species as susceptible in Chapter 1.5.: white leg shrimp (*Penaeus vannamei*) and giant tiger prawn (*Penaeus monodon*).

For the purposes of this chapter, the terms shrimp and prawn are used interchangeably.

Article 9.X.3.

Importation or transit of aquatic animals and aquatic animal products for any purpose from a country, zone or compartment not declared free from acute hepatopancreatic necrosis disease

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* from the species referred to in Article 9.X.2. which are intended for any purpose and which 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);

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

   c) pasteurised crustacean products that have been subjected to heat treatment at 63˚C for at least 30 minutes (or any time/temperature equivalent which has been demonstrated to inactivate Vp_{AHPND});

   d) crustacean oil;

   e) crustacean *meal*;

   f) chemically extracted chitin.]

2) When authorising the importation or transit of *aquatic animals* and *aquatic animal products* of a species referred to in Article 9.X.2., other than those referred to in point 1 of Article 9.X.3., *Competent Authorities* should require the conditions prescribed in Articles 9.X.9 to 9.X.11. relevant to the AHPND status of the *exporting country*, *zone* or *compartment*.

3) When considering the importation or transit of *aquatic animals* and *aquatic animal products* of a species not covered in Article 9.X.2. but which could reasonably be expected to pose a *risk* of spread of 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 assessment.

Annex 10 (contd)

Article 9.X.4.

Country free from acute hepatopancreatic necrosis disease

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.X.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.X.2. are present and *basic biosecurity conditions* have been continuously met for at least the last two years;

OR

2) any of the *susceptible species* referred to in Article 9.X.2. are present and the following conditions have been met:

   a) there has been no observed occurrence of the *disease* for at least the last ten years despite conditions that are conducive to its clinical expression (as described in the corresponding chapter of the *Aquatic Manual*); and

   b) *basic biosecurity conditions* have been continuously met for at least the last two years;

OR

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

   a) *basic biosecurity conditions* have been continuously met for at least the last two years; and

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

OR

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

   a) on detection of the *disease*, the affected area was declared an *infected zone* and a *protection zone* was established; and

   b) infected populations have been destroyed or removed from the *infected zone* by means that minimise the *risk* of further spread of the *disease*, and the appropriate *disinfection* procedures (as described in Chapter 4.3.) have been completed; and

   c) previously existing *basic biosecurity conditions* have been reviewed and modified as necessary and have continuously been in place since eradication of the *disease*; and

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

In the meantime, part or all of the non-affected area may be declared a free *zone* provided that such a part meets the conditions in point 3 of Article 9.X.5.

Article 9.X.5.

Zone or compartment free from acute hepatopancreatic necrosis disease

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

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

1) none of the *susceptible species* referred to in Article 9.X.2. are present in the *zone* or *compartment* and *basic biosecurity conditions* have been continuously met for at least the last two years;

OR

2) any of the *susceptible species* referred to in Article 9.X.2. are present in the *zone* or *compartment* and the following conditions have been met:

   a) there has not been any observed occurrence of the *disease* for at least the last ten years despite conditions that are conducive to its clinical expression (as described in the corresponding chapter of the *Aquatic Manual*); and

   b) *basic biosecurity conditions* have been continuously met for at least the last two years;

OR

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

   a) *basic biosecurity conditions* have been continuously met for at least the last two years; and

   b) *targeted surveillance*, as described in Chapter 1.4., has been in place, in the *zone* or *compartment*, for at least the last two years without detection of AHPND;

OR

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

   a) on detection of the *disease*, the affected area was declared an *infected zone* and a *protection zone* was established; and

   b) infected populations have been destroyed or removed from the *infected zone* by means that minimise the *risk* of further spread of the *disease*, and the appropriate *disinfection* procedures (as described in Chapter 4.3.) have been completed; and

   c) previously existing *basic biosecurity conditions* have been reviewed and modified as necessary and have continuously been in place since eradication of the *disease*; and

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

Article 9.X.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.X.4. or 9.X.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.X.4. or 9.X.5. (as relevant) may discontinue *targeted surveillance* and maintain its status as free from AHPND provided that conditions that are conducive to clinical expression of AHPND, as described in the corresponding chapter of the *Aquatic Manual*, exist, and *basic biosecurity conditions* are continuously maintained.

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

Annex 10 (contd)

Article 9.X.7.

Importation of aquatic animals and aquatic animal products from a country, zone or compartment declared free from acute hepatopancreatic necrosis disease

When importing *aquatic animals* and *aquatic animal products* of species referred to in Article 9.X.2. 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* certifying that, on the basis of the procedures described in Articles 9.X.4. or 9.X.5. (as applicable) and 9.X.6., the place of production of the *aquatic animals* and *aquatic animal products* is a country, *zone* or *compartment* declared free from AHPND.

The *certificate* should be in accordance with the Model Certificate in Chapter 5.11.

This Article does not apply to *commodities* listed in point 1 of Article 9.X.3.

Article 9.X.8.

Importation of live aquatic animals for aquaculture from a country, zone or compartment not declared free from acute hepatopancreatic necrosis disease

1) When importing, for *aquaculture*, live *aquatic animals* of species referred to in Article 9.X.2. 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, apply the following *risk* mitigation measures:

   a) the direct delivery to and lifelong holding of the consignment in biosecure facilities for continuous isolation from the local environment; and

   b) the treatment of water used in transport and of all effluent and waste materials in a manner that ensures inactivation of *Vp*$_{AHPND}$.

2) If the intention of the introduction is the establishment of a new stock, relevant aspects of the Code of Practice on the Introductions and Transfers of Marine Organisms of the International Council for the Exploration of the Seas (ICES) should be considered.

3) For the purposes of the *Aquatic Code*, relevant aspects of the ICES Code (full version see: http://www.ices.dk/publications/our-publications/Pages/Miscellaneous.aspx) may be summarised to the following points:

   a) identify stock of interest (cultured or wild) in its current location;

   b) evaluate stock health and disease history;

   c) take and test samples for *Vp*$_{AHPND}$, pests and general health/disease status;

   d) import of a founder (F-0) population and quarantine in a secure facility;

   e) produce F-1 generation from the F-0 stock in *quarantine*;

   f) culture F-1 stock and at critical times in its development (life cycle) sample and test for *Vp*$_{AHPND}$ and perform general examinations for pests and general health/disease status;

   g) if *Vp*$_{AHPND}$ is not detected, pests are not present, and the general health/disease status of the stock is considered to meet the *basic biosecurity conditions* of the *importing country*, *zone* or *compartment*, the F-1 stock may be defined as AHPND free or specific pathogen free (SPF) for *Vp*$_{AHPND;}$

   h) release SPF F-1 stock from *quarantine* for *aquaculture* or stocking purposes in the country, *zone* or *compartment*.

4) With respect to point 3 e), *quarantine* conditions should be conducive to multiplication of the pathogen and eventually to clinical expression. If *quarantine* conditions are not suitable for pathogen multiplication and development, the recommended diagnostic approach might not be sensitive enough to detect low *infection* level.

This Article does not apply to *aquatic animals* listed in point 1 of Article 9.X.3.

Article 9.X.9.

Importation of aquatic animals and aquatic animal products for processing for human consumption from a country, zone or compartment not declared free from acute hepatopancreatic necrosis disease

When importing, for processing for human consumption, *aquatic animals* or *aquatic animal products* of species referred to in Article 9.X.2. 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 processing into one of the products referred to in point 1 of Article 9.X.3., or products described in point 1 of Article 9.X.11., or other products authorised by the *Competent Authority*; and

2) water used in transport and all effluent and waste materials from the processing are treated in a manner that ensures inactivation of Vp_{AHPND}; or is disposed in a manner that prevents contact of waste with *susceptible species*.

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

Article 9.X.10.

Importation of live aquatic animals intended for use in animal feed, or for agricultural, industrial or pharmaceutical use, from a country, zone or compartment not declared free from acute hepatopancreatic necrosis disease

When importing, for use in animal *feed* or for agricultural, industrial or pharmaceutical use, live *aquatic animals* of species referred to in Article 9.X.2. 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* facilities for slaughter and processing into products authorised by the *Competent Authority*; and

2) water used in transport and all effluent and waste materials from the processing are treated in a manner that ensures inactivation of Vp_{AHPND};.

This Article does not apply to *commodities* referred to in point 1 of Article 9.X.3.

Article 9.X.11.

Importation of aquatic animals and aquatic animal products for retail trade for human consumption from a country, zone or compartment not declared free from acute hepatopancreatic necrosis disease

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 [frozen peeled shrimp or decapod crustacea (shell off, head off)] which have been prepared and packaged for retail trade and which comply with Article 5.4.2.

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

Annex 10 (contd)

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

2) When importing *aquatic animals* or *aquatic animal products*, other than those referred to in point 1 above, of species referred to in Article 9.X.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.

- - - - - - - - - - - - - -

— Text deleted.

Annex 11

CHAPTER 9.2.

INFECTION WITH YELLOW HEAD VIRUS GENOTYPE 1

Article 9.2.1.

For the purposes of the *Aquatic Code*, infection with yellow head virus ~~(YHD)~~ means *infection* with yellow head virus genotype 1 (YHV1). YHV is classified as a species in the genus *Okavirus*, family *Roniviridae* and order *Nidovirales*.

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

Article 9.2.2.

Scope

The recommendations in this chapter apply to the following *susceptible species* which meet the criteria for listing species as susceptible in Chapter 1.5.: giant tiger prawn *(Penaeus monodon)*, white leg shrimp *(Penaeus vannamei)*, blue shrimp *(Penaeus stylirostris)*, dagger blade grass shrimp *(Palaemonetes pugio)* and Jinga shrimp *(Metapenaeus affinis)* ~~giant tiger prawn (*Penaeus monodon*), brown tiger prawn (*P. esculentus*) and Kuruma prawn (*P. japonicus*). These recommendations also apply to any other *susceptible species* referred to in the *Aquatic Manual* when traded internationally.~~

Article 9.2.3.

Importation or transit of aquatic animals and aquatic animal products for any purpose from a country, zone or compartment not declared free from infection with yellow head virus

1) *Competent Authorities* should not require any conditions related to *infection* with ~~YHD~~YHV1, regardless of the *infection* with ~~YHD~~YHV1 status of the *exporting country*, *zone* or *compartment*, when authorising the importation or transit of the following *aquatic animal products* from the species referred to in Article 9.2.2. which are intended for any purpose and which 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 equivalent);

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

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

   d) crustacean oil;

   e) crustacean *meal*;

   f) chemically extracted chitin.

2) When authorising the importation or transit of *aquatic animals* and *aquatic animal products* of a species referred to in Article 9.2.2., other than those referred to in point 1 of Article 9.2.3., *Competent Authorities* should require the conditions prescribed in Articles 9.2.7. to 9.2.11. relevant to the *infection* with ~~YHD~~YHV1 status of the *exporting country*, *zone* or *compartment*.

Annex 11 (contd)

3) When considering the importation or transit of *aquatic animals* and *aquatic animal products* of a species not covered in Article 9.2.2. but which could reasonably be expected to pose a *risk* of spread of *infection* with ~~YHD~~YHV1, the *Competent Authority* should conduct a *risk analysis* in accordance with the recommendations in Chapter 2.1. The *Competent Authority* of the *exporting country* should be informed of the outcome of this assessment.

Article 9.2.4.

Country free from infection with yellow head virus

If a country shares a *zone* with one or more other countries, it can only make a *self-declaration of freedom* from *infection* with ~~YHD~~YHV1 if all the areas covered by the shared water bodies are declared countries or *zones* free from *infection* with ~~YHD~~YHV1 (see Article 9.2.5.).

As described in Article 1.4.6., a country may make a *self-declaration of freedom* from *infection* with ~~YHD~~YHV1 if:

1) none of the *susceptible species* referred to in Article 9.2.2. are present and *basic biosecurity conditions* have been continuously met for at least the last two years;

OR

2) any of the *susceptible species* referred to in Article 9.2.2. are present and the following conditions have been met:

   a) there has been no observed occurrence of the *disease* for at least the last ten years despite conditions that are conducive to its clinical expression, as described in the corresponding chapter of the *Aquatic Manual*; and

   b) *basic biosecurity conditions* have been continuously met for at least the last two years;

OR

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

   a) *basic biosecurity conditions* have been continuously met for at least the last two years; and

   b) *targeted surveillance*, as described in Chapter 1.4., has been in place for at least the last two years without detection of *infection* with ~~YHD~~YHV1;

OR

4) it previously made a *self-declaration of freedom* from *infection* with ~~YHD~~YHV1 and subsequently lost its *disease* free status due to the detection of *infection* with ~~YHD~~YHV1 but the following conditions have been met:

   a) on detection of the *disease*, the affected area was declared an *infected zone* and a *protection zone* was established; and

   b) infected populations have been destroyed or removed from the *infected zone* by means that minimise the *risk* of further spread of the *disease*, and the appropriate *disinfection* procedures (as described in the *Aquatic Manual*) have been completed; and

   c) previously existing *basic biosecurity conditions* have been reviewed and modified as necessary and have continuously been in place since eradication of the *disease*; and

d) *targeted surveillance*, as described in Chapter 1.4., has been in place for at least the last two years without detection of *infection* with ~~YHD~~YHV1.

In the meantime, part or all of the non-affected area may be declared a free *zone* provided that such a part meets the conditions in point 3 of Article 9.2.5.

Article 9.2.5.

Zone or compartment free from infection with yellow head virus

If a *zone* or *compartment* extends over more than one country, it can only be declared a *zone* or *compartment* free from *infection* with ~~YHD~~YHV1 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 ~~YHD~~YHV1 may be declared free by the *Competent Authority(ies)* of the country(ies) concerned if:

1) none of the *susceptible species* referred to in Article 9.2.2. are present in the *zone* or *compartment* and *basic biosecurity conditions* have been continuously met for at least the last two years;

OR

2) any of the *susceptible species* referred to in Article 9.2.2. are present in the *zone* or *compartment* and the following conditions have been met:

   a) there has not been any observed occurrence of the *disease* for at least the last ten years despite conditions that are conducive to its clinical expression (as described in the corresponding chapter of the *Aquatic Manual*); and

   b) *basic biosecurity conditions* have been continuously met for at least the last two years;

OR

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

   a) *basic biosecurity conditions* have been continuously met for at least the last two years; and

   b) *targeted surveillance*, as described in Chapter 1.4., has been in place, in the *zone* or *compartment*, for at least the last two years without detection of *infection* with ~~YHD~~YHV1;

OR

4) it previously made a *self-declaration of freedom* from *infection* with ~~YHD~~YHV1 for a *zone* and subsequently lost its disease status due to the detection of *infection* with ~~YHD~~YHV1 in the *zone* but the following conditions have been met:

   a) on detection of the *disease*, the affected area was declared an *infected zone* and a *protection zone* was established; and

   b) infected populations have been destroyed or removed from the *infected zone* by means that minimise the *risk* of further spread of the *disease*, and the appropriate *disinfection* procedures (as described in the *Aquatic Manual*) have been completed; and

   c) previously existing *basic biosecurity conditions* have been reviewed and modified as necessary and have continuously been in place since eradication of the *disease*; and

Annex 11 (contd)

d) *targeted surveillance*, as described in Chapter 1.4., has been in place for at least the last two years without detection of *infection* with ~~YHD~~YHV1.

Article 9.2.6.

Maintenance of free status

A country, *zone* or *compartment* that is declared free from *infection* with ~~YHD~~YHV1 following the provisions of points 1 or 2 of Articles 9.2.4. or 9.2.5. (as relevant) may maintain its status as free from *infection* with ~~YHD~~YHV1 provided that *basic biosecurity conditions* are continuously maintained.

A country, *zone* or *compartment* that is declared free from *infection* with ~~YHD~~YHV1 following the provisions of point 3 of Articles 9.2.4. or 9.2.5. (as relevant) may discontinue *targeted surveillance* and maintain its status as free from *infection* with ~~YHD~~YHV1 provided that conditions that are conducive to clinical expression of *infection* with ~~YHD~~YHV1, as described in the corresponding chapter of the *Aquatic Manual*, exist, and *basic biosecurity conditions* are continuously maintained.

However, for declared free *zones* or *compartments* in infected countries and in all cases where conditions are not conducive to clinical expression of *infection* with ~~YHD~~YHV1, *targeted surveillance* needs to be continued at a level determined by the *Aquatic Animal Health Service* on the basis of the likelihood of *infection*.

Article 9.2.7.

Importation of aquatic animals and aquatic animal products from a country, zone or compartment declared free from infection with yellow head virus

When importing *aquatic animals* and *aquatic animal products* of species referred to in Article 9.2.2. from a country, *zone* or *compartment* declared free from *infection* with ~~YHD~~YHV1, the *Competent Authority* of the *importing country* should require that the consignment be accompanied by an *international aquatic animal health certificate* issued by the *Competent Authority* of the *exporting country* or a *certifying official* approved by the *importing country* certifying that, on the basis of the procedures described in Articles 9.2.4. or 9.2.5. (as applicable) and 9.2.6., the place of production of the *aquatic animals* and *aquatic animal products* is a country, *zone* or *compartment* declared free from *infection* with ~~YHD~~YHV1.

The *certificate* should be in accordance with the Model Certificate in Chapter 5.11.

This Article does not apply to *commodities* listed in point 1 of Article 9.2.3.

Article 9.2.8.

Importation of live aquatic animals for aquaculture from a country, zone or compartment not declared free from infection with yellow head virus

1) When importing, for *aquaculture*, live *aquatic animals* of species referred to in Article 9.2.2. from a country, *zone* or *compartment* not declared free from *infection* with ~~YHD~~YHV1, the *Competent Authority* of the *importing country* should assess the *risk* and, if justified, apply the following *risk* mitigation measures:

   a) the direct delivery to and lifelong holding of the consignment in biosecure facilities for continuous isolation from the local environment; and

   b) the treatment of water used in transport and all effluent and waste materials in a manner that ensures inactivation of YHV1.

2) If the intention of the introduction is the establishment of a new stock, relevant aspects of the Code of Practice on the Introductions and Transfers of Marine Organisms of the International Council for the Exploration of the Seas (ICES) should be considered.

3) For the purposes of the *Aquatic Code*, relevant aspects of the ICES Code (full version see: http://www.ices.dk/publications/our-publications/Pages/Miscellaneous.aspx) may be summarised to the following points:

   a) identify stock of interest (cultured or wild) in its current location;

   b) evaluate stock health and disease history;

   c) take and test samples for YHV1, pests and general health/disease status;

   d) import of a founder (F-0) population and quarantine in a secure facility;

   e) produce F-1 generation from the F-0 stock in *quarantine*;

   f) culture F-1 stock and at critical times in its development (life cycle) sample and test for *infection* with ~~YHD~~YHV1 and perform general examinations for pests and general health/disease status;

   g) if *infection* with ~~YHD~~YHV1 is not detected, pests are not present, and the general health/disease status of the stock is considered to meet the *basic biosecurity conditions* of the *importing country*, *zone* or *compartment*, the F-1 stock may be defined as *infection* with ~~YHD~~YHV1 free or specific pathogen free (SPF) for *infection* with ~~YHD~~YHV1;

   h) release SPF F-1 stock from *quarantine* for *aquaculture* or stocking purposes in the country, *zone* or *compartment*.

4) With respect to point 3 e), *quarantine* conditions should be conducive to multiplication of the pathogen and eventually to clinical expression. If *quarantine* conditions are not suitable for pathogen multiplication and development, the recommended diagnostic approach might not be sensitive enough to detect low *infection* level.

This Article does not apply to *aquatic animals* listed in point 1 of Article 9.2.3.

Article 9.2.9.

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

When importing, for processing for human consumption, *aquatic animals* or *aquatic animal products* of species referred to in Article 9.2.2. from a country, *zone* or *compartment* not declared free from *infection* with ~~YHD~~YHV1, 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 9.2.3., or products described in point 1 of Article 9.2.11., or other products authorised by the *Competent Authority*; and

2) water used in transport and all effluent and waste materials from the processing are treated in a manner that ensures inactivation of YHV1 or is disposed in a manner that prevents contact of waste with *susceptible species*.

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

Annex 11 (contd)

Article 9.2.10.

Importation of live aquatic animals intended for use in animal feed, or for agricultural, industrial or pharmaceutical use, from a country, zone or compartment not declared free from infection with yellow head virus

When importing, for use in animal *feed* or for agricultural, industrial or pharmaceutical use, live *aquatic animals* of species referred to in Article 9.2.2. from a country, *zone* or *compartment* not declared free from *infection* with ~~YHD~~YHV1, the *Competent Authority* of the *importing country* should require that:

1) the consignment is delivered directly to, and held in, *quarantine* facilities for slaughter and processing into products authorised by the *Competent Authority*; and

2) water used in transport and all effluent and waste materials from the processing are treated in a manner that ensures inactivation of YHV1.

This Article does not apply to *commodities* referred to in point 1 of Article 9.2.3.

Article 9.2.11.

Importation of aquatic animals and aquatic animal products for retail trade for human consumption from a country, zone or compartment not declared free from infection with yellow head virus

1) *Competent Authorities* should not require any conditions related to *infection* with ~~YHD~~YHV1, regardless of the *infection* with ~~YHD~~YHV1 status of the *exporting country*, *zone* or *compartment*, when authorising the importation or transit of frozen peeled shrimp or decapod crustacea (shell off, head off) which have been prepared and packaged for retail trade and which comply with Article 5.4.2.

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

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

2) When importing *aquatic animals* or *aquatic animal products*, other than those referred to in point 1 above, of species referred to in Article 9.2.2. from a country, *zone* or *compartment* not declared free from *infection* with ~~YHD~~YHV1, the *Competent Authority* of the *importing country* should assess the *risk* and apply appropriate *risk* mitigation measures.

- - - - - - - - - - - - - -

Text deleted.

Annex 12

(NB: draft chapters for the *Aquatic Manual* are in English only.
Once adopted, the chapters are then translated into Spanish.)

CHAPTER 2.2.8.

INFECTION WITH YELLOW HEAD VIRUS GENOTYPE 1

1. Scope

For the purpose of this chapter, yellow head disease (YHD) is considered to be infection with yellow head virus genotype 1 (YHV1).

2. Disease information

2.1. Agent factors

2.1.1. Aetiological agent, agent strains

Yellow head virus genotype 1 (YHV1) is one of ~~six~~ eight known genotypes in the yellow head complex of viruses and is the only known agent causing YHD. YHV1 and other genotypes in the yellow head complex are classified by the International Committee on Taxonomy of Viruses as a single species (*Gill-associated virus*) in the genus *Okavirus*, family *Roniviridae*, order *Nidovirales* (Cowley *et al.*, 2012). Gill-associated virus (GAV) is designated as genotype 2. ~~GAV and~~ Four other ~~known~~ genotypes in the complex (genotypes 3–6) occur commonly in healthy *Penaeus monodon* in East Africa, Asia and Australia and are rarely or never associated with disease (Walker *et al.,* 2001, Wijegoonawardane *et al.,* 2008a). Recently, two new YHV-complex genotypes have been reported, one designated YHV7 was detected in diseased *P. monodon* in Australia (Mohr *et al*., 2015) and an eighth genotype was detected in *Fenneropenaeus chinensis* suspected of suffering from acute hepatopancreatic necrosis disease (Liu *et al*., 2014). There is evidence of genetic recombination between genotypes (Wijegoonawardane *et al.*, 2009).

YHV1 forms enveloped, rod-shaped particles 40–50 nm × 150–180 nm (Chantanachookin *et al.,* 1993; Wongteerasupaya *et al*., 1995). Envelopes are studded with prominent peplomers projecting approximately 11 nm from the surface. Nucleocapsids appear as rods (diameter 20–30 nm) and possess a helical symmetry with a periodicity of 5–7 nm. Virions comprise three structural proteins (nucleoprotein p20 and envelope glycoproteins gp64 and gp116) and a ~26 kb positive-sense single-stranded RNA genome.

2.1.2. Survival outside the host

YHV1 remains viable in aerated seawater for up to 72 hours (Flegel *et al.*, 1995b).

2.1.3. Stability of the agent (effective inactivation methods)

YHV1 can be inactivated by heating at 60°C for 15 minutes (Flegel *et al.*, 1995b). Little information is available on other inactivation methods but the virus appears to be susceptible to treatment with chlorine at 30 parts per million (0.03 mg ml^{-1}) (Flegel *et al.,* 1997).

Annex 12 (contd)

2.1.4. Life cycle

High multiplicity YHV1 infections in cell culture have not been reported. Infection at a multiplicity of infection of 0.001 in primary cultures of lymphoid organ cells has indicated that maximum viral titres are obtained 4 days post-infection (Assavalapsakul *et al*., 2003). Clinical signs of YHD occur in *P. monodon* within 7–10 days of exposure. YHV1 replicates in the cytoplasm of infected cells in which long filamentous pre-nucleocapsids are abundant and virions bud into cytoplasmic vesicles in densely packed paracrystalline arrays for egress at the cytoplasmic membrane (Chantanachookin *et al.,* 1993).

2.2. Host factors

2.2.1. Susceptible host species

~~YHD outbreaks have been reported in the~~ 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 ~~black~~ giant tiger prawn (*P. monodon*) and ~~the~~ white leg ~~Pacific~~ shrimp (*P. vannamei*) ~~(~~Chantanachookin *~~et al.~~*, ~~1993; Senapin *et al.*, 2010~~), ~~The Pacific~~ blue shrimp ~~prawn~~ (*P. stylirostris*), ~~the~~ daggerblade grass shrimp (*Palaemonetes pugio*), and ~~the~~ Jinga shrimp (*Metapenaeus affinis*) ~~also fulfil the criteria required for listing a species susceptible to infection with YHV1 according to Chapter 1.5 of *Aquatic Animal Health Code*~~. Natural infections have also been detected in the kuruma prawn (~~*P. japonicus*~~), white banana prawn (~~*P. merguiensis*~~), Pacific blue prawn (~~*P. stylirostris*~~), white prawn (~~*P. setiferus*~~), red endeavour prawn (~~*Metapenaeus ensis*~~), mysid shrimp (~~*Palaemon styliferus*~~) and krill (~~*Acetes* sp.~~). Other species of penaeid and palemonid shrimp and prawns and krill that have been reported to be susceptible to experimental infection include: brown tiger prawn (~~*P. esculentus*~~), brown prawn (~~*P. aztecus*~~); pink prawn, hopper and brown spotted prawn (~~*P. duorarum*~~), greentail prawn (~~*Metapenaeus bennettae*~~), Sunda river prawn (~~*Macrobrachium sintangense*~~), barred estuarine shrimp (~~*Palaemon serrifer*~~), the paste prawn (~~*Ascetes* sp.~~) and the daggerblade grass shrimp (~~*Palaemonetes pugio*~~) (Ma ~~*et al.,* 2009~~). There are variations in the susceptibility of different species to disease. Laboratory trials have shown that YHV can cause high mortality in ~~*P. monodon, P. vannamei, P. stylirostrus, P. aztecus, P. duorarum, M. sintangense, P. styliferus* and *P. serrifer~~ (Lightner ~~*et al.*~~, ~~1998~~; Longyant ~~*et al.*~~, ~~2005; 2006~~; Ma ~~*et al.,* 2009~~). A survey of 16 crab species collected from the vicinity of shrimp farms in Thailand detected no evidence of either natural infection or experimental susceptibility (Longyant ~~*et al.*~~, 2006). A critical review of susceptibility of crustaceans to yellow head disease and implications of inclusion in European legislation has been conducted (Stentiford ~~*et al.*~~, 2009). GAV has been detected in ~~*P. monodon* and *P. esculentus*~~ (Walker ~~*et al.,* 2001~~). To date, infections by other genotypes in the YHV complex have been detected only in ~~*P. monodon*~~ (Wijegoonawardane ~~*et al.*~~, 2008a). ~~*Metapenaeus brevicornis* and *P. aztecus*~~ also fulfil some of the criteria required for listing as susceptible but evidence was lacking to either confirm the identity of the pathogen under study as YHV1, to demonstrate a natural route of infection, or to definitively confirm an ~~'infected' status~~.

2.2.2. Species with incomplete evidence for susceptibility

Species for which there is incomplete evidence to fulfil the criteria for listing a species as susceptible to infection with YHV1 according to Chapter 1.5 of the *Aquatic Code* include: Sunda river prawn (*Macrobrachium sintangense*), yellow shrimp (*Metapenaeus brevicornis*), Carpenter prawn (*Palaemon serrifer*), Pacific blue prawn (*Palaemon styliferus*), northern brown shrimp (*Penaeus aztecus*), pink shrimp (*Penaeus duorarum*), kuruma prawn (*Penaeus japonicas*), white banana prawn (*Penaeus merguiensis*) and northern white shrimp (*Penaeus setiferus*). 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.

2.2.3.~~2.~~ Susceptible stages of the host

Penaeus monodon are susceptible to YHV1 infection beyond PL15 (Khongpradit *et al*., 1995). ~~Experimental infections with GAV indicate that larger (~20 g) *P. 54aponicas* are less susceptible to disease than smaller (~6–13 g) shrimp of the same species (Spann *et al*., 2000).~~

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

YHV1 ~~(genotype 1)~~ infections are usually detected only when disease is evident and whilst they do not occur commonly in healthy *P. monodon*, infections have been detected in healthy wild populations of *P. stylirostris* (Castro-Longoria *et al.,* 2008). During YHD outbreaks in aquaculture ponds, the YHV1 infection prevalence can be assumed to be high. Natural YHV1 infections have been detected in *P. 54aponicas, P. merguiensis, P. setiferus, M. ensis,* and *P. styliferus* (Cowley *et al.,* 2002; Flegel *et al*., 1995a; 1995b), but there is little information available on the natural prevalence. ~~Viruses in yellow head complex genotypes 2–6 are only known to occur commonly (prevalence up to 100%) in *P. monodon*, which appears to be the natural host (Walker *et al*., 2001; Wijegoonawardane *et al*., 2008a; 2009).~~

2.2.5.~~4.~~ Target organs and infected tissue

YHV1 targets tissues of ectodermal and mesodermal origin including lymphoid organ, haemocytes, haematopoietic tissue, gill lamellae and spongy connective tissue of the subcutis, gut, antennal gland, gonads, nerve tracts and ganglia (Chantanachookin *et al.,* 1993; Lightner, 1996).

2.2.6.5. Persistent infection with lifelong carriers

~~GAV persists as a chronic infection for at least 50 days in *P. esculentus* that survive experimental challenge (Spann *et al.*, 2003). The high prevalence of subclinical or chronic infection often found in healthy *P. monodon* infected with GAV (genotype 2) and genotypes 3–6 from postlarval stages onwardssuggests that these infections can perrsist for life (Walker *et al.*, 2001; Wijegoonawardane *et al.*, 2008a). There is also evidence that YHV (genotype 1) can persist in survivors of experimental infection (Longyant *et al.*, 2005; 2006).~~

YHV1 was detected by PCR in clinically normal wild P. stylirostris collected for surveillance purposes in the Gulf of California in 2003 (Casto-Longoria *et al*., 2008). The infectious nature of the YHV1 detected was confirmed by experimental infections.

2.2.7.~~6.~~ Vectors

There are no known vectors of YHV1.

2.2.8.7. Known or suspected wild aquatic animal carriers

There are no known or suspected wild aquatic animal carriers of YHV1. ~~Infection susceptibility and long term persistence indicate the potential for a wide range of wild penaeid and palaemonid shrimp to act as carriers.~~

2.3. Disease pattern

2.3.1. Transmission mechanisms

YHV1 infection can be transmitted horizontally by injection, ingestion of infected tissue, immersion in sea water containing tissue extracts filtered to be free of bacteria, or by co-habitation of naive shrimp with infected shrimp (Flegel *et al.*, 1995b; Lightner, 1996). Infection of shrimp has also been established by injection of extracts of paste prawns (*Acetes* sp.) collected from infected ponds (Flegel *et al.*, 1995a). ~~For GAV, vertical transmission of infection to progeny has been shown to occur from both male and female parents, possibly by surface contamination or infection of tissue surrounding fertilised eggs (Cowley *et al.,* 2002).~~ The dynamics of how YHV1 infection spreads within aquaculture ponds have not been studied. However, the rapid accumulation of mortalities during disease outbreaks suggests that horizontal transmission occurs very effectively.

2.3.2. Prevalence

The infection prevalence of yellow head complex viruses in healthy *P. monodon* (as detected by nested polymerase chain reaction [PCR]) can be high (50–100%) in farmed and wild populations in Australia, Asia and East Africa as well as in *L. vannamei* farmed in Mexico (Castro-Longoria *et al.,* 2008; Cowley *et al*., 2004; Sanchez-Barajas *et al.*, 2009; Walker *et al.*, 2001; Wijegoonawardane *et al.*, 2008a). The prevalence of individual genotypes varies according to the geographical origin of the shrimp. The use of detection methods less sensitive than nested PCR (e.g. histology, immunoblot, dot-blot, *in-situ* hybridisation), is likely in most cases to result in the real infection prevalence amongst populations of shrimp being underestimated.

2.3.3. Geographical distribution

YHD has been reported in Chinese Taipei, Indonesia, Malaysia, the Philippines, Sri Lanka, Thailand and Vietnam (Walker *et al.*, 2001). GAV and other genotypes in the yellow head complex have been detected in healthy *P. monodon* from Australia, Chinese Taipei, India, Indonesia, Malaysia, Mozambique, the Philippines, Thailand and Vietnam (Wijegoonawardane *et al.*, 2008a). YHV1 has also been detected in *P. vannamei* cultured in Mexico (Castro-Longoria *et al.,* 2008; Sanchez-Barajas *et al.*, 2009).

Annex 12 (contd)

2.3.4. Mortality and morbidity

With *P. monodon* being farmed in ponds, disease caused by YHV1 ~~(genotype 1)~~ can cause up to 100% mortality within 3–5 days of the first appearance of clinical signs (Chantanachookin *et al.,* 1993). Whilst mortalities can easily be induced by experimental exposure of *P. monodon* to YHV1 or GAV, bioassays have identified YHV1 to be far more virulent (~10^6-fold by lethal dose [LD_{50}] 50% end-point analysis) (Oanh *et al.,* 2011). Genotypes 3, 4, 5 and 6 have not yet been associated with disease (Wijegoonawardane *et al.*, 2008a). The pathogenicity of new YHV-complex genotypes from Australia and China (People's Rep. of) is still to be determined.

2.3.5. Environmental factors

Elevated virus infection levels accompanied by disease can be precipitated by physiological stress induced by sudden changes in pH or dissolved oxygen levels, or other environmental factors (Flegel *et al*., 1997). ~~The much higher virulence of YHV compared with GAV and other genotypes appears to ensure that the infection threshold required to cause disease is reached far more easily.~~

2.4. Control and prevention

2.4.1. Vaccination

No effective vaccination methods have been developed.

2.4.2. Chemotherapy

No effective commercial anti-viral product is yet available.

2.4.3. Immunostimulation

No scientifically confirmed reports.

2.4.4. Resistance breeding

Not reported.

2.4.5. Restocking with resistant species

All marine shrimp species farmed commercially appear to be susceptible to YHV1.

2.4.6. Blocking agents

Injection of shrimp with double-stranded (ds) RNA homologous to ORF1a/1b gene regions of YHV1 or GAV (thus targeting the genome length viral RNA) can inhibit viral replication and prevent mortalities following experimental challenge. The antiviral action of the dsRNA appears to involve the RNA interference (RNAi) pathway (Tirasophon *et al.,* 2007).

2.4.7. Disinfection of eggs and larvae

Not reported.

2.4.8. General husbandry practices

Specific pathogen free (SPF) or PCR-negative seedstock and biosecure water and culture systems may be used to reduce the risk of disease.

3. Sampling

3.1. Selection of individual specimens

For diagnosis during a disease outbreak, moribund shrimp collected from pond edges are the preferred source of material for examination. Apparently normal shrimp should also be collected from the same ponds. For surveillance for evidence of infection in populations of apparently healthy shrimp, life stages from mysis onwards (mysis, postlarvae [PL], juveniles or adults) can provide tissue sources useful for testing.

3.2. Preservation of samples for submission

Moribund shrimp (or tissue from moribund shrimp) should be snap-frozen on-site in a dry ice/alcohol slurry and preserved frozen in dry ice, liquid nitrogen or in a –80°C freezer. Freezing at or above –20°C is unsuitable.

Tissue samples for PCR screening should be preserved in ~~a minimum 3-fold excess of~~ 80–90% analytical/reagent-grade (absolute) ethanol. At least 10 times the volume of ethanol to tissue should be used. The use of lower grade (laboratory or industrial grade) ethanol is not recommended. Commercial RNA preservatives (e.g. RNAlater) may also be used.

Tissue samples for histology should be preserved in Davidson's fixative. Formalin (10%) in seawater may be a useful alternative. At least 10 times the volume of fixative to tissue should be used.

Tissues for electron microscopy should be sampled from live shrimp.

For guidance on sample preservation methods for the intended test methods, see Chapter 2.2.0.

3.3. Pooling of samples

For detecting YHV1 infection in large populations of shrimp, pooling of tissue samples is acceptable for screening or surveillance of batches of mysis to PL from a hatchery tank or batches of juvenile shrimp in a pond. For PCR analysis, pool size should be determined by tissue mass that can be processed without compromise in a single test. The total numbers of shrimp sampled, either as a single pool or as multiple smaller pools, are selected based on the infection prevalence expected and the required confidence limits of detection. Typically in populations comprising more than a 100,000 shrimp, if the prevalence of infection exceeds 5%, a total of 60 individuals tested in appropriate pool sizes will be required to detect YHV1 at a 95% confidence limit. However, definitive detection may be compromised if the YHV1 loads in the infected shrimp are very low or if tests less sensitive than two-step PCR or real-time PCR are employed. See also Chapter 2.2.0.

3.4. Best organs or tissues

In moribund shrimp suspected to be infected with YHV1, lymphoid organ and gill are the most suitable sample tissues. For screening or surveillance of juvenile or adult shrimp that appear grossly normal, lymphoid organ is preferred. Gills or haemolymph can be used for non-sacrificial sampling.

3.5. Samples/tissues that are not suitable

Not determined.

4. Diagnostic methods

4.1. Field diagnostic methods

4.1.1. Clinical signs

Shrimp from late PL stages onwards can be infected experimentally with YHV1. In cultured shrimp, infection can result in mass mortality occurring, usually in early to late juvenile stages. Moribund shrimp may exhibit a bleached overall appearance and a yellowish discoloration of the cephalothorax caused by the underlying yellow hepatopancreas, which may be exceptionally soft when compared with the brown hepatopancreas of a healthy shrimp. In many cases, the total loss of a pond crop occurs within a few days of the first appearance of shrimp showing gross signs of YHD (Chantanachookin *et al.,* 1993). Cessation of feeding, congregation of moribund shrimp at pond edges and a generally bleached appearance are always seen in YHD outbreaks. However, these disease features are not particularly distinctive for YHD, and in the absence of other more pathognomonic gross signs are not reliable even for preliminary diagnosis of YHD. ~~Gross signs of GAV disease include swimming near the surface and at the pond edges, cessation of feeding, a reddening of body and appendages, and pink to yellow discoloration of the gills (Spann *et al.*, 1997). However, these signs can occur commonly in response to various stressors and thus are not considered pathognomonic for GAV disease. Shrimp chronically infected with YHV or GAV display normal appearance and behaviour.~~

4.1.2. Behavioural changes

Exceptionally high feeding activity followed by an abrupt cessation of feeding may occur within 2–4 days of the appearance of gross clinical signs of disease and mortality. Moribund shrimp may congregate at pond edges near the surface (Chantanachookin *et al.,* 1993).

Annex 12 (contd)

4.2. Clinical methods

4.2.1. Gross pathology

See Section 4.1.

4.2.2. Clinical chemistry

None described.

4.2.3. Microscopic pathology

Fix the cephalothorax tissues of moribund shrimp suspected to be affected by YHD in Davidson's fixative, prepare tissue sections and stain with Meyer's haematoxylin and eosin (H&E) using standard histological procedures (Lightner, 1996). Examine tissues of ectodermal and mesodermal origin by light microscopy for the presence of moderate to large numbers of deeply basophilic, evenly stained, spherical, cytoplasmic inclusions approximately 2 µm in diameter or smaller (Chantanachookin *et al.,* 1993). Tissues of the lymphoid organ, stomach subcuticulum and gills are particularly informative.

4.2.4. Wet mounts

Fix whole shrimp or gill filaments overnight in Davidson's fixative (Lightner, 1996). After fixation, wash some gill filaments thoroughly with tap water to remove the fixative and stain with H&E (Lightner, 1996). After staining and dehydration, when the tissue is in xylene, place a gill filament on a microscope slide in a drop of xylene and, using a fine pair of needles (a stereo microscope is helpful), break off several secondary filaments. Replace the main filament in xylene where it can be stored indefinitely in a sealed vial as a permanent reference. Being careful not to let the xylene dry, tease apart the secondary filaments and remove any large fragments or particles that would thicken the mount unnecessarily. Add a drop of mounting fluid and a cover-slip and use light pressure to flatten the mount as much as possible. This procedure may also be used with thin layers of subcuticular tissue. Examine under a light microscope using a ×40 objective lens. For samples from YHD-affected shrimp, moderate to large numbers of deeply basophilic, evenly stained, spherical, cytoplasmic inclusions (approximately 2 µm in diameter or smaller) will be observed (Flegel *et al.,* 1997). Evidence of such pathology should be used to support results from haemolymph smears (see below) in making a presumptive diagnosis of YHD. As for the fixed tissues and gill filaments preserved in xylene, these whole-mount slides can be preserved as a permanent record.

If rapid results are required, the fixation step can be shortened to only 2 hours by replacing the acetic acid component of Davidson's fixative with a 50% dilution of concentrated HCl. For good fixation, this fixative should not be stored for more than a few days before use. After fixation, wash thoroughly to remove the fixative and check that the pH has returned to near neutral before staining. Do not fix for longer periods or above 25°C as this may result in excessive tissue damage that will make it difficult or impossible to identify specific pathology.

4.2.5. Electron microscopy/cytopathology

For transmission electron microscopy (TEM), the most suitable tissues of shrimp suspected to be infected with YHV1 infection are lymphoid organ and gills. For screening or surveillance of grossly normal shrimp, the most suitable tissue is lymphoid organ.

Stun live shrimp by immersion in iced water until just immobilised or kill by injection of fixative. Quickly dissect and remove small portions of target tissue (no larger than a few mm in diameter) and fix in at least 10 volumes of 6% glutaraldehyde held at 4°C and buffered with sodium cacodylate ($Na[CH_3]_2AsO_2 \cdot 3H_2O$) solution (8.6 g Na cacodylate, 10 g NaCl, distilled water to make 100 ml, adjusted to pH 7 with 0.2 N HCl) or phosphate solution (0.6 g $NaH_2PO_4 \cdot H_2O$, 1.5 g Na_2HPO_4, 1 g NaCl, 0.5 g sucrose, distilled water to make 100 ml, adjusted to pH 7 with 0.2 N HCl). Fix for at least 24 h prior to processing. For long-term storage in fixative at 4°C, reduce glutaraldehyde to 0.5–1.0%. Processing involves post-fixation with 1% osmium tetroxide, dehydration, embedding, sectioning and staining with uranyl acetate and lead citrate according to standard TEM reagents and methods (Lightner, 1996).

In the cytoplasm of cells infected with YHV1, both nucleocapsid precursors and complete enveloped virions are observed. Nucleocapsid precursors appear as long filaments approximately 15 nm in diameter that can vary markedly in length (80–450 nm) and that can sometimes be packed densely in paracrystalline arrays. Virions appear as rod-shaped, enveloped particles 40–50 nm × 150–180 nm with rounded ends and prominent projections (8–11 nm) extending from the surface. In the cell cytoplasm, virions are commonly seen to be localised or packed densely within intracellular vesicles. Virions may also be seen budding at the cytoplasmic membrane and in interstitial spaces. GAV virions and nucleocapsids are indistinguishable from YHV1 by TEM.

Lymphoid organ spheroids are commonly observed in healthy *P. monodon* chronically infected with YHV1 or GAV and lymphoid organ necrosis often accompanies disease (Spann *et al.*, 1997). However, spheroid formation and structural degeneration of lymphoid organ tissue also result from infection by other shrimp viruses (Lightner, 1996).

4.3. Agent detection and identification methods

4.3.1. Direct detection methods

4.3.1.1. Microscopic methods

4.3.1.1.1. Wet mounts

See Section 4.2.4.

4.3.1.1.2. Smears

See Section 4.2.5.

4.3.1.1.3. Fixed sections

See Section 4.2.3.

4.3.1.2. Agent isolation and identification

4.3.1.2.1. Cell culture/artificial media

Although primary shrimp cell culture methods are available, they are not recommended to isolate and identify YHV1 as a routine diagnostic method because of the high risk of them becoming contaminated with adventitious agents. No continuous cell lines suitable for YHV1 culture are yet available.

4.3.1.2.2. Antibody-based antigen detection methods

Reagents and protocols for detecting YHV1 proteins with antibodies have been published (Loh *et al.* 1998; Lu *et al.* 1994). Virions purified from haemolymph of experimentally infected shrimp have been used to produce antiserum in New Zealand white rabbits. From this antiserum, immunoglobulin (IgG) was purified using protein-G-linked columns and cross-reacting normal shrimp antigens were removed by adsorption to acetone-dried, ground shrimp muscle tissue and haemolymph. To detect YHV1 proteins by Western blotting, dilute 0.1 ml haemolymph collected from a live shrimp in an equal volume of citrate buffer and either run immediately or store at –80°C until used. Clarify 200 µl of the sample at 8000 ***g*** for 5 minutes and then pellet virions from the clarified supernatant by ultracentrifugation at 140,000 ***g*** for 5 minutes. Resuspend pellets in 100 µl 2 × loading buffer (2.5 ml 0.5 mM Tris/HCl pH 6.8, 4 ml 10% sodium dodecyl sulphate [SDS], 2 ml glycerol, 1 µl β-mercaptoethanol, 0.5 ml deionised distilled water) and heat at 95°C for 5 minutes. Load 10 µl sample onto a 5% SDS-polyacrylamide gel and electrophorese at 200 V. Blot the gel onto a 0.1 mm pore size nitrocellulose membrane in blotting buffer (3.03 g Tris-base, 14.4 g glycine, 200 ml methanol per litre) at 100 V for 1 hour. Rinse the membrane with phosphate buffered saline (PBS pH 7.4), block in 5% skim milk (in PBS) for 1 hour, and rinse with PBS for 5 minutes. Soak the membrane in a 1/1000 dilution of the anti-YHV1 antibody (IgG) for 1 hour, rinse three times with PBS for 5 minutes, and then soak for 1 hour in a 1/2500 dilution of goat anti-rabbit IgG-horseradish-peroxidase (HRP) conjugate. Rinse membrane three times with PBS for 5 minutes and then soak in HRP substrate 3,3',5,5'-tetramethylbenzidine , until blue-purple colour develops. Stop the reaction by soaking the membrane in distilled water. All incubations should be carried out at 25°C ± 2°C. Use a purified viral preparation as a positive control to identify positions of the YHV1 116 kDa, 64 kDa and 20 kDa structural proteins. The Western blot YHV1 detection sensitivity is approximately 0.4 ng YHV1 protein (≈ 10^6 virions).

Annex 12 (contd)

4.3.1.2.3. Molecular techniques

4.3.1.2.3.1 Reverse-transcription polymerase chain reaction (RT-PCR)

Three RT-PCR protocols are described. The first is a 1-step RT-PCR adapted from Wongteerasupaya *et al.* (1997) that can be used to detect YHV1 in shrimp affected by YHD. This protocol will detect YHV1 (highly virulent genotype first detected in Thailand in association with YHD) but not GAV or any of the other ~~three~~ genotypes currently recognised. The second is a more sensitive multiplex nested RT-PCR protocol adapted from Cowley *et al.* (2004). It can be used to differentiate YHV1 from GAV in diseased shrimp or for screening healthy carriers. ~~This test will not detect all six known genotypes and genotype 3 may generate a PCR product indistinguishable in size from that generated with GAV (genotype 2).~~ .The primary RT-PCR detected YHV7 (Mohr *et al*., 2015) both primary and nested steps detected the novel YHV genotype from China (Liu *et al*., 2014). The test is available in a suitably modified form from a commercial source (YHV/GAV IQ2000, GeneReach Biotechnology Corp., Chinese Taipei). However, this kit is not currently listed as having completed the OIE's formal process for validating and certifying commercial tests (a list of certified test kits and manufacturers is available on the OIE website: http://www.oie.int/en/our-scientific-expertise/registration-of-diagnostic-kits/background-information/). The third is a sensitive multiplex RT-nested PCR protocol described by Wijegoonawardane *et al.* (2008b). This test can be used for screening healthy shrimp for any of the six genotypes of the yellow head complex of viruses ~~(including YHV and GAV)~~, but will not discriminate between genotypes. Assignment of genotype can be achieved by nucleotide sequence analysis of the RT-PCR product.

Sample preparation: For juvenile or adult shrimp, lymphoid organ, gill tissue or haemolymph may be used to prepare total RNA. Fresh tissue is preferred. Lymphoid organ and gill tissue preserved in 95% analytical-grade ethanol or RNAlater (various manufacturers), or stored frozen at –70°C are also suitable for total RNA preparation. Disrupt 10–20 mg lymphoid organ or gill tissue or 50 µl haemolymph in 500 µl Trizol™[1] reagent and extract total RNA according to the product manual. Resuspend RNA in 25 µl water treated with DEPC (diethyl-pyrocarbonate)-, heat at 55°C for 10 minutes, cool on ice and use immediately or store at –70°C until required. Ideally, a 1/200 dilution (i.e. 2.5 µl RNA in 500 µl DEPC-treated water) should be prepared, and UV absorbances at A_{260}nm and A_{280} nm (a UV spectrophotometer is required) should be determined to quantify and check the quality of the RNA (ratio approximately 2:1). RNA yield will vary depending on the type and freshness of tissues, quality of the preservative used, and the length of time tissue has been preserved. However, RNA yields from fresh tissues would be expected to vary from 0.2 to 2.0 µg $µl^{-1}$ and about half these amounts from alcohol-preserved tissues.

From a nursery tank or hatchery tank containing 100,000 PL or more, sample approximately 1000 PL from each of 5 different points. Pool the samples in a basin, gently swirl the water and then select samples of live PL that collect at the centre of the basin. Choose numbers of PL to be pooled and tested according to the assumed or infection prevalence. Homogenise tissue samples in an appropriate volume of Trizol™ reagent and extract RNA according to the product manual. Based on the standard Trizol™ extraction procedure, tissue masses equivalent to 25–30 × PL5, 15 × PL10 and 5 × PL15 are accommodated and produce high quality total RNA free of protein contamination.

For each set of RNA samples to be tested, DEPC-treated water and extracts known to contain YHV RNA and/or GAV RNA (as appropriate to the test) should be included as negative and positive controls, respectively.

Protocol 1: RT-PCR for specific detection of YHV1 in diseased shrimp

To synthesise cDNA, mix 2 µl RNA in 20 µl PCR buffer (10 mM Tris/HCl pH 8.3, 50 mM KCl) containing 2.5 U of M-MLV (Moloney murine leukaemia virus) reverse transcriptase, 1.0 U ribonuclease inhibitor, 0.75 µM antisense primer 144R, 1 mM each of dATP, dTTP, dCTP, and dGTP, and 5 mM $MgCl_2$, and incubate at 42°C for 15 minutes. Incubate the mixture at 100°C for 5 minutes to inactivate the reverse transcriptase and allow the mixture to cool to 5°C. Add PCR mixture (10 mM Tris/HCl pH 8.3, 50 mM KCl) containing 2.5 U *Taq* DNA polymerase, 2 mM $MgCl_2$ and 0.75 µM of sense primer 10F to give a final volume of 100 µl. Unless the instrument is fitted with a heated lid, overlay the tubes with 100 µl of mineral oil and conduct PCR amplification for 40 cycles at 94°C for 30 seconds, 58°C for 30 seconds, 72°C for 30 seconds, and finishing at 72°C for 10 minutes. Alongside a suitable DNA ladder, apply a 20 µl aliquot of the PCR to a 2% agarose/TAE (Tris-acetate-EDTA [ethylene diamine tetra-acetic acid]) gel containing 0.5 µg ml^{-1} ethidium bromide and following electrophoresis, detect the 135 bp DNA band expected for YHV using a UV transilluminator.

[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*.

The sensitivity of the PCR is approximately 0.01 pg of purified YHV RNA (≈ 10^3 genomes).

PCR primer sequences:

10F: 5'-CCG-CTA-ATT-TCA-AAA-ACT-ACG-3'

144R: 5'-AAG-GTG-TTA-TGT-CGA-GGA-AGT-3'

Protocol 2: Nested RT-PCR for differential detection of YHV1 and GAV in healthy or diseased shrimp

For cDNA synthesis, 2 µl RNA (ideally 1.0 µg total RNA, if quantified),0.7 µl 50 pmol μl^{-1} primer GY5 and DEPC-treated water are added to 6 µl total, the mixture , incubated at 70°C for 10 minutes and chilled on ice. Add 2 µl Superscript II buffer × 5 (250 mM Tris/HCl pH 8.3, 375 mM KCl, 15 mM $MgCl_2$), 1 µl 100 mM DTT and 0.5 µl 10 mM dNTP stock mixture (i.e. 10 mM dATP, 10 mM dTTP, 10 mM dCTP, 10 mM dGTP) and mix gently. Preheat to 42°C for 2 minutes, add 0.5 µl 200 U μl^{-1} reverse transcriptase and incubate at 42°C for 1 hour. Heat the reaction at 70°C for 10 minutes, chill on ice and spin briefly in a microcentrifuge to collect the contents of the tube. For the first PCR step, prepare a 50 µl reaction mixture containing 1 × *Taq* buffer (10 mM Tris/HCl pH 8.3, 50 mM KCl, 0.1% Triton X-100), 1.5 mM $MgCl_2$, 35 pmol of each primer GY1 and GY4, 200 µM each of dATP, dTTP, dCTP and dGTP and 2.5 U *Taq* polymerase in a 0.5 ml thin-walled tube. Overlay the reaction mixture with 50 µl liquid paraffin, heat at 85°C for 2–3 minutes and then add 1 µl cDNA. Conduct PCR amplification using 35 cycles at 95°C for 30 seconds, 66°C for 30 seconds, and 72°C for 45 seconds, followed by final extension at 72°C for 7 minutes. For the second PCR step, prepare a 50 µl reaction mixture containing 2 µl of the first step PCR product, 1 × *Taq* buffer (above), 1.5 mM $MgCl_2$, 35 pmol of each primer GY2, Y3 and G6, 200 µM each of dATP, dTTP, dCTP and dGTP and 2.5 U *Taq* polymerase in a 0.5 ml thin-walled tube and overlay with liquid paraffin. Conduct PCR using amplification conditions as described above. Apply a 10 µl aliquot of the PCR to 2% agarose/TAE gels containing 0.5 µg ml^{-1} ethidium bromide alongside a suitable DNA ladder and detect using a UV transilluminator.

If the viral load is sufficiently high, a 794 bp DNA will be amplified from either GAV or YHV1 in the first PCR step. In the second PCR step, a 277 bp product indicates detection of YHV and a 406 bp product indicates detection of GAV. The presence of both 406 bp and 277 bp products indicates a dual infection with GAV and YHV1. The detection sensitivity of the second-step PCR is ~1000-fold greater than the first-step PCR and GAV or YHV1 RNA can be detected to a limit of 10 fg lymphoid organ total RNA.

The sequences of RT-PCR primers generic for GAV and YHV (GY) or specific for GAV (G) or YHV (Y) are as follows:

GY1: 5'-GAC-ATC-ACT-CCA-GAC-AAC-ATC-TG-3'

GY2: 5'-CAT-CTG-TCC-AGA-AGG-CGT-CTA-TGA-3'

GY4: 5'-GTG-AAG-TCC-ATG-TGT-GTG-AGA-CG-3'

GY5: 5'-GAG-CTG-GAA-TTC-AGT-GAG-AGA-ACA-3'

Y3: 5'-ACG-CTC-TGT-GAC-AAG-CAT-GAA-GTT-3'

G6: 5'-GTA-GTA-GAG-ACG-AGT-GAC-ACC-TAT-3'

NB: Due to reported problems with primer specificity for some emerging strains, all PCR products generated using protocol 2 should be sequenced to confirm the virus genotype.

Protocol 3: Nested *RT*-PCR for detection of all currently known genotypes in the yellow head complex (including YHV1 and GAV)

For cDNA synthesis, mix 2 µl RNA (ideally 1.0 µg total RNA, if quantified), 50 ng random hexamer primers and 1.0 µl 10 mM dNTP and make up to a total volume of 14 µl in sterile DEPC-treated water, incubate at 65°C for 5 minutes and chill on ice. Add 4.0 µl Superscript III buffer × 5, 1.0 µl 100 mM DTT, 1.0 µl 40 U μl^{-1} RNaseOUT™ (Invitrogen) and 1.0 µl 200 U μl^{-1} reverse transcriptase and mix gently. Incubate at 25°C for 5 minutes and then at 42°C for 55 minutes, stop the reaction by heating at 70°C for 15 minutes, chill on ice and spin briefly in a microcentrifuge to collect the contents of the tube. For the first PCR step, add 1 µl cDNA to a total 25 µl reaction mixture containing 1 × *Taq* buffer (10 mM Tris/HCl, pH 9.0, 50 mM KCl, 0.1% Triton X-100), 1.5 µl 25 mM $MgCl_2$, 0.35 µl primer mix containing 25 pmol μl^{-1} of each primer pool (see below) YC-F1ab and YC-R1ab, 0.5 µl 10 mM dNTP mix and 0.25 µl 5 U μl^{-1} *Taq* DNA polymerase. Conduct PCR amplification using denaturation at 95°C for 1 minute followed by 35 cycles at 95°C for 30 seconds, 60°C for 30 seconds, 72°C for 40 seconds, followed by a final extension at 72°C for 7 minutes. For the second PCR step, use 1 µl of the first PCR product in the reaction mixture as prepared above but substituting primer pools YC-F2ab and YC-R2ab. Conduct PCR amplification using denaturation at 95°C for 1 minute followed by 35 cycles at 95°C for 30 seconds, 60°C for 30 seconds, 72°C for 30 seconds, followed by a final extension at 72°C for 7 minutes. Apply an 8 µl aliquot of the PCR to 2% agarose/TAE gels containing 0.5 µg ml^{-1} ethidium bromide alongside a suitable DNA ladder and detect using a UV transilluminator.

Annex 12 (contd)

If the viral load is sufficiently high, a 358 bp DNA is amplified in the first PCR step. The second (nested) PCR step amplifies a 146 bp product. The detection of these products indicates detection of one of the six genotypes in the yellow head complex. Further assignment of genotype (if required) is possible by nucleotide sequence analysis of either PCR product followed by comparison with sequences of the known genotypes by multiple sequence alignment and phylogenetic analysis. The detection sensitivity limits of the first PCR step and nested PCR step are 2,500 and 2.5 RNA templates, respectively.

PCR primer sequences (each primer comprises a pool of equal quantities of two related oligonucleotide sequences):

YC-F1ab pool:	5'-ATC-GTC-GTC-AGC-TAC-CGC-AAT-ACT-GC-3'
	5'-ATC-GTC-GTC-AGY-TAY-CGT-AAC-ACC-GC-3'
YC-R1ab pool:	5'-TCT-TCR-CGT-GTG-AAC-ACY-TTC-TTR-GC-3'
	5'-TCT-GCG-TGG-GTG-AAC-ACC-TTC-TTG-GC-3'
YC-F2ab pool:	5'-CGC-TTC-CAA-TGT-ATC-TGY-ATG-CAC-CA-3'
	5'-CGC-TTY-CAR-TGT-ATC-TGC-ATG-CAC-CA-3'
YC-R2ab pool:	5'-RTC-DGT-GTA-CAT-GTT-TGA-GAG-TTT-GTT-3'
	5'-GTC-AGT-GTA-CAT-ATT-GGA-GAG-TTT-RTT-3'
Mixed base codes:	R(AG), Y(CT), M(AC), K(GT), S(GC), W(AT), H(ACT), B(GCT), V(AGC), D(AGT), N(AGCT).

4.3.1.2.3. *In-situ* hybridisation

The protocol of Tang *et al.* (2002) described is suitable for detecting YHV1 or GAV (Tang & Lightner, 1999). To preserve viral RNA accessibility, fix tissues sampled from live shrimp in neutral-buffered, modified Davidson's fixative without acetic acid (RF-fixative) (Hasson *et al.*, 1997). To achieve good tissue preservation whilst also preserving RNA accessibility, normal Davidson's fixative can be used as long as the fixation time is limited to 24 hours (maximum of 48 hours). Process the fixed tissue using standard histological methods and prepare 4 µm thick sections on Superfrost Plus slides (Fisher Scientific, Pennsylvania, USA). Prior to hybridisation, incubate sections at 65°C for 45 minutes, remove paraffin with Hemo-De (Fisher Scientific, Pennsylvania, USA), and rehydrate through a reducing ethanol concentration series to water. Digest sections with proteinase K (100 µg ml^{-1}, in 50 mM Tris/HCl pH 7.4, 10 mM NaCl, 1 mM EDTA) for 15 minutes at 37°C, followed by post-fixation in 0.4% formaldehyde for 5 minutes. Rinse in 2 × SSC (standard saline citrate), then pre-hybridise with 500 µl pre-hybridisation solution (4 × SSC, 50% formamide, 1 × Denhardt's, 0.25 mg ml^{-1} yeast RNA, 0.5 mg m^{-1} sheared salmon sperm DNA, 5% dextran sulphate) at 42°C for 30 minutes. For hybridisation, overlay the sections with 250 µl hybridisation solution containing a digoxigenin-labelled DNA probe (20–40 ng ml^{-1}) at 42°C overnight. The next day, wash the sections as follows: 2 × SSC once for 30 minutes at room temperature; 1 × SSC twice for 5 minutes at 37°C; 0.5 × SSC twice for 5 minutes at 37°C. Incubate the sections with sheep anti-digoxigenin-alkaline phosphatase conjugate (Roche) at 37°C for 30 minutes. Wash with 0.1 M Tris/HCl pH 7.5, 0.15 M NaCl twice for 10 minutes at room temperature and rinse with 0.1 M Tris/HCl pH 9.5, 0.1 M NaCl. Incubate with nitroblue tetrazolium and 5-bromo-4-chloro-3-indoyl phosphate in the dark for 1–2 h for colour development. Counterstain with Bismarck Brown Y (0.5%), dehydrate through a series of ethanol and Hemo-De, add Permount (Fisher Scientific, Pennsylvania, USA) and cover with a cover-slip. YHV-infected cells give a blue to purple-black colour against the brown counter stain. Include positive controls of YHV-infected tissue and negative controls of uninfected shrimp tissue. The digoxigenin-labelled DNA probe can be prepared by PCR labelling using the following primers:

YHV1051F:	5'-ACA-TCT-GTC-CAG-AAG-GCG-TC-3'
YHV1051R:	5'-GGG-GGT-GTA-GAG-GGA-GAG-AG-3'

4.3.1.2.3 Agent purification

A YHV1 purification method based on density gradient ultracentrifugation is described (Wongteersupaya *et al.* 1995). Approximately 250 healthy juvenile *P. monodon* shrimp (approximately 10 g) should ideally be used as a source of virus for purification. After acclimatising for several days in 1500 litre tanks (approximately 80 shrimp/tank) at a salinity of 3.5 parts per thousand (mg ml^{-1}), inoculate each shrimp intramuscularly with 100 µl of a 1/100 gill extract

suspension prepared from YHV-infected shrimp. At 2 days post-infection, harvest moribund shrimp showing typical signs of YHD. Use a syringe to draw haemolymph from the sinuses at the base of the walking legs and mix carefully on ice with the same volume of lobster haemolymph medium (LHM) (486 mM NaCl, 15 mM $CaCl_2$, 10 mM KCl, 5 mM $MgCl_2$, 0.5 mM Na_2HPO_4 8.1 mM $MgSO_4$, 36 mM $NaHCO_3$, 0.05% dextrose in Minimal Eagle's Medium, adjusted pH 7.6 with 1 N NaOH). Centrifuge the mixture at 480 ***g*** for 30 minutes at 4°C to remove cellular debris. Ultracentrifuge the supernatant at 100,000 ***g*** for 1 hour at 4°C. Discard the supernatant and gently resuspend the pellet overnight at 4°C in 1 ml LHM. Layer this suspension over a continuous gradient of 20–40% Urografin and ultracentrifuge at 100,000 ***g*** for 1 hour at 4°C. After centrifugation, collect the viral band by using a Pasteur pipette and dilute with NTE buffer (0.02 M EDTA, 0.2 M NaCl, 0.2 M Tris/HCl [pH 7.4]) to a final volume of 12 ml. Ultracentrifuge the suspension at 100,000 ***g*** for 1 hour at4°C and resuspend the pellet (purified virus) in 100 µl TE buffer (10 mM Tris/HCl, 1 mM EDTA [pH 7.4]) and store in 20 µl aliquots at –80°C until required.

4.3.1.2.4 Bioassay

The bioassay procedure is based on that described by Spann *et al.* (1997), but similar procedures have been described by several other authors (Lu *et al.,* 1994). The bioassay should be conducted in susceptible shrimp (see Section 2.2 above) ideally that have been certified as SPF and have been obtained from a biosecure breeding facility. Alternatively, susceptible wild or farmed shrimp to be used for bioassay should be screened by nested RT-PCR using RNA extracted from haemolymph to confirm the absence of pre-existing chronic infections with YHV1, GAV or related viruses. Throughout the procedure, shrimp should be maintained under optimal conditions for survival of the species in laboratory tank systems.

Collect moribund shrimp from a YHD-affected ponds or shrimp suspected of being carriers of infection and maintain at 4°C or on ice. Remove and discard the tail and appendages. If necessary, the whole shrimp or the retained cephalothorax may be snap-frozen and stored at –80°C or in liquid nitrogen until required. Thaw stored samples rapidly in a 37°C water bath within two snap-seal plastic bags and then maintain at 4°C or on ice during all procedures. Remove the carapace and calciferous mouth-parts. Suspend the remaining tissues in six volumes of TN buffer (0.02 M Tris/HCl, pH 7.4, 0.4 M NaCl) and homogenise in a tissue grinder to form a smooth suspension. Clarify the homogenate at 1300 ***g*** for 20 minutes at 4°C. Remove the supernatant fluid below the lipid layer and pass through a 0.45 µm filter. Maintain the filtrate at 4°C for immediate use or snap-freeze and store in aliquots at –80°C or in liquid nitrogen. Thaw the filtrate rapidly at 37°C and maintain on ice prior to use.

Inject at least 12 juvenile (1–5 g) shrimp of a known susceptible species (*P. monodon*, *P. esculentus*, *P. japonicus*, *P. merguiensis*, *P. vannamei*, *P. stylirostris*), with 5 µl of filtrate per gram body weight into the second abdominal segment using a 26-gauge needle. Inject two equivalent groups of at least 12 shrimp with TN buffer and a filtered tissue extract prepared from uninfected shrimp. One additional group of at least 12 shrimp should be injected last with a known and calibrated positive control inoculum from shrimp infected with YHV~~ or GAV (as required)~~. Maintain each group of shrimp in a separate covered tank with a separate water supply for the duration of the bioassay. Ensure no inadvertent transfer of water between tanks by good laboratory practice. Observe the shrimp and record mortalities for at least 21 days or until the test and positive control groups reach 100% mortality. Collect at least one moribund shrimp from each of the four groups for examination by histology, TEM, *in situ* nucleic acid hybridisation, and PCR or Western-blot analysis to confirm the presence of YHV1 ~~or GAV (as required)~~ in the sample (refer to the Sections above for test procedures).

NOTE: shrimp to be tested that are suspected of being carriers of low level chronic infections may produce an inoculum containing a very low dose of virus. In bioassay, such an inoculum may not necessarily cause mortalities, gross signs of disease or histology characteristic of a lethal infection. In this event, molecular tests (PCR or ISH) or TEM must be applied to the bioassay shrimp.

Annex 12 (contd)

4.3.2. Serological methods

Not applicable.

5. Rating of tests against purpose of use

The methods currently available for targeted surveillance and diagnosis of YHD are listed in Table 5.1. The designations used in the Table indicate: a = the method is the recommended method for reasons of availability, utility, and diagnostic specificity and sensitivity; b = the method is a standard method with good diagnostic sensitivity and specificity; c = the method has application in some situations, but cost, accuracy, or other factors severely limits its application; and d = the method is presently not recommended for this purpose. These are somewhat subjective as suitability involves issues of reliability, sensitivity, specificity and utility. Although not all of the tests listed as category a or b have undergone formal standardisation and validation, their routine nature and the fact that they have been used widely without dubious results, makes them acceptable.

Table 5.1. *Methods for targeted surveillance and diagnosis*

Method	Targeted surveillance				Presumptive diagnosis	Confirmatory diagnosis
	Larvae	PLs	Juveniles	Adults		
Gross signs	d	d	c	c	c	d
Bioassay	d	d	d	d	c	b
Direct LM	d	d	d	d	a	d
Histopathology	d	d	c	c	a	d
Transmission EM	d	d	c	c	d	b
Antibody-based assays	d	d	c	c	a	b
***In-situ* DNA probes**	d	d	c	c	b	a
PCR	a	a	a	a	a	a
Sequence	a	a	a	a	d	a

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

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

Nested RT-PCR (Section 4.3.1.2.3.1; Protocol 3) followed by confirmatory sequencing of the amplified PCR product is the prescribed method for declaring freedom. Two-step PCR negative results are required. ~~The very rare case when a two-step PCR positive result cannot be confirmed by sequencing is also considered to be a negative result.~~

7. Corroborative diagnostic criteria

7.1. Definition of suspect case

A suspect case of YHV1 ~~genotype 1~~ is defined as a disease outbreak in marine shrimp with rapidly accumulating mortalities (up to 100%) in the early to late juvenile stages, which may be preceded by cessation of feeding and congregation of shrimp at pond edges. Moribund shrimp may exhibit a bleached overall appearance and a yellowish discoloration of the cephalothorax caused by the underlying yellow hepatopancreas. Histological examination of fixed lymphoid organ tissues should reveal moderate to large numbers of deeply basophilic, evenly stained, spherical, cytoplasmic inclusions (approximately 2 µm in diameter or smaller).

7.2. Definition of confirmed case

YHV1 may be confirmed by the detection of high levels of disseminated infection in tissues of ectodermal and mesodermal origin by *in situ* hybridisation in conjunction with the detection of amplified products of the prescribed size using discriminatory RT-PCR assays and sequencing, as described in Section 4.3 of this chapter. ~~As low-level chronic infections with yellow head complex viruses are common in some regions, detection of the presence of virus is not, in itself, evidence of aetiology.~~

8. References

ASSAVALAPSAKUL W., SMITH D.R. & PANYIM S. (2003). Propagation of infectious yellow head virus particles prior to cytopathic effect in primary lymphoid cell cultures of *Penaeus monodon*. *Dis. Aquat. Org*., **55**, 253–258.

CASTRO-LONGORIA R., QUINTERO-ARREDONDO N., GRIJALVA-CHON J.M. & RAMOS-PAREDES J. (2008). Detection of the yellow-head virus (YHV) in wild blue shrimp, *Penaeus stylirostris*, from the Gulf of California and its experimental transmission to the Pacific white shrimp, *Penaeus vannamei*. *J. Fish Dis.,* **31** (12), 953–956.

CHANTANACHOOKIN C., BOONYARATPALIN S., KASORNCHANDRA J., DIREKBUSARAKOM S., AEKPANITHANPONG U., SUPAMATTAYA K., SRIURAITANA S. & FLEGEL T.W. (1993). Histology and ultrastructure reveal a new granulosis-like virus in *Penaeus monodon* affected by yellow-head disease. *Dis. Aquat. Org.*, **17**, 145–157.

COWLEY J.A., CADOGAN L.C., WONGTEERASUPAYA C., HODGSON R.A.J., SPANN K.M., BOONSAENG V. & WALKER P.J. (2004). Differential detection of gill-associated virus (GAV) from Australia and yellow head virus (YHV) from Thailand by multiplex RT-nested PCR. *J. Virol. Methods*, **117**, 49–59.

COWLEY J.A., HALL M.R., CADOGAN L.C., SPANN K.M. & WALKER P.J. (2002). Vertical transmission of gill-associated virus (GAV) in the black tiger prawn *Penaeus monodon. Dis. Aquat. Org*., **50**, 95–104.

COWLEY J.A., WALKER P.J., FLEGEL T.W., LIGHTNER D.V., BONAMI J.R., SNIJDER E.J. & DE GROOT R.J. (2012). Family Roniviridae. *In*: Virus Taxonomy, IXth Report of the International Committee on Taxonomy of Viruses, King A., Adams M., Carstens E. & Lefkowitz E.J., eds. Elsevier, Academic Press, London, UK, 797–801.

FLEGEL T.W., BOONYARATPALIN S. & WITHYACHUMNARNKUL B. (1997). Current status of research on yellow-head virus and white-spot virus in Thailand. *In:* Diseases in Asian Aquaculture III, Flegel T.W. & MacRae I.H., eds. Fish Health Section, Asian Fisheries Society, Manila, the Philippines, 285–296.

FLEGEL T.W., FEGAN D.F. & SRIURAIRATANA S. (1995a). Environmental control of infectious shrimp diseases in Thailand. *In:* Diseases in Asian Aquaculture II, Shariff M., Subasinghe R.P. & Arthur J.R., eds. Asian Fisheries Society, Manila, the Philippines, 65–79.

FLEGEL T.W., SRIURAIRATANA S., WONGTERRASUPAYA C., BOONSAENG V., PANYIM S. & WITHYACHUMNARNKUL B. (1995b). Progress in characterization and control of yellow-head virus of Penaeus monodon. *In:* Swimming Through Troubled Water, Proceedings of the Special Session on Shrimp Farming, Aquaculture '95, Browdy C.L. & Hopkins J.S., eds. World Aquaculture Society, Baton Rouge, USA, 76–83.

HASSON K.W., HASSON J., AUBERT H., REDMAN R.M. & LIGHTNER D.V. (1997). A new RNA-friendly fixative for the preservation of penaeid shrimp samples for virological assay using cDNA probes. *J. Virol. Methods,* **66**, 227–236.

KHONGPRADIT R., KASORNCHANDRA J. & BOONYARATALIN S. (1995). Susceptibility of the postlarval stages of black tiger shrimp (*Penaeus monodon*) to yellow-head baculovirus (YBV). *In:* Diseases in Asian Aquaculture II, Shariff M., Subasinghe R.P. & Arthur J.R., eds. Asian Fisheries Society, Manila, the Philippines, p. 6.

LIGHTNER D.V. (ED.) (1996). Handbook of Pathology and Diagnostic Procedures for Diseases of Penaeid Shrimp. World Aquaculture Society, Baton Rouge, USA.

LIU Q., HUANG J., YANG H.-L., YANG B., WANG H.-L., WANG Q.-T., LIU F. & ZHANG Q.-L. (2014) Detection of a new genotype of yellow-head virus in farmed shrimp suspicious of EMS/AHPNS infection. *Oceanol. Limnol. Sin.*, **45** (4), 703–709.

Annex 12 (contd)

LIGHTNER D.V., HASSON, K.W., WHITE, B.L. & REDMAN R.M. (1998). Experimental infection of western hemisphere penaeid shrimp with Asian white spot syndrome virus and Asian yellow head virus. *J. Aquat. Anim. Health*, **10**, 271–281.

LOH P.C., CESAR E., NADALA B. JR, TAPAY L.M. & LU Y. (1998). Recent developments in immunologically-based and cell culture protocols for the specific detection of shrimp viral pathogens. In: Advances in Shrimp Biotechnology, Flegel T.W., ed. National Center for Genetic Engineering and Biotechnology, Bangkok, Thailand, 255–259.

LONGYANT S., SATTAMAN S., CHAIVISUTHANGKURA P., RUKPRATANPORN S., SITHIGORNGUL W. & SITHIGORNGUL P. (2006). Experimental infection of some penaeid shrimps and crabs by yellow head virus (YHV). *Aquaculture,* **257**, 83–91.

LONGYANT S., SITHIGORNGUL P., CHAIVISUTHANGKURA P., RUKPRATANPORN S., SITHIGORNGUL W. & MENASVETA P. (2005). Differences in the susceptibility of palaemonid shrimp species to yellow head virus (YHV) infection. *Dis. Aquat. Org.*, **64**, 5–12.

LU Y., TAPAY L.M., BROCK J.A. & LOH P.C. (1994). Infection of the yellow head baculo-like virus (YBV) in two species of penaeid shrimp *Penaeus stylirostris* (Stimpson) and *Penaeus vannamei* (Boone). *J. Fish Dis.*, **17**, 649–656.

MA H., OVERSTREET R.M. & JOVONOVICH J.A. (2009). Daggerblade grass shrimp (*Palaemonetes pugio*): A reservoir host for yellow head virus (YHV). *J. Invert. Pathol.* **101**, 112–118.

MOHR P.G., MOODY N.J.G, HOAD J., WILLIAMS L.M., BOWATER R.O., CUMMINS D.M., COWLEY J.A. & CRANE M.STJ. (2015) New yellow head virus genotype (YHV7) in giant tiger shrimp *Penaeus monodon* indigenous to northern Australia. *Dis. Aquat. Org.*, **115,** 263–268.

OANH D.T., VAN HULTEN M.C., COWLEY J.A. & WALKER P.J. (2011). Pathogenicity of gill-associated virus and Mourilyan virus during mixed infections of black tiger shrimp (*Penaeus monodon*). *J. Gen. Virol.*, **92**, 893–901.

SANCHEZ-BARAJAS M., LINAN-CABELLO M.A. & MENA-HERRERA A. (2009). Detection of yellow-head disease in intensive freshwater production systems of *Litopenaeus vannamei*. *Aquacult. Internatn.,* **17,** 101–112.

SENAPIN S., THAOWBUT Y., GANGNONNGIW W., CHUCHIRD N., SRIURAIRATANA S. & FLEGEL TW. (2010). Impact of yellow head virus outbreaks in the whiteleg shrimp, *Penaeus vannamei* (Boone), in Thailand. *J. Fish Dis.*, **33** (5), 421–430.

SPANN K.M., COWLEY J.A., WALKER P.J. & LESTER R.J.G. (1997). A yellow-head-like virus from *Penaeus monodon* cultured in Australia. *Dis. Aquat. Org.*, **31**, 169–179.

SPANN K.M. DONALDSON R.A. COWLEY J.A. & WALKER P.J. (2000). Differences in susceptibility of some penaeid prawn species to gill associated virus (GAV) infection. *Dis. Aquat. Org.*, **42**, 221–225.

SPANN K.M., MCCULLOCH R.J., COWLEY J.A. & WALKER P.J. (2003). Detection of gill-associated virus (GAV) by *in situ* hybridisation during acute and chronic infections in *Penaeus monodon* and *Penaeus esculentus* shrimp. *Dis. Aquat. Org.*, **56**, 1–10.

STENTIFORD G.D., BONAMI J.R. & ALDAY SANZ V. (2009). A critical review of susceptibility of crustaceans to Taura syndrome, Yellowhead disease and White Spot Disease and implications of inclusion of these diseases in European leglislation. *Aquaculture,* **291**, 1–17.

TANG K.F.J. & LIGHTNER D.V. (1999). A yellow head virus gene probe: nucleotide sequence and application for *in situ* hybridization. *Dis. Aquat. Org.*, **35**, 165–173.

TANG K.F.J., SPANN K.M., OWENS L. & LIGHTNER D.V. (2002). *In situ* detection of Australian gill-associated virus with a yellow head virus gene probe. *Aquaculture,* **205**, 1–5.

Annex 12 (contd)

TIRASOPHON W., YODMUANG S., CHINNIRUNVONG W., PLONGTHONGKUM & PANYIM S. (2007). Therapeutic inhibition of yellow head virus multiplication in infected shrimps by YHV-protease dsRNA. *Antiviral Res*., **74**, 150–155.

WALKER P.J., COWLEY J.A. SPANN K.M., HODGSON R.A.J. HALL M.R & WITHYACHUMNARNKUL B. (2001). Yellow head complex viruses: Transmission cycles and topographical distribution in the Asia-Pacific Region. *In:* The New Wave, Proceedings of the Special Session on Sustainable Shrimp Culture, Aquaculture 2001, Browdy C.L. & Jory D.E., eds. The World Aquaculture Society, Baton Rouge, LA, USA, 292–302.

WIJEGOONAWARDANE P.K.M., COWLEY J.A., SITTIDILOKRATNA, N., PHETCHAMPAI, N., COWLEY, J.A., GUDKOVS, N. & WALKER P.J. (2009). Homologous genetic recombination in the yellow head complex of nidoviruses infecting *Penaeus monodon shrimp*. Virology doi: 1016/j.virol.2009.04.015.

WIJEGOONAWARDANE P.K.M., COWLEY J.A., PHAN T., HODGSON R.A.J., NIELSEN L., KIATPATHOMCHAI W. & WALKER P.J. (2008a). Genetic diversity in the yellow head nidovirus complex. Virology **380**, 213–225.

WIJEGOONAWARDANE P.K.M., COWLEY J.A. & WALKER P.J. (2008b). Consensus RT-nested PCR to detect yellow head virus genotypes in penaeid shrimp. *J. Virol. Methods*, **153**, 168–175.

WONGTEERASUPAYA C., BOONSAENG V., PANYIM S., TASSANAKAJON A., WITHYACHUMNARNKUL B. & FLEGEL T.W. (1997). Detection of yellow-head virus (YHV) of *Penaeus monodon* by RT-PCR amplification. *Dis. Aquat. Org.,* **31**, 181–186.

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

Annex 13

AQUATIC ANIMAL HEALTH STANDARDS COMMISSION WORK PLAN 2015–2016

Aquatic Animal Health Code

Task	Oct 2015	Feb 2016
Glossary	Propose new definitions and circulate for comment	Review Member comments
Chapter 1.1. – Notification of diseases, and provision of epidemiological information	Review Member comments, amend and circulate for comment	Review Member comments
Chapter 1.2. – Criteria for listing	Review Member comments, amend and circulate for comment	Review Member comments, amend and circulate for comment
Chapter 1.3. – List of diseases	AAHSC to develop assessments using the criteria for listing for *Batrachochytrium salamandrivorans* and *Marteilia cochillia*	AAC to review assessments for *Batrachochytrium salamandrivorans* and *Marteilia cochillia.* Review Member comments on amended YHD name and recirculate for comment
Revision of Section 4 to improve guidance on the control of disease	Circulate revised structure for member comment	Review Member comments and develop approach to the work
Chapter 4.3. – General recommendations on disinfection	Review AHG draft chapter and circulate for member comments.	Review Member comments and circulate for comment
Possible development of other eggs and larvae disinfection chapters	AAC to consider this in light of AHG report	
Revise X.X.8. to remove reference to ICES		Finalise revised text and circulate for comment
Develop concept for a possible guidance document on how to use the *Aquatic Code* to facilitate trade.		Consider developing a concept note
Chapter 9.2. – YHD (Listing of susceptible species in disease-specific chapters)	Review Member Countries' comments and circulated for Member comments	Review Member comments, amend and circulate for comment
AHPND (Chapter 9.X.)	AAC developed a new chapter and circulated for Member comments	Review Member comments, amend and circulate for comment
Rana virus genus question	Review Reference Laboratory expert opinion	Reconsider once ICTV position is clear
Develop revised lists of susceptible species – crustacean	AHG to meet 13–15 October 2015 to develop susceptible species lists for all crustacean diseases except WSD. Requested AHG to develop a strategy as to how to address the susceptible species list for WSD.	Review AHG report and amend relevant chapters and circulate for comment
Periods to claim/reclaim freedom (in relation to Chapter 1.4.) Develop principles for determining surveillance periods in disease-specific chapters and provide advice on amendments for Chapter 1.4.		AAC to commence this work
Aquatic animal definition		AAC to commence this work

Annex 13 (contd)

***Manual* tasks**	**Oct 2015**	**Feb 2016**
YHD chapter	AAC amended text and circulated for MC	Review Member comments, amend and circulate for comment
AHPND chapter	AAC proposed an AHG to review and amend	Review AHG report and amend and circulate to MC
Manual–complete within three years Case definition Validation tests Model template (more concise) Test performance table Sections on agent stability (in connection with disinfection)	AAC proposed an AHG be convened to commence this work	Review progress on this work
Develop revised lists of susceptible species – crustacean	AHG to meet on 13–15 October 2015	Review AHG report and amend relevant chapters and circulate for MC

Other items

Fish-borne Zoonotic Trematodes (FZT)

Annex 14

Organisation Mondiale de la Santé Animale | World Organisation for Animal Health | Organización Mundial de Sanidad Animal

Original : anglais
May 2015

REPORT OT THE MEETING OF THE OIE *AD HOC* GROUP ON DISINFECTION OF AQUACULTURE ESTABLISHMENTS AND EQUIPMENT

Paris, 19–21 May 2015

———

The OIE *ad hoc* Group on Disinfection of Aquaculture Establishments and Equipment (the *ad hoc* Group) met at OIE Headquarters in Paris from 19 to 21 May 2015.

The members of the *ad hoc* Group are listed at Annex 1 and the Terms of Reference are provided at Annex 2.

During this meeting the *ad hoc* Group finalised work on the development of a revised draft Chapter 4.3. 'Disinfection of aquaculture establishments and equipment', that they had commenced work on during their first meeting in August 2014.

The *ad hoc* Group drafted a revised Chapter 4.3. that includes recommendations for disinfection procedures for aquaculture establishments and equipment used during routine biosecurity activities and emergency response. Guidance is also provided on general principles for the planning and implementation of disinfection activities.

The *ad hoc* Group agreed that the general principles included in the new draft chapter apply to the transport of aquatic animals and suggested that once the new draft Chapter 4.3. is adopted, a cross-reference should be included in Chapter 5.5. 'Control of aquatic animal health risks associated with transport of aquatic animals', and text reviewed to ensure consistency between the two chapters.

The *ad hoc* Group noted that a new Chapter 4.4. 'Recommendations for surface disinfection of salmonid eggs' would be proposed for adoption at the 83rd General Session in May 2015 and recommended consideration be given to the development of protocols for the disinfection of eggs from non-salmonid fish species; mollusc eggs and larvae; and crustaceans eggs and larvae.

The *ad hoc* Group noted the lack of good references regarding disinfection in aquaculture and recommended that a scientific review be undertaken for disinfection of aquaculture establishments and equipment.

The *ad hoc* Group wished to remind Member Countries of two useful OIE publications:

1. Disinfection in aquaculture, Maris P., *Rev. sci. tech. Off. int. Epiz.,* 1995, Vol. 14 (1), p. 47–55. http://www.oie.int/doc/ged/D8965.PDF

2. Modes of actions of disinfectants, Torgersen Y. and Hastein T., *Rev. sci. tech. Off. int. Epiz.,* 1995, Vol. 14 (2), p. 419–434. http://www.oie.int/doc/ged/D8957.PDF

.../Annexes

Annex 14 (contd)

Annex 1

REPORT OT THE MEETING OF THE OIE *AD HOC* GROUP ON DISINFECTION OF AQUACULTURE ESTABLISHMENTS AND EQUIPMENT

Paris, 19–21 May 2015

———

List of participants

MEMBERS OF THE *AD HOC* GROUP

Dr Kevin Ellard
Senior Veterinary Officer
Biosecurity Tasmania
Department of Primary Industries
Parks Water & Environment
13 St Johns Street, New Town
Tasmania 7008
AUSTRALIA
Kevin.Ellard@dpipwe.tas.gov.au
Tel.: +613 616 532 60

Dr Marc Le Groumellec
Villa 30 Plateau Des Tombes
Mangarivotra
401 Majunga
MADAGASCAR
le.groumellec@gmail.com
Tel.: +261 320 719 581
+261 344 919 581

Dr Pamela Cañas
Dardo Regulez 2449, Vitacura
Santiago, Region Metropolitana,
CHILE
pamela.canas@masterquality.cl
mpamelac@gmail.com
Tel.: +56 2 22187789

Dr Semir Loncarevic
Norwegian Veterinary Institute
Pb 750 Sentrum, N-0106 Oslo
NORWAY
semir.loncarevic@vetinst.no
Tel.: +47 23 21 62 48

Dr Ingo Ernst
Director, Aquatic Pest and Health Policy
Australian Government Department of Agriculture
GPO Box 858
Canberra ACT 2601
AUSTRALIA
Ingo.Ernst@agriculture.gov.au
Tel.: +61 2 627 256 15

OIE HEADQUARTERS

Dr Gillian Mylrea
Deputy Head
International Trade Department
OIE
g.mylrea@oie.int

Annex 14 (contd)

Annex 2

REPORT OT THE MEETING OF THE OIE *AD HOC* GROUP ON DISINFECTION OF AQUACULTURE ESTABLISHMENTS AND EQUIPMENT

———

Terms of Reference

Purpose of the meeting

The *ad hoc* Group on Disinfection of aquaculture establishments and equipment is to complete the development of a new draft chapter that will provide guidance on disinfection of aquaculture establishments and equipment to be included in Section 4 of the *Aquatic Animal Health Code (Aquatic Code).*

Background

The Aquatic Animals Commission, during their February 2014 meeting, recommended that a new *ad hoc* Group (AHG) be convened to review and revise the *Aquatic Code* Chapter 4.3. 'General recommendations on disinfection' to encompass the topics currently contained in the *Manual of Diagnostic Tests for Aquatic Animals (Aquatic Manual)* Chapter 1.1.3. 'Methods for disinfection of aquaculture establishments' as they agreed that this chapter was misplaced in the *Aquatic Manual* since the scope of the *Manual* is diagnosis.

Relevant considerations:

The *ad hoc* Group should:

1) Determine the elements of the *Aquatic Code* that require supporting guidance on disinfection of aquaculture establishments, water, fish eggs and transport vehicles (i.e. any method used for transport).

2) Consider the relevance of information in the current Chapter 1.1.3. 'Methods for Disinfection of Aquaculture Establishments' in the *Aquatic Manual.*

3) Design an appropriate structure for the chapter (or chapters) and, the appropriate level of guidance to be included in the new chapter (s) to support other recommendations in the *Aquatic Code* (i.e. whether guidance should be in the form of general principles or more detailed technical recommendations).

4) Consider any recommendations on complementarity with other elements of the *Aquatic Code*, and any need for consistency with relevant information in the *Terrestrial Animal Health Code*.

5) Format the new chapter following the style of existing *Aquatic Code* chapters.

6) Prepare a report for submission to the next meeting of the Aquatic Animal Health Standards Commission.