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**REPORT OF THE MEETING OF THE
OIE AQUATIC ANIMAL HEALTH STANDARDS COMMISSION
Paris, 5-9 March 2007**

The OIE Aquatic Animal Health Standards Commission (hereafter referred to as the Aquatic Animals Commission) met at the OIE Headquarters from 5 to 9 March 2007. The meeting was chaired by Dr Eva-Maria Bernoth, President of the Commission, and Dr Ricardo Enriquez, Secretary General, acted as Rapporteur. Participants are listed at [Appendix I](#). The adopted Agenda is given at [Appendix II](#).

Dr Bernard Vallat, Director General of the OIE, welcomed the participants of the meeting and thanked them for their dedicated and continuing good work. He informed the Aquatic Animals Commission on the ongoing discussions in regard to closer collaboration between the OIE and the Food and Agriculture Organization of the United Nations (FAO). A chart describing the respective roles and competencies has been prepared by the OIE and endorsed by the FAO Director General after discussion with FAO staff and the OIE Administrative Commission.

Dr Vallat illustrated the progress the OIE is making in assisting Member Countries in capacity building activities through the use of the Performance, Vision and Strategy (PVS) tool. More than 70 certified experts have been trained by the OIE to conduct PVS evaluations in Member Countries. He explained that the OIE could perform an evaluation in a Country only after receiving an invitation from its OIE Delegate; 40 Member Countries have already requested an evaluation by the OIE. Dr Vallat explained that the OIE proposed to extend the application of the PVS to aquatic animal health services and invited the Aquatic Animals Commission to involve itself in making proposals for this work.

Dr Vallat explained that the OIE intends to submit to the next International Committee a list of antimicrobials of veterinary importance. This list includes antimicrobials used in aquaculture. Dr Vallat thanked the Aquatic Animals Commission for its contribution and stated that the OIE will recommend prudent use of these antimicrobials in order to minimise the risk of development of antimicrobial resistance while safeguarding access to products needed for animal health.

Dr Vallat noted the significant number of responses from Member Countries on the questionnaire on amphibian trade and diseases and the generally positive support for including amphibians in the remit of the OIE (despite the opposition of some countries). Dr Vallat recommended the Aquatic Animals Commission bring recommendations on this issue to the May 2007 OIE General Session.

Finally Dr Vallat thanked the Aquatic Animals Commission's members for their contribution to the OIE Regional Conferences and underlined the importance of continuously updating the presentations so to convey relevant political messages. He encouraged the continuation of this practice to inform OIE Delegates about OIE activities in the field of aquatic animal health and trade.

The Aquatic Animals Commission recognised the contribution of the following Member Countries in providing comments: Argentina, Australia, Canada, Colombia, the European Community (EC), Japan, Madagascar, Nicaragua, Norway, Switzerland, Taipei China, Thailand and the United States of America (USA).

The Aquatic Animals Commission examined various *Aquatic Animal Health Code* (hereafter referred to as the *Aquatic Code*) draft texts from its October 2006 report in the light of Member Countries' comments. The outcome of the Aquatic Animals Commission's work is presented as Appendices III to XXX to this report. Additions made during the October 2006 meeting are shown as double underlined text, with deleted text in strikeout, and those made at this meeting (March 2007) in a similar fashion but with a coloured background to distinguish the two groups of proposals.

Member Countries are invited to submit their comments to the OIE on Appendices XXIII to XXX of this report prior to 6 August 2007. The comments should be sent preferably by electronic mail to the following address: trade.dept@oie.int. The Aquatic Animals Commission will address the comments received at its next meeting.

The table below summarises the texts that will be proposed – as presented in the appendices – to the OIE International Committee for adoption at the 75th General Session (first part), the texts that are presented for Member Countries' comment (second part), and texts for Member Countries' information (third part). A blank appendix was inserted to keep the numbering of appendices consistent with that of the October 2006 report.

Appendices proposed for adoption at the 75th General Session	Appendix number
Definitions (Ch. 1.1.1.)	Appendix III
Diseases listed by the OIE (Ch. 1.2.3.)	Appendix IV
Zoning and compartmentalisation (Ch. 1.4.4.)	Appendix V
Infection with <i>Bonamia ostreae</i> (Ch. 2.2.1.)	Appendix VI
Infection with <i>Bonamia exitiosa</i> (Ch. 2.2.2.)	Appendix VII
Infection with <i>Haplosporidium nelsoni</i> (Ch. 2.2.3.)	Appendix VIII
Infection with <i>Marteilia refringens</i> (Ch. 2.2.4.)	Appendix IX
Infection with <i>Mikrocytos mackini</i> (Ch. 2.2.5.)	Appendix X
Infection with <i>Xenohaliotis californiensis</i> (Ch. 2.2.8.)	Appendix XI
Recommendations for transport (Ch. 1.5.1.)	Appendix XII
Blank Appendix	Appendix XIII
Koi herpesvirus disease (Ch. 2.1.17.)	Appendix XIV
Taura syndrome (Ch. 2.3.1.)	Appendix XV
White spot disease (Ch. 2.3.2.)	Appendix XVI
Yellowhead disease (Ch. 2.3.3.)	Appendix XVII
Tetrahedral baculovirus (Ch. 2.3.4.)	Appendix XVIII
Spherical baculovirus (Ch. 2.3.5.)	Appendix XIX
Infectious hypodermal and haematopoietic necrosis (Ch. 2.3.6.)	Appendix XX
Crayfish plague (Ch. 2.3.7.)	Appendix XXI
Koi herpesvirus disease (<i>Aquatic Manual</i> Chapter)	Appendix XXII
Appendices for Member Countries' comments (deadline 6 August 2007)	Appendix number
Infectious myonecrosis (Ch. 2.3.9.)	Appendix XXIII
Necrotising hepatopancreatitis (Ch. 2.3.10.)	Appendix XXIV
White tail disease (Ch. 2.3.11.)	Appendix XXV
Hepatopancreatic parvovirus disease (Ch. 2.3.12.)	Appendix XXVI
Mourilyan virus disease (Ch. 2.3.13.)	Appendix XXVII
Guidelines for the control of aquatic animal health hazards in aquatic animal feeds	Appendix XXVIII
General guidelines for aquatic animal health surveillance (<i>Aquatic Code</i> Appendix)	Appendix XXIX
Guidelines for aquatic animal health surveillance (<i>Aquatic Manual</i> Chapter)	Appendix XXX

Appendices for Member Countries' information	Appendix number
Report of the <i>ad hoc</i> Group on Aquatic Animal Feeds	Appendix XXXI
Report of the <i>ad hoc</i> Group on Aquatic Animal Health Surveillance	Appendix XXXII
Conclusions and abstracts from workshop in Florianopolis	Appendix XXXIII
Work plan	Appendix XXXIV

1. Activities and progress of *ad hoc* Groups

The Aquatic Animals Commission reviewed the progress made by those *ad hoc* Groups that have met since the previous meeting of the Commission:

- I. OIE *ad hoc* Group on Aquatic Animal Health Surveillance, 24-26 July 2006 and 29-31 January 2007.
- II. OIE *ad hoc* Group on Aquatic Animal Feeds, 12-14 December 2006.

The Commission noted the overall progress made by the *ad hoc* Groups against their terms of reference and expressed its appreciation for the excellent work of the experts involved. The Commission recognised the efficiency of face-to-face meetings and agreed that this way of working should be continued.

Specific items related to the above *ad hoc* Groups will be dealt with in specific agenda items below.

2. Aquatic Animal Health Code

2.1. General comments on the October 2006 report

Nicaragua had suggested using the name *Litopenaeus vannamei* instead of *Penaeus vannamei* in Article 2 of the crustacean disease chapters. The Aquatic Animals Commission discussed this request but decided to maintain the previous nomenclature as recommended by leading experts. See following references:

ALDERMAN D.J., COSTA-PIERCE B.A., DONALDSON E.M., HULATA G. & WILSON R.P. (2007). - Editorial: Use of the generic name *Penaeus*. *Aquaculture*, in press.

FLEGEL T.W. (2007). - The right to refuse revision in the genus *Penaeus*. *Aquaculture*, 264, 2–8.

Colombia drew the Commission's attention to the rapid expansion of aquaculture of warm water fish species (e.g. tilapia) and suggested that the *Aquatic Code* refer to diseases of those species and also that health conditions be revised. The Commission pointed out that the *Aquatic Code* chapters on individual diseases apply to all species listed as susceptible in the scope of those chapters, including warm water or ornamental varieties as applicable.

The Commission welcomed the suggestion made by the EC on the pathways for infected compartments to again be declared free from the disease in question. Considering the ongoing discussions related to the Chapter on zoning and compartmentalisation (see point 2.5 below), the Commission decided to await the adoption of the draft chapter prior to formulating any specific recommendations on this topic.

The EC queried the need for animal health certificates for dead molluscs, fish and crustacean products. The Commission points out that for those commodities that are considered safe, and therefore listed in Article 3, point 1) of each disease chapter, there is no need to provide an animal health certificate. Furthermore a health certificate is currently recommended only for those products originating from a country, zone or compartment declared free from the diseases under consideration, to provide confirmation of the claim of free status to the importing country.

The EC also suggested combining the articles "Importation of aquatic animal products from an area not declared free" and "Importation of aquatic animal products from an area declared free" into one single article. In line with the above, the Commission points out that maintaining two separate articles makes the different requirements of each easier to understand.

In support of their previous request to consider disinfected fish eggs as safe commodities for some diseases, the EC has provided the report of the EU funded study “Fish Egg Trade”. The Commission agreed with the EC comment that it would be useful to have in the *OIE Manual of Diagnostic Tests for Aquatic Animals* (hereafter referred to as the *Aquatic Manual*) full details of step by step procedures for the disinfection of eggs. The Commission will ask the consultant editor for the *Aquatic Manual* to redraft the egg disinfection section on Chapter 1.1.5. Once this task is completed, the Commission will forward the consultant editor’s revision as well as the report of the EU funded study “Fish Egg Trade” to the OIE *ad hoc* Group on Chapters for Fish Diseases for the OIE *Aquatic Animal Health Code* for consideration and formulation of a recommendation on whether disinfected fish eggs could be listed under Article 3 of the specific disease chapter.

The EC raised concerns about the suggested references in the *Aquatic Code* to the ICES Code of Practice on the Introductions and Transfers of Marine Organisms. The Commission stressed that the ICES Code is an internationally recognised document which has been in existence for many years and has been successfully applied globally. The Commission noted that the practice of cross referencing to non OIE international standards is used in other OIE texts. The Commission noted that methods for disease prevention and control are within the mandate of the OIE; it also noted that the scope of the ICES Code goes beyond development of specific pathogen free populations and includes procedures for introducing new species while mitigating the risk of introduction of disease. Hence the Commission considers it appropriate to refer to the ICES Code. Furthermore, given the relevance of the ICES Code to the OIE mandate, the OIE Central Bureau may wish to look at the possibility of establishing more formal arrangements between ICES and the OIE.

The EC and Canada commented on the explanatory note for disease chapters on diseases that have been removed from the OIE list. To better identify these diseases for which specific disease chapters are nevertheless retained in the *Aquatic Code*, despite their removal from the list of diseases in Chapter 1.2.3., the Aquatic Animals Commission proposes to amend the explanatory note as follows: “NB: This disease does not meet the listing criteria in Chapter 1.2.2. Nevertheless, reporting requirements for non listed diseases apply in regard to significant epidemiological events (Article 1.2.1.3, point 1e)”.

2.2. Definitions (Chapter 1.1.1.)

Several Member Countries expressed concern with the proposed definition of “veterinary paraprofessionals”. The Aquatic Animals Commission noted that the proposed definition is based on the current definition in the OIE *Terrestrial Animal Health Code* (hereafter referred to as the *Terrestrial Code*) and will liaise with the Terrestrial Code Commission prior to any modifications to the proposed definition. The draft definition will therefore not be submitted for adoption at the 75th General Session in May 2007.

In response to a suggestion from Australia, the Commission modified several other definitions that cross reference to the term “infection” to accommodate the newly proposed term “infestation”.

Some comments were received on the French and Spanish translations of certain proposed definitions. The Commission referred these comments to the OIE Central Bureau.

Several other comments received were addressed by the Commission in their review of the proposed definitions.

The definitions that will be proposed to the OIE International Committee for adoption at the 75th General Session in May 2007 are attached at [Appendix III](#).

2.3. Criteria for listing aquatic animal diseases (Chapter 1.2.2.)

The Aquatic Animals Commission addressed a comment from Chile proposing an amendment to criterion 7 (geographic distribution) of the disease listing criteria so that it specifies the number of countries that must be free of the disease for this criterion to apply. The Commission discussed this issue but is of the opinion that the application of that criterion needs to take into account the different variables of specific situations (distribution of the susceptible species, climate) on a case by case approach. Therefore, the Commission does not propose any changes to this Chapter.

2.4. Revision of the list of diseases (Chapter 1.2.3.)

Argentina expressed concerns about the removal of infectious pancreatic necrosis (IPN) from the OIE list of diseases arguing that IPN is one of the diseases with the highest impact on aquatic animal trade, both in terms of economics and production. The Aquatic Animals Commission reminds Argentina that the OIE International Committee adopted the removal of IPN from the list of diseases in May 2006; however, an *Aquatic Code* chapter on IPN has been retained to provide guidance for trade.

Japan recommended the delisting of abalone viral mortality because of the absence of a specific diagnostic test and consequent difficulties with reporting. The Commission pointed out that the OIE International Committee adopted the listing of abalone viral mortality in May 2006. The Commission acknowledges that the diagnosis of diseases in this syndrome is problematic, but the only way to gather additional information is by making the disease notifiable. As previously stated, all diseases listed as emerging diseases will be reviewed within three years of their listing to decide whether they now meet the full listing criteria or whether they should be recommended for deletion.

The Commission agreed that further scientific information has become available since the last meeting of the OIE *ad hoc* Group on the OIE List of Aquatic Animal Diseases and that a revision of the disease card on this disease may be necessary (see point 9.1 below). The Commission pointed out that, at this stage, the *Aquatic Code* does not contain any recommendations related to trade and abalone viral mortality.

Nicaragua commented against the proposed listing of necrotising hepatopancreatitis (NHP) since therapeutic methods are readily available. The Commission pointed out that the availability of therapeutic treatment is not part of the listing criteria. On the other hand managerial procedures can mitigate the losses due to the pathogen; therefore the disease will need to be reassessed against Criterion 1 by the OIE *ad hoc* Group on the OIE List of Aquatic Animal Diseases. The Commission therefore proposes to maintain NHP as under study pending the recommendations of the *ad hoc* Group.

Madagascar commented that hepatopancreatic parvovirus (HPV) disease should not be listed because it causes neither massive mortality nor significant production losses in well managed systems. Because multiple strains of HPV exist that are not detectable by the available diagnostic methods, a robust diagnostic test still needs to be developed. The Commission decided that the disease will need to be reassessed against the listing criteria by the OIE *ad hoc* Group on the OIE List of Aquatic Animal Diseases. The Commission therefore proposes to list HPV disease as under study pending the recommendations of the *ad hoc* Group.

The USA considers the listing of Mourilyan virus disease premature due to the paucity of scientific literature linking the virus to a distinct disease (see Criterion 2 of Article 1.2.2.2.). The Commission decided that the disease will need to be reassessed against the listing criteria by the OIE *ad hoc* Group on the OIE List of Aquatic Animal Diseases. The Commission therefore proposes to list Mourilyan virus disease as under study pending the recommendations of the *ad hoc* Group.

The updated Chapter on the Diseases Listed by the OIE that will be proposed to the OIE International Committee for adoption at the 75th General Session in May 2007 is attached at [Appendix IV](#).

2.5. Zoning and compartmentalisation (Chapter 1.4.4.)

The Aquatic Animals Commission revised the draft chapter taking into account Member Countries' comments.

The updated Chapter on Zoning and Compartmentalisation that will be proposed to the OIE International Committee for adoption at the 75th General Session in May 2007 is attached at [Appendix V](#).

2.6. Recommendations for transport (Chapter 1.5.1.)

The Aquatic Animals Commission revised the draft chapter taking into account Member Countries' comments.

Norway suggested animal welfare be taken into account in relation to transport of fish. The Commission noted the ongoing work on aquatic animal welfare during transport that is being addressed by the OIE Working Group on Animal Welfare (see point 2.9 below for more details).

Norway and the EC suggested further work be done on biosecurity risks associated with transport by sea. The Commission agreed with this suggestion, accepted Norway's offer to assist and invited Norway to submit a draft text for consideration by the Commission.

The updated Chapter on recommendations for transport that will be proposed to the OIE International Committee for adoption at the 75th General Session in May 2007 is attached at [Appendix XII](#).

2.7. Disease chapters (Part 2)

Australia and the OIE Reference Laboratory for Infection with *Bonamia exitiosa*, Infection with *Bonamia ostreae*, Infection with *Mikrocytos roughleyi*, Infection with *Marteilia refringens* and Infection with *Marteilia sydneyi* commented on the basis for establishing timeframes recommended for basic biosecurity conditions in mollusc disease chapters. The Aquatic Animals Commission explained that the timeframes for these conditions were proposed on the basis of the biology and lifecycles of the agent and susceptible species, the requirement for and presence of intermediate hosts, and direct transmission and incubation periods. However, the Commission acknowledges that other factors could be considered and agreed to ask the OIE *ad hoc* Group on Aquatic Animal Health Surveillance to propose what criteria should be used for establishing timeframes for all disease chapters.

Several comments suggested that larvae should not be listed in Article 3 point 1a) of some mollusc disease chapters. The Commission noted that the argument put forward is more relevant to spat and juvenile stages than larvae. The Commission decided to maintain the current recommendation for the time being but, realising the complexity of this issue, agreed to forward these comments to the OIE *ad hoc* Group on Chapters for Mollusc Diseases for the OIE *Aquatic Animal Health Code* for disease-by-disease consideration.

Taipei China asked to specify the inactivation temperatures for "heat treated products" in Article 3 point 1a) of all mollusc disease chapters. The Commission pointed out that the comment referred to an already approved text in the *Aquatic Code* but proposed, based on expert opinion, to replace the reference to "heat treated" with "pasteurised".

The Commission reviewed further Member Countries' comments on the proposed disease chapters on mollusc diseases and amended the draft chapters where necessary.

The updated Chapters on mollusc diseases that will be proposed to the OIE International Committee for adoption at the 75th General Session in May 2007 are attached at [Appendices VI to XI](#).

The Commission reviewed Member Countries' comments on the draft disease chapter on gyrodactylosis and agreed that there was merit with many of the points raised, but due to the highly technical, scientific nature of these comments, decided to refer them to the OIE *ad hoc* Group on Chapters for Fish Diseases for the OIE *Aquatic Animal Health Code*. This draft chapter will not be proposed therefore for adoption at the May 2007 General Session.

The Commission reviewed Member Countries' comments on the draft disease chapter on koi herpesvirus (KHV) disease, agreed with many of the points raised and made some changes to several articles accordingly. For example, Article 2.1.17.3. was changed to clarify the nature of some commodities, e.g. fish meal intended for use in animal feeds. The updated Chapter on KHV disease that will be proposed to the OIE International Committee for adoption at the 75th General Session in May 2007 is attached at [Appendix XIV](#).

Taipei China pointed out a discrepancy regarding the causative agent for white tail disease between the *Aquatic Code* and the disease card. The Commission clarified that the text in the *Aquatic Code* is the correct one and asked the OIE Central Bureau to amend the disease card.

Taipei China asked for more detail on the inactivation parameters for dry feeds mentioned in Articles 3 of the crustacean disease chapters. The Commission indicated that more details are provided in the report of the *ad hoc* Group on Aquatic Animal Feeds (see point 2.11 below).

Dr David Alderman, designated OIE expert for the OIE Reference Laboratory on Crayfish plague, joined the meeting for this item. The Commission reviewed in detail the draft chapter on crayfish plague and agreed on several changes due to the different nature of this disease in comparison to the other crustacean diseases.

The updated Chapters on crustacean diseases that will be proposed to the OIE International Committee for adoption at the 75th General Session in May 2007 are attached at Appendices XV to XXI.

The draft Chapters on Infectious myonecrosis, white tail disease, NHP, HPV and Mourilyan virus disease are presented at Appendices XXIII to XXVII for Member Countries' comment.

2.8. New appendix on general guidelines for aquatic animal health surveillance

See point 7.3 below.

2.9. Aquatic animal welfare

The Aquatic Animals Commission awaits feedback from the OIE Animal Welfare Working Group on issues previously raised by Member Countries.

2.10. Antimicrobial resistance in the field of aquatic animals

The Aquatic Animals Commission reviewed the list of antimicrobials of veterinary importance that had been compiled by the OIE *ad hoc* Group on Antimicrobial Resistance (which reports to the Biological Standards Commission) and the comments on it that had been sought from aquatic experts. The Commission made some minor changes to better reflect the relative importance of some groups on antimicrobials for aquatic animals. The amended document will be referred back to the Biological Standards Commission for consideration prior to presentation at the 75th General Session.

2.11. Aquatic animal feeds

Prof. Eli Katunguka-Rwakishaya, Member of the Aquatic Animals Commission, reported on the progress made by the OIE *ad hoc* Group on Aquatic Animal Feeds (the report is appended at Appendix XXXI for Member Countries' information). The Aquatic Animals Commission was impressed by the useful work of the *ad hoc* Group on these complex issues.

The Commission discussed the Draft Guidelines for the Control of Aquatic Animal Health Hazards in Aquatic Animal Feeds and made some minor modifications to the text which is appended at Appendix XXVIII for Member Countries' comments.

The Commission noted the *ad hoc* Group's query about the proposed scope of the draft Guidelines. The Commission agreed that the *ad hoc* Group should – as a priority – complete its work on aquatic animal pathogens through a further meeting. The timing of this meeting should be organised to allow for the *ad hoc* Group to consider Member Countries' comments received after the May 2007 General Session. Additional work could take place on hazards of public health significance, but the Commission recommended that this be done under the auspices of the OIE Animal Production Food Safety Working Group.

2.12. Diseases of amphibians

The Aquatic Animals Commission reviewed the outcomes of the questionnaire on amphibian diseases and was pleased with the replies received. The Commission noted that of the 65 countries that had replied, 46 supported the inclusion of amphibian diseases in the remit of the OIE. In view of this supportive majority, the Commission proposes to ask the OIE International Committee at the 75th General Session in May 2007 for in-principle agreement to this expansion of the OIE's remit. If agreement is reached, the Commission proposes to reconvene the OIE *ad hoc* Group on Amphibian Diseases, with revised terms of reference that include the development of a list of diseases and draft chapters for the *Aquatic Code* and the *Aquatic Manual*.

3. Joint meeting with the President of the OIE Terrestrial Animal Health Standards Commission

Dr Alex Thiermann, President of the OIE Terrestrial Animal Health Standards Commission (hereafter referred to as the Terrestrial Code Commission), joined the meeting for this agenda item.

3.1. Model certificates

Dr Thiermann reported on the progress made by the OIE *ad hoc* Group on the Revision of the Model Certificates. The revised models will be discussed at the next meeting of the Terrestrial Code Commission, with the view of circulating them for Member Countries' comments.

The Aquatic Animals Commission reviewed the revised model certificates and expressed support for the approach taken. The Commission will follow developments (including the feedback from Member Countries) of these models with a view to revising the aquatic model certificates along similar lines.

3.2. Handling and disposal of carcasses and wastes of aquatic animals

Prof. Katunguka-Rwakishaya presented to the Aquatic Animals Commission a new draft Appendix for the *Aquatic Code* on General Guidelines for Disposal of Dead Aquatic Animals and Wastes of Aquatic Animals. This draft takes into account the current Appendix 3.6.6. in the *Terrestrial Code*. Issues relating to welfare will be handled in due course based on the recommendations of the Animal Welfare Working Group. The Commission thanked Prof. Katunguka-Rwakishaya for his work and provided some comments. Prof. Katunguka-Rwakishaya will provide a revised draft for consideration for the next meeting of the Commission, with a view to then circulating it for Member Countries' comments.

3.3. Future evolution of both Codes

Dr Thiermann explained that due to the size of the printed edition of the *Terrestrial Code*, OIE plans to publish the next edition as two volumes. This separation requires a structural reorganisation of the chapters rather than just splitting the book in two. Representative examples will be presented to the next General Session for demonstration.

While future editions of the horizontal chapters of both the *Aquatic* and *Terrestrial Codes* could be merged into one volume, the Aquatic Animals Commission is firmly of the view that harmonising the content of both Codes is a higher priority than merging into one single volume; first there are concepts that are sufficiently different in application (e.g. zones) to make merging of horizontal chapters unnecessarily complicated and difficult to apply. Furthermore, the benefit to the different groups of end users (i.e. aquatic or terrestrial) of such merging is not apparent. Where appropriate, the contents of the horizontal chapters of both Codes will be identical.

3.4. Performance, Vision and Strategy tool

The Aquatic Animals Commission was updated on the OIE activities related to the PVS and received a copy of the PVS tool and manual. The Aquatic Animals Commission acknowledged the intent of the OIE to extend the application of the PVS to aquatic animal health services and a proposal to offer training to potential assessors of aquatic animal health services. The Commission noted the training could take place later this year.

The PVS tool is designed to assist Veterinary Services to establish their current level of performance, to identify gaps and weaknesses regarding their ability to comply with OIE international standards, to form a shared vision with stakeholders (including the private sector) and to establish priorities and carry out strategic initiatives.

The Aquatic Animals Commission welcomed the principles expressed in the PVS and its future extension to aquatic animal health but recommended that due consideration be given to adapting the tool to make it broadly applicable to aquatic animal health (e.g. the central role of the veterinarians, the issue of accreditation of laboratories and experts, application of zones and compartments, traceability of animals, food safety, certification).

The Commission suggested that in adapting the tool, the provisions of the *Aquatic Code* (e.g. Evaluation of the Competent Authorities) need to be used as a legal basis for the aquatic PVS.

The OIE *ad hoc* Group on the Evaluation of Veterinary Services will meet in July 2007 and will continue the work on the development of the PVS. The Aquatic Animals Commission will suggest to the OIE Central Bureau to send a representative to this meeting and recommended that the OIE convene a specific *ad hoc* Group to develop the aquatic PVS. The Aquatic Animals Commission will also provide a list of potential candidates acquainted with aquatic animal health services as potential trainees as PVS evaluators.

4. **Feedback from the Commission on the OIE World Animal Health Information Database (WAHID)**

Dr Karim Ben Jebara, Head of the OIE Information Department, joined the meeting for this agenda item.

The Aquatic Animals Commission provided feedback on its experience on using the various features of WAHID for information on the occurrence of aquatic diseases in Member Countries. The Commission was impressed by the appearance and the ease of use of the new system.

Dr Ben Jebara demonstrated several features of WAHID and explained that further refinement is taking place in response to feedback from users. For example the Information Department is working on the possibility for displaying information on aquatic diseases separately from that on terrestrial diseases in addition to the current combined manner.

The Commission noted that although links to disease cards are provided for some of the terrestrial animal diseases, no such links are yet provided for the aquatic animal diseases. The Commission requested that links be provided and Dr Ben Jebara agreed that this would be done as soon as practicable.

5. **OIE Scientific and Technical Review: Issue on aquatic animal health**

Dr Paul-Pierre Pastoret, Head of the OIE Publications Department, joined the meeting for this agenda item.

He reported good progress on the preparation of the OIE *Scientific and Technical Review: Changing trends in managing aquatic animal disease emergencies*, which is due to be published in April 2008.

6. **The role and activities of the OIE in the field of aquatic animal health**

6.1. International meetings

6.1.1. Regional Commissions Conferences

The Conference of the OIE Regional Commission for Africa took place in Eritrea, Asmara from 26 February to 1 March 2007. Prof. Katunguka-Rwakishaya represented the Aquatic Animals Commission and presented a paper "Update on the activities of the Aquatic Animal Health Standards Commission". The paper emphasized the following areas:

- Importance of aquaculture as the fastest growing animal food producing industry

- The need to control and prevent spread of listed diseases (*Aquatic Code* and *Aquatic Manual*)
- The need for veterinary authorities of Member Countries to take a keener interest in aquatic animal diseases
- Better cooperation between veterinary and fisheries authorities in the control and reporting of aquatic diseases.

The paper was well received and discussed broadly. Dr Barry O'Neil, President of the OIE International Committee, Dr Robert Thwala, President of the OIE Regional Commission for Africa, and Dr Dewan Sibartie, Head of the OIE Regional Activities Department, called upon Delegates to seriously consider the issues raised in the presentation.

The 18th Conference of the OIE Regional Commission for the Americas took place in Brazil, Florianopolis from 28 November – 2 December 2006. Dr Enriquez presented an update of the activities of the OIE related to the Aquatic Animals.

Dr Enriquez underlined the importance of receiving comments from Member Countries on the proposals of the Commission especially those regarding the amendments to be made in the *Aquatic Code* and the *Aquatic Manual*. He pointed out that a national focal point for aquatic animal diseases was imperative to improve such exchange of information.

At the end of his presentation, Dr Enriquez stated that the communication with the Animal Health Information Department on the OIE World Animal Health Information System has been particularly fruitful.

The Commission committed to provide input at the upcoming Conferences of the OIE Regional Commission for the Middle-East (October 2007) and the OIE Regional Commission for Asia, the Far East and Oceania (November 2007).

6.1.2. Fifth Annual General Meeting of NACA's Asia Regional Advisory Group on Aquatic Animal Health, 22-24 November 2006, Bangkok, Thailand

Dr Bernoth attended this three-day meeting which addressed global and regional aquatic animal health issues. She presented a report on the outcomes of the OIE General Session in May 2006 and new initiatives underway in aquatic animal health. Topics covered at the meeting included an update on emerging crustacean, fish and mollusc diseases in the region and regional and international cooperation in Asian aquatic animal health management. The NACA/OIE quarterly aquatic animal disease reporting system was reviewed.

The full report of the meeting has been sent to National Coordinators and OIE Aquatic Focal Points and OIE Delegates in the 21 participating countries in the Asia Pacific. The meeting acknowledged the collaboration with the OIE Central Bureau and with the OIE Regional Official as well as the Commission, who all have contributed to significantly strengthening aquatic animal disease control and management in the Asian region.

6.1.3. First International Conference of OIE Reference Laboratories and Collaborating Centres, 3-5 December 2006, Florianopolis, Brazil

Dr Gideon Brückner, Head of the OIE Scientific and Technical Department, joined the meeting for this and the following agenda item.

The Conference had been a success; over 300 participants from 35 countries had attended. Dr Brückner presented the recommendations, which would be published in the Conference proceedings.

The new OIE laboratory twinning concept had been launched at the Conference and was well received. However, participants stressed that for the twinning to be successful, funding would need to be found for both the twinned laboratory and to cover the additional costs for OIE Reference Laboratories. The Commission welcomed the laboratory twinning initiative and encouraged Member Countries to consider their twinning opportunities. Dr Brückner informed the Commission that the Delegates would shortly be sent documentation outlining procedures for applications to the OIE. The Commission recommended that the OIE Reference Laboratories and Collaborating Centres also be sent this information.

6.1.4. OIE Global Conference on Aquatic Animal Health, 9-12 October 2006, Bergen, Norway

The OIE, in collaboration with the Norwegian Government, organised the first Global Conference on Aquatic Animal Health dedicated to reinforcing the commitment of OIE Member Countries to their rights and obligations regarding disease notification and implementation of OIE standards.

The Commission endorsed the draft recommendations to Member Countries and to the OIE on aquatic animal health issues, which had been formulated at the Conference. These recommendations will be included in the proceedings and will be reported to the OIE International Committee at the 75th General Session in May 2007.

6.2. Cooperation with FAO

The Aquatic Animals Commission noted the exchange of correspondence between the OIE and the FAO on the topic of aquatic animal health. The Commission will continue to work with the OIE Central Bureau to support further strengthening of the collaboration between the OIE and the FAO.

7. *Manual of Diagnostic Tests for Aquatic Animals*

7.1. Koi herpesvirus disease

Comments had been received on the draft chapter on KHV disease that had been appended to the last meeting's report. The Aquatic Animals Commission was grateful for the constructive and helpful comments which were referred to the author who made a number of amendments to the chapter. This amended version will be proposed for adoption at the General Session in May and, if adopted, will be added to the web version of the *Aquatic Manual*.

The new Chapter on KHV disease that will be proposed to the OIE International Committee for adoption at the 75th General Session in May 2007 is attached at [Appendix XXII](#).

7.2. Update from the Consultant Editor

Dr David Alderman, the newly appointed Consultant Editor for the *Aquatic Manual*, joined the meeting for this agenda item.

One of the Terms of Reference for the Consultant Editor is to revise the design of the disease chapter template. Dr Alderman agreed to reformat the template in time for the next meeting of the Commission in the second half of 2007, taking into account the updated template prepared by the OIE *ad hoc* Group on Aquatic Animal Health Surveillance (see point 2.8 above). Once the Commission approves the new template, it would be sent to the authors with the request to use it to update their chapters. The next edition of the *Aquatic Manual* is scheduled to be published in 2009. It is planned to include in this edition updates of all the chapters, including those that were not updated in the 2006 edition.

7.3. Report of the meetings of the OIE *ad hoc* Group on Aquatic Animal Health Surveillance

The Aquatic Animals Commission noted the report of the *ad hoc* Group (which is appended at [Appendix XXXII](#) for Member Countries' information). The Commission was very impressed with the amount of progress made by the Group at its two meetings and the quality of the output.

Appendices VI and VII of the report of the *ad hoc* Group represent work in progress; the Commission will submit its comments to the *ad hoc* Group with the view to preparing texts for Member Country comment at the next meeting of the Commission.

Member Country comments are sought on Appendices XXIX and XXX.

7.4. Review of Chapter 1.1.5. on disinfection of aquaculture establishments

The current *Aquatic Manual* chapter on disinfection of aquaculture establishments is divided into three sections: one each for fish, mollusc and crustacean farms. This means that there is some repetition as the principles and some procedures are common to all three groups. Dr Alderman agreed to rearrange the chapter such that it begins with the general principles and procedures followed by specific procedures for fish, molluscs and crustaceans, e.g. fish eggs, crustacean broodstock, etc. The revised chapter will be reviewed by the Aquatic Animals Commission at its next meeting.

8. OIE Reference Laboratories

8.1. Review of list of Reference Laboratories

The Commission recommends acceptance of the following new applications for OIE Reference Laboratory status:

OIE Reference Laboratory for Koi herpesvirus disease

Fisheries Research Agency, Research Promotion & Development Department, Yokohama 220-6115, JAPAN Tel.: (+81-45) 227.2677; Fax: (+81-45) 227.2703; sanogen@fra.affrc.go.jp
Designated Reference Expert: Dr Motohiko Sano.

OIE Reference Laboratory for Koi herpesvirus disease

Centre for Environment, Fisheries and Aquaculture Science (CEFAS), the Nothe, Weymouth, Dorset DT4 8UB, UNITED KINGDOM Tel.: (+44-1305) 206.639; Fax: (+44-1305) 206.601; keith.way@cefasc.co.uk Designated Reference Expert: Dr Keith Way.

The OIE has been notified of the following changes of experts at OIE Reference Laboratories. The Commission recommends their acceptance:

Spring viraemia of carp

Dr Peter Dixon to replace Prof. Barry Hill at CEFAS, Weymouth, United Kingdom.

Crayfish plague (Aphanomyces astaci)

Dr Birgit Oidtmann to replace Dr David Alderman at CEFAS, Weymouth, United Kingdom.

The Commission acknowledged a request from the Delegate of the United Kingdom that the OIE Reference Laboratory for infectious pancreatic necrosis, at CEFAS, Weymouth be removed from the list.

The Commission was disappointed that it had not received any applications for OIE Reference Laboratory status for abalone viral mortality and once again encourages applications to be submitted through the OIE Delegate.

If the OIE International Committee adopts the listing of the crustacean diseases infectious myonecrosis and white tail disease in May 2007, OIE Delegates will be encouraged to submit applications for Reference Laboratories for these two diseases.

8.2. Concept paper on pathogen strain differentiation

Comments had been received from the USA on the concept paper (that was appended to the October 2006 report) strongly supporting this initiative and proposing guidelines to be included in the *Aquatic Manual*. The EC had also welcomed the concept paper and encourages OIE to pursue this issue.

The paper was presented at a special workshop held in conjunction with the First International Conference of OIE Reference Laboratories and Collaborating Centres (see point 6.1.3 above). The workshop reached the conclusion, endorsed in the recommendations of the conference, that this issue should be discussed in a wider forum at the next conference of OIE Reference Laboratories and Collaborating Centres and that the implications of differentiating between genotypes for OIE notification and reporting criteria should be considered by the Aquatic Animals Commission. The conclusions and abstracts from this workshop can be found at [Appendix XXXIII](#) for information of Member Countries.

8.3. Review of annual reports of activities (2006)

Reports had been received from all 28 Reference Laboratories and from the Collaborating Centre. The Aquatic Animals Commission was very impressed with the quality of the work carried out by the laboratories and appreciates the contributions they make towards achieving the objectives of the OIE.

9. Any other business

9.1. Disease cards

All the disease cards are available on the Aquatic Animals Commission web page under 'Disease Information'. Whilst discussing the format of the diseases cards, the Aquatic Animals Commission questioned the usefulness of disease cards for diseases for which an *Aquatic Manual* chapter already exists in print and on the web. The Commission is leaning towards having disease cards only for emerging and recently listed diseases for which there are not yet an *Aquatic Manual* chapter, and discontinuing cards for all other diseases. The Commission invites Member Country views on this proposition.

Australia queried the inclusion of abalone viral ganglioneuritis that occurred in Australia in the disease card on abalone viral mortality. The Commission acknowledged that since the update of that disease card, new research findings have become available on the Australian situation. The Commission invites Australia to submit such information to the Commission for consideration by the OIE *ad hoc* Group on the OIE List of Aquatic Animal Diseases, with the view to revise the disease card (in particular the case definition).

Comments had been received from Australia and the USA on the Mourilyan virus disease card. The USA queried the listing of this disease while Australia commented on the lack of specificity in the description of gross signs in the card. The Commission decided to propose the listing of Mourilyan disease as 'under study', but to keep the disease card until more information becomes available.

9.2. Work plan

The Aquatic Animals Commission reviewed its work plan for 2007–2008. The work plan is appended at [Appendix XXXIV](#) for Member Countries' information.

10. **Date of the next meeting**

The Aquatic Animals Commission proposed to meet on 1–5 October 2007.

../Appendices

**MEETING OF THE OIE
AQUATIC ANIMAL HEALTH STANDARDS COMMISSION
Paris, 5–9 March 2007**

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**MEETING OF THE OIE
AQUATIC ANIMAL HEALTH STANDARDS COMMISSION
Paris, 5-9 March 2007**

Adopted agenda

1. **Activities and progress of *ad hoc* Groups**
2. ***Aquatic Animal Health Code***
 - 2.1. General comments on the October 2006 report
 - 2.2. Definitions (Chapter 1.1.1.)
 - 2.3. Criteria for listing aquatic animal diseases (Chapter 1.2.2.)
 - 2.4. Revision of the list of diseases (Chapter 1.2.3.)
 - 2.5. Zoning and compartmentalisation (Chapter 1.4.4.)
 - 2.6. Recommendations for transport (Chapter 1.5.1.)
 - 2.7. Disease chapters (Part 2)
 - 2.8. New appendix on general guidelines for aquatic animal health surveillance
 - 2.9. Aquatic animal welfare
 - 2.10. Antimicrobial resistance in the field of aquatic animals
 - 2.11. Aquatic animal feeds
 - 2.12. Diseases of amphibians
3. **Joint meeting with the President of the Terrestrial Animal Health Standards Commission**
 - 3.1. Model certificates
 - 3.2. Handling and disposal of carcasses and wastes of aquatic animals
 - 3.3. Future evolution of both Codes
 - 3.4. Performance, Vision and Strategy tool
4. **Feedback from the Commission on the OIE World Animal Health Information Database (WAHID)**
5. **OIE *Scientific and Technical Review*: Issue on aquatic animal health**
6. **The role and activities of the OIE in the field of aquatic animal health**
 - 6.1. International meetings
 - 6.1.1. Regional Commission Conferences
 - 6.1.2. Fifth Annual General Meeting of NACA's Asia Regional Advisory Group on Aquatic Animal Health, 22-24 November 2006, Bangkok, Thailand
 - 6.1.3. First International Conference of OIE Reference Laboratories and Collaborating Centres, 3-5 December 2006, Florianopolis, Brazil
 - 6.1.4. First OIE Global Conference on Aquatic Animal Health, 9-12 October 2006, Bergen, Norway
 - 6.2. Cooperation with FAO

Appendix II (contd)

7. ***Manual of Diagnostic Tests for Aquatic Animals***

7.1. Koi herpesvirus disease

7.2. Update from the Consultant Editor

7.3. Report of the meetings of the OIE *ad hoc* Group on Aquatic Animal Health Surveillance

7.4. Review of Chapter 1.1.5 on disinfection of aquaculture establishments

8. **OIE Reference Laboratories**

8.1. Review of list of Reference Laboratories

8.2. Concept paper on pathogen strain differentiation

8.3. Review of annual reports of activities (2006)

9. **Any other business**

9.1. Disease cards

9.2. Review of the Aquatic Animals Commission's work plan for 2007-2008

10. **Date of the next meeting**

CHAPTER 1.1.1. DEFINITIONS

Article 1.1.1.1.

Aquatic animal health status

means the status of a country, zone or compartment with respect to an aquatic animal disease, according to the criteria listed in the relevant chapter of the Aquatic Code dealing with the disease.

Biosecurity plan

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

Compartmentalisation

means identifying compartments for disease control or international trade purposes.

Disease

means clinical or non clinical *infection* or *infestation* with one or more of the aetiological agents of the diseases referred to in the *Aquatic Code*.

Infection

means the presence of a multiplying or otherwise developing or latent *disease agent* in ~~or, for~~ ectoparasites, ~~on~~ a host.

Infestation

means the presence in large sufficient numbers of a multiplying parasitic, or commensal, agent on a host so as to cause damage or disease.

Inspection

means the control carried out by the Competent Authority in order to ensure that an aquatic animal is/ aquatic animals are free from the diseases/infections considered in the Aquatic Code, the inspection may call for clinical examination, laboratory tests and, generally, the application of other procedures that could reveal an infection or infestation that may be present in an aquatic animal population.

Subpopulation

means a distinct part of a population identifiable according to specific common aquatic animal health characteristics.

Stamping-out policy

means the carrying out under the authority of the Competent Authority, on confirmation of a disease, of preventive aquatic animal health measures, consisting of killing the aquatic animals that are affected, those suspected of being affected in the population and those in other populations that have been exposed to infection or infestation by direct or indirect contact of a kind likely to cause the transmission of the disease agent. All these aquatic animals, vaccinated or unvaccinated, on an infected site should be killed and the carcasses destroyed by burning or burial, or by any other method that will eliminate the spread of infection or infestation through the carcasses or products of the aquatic animals destroyed.

Appendix III (contd)

This policy should be accompanied by cleansing and *disinfection* procedures as defined in the *Aquatic Code*. *Fallowing* should be for an appropriate period determined by *risk assessment*.

Subclinical

means without clinical manifestations, for example a stage of *infection* or *infestation* at which signs are not apparent or detectable by clinical examination.

Susceptible species

means a species of *aquatic animal* in which *infection* or *infestation* ~~by a disease~~ has been demonstrated by natural cases or by experimental exposure to the *disease agent* that mimics the natural pathways for *infection* or *infestation*. Each disease chapter in the *Aquatic Manual* contains a list of currently known *susceptible species*.

Targeted surveillance

means *surveillance* targeted at a specific *disease* ~~or~~ *infection* or *infestation*.

Veterinary para professional

means a person who, for the purposes of the *Aquatic Code* is authorised by the *veterinary statutory body* to carry out certain designated tasks (dependent upon the category of *veterinary para professional*) in a country, and delegated to them under the responsibility and direction of a *veterinarian*. The tasks authorized for each category of *veterinary para professional* should be defined by the *veterinary statutory body* depending on qualifications and training, and according to need.

Zoning

means identifying zones for disease control or international trade purposes.

— text deleted

CHAPTER 1.2.3.
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 1.2.2.1.) or criteria for listing an emerging aquatic animal disease (see Article 1.2.2.2.)

Article 1.2.3.1.

The following diseases of fish are listed by the OIE:

- Epizootic haematopoietic necrosis
- Infectious haematopoietic necrosis
- Spring viraemia of carp
- Viral haemorrhagic septicaemia
- Infectious salmon anaemia
- Epizootic ulcerative syndrome
- Gyrodactylosis (*Gyrodactylus salaris*)
- Red sea bream iridoviral disease
- Koi herpesvirus disease.

Article 1.2.3.2.

The following diseases of molluscs are listed by the OIE:

- Infection with *Bonamia ostreae*
- Infection with *Bonamia exitiosa*
- Infection with *Marteilia refringens*
- Infection with *Perkinsus marinus*
- Infection with *Perkinsus olseni*
- Infection with *Xenohaliotis californiensis*.
- Abalone viral mortality μ .

Article 1.2.3.3.

The following diseases of crustaceans are listed by the OIE:

- Taura syndrome

Appendix IV (contd)

- White spot disease
- Yellowhead disease
- Tetrahedral baculovirus (*Baculovirus penaei*)
- Spherical baculovirus (*Penaeus monodon*-type baculovirus)
- Infectious hypodermal and haematopoietic necrosis
- Crayfish plague (*Aphanomyces astaci*)
- Necrotising hepatopancreatitis ⁽²⁾
- Infectious myonecrosis⁽²⁾
- = White tail disease ⁽¹⁾
- = Hepatopancreatic parvovirus disease ^{(1) (2)}
- = Mourilyan virus disease ^{(1) (2)}

1 Listed according to Article 1.2.2.2.

2 Listing of this disease is under study.

— text deleted

CHAPTER 1.4.4.

ZONING AND COMPARTMENTALISATION

Article 1.4.4.1.

Introduction

Given the difficulty of establishing and maintaining freedom from a particular disease for an entire country ~~the status of free country for a particular disease~~, especially for *diseases the entry of which whose entry* is difficult to control ~~through measures at national boundaries~~, there may be benefits to one or more Member Countries in establishing and maintaining a *subpopulation* with a distinct *aquatic animal health status*. *Subpopulations* may be separated by natural or artificial geographical barriers or, in certain situations, by the application of appropriate management systems practices.

Zoning and *compartmentalisation* are procedures implemented by a country under the provisions of this chapter with a view to defining define *subpopulations* of distinct *aquatic animal health status* for the purpose of disease control or *international trade*. *Compartmentalisation* applies to a *subpopulation* when management practices related to biosecurity are the defining factors, while *zoning* applies when a *subpopulation* is defined on a geographical basis. In practice, spatial considerations and good management play important roles in the application of both concepts.

This chapter is to assist OIE Member Countries wishing to establish and maintain different *subpopulations*, using the principles of *compartmentalisation* and *zoning*. These principles should be applied in accordance with the measures recommended in the relevant *disease* chapter(s). This chapter also outlines a process through which trading partners may recognise such *subpopulations*. This process is best implemented by trading partners through establishing parameters and gaining agreement on the necessary measures prior to *outbreaks of disease*.

Before trade in *aquatic animals* or *aquatic animal products* may occur, an *importing country* needs to be satisfied that its *aquatic animal health status* will be appropriately protected. In most cases, the import regulations developed will rely in part on judgements made about the effectiveness of sanitary procedures undertaken by the *exporting country*, both at its borders and within its *territory*.

In addition to As well as contributing to the safety of *international trade*, *zoning* and *compartmentalisation* may assist *disease* control or eradication within Member Countries. *Zoning* may encourage the more efficient use of resources, and *compartmentalisation* may allow the functional separation of a *subpopulation* from other domestic or wild *aquatic animals* through biosecurity measures, which a *zone* (through geographical separation) would not achieve. Following an *outbreak of disease*, *compartmentalisation* may allow a Member Country be able to take advantage of epidemiological links among *subpopulations* or common practices relating to biosecurity, despite diverse geographical locations, to facilitate *disease* control and/or the resumption of trade.

Zoning and *compartmentalisation* may not be applicable to all *diseases*, but separate requirements will be developed for each *disease* for which the application of *zoning* or *compartmentalisation* is considered appropriate.

To regain the status of a *free zone* or *free compartment* following an *outbreak of disease*, Member Countries should follow the recommendations in the relevant *disease* chapter in the *Aquatic Code*.

Appendix V (contd)

Article 1.4.4.2.

General considerations

The *Competent Authority* of an *exporting country* that is establishing a *zone* or *compartment* for *international trade* purposes should clearly define the *subpopulation* in accordance with the recommendations in the relevant chapters in the *Aquatic Code*, including those on *surveillance*, and the identification and traceability of *aquatic animals*. The *Competent Authority* of an *exporting country* should be able to explain to the *Competent Authority* of an *importing country* the basis for its claim of a distinct *aquatic animal health status* for the *zone* or *compartment* in such terms.

The procedures used to establish and maintain the distinct *aquatic animal health status* of a *zone* or *compartment* should be appropriate to the particular circumstances and will depend on the epidemiology of the *disease*, environmental factors, risk of introduction and establishment of disease and applicable biosecurity measures. The *exporting country* should be able to demonstrate, through detailed documentation supplied to the importing country, published through official channels, that it has implemented the recommendations in the *Aquatic Code* for establishing and maintaining such a *zone* or *compartment*.

An *importing country* should recognise the existence of this *zone* or *compartment* when the appropriate measures recommended in the *Aquatic Code* are applied, and the *Competent Authority* of the *exporting country* certifies that this is the case. Note that an importing country may adopt a higher level of protection where it is scientifically justified and the obligations referred to in Article 1.4.1.2. are met. Article 1.4.4.4. is also relevant.

Where countries share a *zone* or *compartment*, the *Competent Authority* of each country should collaborate to define and fulfil their respective responsibilities.

Article 1.4.4.3.**Prerequisite considerations in defining a zone or compartment**

The *exporting country* should conduct an assessment of the resources needed and available to establish and maintain a *zone* or *compartment* for *international trade* purposes. These include the human and financial resources and the technical capability of the *Competent Authority* (and of the relevant industry, in the case of a *compartment*) including on *disease surveillance* and *diagnosis*.

Article 1.4.4.4³.**Principles for defining a zone or compartment**

In conjunction with the above considerations and the definitions of *zone* and *compartment*, the following principles should apply when Member Countries define a *zone* or *compartment*:

1. The extent of a *zone* should be established by the *Competent Authority* on the basis of the definition of *zone* and made public through official channels.
2. The factors defining a *compartment* should be established by the *Competent Authority* on the basis of relevant criteria such as management and husbandry practices related to biosecurity, and made public through official channels.
3. *Aquatic animals* belonging to such *subpopulations* need to be recognizable as such through a clear epidemiological separation from other *aquatic animals* and all things presenting a *disease* risk.

4. For a *zone* or *compartment*, the *Competent Authority* should document in detail the measures taken to ensure the identification of the *subpopulation*, for example by means of registration of all the *aquaculture establishments* located in such a *zone* or *compartment* and the establishment and maintenance of its *aquatic animal health status* through a *biosecurity plan*. The measures used to establish and maintain the distinct *aquatic animal health status* of a *zone* or *compartment* should be appropriate to the particular circumstances and will depend on the epidemiology of the *disease*, environmental factors, the *aquatic animal health status* in adjacent areas, applicable biosecurity measures (including movement controls, use of natural and artificial boundaries, the spatial separation of *aquatic animals*, and commercial management and husbandry practices), and *surveillance*.
5. For a *compartment*, the *biosecurity plan* should describe the partnership between the relevant enterprise/industry and the *Competent Authority*, and their respective responsibilities, including the procedures for oversight of the operation of the *compartment* by the *Competent Authority*.
6. For a *compartment*, the *biosecurity plan* should also describe the routine operating procedures to provide clear evidence that the *surveillance* conducted and the management practices are adequate to meet the definition of the *compartment*. In addition to information on *aquatic animal* movements, the *biosecurity plan* should include production and stock records, feed sources, traceability, *surveillance* results, visitor logbook, morbidity and mortality history, medications, vaccinations, documentation of training and any other criteria necessary for evaluation of risk mitigation. The information required may vary according to the *aquatic animal* species and *disease(s)* under consideration. The *biosecurity plan* should also describe how the measures will be audited to ensure that the risks are regularly re-assessed and the measures adjusted accordingly.
7. Thus defined, the *zones* and *compartments* constitute the relevant *subpopulations* for the application of the recommendations in Part 2 of the *Aquatic Code*.

Article 1.4.4 54.

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

There is no single sequence of steps which should be followed in defining establishing a *zone* or a *compartment*. The steps that the *Competent Authority* of the *importing country* and the *exporting country* choose and implement will generally depend on the circumstances existing within the countries and at their borders, and their trading history. The recommended steps are:

1. For zoning
 - a) The *exporting country* identifies a geographical area, which it considers to contain an *aquatic animal subpopulation* with a distinct *aquatic animal health status* with respect to a specific *disease/specific diseases*, based on *surveillance*.
 - b) The *exporting country* describes in the *biosecurity plan* for the *zone* the measures which are being, or will be, applied to distinguish such an area epidemiologically from other parts of its *territory*, in accordance with the recommendations in the *Aquatic Code*.
 - c) The *exporting country* provides the above information to the *importing country*, with an explanation of why the area can be treated as an epidemiologically separated *zone* for *international trade* purposes.
 - d) The *importing country* determines whether it accepts such an area as a *zone* for the importation of *aquatic animals* and *aquatic animal products*, taking into account:

Appendix V (contd)

- i) an evaluation of the *exporting country's Competent Authority*;
 - ii) the result of a *risk assessment* based on the information provided by the *exporting country* and its own research;
 - iii) its own *aquatic animal* health situation with respect to the *disease(s)* concerned; and
 - iv) other relevant OIE standards.
- e) The *importing country* notifies the *exporting country* of the result of its determination and the underlying reasons, within a reasonable period of time, being either:
- i) recognition of the *zone*;
 - ii) request for further information; or
 - iii) rejection of the area as a *zone* for *international trade* purposes.
- f) An attempt should be made to resolve any differences over the **definition recognition** of the *zone*, either in the interim or finally, by using an agreed mechanism to reach consensus (such as the OIE dispute settlement mechanism).
- g) The *importing country* and the *exporting country* **may should** enter into a formal agreement **defining recognising** the *zone*.
2. For compartmentalisation
- a) Based on discussions with the relevant enterprise/industry, the *exporting country* identifies a *compartment* of one or more *aquaculture establishments* or other premises **owned by an enterprise(s) which that** operates under common management practices related to biosecurity, and which contains an identifiable *aquatic animal subpopulation* with a distinct *aquatic animal health status* with respect to a specific *disease/specific diseases*; the *exporting country* describes how this status is maintained through a partnership between the relevant enterprise/industry and the *Competent Authority* of the *exporting country*.
 - b) The *exporting country* examines the *compartment's biosecurity plan* and confirms through an audit that:
 - i) the *compartment* is epidemiologically closed throughout its routine operating procedures as a result of effective implementation of its *biosecurity plan*; and
 - ii) the *surveillance* programme in place is appropriate to verify the status of such *aquaculture establishment(s)* with respect to such *disease(s)*.
 - c) The *exporting country* describes the *compartment*, in accordance with the recommendations in the *Aquatic Code*.
 - d) The *exporting country* provides the above information to the *importing country*, with an explanation of why such an enterprise can be treated as an epidemiologically separated *compartment* for *international trade* purposes.
 - e) The *importing country* determines whether it accepts such an enterprise as a *compartment* for the importation of *aquatic animals* and *aquatic animal products*, taking into account:
 - i) an evaluation of the *exporting country's Competent Authority*;

Appendix V (contd)

- ii) the result of a *risk assessment* based on the information provided by the *exporting country* and its own research;
 - iii) its own *aquatic animal* health situation with respect to the *disease(s)* concerned; and
 - iv) other relevant OIE standards.
- f) The *importing country* notifies the *exporting country* of the result of its examination and the underlying reasons, within a reasonable period of time, being either:
- i) recognition of the *compartment*;
 - ii) request for further information; or
 - iii) rejection of such an enterprise as a *compartment* for *international trade* purposes.
- g) An attempt should be made to resolve any differences over the definition recognition of the *compartment*, either in the interim or finally, by using an agreed mechanism to reach consensus (such as the OIE dispute settlement mechanism).
- h) The *importing country* and the *exporting country* may should enter into a formal agreement definition recognising the *compartment*.

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CHAPTER 2.2.1.

INFECTION WITH *BONAMIA OSTREAE*

Article 2.2.1.1.

For the purposes of the *Aquatic Code*, infection with *Bonamia ostreae* means infection only with *Bonamia ostreae*.

Methods for conducting surveillance, diagnosis and confirmatory identification of infection with *Bonamia ostreae* are provided in the *Aquatic Manual*.

Article 2.2.1.2.

Scope

The recommendations in this Chapter apply to: European flat oyster (*Ostrea edulis*), Australian mud oyster (*O. angasi*), Argentinean flat oyster (*O. puelchana*), Chilean flat oyster (*O. chilensis*), Asiatic oyster (*O. denselammellosa*) and Suminoe oyster (*Crassostrea ariakensis*). These recommendations also apply to any other susceptible species referred to in the *Aquatic Manual* when traded internationally.

Article 2.2.1.3.

Commodities

1. When authorising the importation or transit of the following commodities, the Competent Authorities should not require any *Bonamia ostreae* related conditions, regardless of the *Bonamia ostreae* status of the exporting country, zone or compartment:
 - a) For the species referred to in Article 2.2.1.2. being used for any purpose:
 - i) commodities treated in a manner that kills the host (and thereby inactivates the disease agent) e.g. commercially sterile canned or pasteurised products or other heat treated;
 - ii) ~~gametes, eggs and~~ larvae;
 - iii) biological samples preserved for diagnostic applications in such a manner as to inactivate the disease agent.
 - b) All commodities from *Crassostrea gigas*, *C. virginica*, *Ruditapes decussatus*, *R. philippinarum*, *M. galloprovincialis* and *M. edulis*, including the live aquatic animal.
 - cb) The following commodities destined for human consumption from the species referred to in Article 2.2.1.2. which have been prepared and packaged for direct retail trade in such a way as to minimise the likelihood of alternative uses:
 - i) ~~chemically preserved products (e.g. smoked, salted, pickled, marinated, etc.);~~
 - ii) ~~non commercially sterile products (e.g. ready prepared meals) that have been heat treated in a manner to ensure the inactivation of the parasite;~~
 - iii) ~~off the shell (chilled or frozen) packaged for direct retail trade;~~
 - iv) ~~half-shell (chilled).~~

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e) ~~All commodities from *Crassostrea gigas*, *C. virginica*, *Ruditapes decussatus*, *R. philippinarum*, *Mytilus galloprovincialis* and *M. edulis*, including the live aquatic animal.~~

For the *commodities* referred to in point 1**b)c)**, Member Countries should consider introducing internal measures to prevent the *commodity* being used for any purpose other than for human consumption.

2. When authorising the importation or transit of *commodities* of a species referred to in Article 2.2.1.2., other than *commodities* referred to in point 1 of Article 2.2.1.3., the *Competent Authorities* should require the conditions prescribed in Articles 2.2.1.7. to 2.2.1.11. relevant to the *Bonamia ostreae* status of the *exporting country, zone or compartment*.
3. When considering the importation/transit from an *exporting country, zone or compartment* not declared free of infection with *Bonamia ostreae* of a *commodity* from bivalve species not referred to covered in Article 2.2.1.2. ~~(especially those of the genus *Ostrea*)~~ nor in point 1**e)b)** of Article 2.2.1.3. **but which could reasonably be expected to be a potential *Bonamia ostreae* vector from an *exporting country, zone or compartment* not declared free of *Bonamia ostreae*, the *Competent Authorities* should conduct a *risk analysis in accordance with the recommendations in the Aquatic Code of the risk of introduction, establishment and spread of *Bonamia ostreae*, and the potential consequences, associated with the importation of the commodity prior to a decision.* The *exporting country* should be informed of the outcome of this assessment.**

Article 2.2.1.4.

***Bonamia ostreae* free country**

A country may make a *self-declaration of freedom* from *Bonamia ostreae* if it meets the conditions in points 1, 2, 3 or 4 below.

If a country shares a *zone* with one or more other countries, it can only make a *self-declaration of freedom* from *Bonamia ostreae* if all the areas covered by the shared water are declared *Bonamia ostreae* free zones (see Article 2.2.1.5.).

1. A country where none of the *susceptible species* referred to in Article 2.2.1.2. is present may make a *self-declaration of freedom* from *Bonamia ostreae* when *basic biosecurity conditions* have been continuously met in the country for at least the past 2 years.

OR

2. A country where any *susceptible species* referred to in Article 2.2.1.2. are present but there has never been any observed occurrence of the *disease* for at least the past 10 years despite conditions – in all areas where the species are present – that are conducive to its clinical expression, as described in Chapter 2.2.1. of the *Aquatic Manual*, may make a *self-declaration of freedom* from *Bonamia ostreae* when *basic biosecurity conditions* have been continuously met in the country for at least the past 2 years and infection with *Bonamia ostreae* is not known to be established in wild populations.

OR

3. A country where the last known clinical occurrence was within the past 10 years, or where the *infection* status prior to *targeted surveillance* was unknown, **for example (e.g. because of the absence of conditions conducive to clinical expression, as described in Chapter 2.2.1. of the *Aquatic Manual*),** may make a *self-declaration of freedom* from *Bonamia ostreae* when:

- a) *basic biosecurity conditions* have been continuously met for at least the past 2 years; and
- b) *targeted surveillance*, as described in Chapters 1.1.4. and 2.2.1. of the *Aquatic Manual*, has been in place for at least the past 2 years without detection of *Bonamia ostreae*.

OR

4. A country that has previously made a *self-declaration of freedom* from *Bonamia ostreae* but in which the *disease* is subsequently detected may **not** make a *self-declaration of freedom* from *Bonamia ostreae* again **until when** the following conditions have been met:
 - a) on detection of the *disease*, the affected area was declared an *infected zone* and a *buffer 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 (see *Aquatic Manual*) have been completed; and
 - c) *targeted surveillance*, as described in Chapters 1.1.4. and 2.2.1. of the *Aquatic Manual*, has been in place for at least the past 2 years without detection of *Bonamia ostreae* **and**
 - d) previously existing *basic biosecurity conditions* have been reviewed and modified as necessary and have continuously been in place for at least the past 2 years.**

In the meantime, part of the non-affected area may be declared a free *zone* provided that **such part** meets the conditions in point 3 of Article 2.2.1.5.

Article 2.2.1.5.

***Bonamia ostreae* free zone or free compartment**

A *zone* or *compartment* free from *Bonamia ostreae* may be established within the *territory* of one or more countries of infected or unknown status for infection with *Bonamia ostreae* and declared free by the *Competent Authority(ies)* of the country(ies) concerned if the *zone* or *compartment* meets the conditions referred to in points 1, 2, 3 or 4 below.

If a *zone* or *compartment* extends over more than one country, it can only be declared a *Bonamia ostreae* free *zone* or *compartment* if the conditions outlined below apply to all areas of the *zone* or *compartment*.

1. In a country of unknown status for *Bonamia ostreae*, a *zone* or *compartment* where none of the *susceptible species* referred to in Article 2.2.1.2. is present may be declared free from *Bonamia ostreae* when *basic biosecurity conditions* have been continuously met in the *zone* or *compartment* for at least the past 2 years.

OR

2. In a country of unknown status for *Bonamia ostreae*, a *zone* or *compartment* where any *susceptible species* referred to in Article 2.2.1.2. are present but there has never been any observed occurrence of the *disease* for at least the past 10 years despite conditions – in all areas where the species are present – that are conducive to its clinical expression, as described in Chapter 2.2.1. of the *Aquatic Manual*, may be declared free from *Bonamia ostreae* when *basic biosecurity conditions* have been continuously met in the *zone* or *compartment* for at least the past 2 years and infection with *Bonamia ostreae* is not known to be established in wild populations.

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OR

3. A *zone* or *compartment* where the last known clinical occurrence was within the past 10 years, or where the *infection* status prior to *targeted surveillance* was unknown, for example (e.g. because of the absence of conditions conducive to clinical expression, as described in Chapter 2.2.1. of the *Aquatic Manual*), may be declared free from *Bonamia ostreae* when:
- a) *basic biosecurity conditions* have been continuously met for at least the past 2 years; and
 - b) *targeted surveillance*, as described in Chapters 1.1.4. and 2.2.1. of the *Aquatic Manual*, has been in place for at least the past 2 years without detection of *Bonamia ostreae*.

OR

4. A *zone* previously declared free from *Bonamia ostreae* but in which the *disease* is subsequently detected may ~~not~~ be declared free from *Bonamia ostreae* again until when the following conditions have been met:
- a) on detection of the *disease*, the affected area was declared an *infected zone* and a *buffer 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 (see *Aquatic Manual*) have been completed; and
 - c) *targeted surveillance*, as described in Chapters 1.1.4. and 2.2.1. of the *Aquatic Manual*, has been in place for at least the past 2 years without detection of *Bonamia ostreae*, and
 - d) previously existing *basic biosecurity conditions* have been reviewed and modified as necessary and have continuously been in place for at least the past 2 years.

Article 2.2.1.6.

Maintenance of free status

A country, *zone* or *compartment* that is declared free from *Bonamia ostreae* following the provisions of points 1 or 2 of Articles 2.2.1.4. or 2.2.1.5. (as relevant) may maintain its status as *Bonamia ostreae* free provided that *basic biosecurity conditions* are continuously maintained.

A country, *zone* or *compartment* that is declared free from *Bonamia ostreae* following the provisions of point 3 of Articles 2.2.1.4. or 2.2.1.5. (as relevant) may discontinue *targeted surveillance* and maintain its status as *Bonamia ostreae* free provided that conditions that are conducive to clinical expression of infection with *Bonamia ostreae*, as described in Chapter 2.2.1. 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 *Bonamia ostreae*, *targeted surveillance* needs to be continued at a level determined by the *Competent Authority* on the basis of the likelihood of *infection*.

Article 2.2.1.7.

Importation of live aquatic animals from a country, zone or compartment declared free from *Bonamia ostreae*

When importing live *aquatic animals* of species referred to in Article 2.2.1.2. from a country, *zone* or *compartment* declared free from *Bonamia ostreae*, the *Competent Authority* of the *importing country* should require an *international aquatic animal health certificate* issued by the *Competent Authority* of the *exporting country* or a *certifying official* approved by the *importing country*.

This *certificate* must certify, on the basis of the procedures described in Articles 2.2.1.4. or 2.2.1.5. (as applicable), whether the place of production of the commodity consignment is a country, *zone* or *compartment* declared free from *Bonamia ostreae*.

The *certificate* should be in accordance with the Model Certificate in Appendix 4.1.2.

This Article does not apply to *commodities* referred to in point 1 of Article 2.2.1.3.

Article 2.2.1.8.

Importation of live aquatic animals for aquaculture from a country, zone or compartment not declared free from *Bonamia ostreae*

1. When importing, for *aquaculture*, live *aquatic animals* of species referred to in Article 2.2.1.2. from a country, *zone* or *compartment* not declared free from *Bonamia ostreae*, the *Competent Authority* of the *importing country* should assess the *risk* and, if justified, apply the following risk mitigation measures such as:
 - a) the direct delivery into and lifelong holding of the consignment in biosecure quarantine facilities for:
 2. the continuous isolation of the imported aquatic animals from the local environment; and
 - b) the treatment of all effluent and waste material from the processing in a manner that ensures inactivation of *Bonamia ostreae*.
2. If the intention of the introduction is the establishment of a new stock, international standards, such as 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 followed.
3. For the purposes of the Aquatic Code, the ICES Code may be summarised to the following main points:
 - a) identify stock of interest (cultured or wild) in its current location;
 - b) evaluate stock health/disease history;
 - c) take and test samples for *Bonamia ostreae*, pests and general health/disease status;
 - d) import and quarantine in a secure facility a founder (F-0) population;
 - e) produce F-1 generation from the F-0 stock in quarantine;

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- f) culture F-1 stock and at critical times in its development (life cycle) sample and test for *Bonamia ostreae* and perform general examinations for pests and general health/disease status;
- g) if *Bonamia ostreae* 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 free of infection with *Bonamia ostreae* or specific pathogen free (SPF) for *Bonamia ostreae*;
- h) release SPF F-1 stock from *quarantine* for *aquaculture* or stocking purposes in the *country, zone or compartment*.

This Article does not apply to *commodities* referred to in point 1 of Article 2.2.1.3.

Article 2.2.1.9.

Importation of live aquatic animals for processing for human consumption from a country, zone or compartment not declared free from *Bonamia ostreae*

When importing, for processing for human consumption, live *aquatic animals* of species referred to in Article 2.2.1.2. from a country, *zone* or *compartment* not declared free from *Bonamia ostreae*, the *Competent Authority* of the *importing country* should assess the risk and, if justified, require that:

1. the consignment be delivered directly to and held in *quarantine* facilities until processing and/or consumption; and
2. all effluent and waste material from the processing be treated in a manner that ensures inactivation of *Bonamia ostreae*.

This Article does not apply to *commodities* referred to in point 1 of Article 2.2.1.3.

Article 2.2.1.10.

Importation of aquatic animal products from a country, zone or compartment declared free from *Bonamia ostreae*

When importing *aquatic animal products* of species referred to in Article 2.2.1.2. from a country, *zone* or *compartment* declared free from *Bonamia ostreae*, 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*.

This *certificate* must certify, on the basis of the procedures described in Articles 2.2.1.4. or 2.2.1.5. (as applicable), whether or not the place of production of the consignment is a country, *zone* or *compartment* declared free from *Bonamia ostreae*.

The *certificate* should be in accordance with the Model Certificate in Appendix X.X.X. (under study).

This Article does not apply to *commodities* referred to in point 1 of Article 2.2.1.3.

Article 2.2.1.11.

Importation of aquatic animal products from a country, zone or compartment not declared free from *Bonamia ostreae*

When importing *aquatic animal products* of species referred to in Article 2.2.1.2. from a country, *zone* or *compartment* not declared free from *Bonamia ostreae*, the *Competent Authority* of the *importing country* should assess the risk and apply appropriate risk mitigation measures.

This Article does not apply to *commodities* referred to in point 1 of Article 2.2.1.3.

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CHAPTER 2.2.2.

INFECTION WITH *BONAMIA EXITIOSA*

Article 2.2.2.1.

For the purposes of the *Aquatic Code*, infection with *Bonamia exitiosa* means infection only with *Bonamia exitiosa*.

Methods for conducting surveillance, diagnosis and confirmatory identification of infection with *Bonamia exitiosa* are provided in the *Aquatic Manual*.

Article 2.2.2.2.

Scope

The recommendations in this Chapter apply to: Australian mud oyster (*Ostrea angasi*) and Chilean flat oyster (*O. chilensis*). These recommendations also apply to any other susceptible species referred to in the *Aquatic Manual* when traded internationally.

Article 2.2.2.3.

Commodities

1. When authorising the importation or transit of the following commodities, the Competent Authorities should not require any *Bonamia exitiosa* related conditions, regardless of the *Bonamia exitiosa* status of the exporting country, zone or compartment:

a) For the species referred to in Article 2.2.2.2. being used for any purpose:

- i) commodities treated in a manner that kills the host (and thereby inactivates the disease agent) e.g. commercially sterile canned or pasteurised products or other heat treated products;
- ii) ~~gametes, eggs and~~ larvae;
- iii) biological samples preserved for diagnostic applications in such a manner as to inactivate the disease agent.

b) All commodities from *Crassostrea gigas* and *Saccostrea glomerata*, including the live aquatic animal.

~~c)~~ The following commodities destined for human consumption from the species referred to in Article 2.2.2.2. which have been prepared and packaged for direct retail trade in such a way as to minimise the likelihood of alternative uses:

- i) chemically preserved products (e.g. smoked, salted, pickled, marinated, etc.);
- ii) non commercially sterile products (e.g. ready prepared meals) that have been heat treated in a manner to ensure the inactivation of the parasite;
- iii) off the shell (chilled or frozen) packaged for direct retail trade;
- iv) half-shell (chilled).

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e) ~~All commodities from *Crassostrea gigas*, *C. virginica* and *Saccostrea glomerata*, including the live aquatic animal.~~

For the *commodities* referred to in point 1 ~~b)c)~~, Member Countries should consider introducing internal measures to prevent the *commodity* being used for any purpose other than for human consumption.

2. When authorising the importation or transit of *commodities* of a species referred to in Article 2.2.2.2., other than *commodities* referred to in point 1 of Article 2.2.2.3., the *Competent Authorities* should require the conditions prescribed in Articles 2.2.2.7. to 2.2.2.11. relevant to the *Bonamia exitiosa* status of the *exporting country, zone* or *compartment*.
3. When considering the importation/transit from an *exporting country, zone* or *compartment* not declared free of infection with *Bonamia exitiosa* of a *commodity* from bivalve species not covered in Article 2.2.2.2. ~~(especially those of the genus *Ostrea*)~~ nor in point 1 ~~e)b)~~ of Article 2.2.2.3. but which could reasonably be expected to be a potential *Bonamia exitiosa* vector, the *Competent Authorities* should conduct a risk analysis in accordance with the recommendations in the *Aquatic Code* of the risk of introduction, establishment and spread of *Bonamia exitiosa*, and the potential consequences, associated with the importation of the *commodity* prior to a decision. The *exporting country* should be informed of the outcome of this assessment.

Article 2.2.2.4.

***Bonamia exitiosa* free country**

A country may make a *self-declaration of freedom* from *Bonamia exitiosa* if it meets the conditions in points 1, 2, 3 or 4 below.

If a country shares a *zone* with one or more other countries, it can only make a *self-declaration of freedom* from *Bonamia exitiosa* if all the areas covered by the shared water are declared *Bonamia exitiosa* free zones (see Article 2.2.2.5.).

1. A country where none of the *susceptible species* referred to in Article 2.2.2.2. is present may make a *self-declaration of freedom* from *Bonamia exitiosa* when *basic biosecurity conditions* have been continuously met in the country for at least the past 2 years.

OR

2. A country where any *susceptible species* referred to in Article 2.2.2.2. are present but there has never been any observed occurrence of the *disease* for at least the past 10 years despite conditions – in all areas where the species are present – that are conducive to its clinical expression, as described in Chapter 2.2.2. of the *Aquatic Manual*, may make a *self-declaration of freedom* from *Bonamia exitiosa* when *basic biosecurity conditions* have been continuously met in the country for at least the past 2 years and infection with *Bonamia exitiosa* is not known to be established in wild populations.

OR

3. A country where the last known clinical occurrence was within the past 10 years, or where the *infection* status prior to *targeted surveillance* was unknown, for example (e.g.) because of the absence of conditions conducive to clinical expression, as described in Chapter 2.2.2. of the *Aquatic Manual*, may make a *self-declaration of freedom* from *Bonamia exitiosa* when:
 - a) *basic biosecurity conditions* have been continuously met for at least the past 2 years; and
 - b) *targeted surveillance*, as described in Chapters 1.1.4. and 2.2.2. of the *Aquatic Manual*, has been in place for at least the past 2 years without detection of *Bonamia exitiosa*.

OR

4. A country that has previously made a *self-declaration of freedom* from *Bonamia exitiosa* but in which the *disease* is subsequently detected may ~~not~~ make a *self-declaration of freedom* from *Bonamia exitiosa* again ~~until~~ when the following conditions have been met:
- on detection of the *disease*, the affected area was declared an *infected zone* and a *buffer zone* was established; and
 - 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 (see *Aquatic Manual*) have been completed; and
 - targeted surveillance*, as described in Chapters 1.1.4. and 2.2.2. of the *Aquatic Manual*, has been in place for at least the past 2 years without detection of *Bonamia exitiosa*; and
 - previously existing basic biosecurity conditions have been reviewed and modified as necessary and have continuously been in place for at least the past 2 years.

In the meantime, part of the non-affected area may be declared a free *zone* provided that ~~it~~ such part meets the conditions in point 3 of Article 2.2.2.5.

Article 2.2.2.5.

***Bonamia exitiosa* free zone or free compartment**

A *zone* or *compartment* free from *Bonamia exitiosa* may be established within the *territory* of one or more countries of infected or unknown status for infection with *Bonamia exitiosa* and declared free by the *Competent Authority(ies)* of the country(ies) concerned if the *zone* or *compartment* meets the conditions referred to in points 1, 2, 3 or 4 below.

If a *zone* or *compartment* extends over more than one country, it can only be declared a *Bonamia exitiosa* free *zone* or *compartment* if the conditions outlined below apply to all areas of the *zone* or *compartment*.

- In a country of unknown status for *Bonamia exitiosa*, a *zone* or *compartment* where none of the *susceptible species* referred to in Article 2.2.2.2. is present may be declared free from *Bonamia exitiosa* when *basic biosecurity conditions* have been continuously met in the *zone* or *compartment* for at least the past 2 years.

OR

- In a country of unknown status for *Bonamia exitiosa*, a *zone* or *compartment* where any *susceptible species* referred to in Article 2.2.2.2. are present but there has never been any observed occurrence of the *disease* for at least the past 10 years despite conditions – in all areas where the species are present – that are conducive to its clinical expression, as described in Chapter 2.2.2. of the *Aquatic Manual*, may be declared free from *Bonamia exitiosa* when *basic biosecurity conditions* have been continuously met in the *zone* or *compartment* for at least the past 2 years and infection with *Bonamia exitiosa* is not known to be established in wild populations.

OR

- A *zone* or *compartment* where the last known clinical occurrence was within the past 10 years, or where the *infection status* prior to *targeted surveillance* was unknown, ~~for example~~ (e.g. because of the absence of conditions conducive to clinical expression, as described in Chapter 2.2.2. of the *Aquatic Manual*), may be declared free from *Bonamia exitiosa* when:

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- a) *basic biosecurity conditions* have been continuously met for at least the past 2 years; and
- b) *targeted surveillance*, as described in Chapters 1.1.4. and 2.2.2. of the *Aquatic Manual*, has been in place for at least the past 2 years without detection of *Bonamia exitiosa*.

OR

4. A *zone* previously declared free from *Bonamia exitiosa* but in which the *disease* is subsequently detected may **not** be declared free from *Bonamia exitiosa* again **until when** the following conditions have been met:
- a) on detection of the *disease*, the affected area was declared an *infected zone* and a *buffer 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 (see *Aquatic Manual*) have been completed; and
 - c) *targeted surveillance*, as described in Chapters 1.1.4. and 2.2.2. of the *Aquatic Manual*, has been in place for at least the past 2 years without detection of *Bonamia exitiosa*; **and**
 - d) previously existing *basic biosecurity conditions* have been reviewed and modified as necessary and have continuously been in place for at least the past 2 years.**

Article 2.2.2.6.

Maintenance of free status

A country, *zone* or *compartment* that is declared free from *Bonamia exitiosa* following the provisions of points 1 or 2 of Articles 2.2.2.4. or 2.2.2.5. (as relevant) may maintain its status as *Bonamia exitiosa* free provided that *basic biosecurity conditions* are continuously maintained.

A country, *zone* or *compartment* that is declared free from *Bonamia exitiosa* following the provisions of point 3 of Articles 2.2.2.4. or 2.2.2.5. (as relevant) may discontinue *targeted surveillance* and maintain its status as *Bonamia exitiosa* free provided that conditions that are conducive to clinical expression of infection with *Bonamia exitiosa*, as described in Chapter 2.2.2. 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 *Bonamia exitiosa*, *targeted surveillance* needs to be continued at a level determined by the *Competent Authority* on the basis of the likelihood of *infection*.

Article 2.2.2.7.

Importation of live aquatic animals from a country, zone or compartment declared free from *Bonamia exitiosa*

When importing live *aquatic animals* of species referred to in Article 2.2.2.2. from a country, *zone* or *compartment* declared free from *Bonamia exitiosa*, the *Competent Authority* of the *importing country* should require an *international aquatic animal health certificate* issued by the *Competent Authority* of the *exporting country* or a *certifying official* approved by the *importing country*.

This *certificate* must certify, on the basis of the procedures described in Articles 2.2.2.4. or 2.2.2.5. (as applicable), whether the place of production of the commodity consignment is a country, *zone* or *compartment* declared free from *Bonamia exitiosa*.

The *certificate* should be in accordance with the Model Certificate in Appendix 4.1.2.

This Article does not apply to *commodities* referred to in point 1 of Article 2.2.2.3.

Article 2.2.2.8.

Importation of live aquatic animals for aquaculture from a country, zone or compartment not declared free from *Bonamia exitiosa*

- 1.** When importing, for *aquaculture*, live *aquatic animals* of species referred to in Article 2.2.2.2. from a country, *zone* or *compartment* not declared free from *Bonamia exitiosa*, the *Competent Authority* of the *importing country* should assess the *risk* and, if justified, apply the following risk mitigation measures such as:
 - a)1. the direct delivery into and lifelong holding of the consignment in biosecure quarantine facilities for;
 2. the continuous isolation of the imported aquatic animals from the local environment; and
 - b)3. the treatment of all effluent and waste material from the processing in a manner that ensures inactivation of *Bonamia exitiosa*.
- 2.** If the intention of the introduction is the establishment of a new stock, international standards, such as 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 followed.
- 3.** For the purposes of the *Aquatic Code*, the ICES Code may be summarised to the following main points:
 - a) identify stock of interest (cultured or wild) in its current location;
 - b) evaluate stock health/disease history;
 - c) take and test samples for *Bonamia exitiosa*, pests and general health/disease status;
 - d) import and quarantine in a secure facility a founder (F-0) population;
 - 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 *Bonamia exitiosa* and perform general examinations for pests and general health/disease status;
 - g) if *Bonamia exitiosa* 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 free of infection with *Bonamia exitiosa* or specific pathogen free (SPF) for *Bonamia exitiosa*;
 - h) release SPF F-1 stock from *quarantine* for *aquaculture* or stocking purposes in the *country, zone or compartment*.

This Article does not apply to *commodities* referred to in point 1 of Article 2.2.2.3.

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Article 2.2.2.9.

Importation of live aquatic animals for processing for human consumption from a country, zone or compartment not declared free from *Bonamia exitiosa*

When importing, for processing for human consumption, live *aquatic animals* of species referred to in Article 2.2.2.2. from a country, *zone* or *compartment* not declared free from *Bonamia exitiosa*, the *Competent Authority* of the *importing country* should assess the risk and, if justified, require that:

1. the consignment be delivered directly to and held in *quarantine* facilities until processing and/or consumption; and
2. all effluent and waste material from the processing be treated in a manner that ensures inactivation of *Bonamia exitiosa*.

This Article does not apply to *commodities* referred to in point 1 of Article 2.2.2.3.

Article 2.2.2.10.

Importation of aquatic animal products from a country, zone or compartment declared free from *Bonamia exitiosa*

When importing *aquatic animal products* of species referred to in Article 2.2.2.2. from a country, *zone* or *compartment* declared free from *Bonamia exitiosa*, 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*.

This *certificate* must certify, on the basis of the procedures described in Articles 2.2.2.4. or 2.2.2.5. (as applicable), whether or not the place of production of the consignment is a country, *zone* or *compartment* declared free from *Bonamia exitiosa*.

The *certificate* should be in accordance with the Model Certificate in Appendix X.X.X. (under study).

This Article does not apply to *commodities* referred to in point 1 of Article 2.2.2.3.

Article 2.2.2.11.

Importation of aquatic animal products from a country, zone or compartment not declared free from *Bonamia exitiosa*

When importing *aquatic animal products* of species referred to in Article 2.2.2.2. from a country, *zone* or *compartment* not declared free from *Bonamia exitiosa*, the *Competent Authority* of the *importing country* should assess the risk and apply appropriate risk mitigation measures.

This Article does not apply to *commodities* referred to in point 1 of Article 2.2.2.3.

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CHAPTER 2.2.3.

INFECTION WITH *HAPLOSPORIDIUM NELSONI*

Article 2.2.3.1.

For the purposes of the *Aquatic Code*, infection with *Haplosporidium nelsoni* means infection only with *Haplosporidium nelsoni*.

Methods for conducting surveillance, diagnosis and confirmatory identification of infection with *Haplosporidium nelsoni* are provided in the *Aquatic Manual* (under study).

Article 2.2.3.2.

Scope

The recommendations in this Chapter apply to: Pacific oyster (*Crassostrea gigas*) and Eastern oyster (*C. virginica*). These recommendations also apply to any other *susceptible species* referred to in the *Aquatic Manual* when traded internationally.

Article 2.2.3.3.

Commodities

1. When authorising the importation or transit of the following *commodities*, the *Competent Authorities* should not require any *Haplosporidium nelsoni* related conditions, regardless of the *Haplosporidium nelsoni* status of the *exporting country, zone or compartment*:
 - a) For the species referred to in Article 2.2.3.2. being used for any purpose:
 - i) commodities treated in a manner that kills the host (and thereby inactivates the disease agent) e.g. commercially sterile canned or pasteurised products or cooked products;
 - ii) ~~gametes, eggs and larvae;~~
 - iii) biological samples preserved for diagnostic applications in such a manner as to inactivate the disease agent.
 - b) All commodities from *Crassostrea ariakensis*, including the live aquatic animal.
 - cb) The following *commodities* destined for human consumption from the species referred to in Article 2.2.3.2. which have been prepared and packaged for direct retail trade in such a way as to minimise the likelihood of alternative uses:
 - i) ~~chemically preserved products (e.g. smoked, salted, pickled, marinated, etc.);~~
 - ii) ~~products (e.g. ready prepared meals) that have been heat treated in a manner to ensure the inactivation of the parasite;~~
 - iii) ~~off the shell (chilled or frozen) packaged for direct retail trade;~~
 - iv) ~~half-shell (chilled).~~

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e) ~~All commodities from *Crassostrea ariakensis*, including the live aquatic animal.~~

For the *commodities* referred to in point 1**b)c)**, Member Countries should consider introducing internal measures to prevent the *commodity* being used for any purpose other than for human consumption.

2. When authorising the importation or transit of the *commodities* of a species referred to in Article 2.2.3.2., other than *commodities* referred to in point 1 of Article 2.2.3.3., the *Competent Authorities* should require the conditions prescribed in Articles 2.2.3.7. to 2.2.3.11. relevant to the *Haplosporidium nelsoni* status of the *exporting country, zone* or *compartment*.
3. When considering the importation/transit from an *exporting country, zone* or *compartment* not declared free of infection with *Haplosporidium nelsoni* of a *commodity* from bivalve species not covered in Article 2.2.3.2. nor in point 1**e)b)** of Article 2.2.3.3. but which could reasonably be expected to be a potential *Haplosporidium nelsoni* vector, the *Competent Authorities* should conduct a risk analysis in accordance with the recommendations in the *Aquatic Code* of the risk of introduction, establishment and spread of *Haplosporidium nelsoni*, and the potential consequences, associated with the importation of the *commodity* prior to a decision. The *exporting country* should be informed of the outcome of this assessment.

Article 2.2.3.4.

***Haplosporidium nelsoni* free country**

A country may make a *self-declaration of freedom* from *Haplosporidium nelsoni* if it meets the conditions in points 1, 2, 3 or 4 below.

If a country shares a *zone* with one or more other countries, it can only make a *self-declaration of freedom* from *Haplosporidium nelsoni* if all the areas covered by the shared water are declared *Haplosporidium nelsoni* free zones (see Article 2.2.3.5.).

1. A country where none of the *susceptible species* referred to in Article 2.2.3.2. is present may make a *self-declaration of freedom* from *Haplosporidium nelsoni* when *basic biosecurity conditions* have been continuously met in the country for at least the past 2 years.

OR

2. A country where any *susceptible species* referred to in Article 2.2.3.2. are present but there has never been any observed occurrence of the *disease* for at least the past 10 years despite conditions – in all areas where the species are present – that are conducive to its clinical expression, as described in Chapter 2.2.3. of the *Aquatic Manual*, may make a *self-declaration of freedom* from *Haplosporidium nelsoni* when *basic biosecurity conditions* have been continuously met in the country for at least the past 2 years and infection with *Haplosporidium nelsoni* is not known to be established in wild populations.

OR

3. A country where the last known clinical occurrence was within the past 10 years, or where the *infection* status prior to *targeted surveillance* was unknown, for example (e.g.) because of the absence of conditions conducive to clinical expression, as described in Chapter 2.2.3. of the *Aquatic Manual*, may make a *self-declaration of freedom* from *Haplosporidium nelsoni* when:
 - a) *basic biosecurity conditions* have been continuously met for at least the past 2 years; and
 - b) *targeted surveillance*, as described in Chapters 1.1.4. and 2.2.3. of the *Aquatic Manual*, has been in place for at least the past 2 years without detection of *Haplosporidium nelsoni*.

OR

4. A country that has previously made a *self-declaration of freedom* from *Haplosporidium nelsoni* but in which the *disease* is subsequently detected may **not** make a *self-declaration of freedom* from *Haplosporidium nelsoni* again **until** **when** the following conditions have been met:
- on detection of the *disease*, the affected area was declared an *infected zone* and a *buffer zone* was established; and
 - 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 (see *Aquatic Manual*) have been completed; and
 - targeted surveillance*, as described in Chapters 1.1.4. and 2.2.3. of the *Aquatic Manual*, has been in place for at least the past 2 years without detection of *Haplosporidium nelsoni*; **and**
 - previously existing basic biosecurity conditions have been reviewed and modified as necessary and have continuously been in place for at least the past 2 years.**

In the meantime, part of the non-affected area may be declared a free *zone* provided that **such part** meets the conditions in point 3 of Article 2.2.3.5.

Article 2.2.3.5.

***Haplosporidium nelsoni* free zone or free compartment**

A *zone* or *compartment* free from *Haplosporidium nelsoni* may be established within the *territory* of one or more countries of infected or unknown status for infection with *Haplosporidium nelsoni* and declared free by the *Competent Authority(ies)* of the country(ies) concerned if the *zone* or *compartment* meets the conditions referred to in points 1, 2, 3 or 4 below.

If a *zone* or *compartment* extends over more than one country, it can only be declared a *Haplosporidium nelsoni* free *zone* or *compartment* if the conditions outlined below apply to all areas of the *zone* or *compartment*.

- In a country of unknown status for *Haplosporidium nelsoni*, a *zone* or *compartment* where none of the *susceptible species* referred to in Article 2.2.3.2. is present may be declared free from *Haplosporidium nelsoni* when *basic biosecurity conditions* have been continuously met in the *zone* or *compartment* for at least the past 2 years.

OR

- In a country of unknown status for *Haplosporidium nelsoni*, a *zone* or *compartment* where any species referred to in Article 2.2.3.2. are present but there has never been any observed occurrence of the *disease* for at least the past 10 years despite conditions – in all areas where the species are present – that are conducive to its clinical expression, as described in Chapter 2.2.3. of the *Aquatic Manual*, may be declared free from *Haplosporidium nelsoni* when *basic biosecurity conditions* have been continuously met in the *zone* or *compartment* for at least the past 2 years and infection with *Haplosporidium nelsoni* is not known to be established in wild populations.

OR

- A *zone* or *compartment* where the last known clinical occurrence was within the past 10 years, or where the *infection* status prior to *targeted surveillance* was unknown, **for example (e.g.)** because of the absence of conditions conducive to clinical expression, as described in Chapter 2.2.3. of the *Aquatic Manual*, may be declared free from *Haplosporidium nelsoni* when:

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- a) *basic biosecurity conditions* have been continuously met for at least the past 2 years; and
- b) *targeted surveillance*, as described in Chapters 1.1.4. and 2.2.3. of the *Aquatic Manual*, has been in place for at least the past 2 years without detection of *Haplosporidium nelsoni*.

OR

4. A *zone* previously declared free from *Haplosporidium nelsoni* but in which the *disease* is subsequently detected may **not** be declared free from *Haplosporidium nelsoni* again **until when** the following conditions have been met:
 - a) on detection of the *disease*, the affected area was declared an *infected zone* and a *buffer 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 (see *Aquatic Manual*) have been completed; and
 - c) *targeted surveillance*, as described in Chapters 1.1.4. and 2.2.3. of the *Aquatic Manual*, has been in place for at least the past 2 years without detection of *Haplosporidium nelsoni*; **and**
 - d) previously existing *basic biosecurity conditions* have been reviewed and modified as necessary and have continuously been in place for at least the past 2 years.**

Article 2.2.3.6.

Maintenance of free status

A country, *zone* or *compartment* that is declared free from *Haplosporidium nelsoni* following the provisions of points 1 or 2 of Articles 2.2.3.4. or 2.2.3.5. (as relevant) may maintain its status as *Haplosporidium nelsoni* free provided that *basic biosecurity conditions* are continuously maintained.

A country, *zone* or *compartment* that is declared free from *Haplosporidium nelsoni* following the provisions of point 3 of Articles 2.2.3.4. or 2.2.3.5. (as relevant) may discontinue *targeted surveillance* and maintain its status as *Haplosporidium nelsoni* free provided that conditions that are conducive to clinical expression of infection with *Haplosporidium nelsoni*, as described in Chapter 2.2.3. 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 *Haplosporidium nelsoni*, *targeted surveillance* needs to be continued at a level determined by the *Competent Authority* on the basis of the likelihood of *infection*.

Article 2.2.3.7.

Importation of live aquatic animals from a country, zone or compartment declared free from *Haplosporidium nelsoni*

When importing live *aquatic animals* of species referred to in Article 2.2.3.2. from a country, *zone* or *compartment* declared free from *Haplosporidium nelsoni*, the *Competent Authority* of the *importing country* should require an *international aquatic animal health certificate* issued by the *Competent Authority* of the *exporting country* or a *certifying official* approved by the *importing country*.

This *certificate* must certify, on the basis of the procedures described in Articles 2.2.3.4. or 2.2.3.5. (as applicable), whether the place of production of the commodity consignment is a country, *zone* or *compartment* declared free from *Haplosporidium nelsoni*.

The *certificate* should be in accordance with the Model Certificate in Appendix 4.1.2.

This Article does not apply to *commodities* referred to in point 1 of Article 2.2.3.3.

Article 2.2.3.8.

Importation of live aquatic animals for aquaculture from a country, zone or compartment not declared free from *Haplosporidium nelsoni*

1. When importing, for *aquaculture*, live *aquatic animals* of species referred to in Article 2.2.3.2. from a country, *zone* or *compartment* not declared free from *Haplosporidium nelsoni*, the *Competent Authority* of the *importing country* should assess the *risk* and, if justified, apply the following risk mitigation measures such as:
 - a)1. the direct delivery into and lifelong holding of the consignment in biosecure quarantine facilities for;
 2. the continuous isolation of the imported aquatic animals from the local environment; and
 - b)3. the treatment of all effluent and waste material from the processing in a manner that ensures inactivation of *Haplosporidium nelsoni*.
2. If the intention of the introduction is the establishment of a new stock, international standards, such as 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 followed.
3. For the purposes of the Aquatic Code, the ICES Code may be summarised to the following main points:
 - a) identify stock of interest (cultured or wild) in its current location;
 - b) evaluate stock health/disease history;
 - c) take and test samples for *Haplosporidium nelsoni*, pests and general health/disease status;
 - d) import and quarantine in a secure facility a founder (F-0) population;
 - e) produce F-1 generation from the F-0 stock in quarantine;
 - f) culture F1 stock and at critical times in its development (life cycle) sample and test for *Haplosporidium nelsoni* and perform general examinations for pests and general health/disease status;
 - g) if *Haplosporidium nelsoni* 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 free of infection with *Haplosporidium nelsoni* or specific pathogen free (SPF) for *Haplosporidium nelsoni*;
 - h) release SPF F-1 stock from quarantine for aquaculture or stocking purposes in the country, zone or compartment.

This Article does not apply to *commodities* referred to in point 1 of Article 2.2.3.3.

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Article 2.2.3.9.

Importation of live aquatic animals for processing for human consumption from a country, zone or compartment not declared free from *Haplosporidium nelsoni*

When importing, for processing for human consumption, live *aquatic animals* of species referred to in Article 2.2.3.2. from a country, *zone* or *compartment* not declared free from *Haplosporidium nelsoni*, the *Competent Authority* of the *importing country* should assess the risk and, if justified, require that:

1. the consignment be delivered directly to and held in *quarantine* facilities until processing and/or consumption; and
2. all effluent and waste material from the processing be treated in a manner that ensures inactivation of *Haplosporidium nelsoni*.

This Article does not apply to *commodities* referred to in point 1 of Article 2.2.3.3.

Article 2.2.3.10.

Importation of aquatic animal products from a country, zone or compartment declared free from *Haplosporidium nelsoni*

When importing *aquatic animal products* of species referred to in Article 2.2.3.2. from a country, *zone* or *compartment* declared free from *Haplosporidium nelsoni*, 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*.

This *certificate* must certify, on the basis of the procedures described in Articles 2.2.3.4. or 2.2.3.5. (as applicable), whether or not the place of production of the consignment is a country, *zone* or *compartment* declared free from *Haplosporidium nelsoni*.

The *certificate* should be in accordance with the Model Certificate in Appendix X.X.X. (under study).

This Article does not apply to *commodities* referred to in point 1 of Article 2.2.3.3.

Article 2.2.3.11.

Importation of aquatic animal products from a country, zone or compartment not declared free from *Haplosporidium nelsoni*

When importing *aquatic animal products* of species referred to in Article 2.2.3.2. from a country, *zone* or *compartment* not declared free from *Haplosporidium nelsoni*, the *Competent Authority* of the *importing country* should assess the risk and apply appropriate risk mitigation measures.

This Article does not apply to *commodities* referred to in point 1 of Article 2.2.3.3.

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CHAPTER 2.2.4.

INFECTION WITH *MARTEILIA REFRINGENS*

Article 2.2.4.1.

For the purposes of the *Aquatic Code*, infection with *Marteilia refringens* means infection only with *Marteilia refringens*.

Methods for conducting surveillance, diagnosis and confirmatory identification of infection with *Marteilia refringens* are provided in the *Aquatic Manual*.

Article 2.2.4.2.

Scope

The recommendations in this Chapter apply to: European flat oyster (*Ostrea edulis*), Australian mud oyster (*O. angasi*), Argentinean oyster (*O. puelchana*) and Chilean flat oyster (*O. chilensis*), ~~as well as~~ blue mussel (*Mytilus edulis*) and Mediterranean mussel (*M. galloprovincialis*). These recommendations also apply to any other *susceptible species* referred to in the *Aquatic Manual* when traded internationally.

Article 2.2.4.3.

Commodities

1. When authorising the importation or transit of the following *commodities*, the *Competent Authorities* should not require any *Marteilia refringens* related conditions, regardless of the *Marteilia refringens* status of the *exporting country, zone or compartment*:
 - a) For the species referred to in Article 2.2.4.2. being used for any purpose:
 - i) commodities treated in a manner that kills the host (and thereby inactivates the disease agent) e.g. commercially sterile canned or pasteurised products or other heat treated products;
 - ii) ~~gametes, eggs and larvae;~~
 - iii) biological samples preserved for diagnostic applications in such a manner as to inactivate the disease agent.
 - b) All commodities from *Crassostrea gigas*, including the live aquatic animal.
 - cb) The following *commodities* destined for human consumption from the species referred to in Article 2.2.4.2. which have been prepared and packaged for direct retail trade in such a way as to minimise the likelihood of alternative uses:
 - i) chemically preserved products (e.g. smoked, salted, pickled, marinated, etc.);
 - ii) non commercially sterile products (e.g. ready prepared meals) that have been heat treated in a manner to ensure the inactivation of the parasite;
 - iii) off the shell (chilled or frozen) packaged for direct retail trade;
 - iv) half-shell (chilled).
 - e) All commodities from *Crassostrea gigas*, including the live aquatic animal.

For the *commodities* referred to in point 1b), Member Countries should consider introducing internal measures to prevent the *commodity* being used for any purpose other than for human consumption.

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2. When authorising the importation or transit of *commodities* of a species referred to in Article 2.2.4.2., other than *commodities* referred to in point 1 of Article 2.2.4.3., the *Competent Authorities* should require the conditions prescribed in Articles 2.2.4.7. to 2.2.4.11. relevant to the *Marteilia refringens* status of the *exporting country, zone or compartment*.
3. When considering the importation/transit from an *exporting country, zone or compartment* not declared free of infection with *Marteilia refringens* of a *commodity* from bivalve species not covered in Article 2.2.4.2. (especially those the other species of the genera *Ostrea* and *Mytilus*) nor in point 1(e)b) of Article 2.2.4.3. but which could reasonably be expected to be a potential *Marteilia refringens* vector, the *Competent Authorities* should conduct an risk analysis in accordance with the recommendations in the Aquatic Code of the risk of introduction, establishment and spread of *Haplosporidium nelsoni*, and the potential consequences, associated with the importation of the *commodity* prior to a decision. The *exporting country* should be informed of the outcome of this assessment.

Article 2.2.4.4.

***Marteilia refringens* free country**

A country may make a *self-declaration of freedom* from *Marteilia refringens* if it meets the conditions in points 1, 2, 3 or 4 below.

If a country shares a *zone* with one or more other countries, it can only make a *self-declaration of freedom* from *Marteilia refringens* if all the areas covered by the shared water are declared *Marteilia refringens* free zones (see Article 2.2.4.5.).

1. A country where none of the *susceptible species* referred to in Article 2.2.4.2. is present may make a *self-declaration of freedom* from *Marteilia refringens* when *basic biosecurity conditions* have been continuously met in the country for at least the past 3 years.

OR

2. A country where any *susceptible species* referred to in Article 2.2.4.2. is present but there has never been any observed occurrence of the *disease* for at least the past 10 years despite conditions – in all areas where the species are present – that are conducive to its clinical expression, as described in Chapter 2.2.4. of the *Aquatic Manual*, may make a *self-declaration of freedom* from *Marteilia refringens* when *basic biosecurity conditions* have been continuously met in the country for at least the past 3 years and infection with *Marteilia refringens* is not known to be established in wild populations.

OR

3. A country where the last known clinical occurrence was within the past 10 years, or where the *infection* status prior to *targeted surveillance* was unknown, for example (e.g) because of the absence of conditions conducive to clinical expression, as described in Chapter 2.2.4. of the *Aquatic Manual*, may make a *self-declaration of freedom* from *Marteilia refringens* when:
 - a) *basic biosecurity conditions* have been continuously met for at least the past 3 years; and
 - b) *targeted surveillance*, as described in Chapters 1.1.4. and 2.2.4. of the *Aquatic Manual*, has been in place for at least the last 2 of the past 3 years without detection of *Marteilia refringens*.

OR

4. A country that has previously made a *self-declaration of freedom* from *Marteilia refringens* but in which the *disease* is subsequently detected may **not** make a *self-declaration of freedom* from *Marteilia refringens* again **until when** the following conditions have been met:

- a) on detection of the *disease*, the affected area was declared an *infected zone* and a *buffer 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 (see *Aquatic Manual*) have been completed; and
- c) *targeted surveillance*, as described in Chapters 1.1.4. and 2.2.4. of the *Aquatic Manual*, has been in place for at least the last 2 of the past 3 years without detection of *Marteilia refringens*; and
- d) previously existing basic biosecurity conditions have been reviewed and modified as necessary and have continuously been in place for at least the past 3 years.

In the meantime, part of the non-affected area may be declared a free *zone* provided that it such part meets the conditions in point 3 of Article 2.2.4.5.

Article 2.2.4.5.

***Marteilia refringens* free zone or free compartment**

A *zone* or *compartment* free from *Marteilia refringens* may be established within the *territory* of one or more countries of infected or unknown status for infection with *Marteilia refringens* and declared free by the *Competent Authority(ies)* of the country(ies) concerned if the *zone* or *compartment* meets the conditions referred to in points 1, 2, 3 or 4 below.

If a *zone* or *compartment* extends over more than one country, it can only be declared a *Marteilia refringens* free *zone* or *compartment* if the conditions outlined below apply to all areas of the *zone* or *compartment*.

1. In a country of unknown status for *Marteilia refringens*, a *zone* or *compartment* where none of the *susceptible species* referred to in Article 2.2.4.2. is present may be declared free from *Marteilia refringens* when *basic biosecurity conditions* have been continuously met in the *zone* or *compartment* for at least the past 3 years.

OR

2. In a country of unknown status for *Marteilia refringens*, a *zone* or *compartment* where any *susceptible species* referred to in Article 2.2.4.2. is present but there has never been any observed occurrence of the *disease* for at least the past 10 years despite conditions – in all areas where the species are present – that are conducive to its clinical expression, as described in Chapter 2.2.4. of the *Aquatic Manual*, may be declared free from *Marteilia refringens* when *basic biosecurity conditions* have been continuously met in the *zone* or *compartment* for at least the past 3 years and infection with *Marteilia refringens* is not known to be established in wild populations.

OR

3. A *zone* or *compartment* where the last known clinical occurrence was within the past 10 years, or where the *infection* status prior to *targeted surveillance* was unknown, for example (e.g. because of the absence of conditions conducive to clinical expression, as described in Chapter 2.2.4. of the *Aquatic Manual*), may be declared free from *Marteilia refringens* when:
 - a) *basic biosecurity conditions* have been continuously met for at least the past 3 years; and
 - b) *targeted surveillance*, as described in Chapters 1.1.4. and 2.2.4. of the *Aquatic Manual*, has been in place for at least the last 2 of the past 3 years without detection of *Marteilia refringens*.

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OR

4. A *zone* previously declared free from *Marteilia refringens* but in which the *disease* is subsequently detected may **not** be declared free from *Marteilia refringens* again **until when** the following conditions have been met:
- a) on detection of the *disease*, the affected area was declared an *infected zone* and a *buffer 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 (see *Aquatic Manual*) have been completed; and
 - c) *targeted surveillance*, as described in Chapters 1.1.4. and 2.2.4. of the *Aquatic Manual*, has been in place for at least the last 2 of the past 3 years without detection of *Marteilia refringens*; **and**
 - d) previously existing basic biosecurity conditions have been reviewed and modified as necessary and have continuously been in place for at least the past 3 years.**

Article 2.2.4.6.

Maintenance of free status

A country, *zone* or *compartment* that is declared free from *Marteilia refringens* following the provisions of points 1 or 2 of Articles 2.2.4.4. or 2.2.4.5. (as relevant) may maintain its status as *Marteilia refringens* free provided that *basic biosecurity conditions* are continuously maintained.

A country, *zone* or *compartment* that is declared free from *Marteilia refringens* following the provisions of point 3 of Articles 2.2.4.4. or 2.2.4.5. (as relevant) may discontinue *targeted surveillance* and maintain its status as *Marteilia refringens* free provided that conditions that are conducive to clinical expression of infection with *Marteilia refringens*, as described in Chapter 2.2.4. 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 *Marteilia refringens*, *targeted surveillance* needs to be continued at a level determined by the *Competent Authority* on the basis of the likelihood of *infection*.

Article 2.2.4.7.

Importation of live aquatic animals from a country, zone or compartment declared free from *Marteilia refringens*

When importing live *aquatic animals* of species referred to in Article 2.2.4.2. from a country, *zone* or *compartment* declared free from *Marteilia refringens*, the *Competent Authority* of the *importing country* should require an *international aquatic animal health certificate* issued by the *Competent Authority* of the *exporting country* or a *certifying official* approved by the *importing country*.

This *certificate* must certify, on the basis of the procedures described in Articles 2.2.4.4. or 2.2.4.5. (as applicable), whether the place of production of the commodity consignment is a country, *zone* or *compartment* declared free from *Marteilia refringens*.

The *certificate* should be in accordance with the Model Certificate in Appendix 4.1.2.

This Article does not apply to *commodities* referred to in point 1 of Article 2.2.4.3.

Article 2.2.4.8.

Importation of live aquatic animals for aquaculture from a country, zone or compartment not declared free from *Marteilia refringens*

1. When importing, for *aquaculture*, live *aquatic animals* of species referred to in Article 2.2.4.2. from a country, *zone* or *compartment* not declared free from *Marteilia refringens*, the *Competent Authority* of the *importing country* should assess the *risk* and, if justified, apply the following risk mitigation measures such as:
 - ~~a)1.~~ the direct delivery into and lifelong holding of the consignment in biosecure quarantine facilities for;
 - ~~2.~~ the continuous isolation of the imported aquatic animals from the local environment; and
 - ~~b)3.~~ the treatment of all effluent and waste material from the processing in a manner that ensures inactivation of *Marteilia refringens*.
2. If the intention of the introduction is the establishment of a new stock, international standards, such as 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 followed.
3. For the purposes of the Aquatic Code, the ICES Code may be summarised to the following main points:
 - a) identify stock of interest (cultured or wild) in its current location;
 - b) evaluate stock health/disease history;
 - c) take and test samples for *Marteilia refringens*, pests and general health/disease status;
 - d) import and quarantine in a secure facility a founder (F-0) population;
 - 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 *Marteilia refringens* and perform general examinations for pests and general health/disease status;
 - g) if *Marteilia refringens* 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 free of infection with *Marteilia refringens* or specific pathogen free (SPF) for *Marteilia refringens*;
 - h) release SPF F-1 stock from quarantine for aquaculture or stocking purposes in the country, zone or compartment.

This Article does not apply to *commodities* referred to in point 1 of Article 2.2.4.3.

Article 2.2.4.9.

Importation of live aquatic animals for processing for human consumption from a country, zone or compartment not declared free from *Marteilia refringens*

When importing, for processing for human consumption, live *aquatic animals* of species referred to in Article 2.2.4.2. from a country, *zone* or *compartment* not declared free from *Marteilia refringens*, the *Competent Authority* of the *importing country* assess the risk and, if justified, require that:

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1. the consignment be delivered directly to and held in *quarantine* facilities until processing and/or consumption; and
2. all effluent and waste material from the processing be treated in a manner that ensures inactivation of *Marteilia refringens*.

This Article does not apply to *commodities* referred to in point 1 of Article 2.2.4.3.

Article 2.2.4.10.

Importation of aquatic animal products from a country, zone or compartment declared free from *Marteilia refringens*

When importing *aquatic animal products* of species referred to in Article 2.2.4.2. from a country, *zone* or *compartment* declared free from *Marteilia refringens*, 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*.

This *certificate* must certify, on the basis of the procedures described in Articles 2.2.4.4. or 2.2.4.5. (as applicable), whether or not the place of production of the consignment is a country, *zone* or *compartment* declared free from *Marteilia refringens*.

The *certificate* should be in accordance with the Model Certificate in Appendix X.X.X. (under study).

This Article does not apply to *commodities* referred to in point 1 of Article 2.2.4.3.

Article 2.2.4.11.

Importation of aquatic animal products from a country, zone or compartment not declared free from *Marteilia refringens*

When importing *aquatic animal products* of species referred to in Article 2.2.4.2. from a country, *zone* or *compartment* not declared free from *Marteilia refringens*, the *Competent Authority* of the *importing country* should assess the risk and apply appropriate risk mitigation measures.

This Article does not apply to *commodities* referred to in point 1 of Article 2.2.4.3.

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CHAPTER 2.2.5.

INFECTION WITH *MIKROCYTOS MACKINI*

Article 2.2.5.1.

For the purposes of the *Aquatic Code*, infection with *Mikrocytos mackini* means infection only with *Mikrocytos mackini*.

Methods for conducting surveillance, diagnosis and confirmatory identification of infection with *Mikrocytos mackini* are provided in the *Aquatic Manual* (under study).

Article 2.2.5.2.

Scope

The recommendations in this Chapter apply to: European flat oyster (*Ostrea edulis*), Olympia oyster (*O. conchaphila*), Pacific oyster (*Crassostrea gigas*) and Eastern oyster (*C. virginica*). These recommendations also apply to any other *susceptible species* referred to in the *Aquatic Manual* when traded internationally.

Article 2.2.5.3.

Commodities

1. When authorising the importation or transit of the following *commodities*, the *Competent Authorities* should not require any *Mikrocytos mackini* related conditions, regardless of the *Mikrocytos mackini* status of the *exporting country, zone* or *compartment*:

a) For the species referred to in Article 2.2.5.2. being used for any purpose:

i) commodities treated in a manner that kills the host (and thereby inactivates the disease agent) e.g. commercially sterile canned or pasteurised products or other heat treated products;

ii) ~~gametes, eggs and larvae;~~

iii) biological samples preserved for diagnostic applications in such a manner as to inactivate the disease agent.

b) All commodities from *Panope abrupta*, including the live aquatic animal.

cb) The following commodities destined for human consumption from the species referred to in Article 2.2.5.2. which have been prepared and packaged for direct retail trade in such a way as to minimise the likelihood of alternative uses:

i) chemically preserved products (e.g. smoked, salted, pickled, marinated, etc.);

ii) non commercially sterile products (e.g. ready prepared meals) that have been heat treated in a manner to ensure the inactivation of the parasite;

iii) off the shell (chilled or frozen) packaged for direct retail trade.

c) All commodities from *Panope abrupta*, including the live aquatic animal.

For the *commodities* referred to in point 1b), Member Countries should consider introducing internal measures to prevent the *commodity* being used for any purpose other than for human consumption.

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2. When authorising the importation or transit of *commodities* of a species referred to in Article 2.2.5.2., other than *commodities* referred to in point 1 of Article 2.2.5.3., the *Competent Authorities* should require the conditions prescribed in Articles 2.2.5.7. to 2.2.5.11. relevant to the *Mikrocytos mackini* status of the *exporting country, zone* or *compartment*.
3. When considering the importation/transit from an *exporting country, zone* or *compartment* not declared free of infection with *Mikrocytos mackini* of a *commodity* from bivalve species not covered in Article 2.2.5.2. ~~nor in point 11(e)b) of Article 2.2.5.3.~~ **but which could reasonably be expected to be a potential *Mikrocytos mackini* vector,** the *Competent Authorities* should conduct an **risk analysis in accordance with the recommendations in the *Aquatic Code* of the risk of introduction, establishment and spread of *Haplosporidium nelsoni*, and the potential consequences, associated with the importation of the *commodity* prior to a decision.** The *exporting country* should be informed of the outcome of this assessment.

Article 2.2.5.4.

***Mikrocytos mackini* free country**

A country may make a *self-declaration of freedom* from *Mikrocytos mackini* if it meets the conditions in points 1, 2, 3 or 4 below.

If a country shares a *zone* with one or more other countries, it can only make a *self-declaration of freedom* from *Mikrocytos mackini* if all the areas covered by the shared water are declared *Mikrocytos mackini* free *zones* (see Article 2.2.5.5.).

1. A country where none of the *susceptible species* referred to in Article 2.2.5.2. is present may make a *self-declaration of freedom* from *Mikrocytos mackini* when *basic biosecurity conditions* have been continuously met in the country for at least the past 2 years.

OR

2. A country where any *susceptible species* referred to in Article 2.2.5.2. are present but there has never been any observed occurrence of the *disease* for at least the past 10 years despite conditions – in all areas where the species are present – that are conducive to its clinical expression, as described in Chapter 2.2.5. of the *Aquatic Manual*, may make a *self-declaration of freedom* from *Mikrocytos mackini* when *basic biosecurity conditions* have been continuously met in the country for at least the past 2 years and infection with *Mikrocytos mackini* is not known to be established in wild populations.

OR

3. A country where the last known clinical occurrence was within the past 10 years, **or where the *infection* status prior to *targeted surveillance* was unknown, for example (e.g. because of the absence of conditions conducive to clinical expression, as described in Chapter 2.2.5. of the *Aquatic Manual*),** may make a *self-declaration of freedom* from *Mikrocytos mackini* when:
 - a) *basic biosecurity conditions* have been continuously met for at least the past 2 years; and
 - b) *targeted surveillance*, as described in Chapters 1.1.4. and 2.2.5. of the *Aquatic Manual*, has been in place for at least the past 2 years without detection of *Mikrocytos mackini*.

OR

4. A country that has previously made a *self-declaration of freedom* from *Mikrocytos mackini* but in which the *disease* is subsequently detected may **not** make a *self-declaration of freedom* from *Mikrocytos mackini* again **until when** the following conditions have been met:

- a) on detection of the *disease*, the affected area was declared an *infected zone* and a *buffer 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 (see *Aquatic Manual*) have been completed; and
- c) *targeted surveillance*, as described in Chapters 1.1.4. and 2.2.5. of the *Aquatic Manual*, has been in place for at least the past 2 years without detection of *Mikrocytos mackini*; and

d) previously existing *basic biosecurity conditions* have been reviewed and modified as necessary and have continuously been in place for at least the past 2 years.

In the meantime, part of the non-affected area may be declared a free *zone* provided that it such part meets the conditions in point 3 of Article 2.2.5.5.

Article 2.2.5.5.

***Mikrocytos mackini* free zone or free compartment**

A *zone* or *compartment* free from *Mikrocytos mackini* may be established within the *territory* of one or more countries of infected or unknown status for infection with *Mikrocytos mackini* and declared free by the *Competent Authority(ies)* of the country(ies) concerned, if the *zone* or *compartment* meets the conditions referred to in points 1, 2, 3 or 4 below.

If a *zone* or *compartment* extends over more than one country, it can only be declared a *Mikrocytos mackini* free *zone* or *compartment* if the conditions outlined below apply to all areas of the *zone* or *compartment*.

1. In a country of unknown status for *Mikrocytos mackini*, a *zone* or *compartment* where none of the *susceptible species* referred to in Article 2.2.5.2. is present may be declared free from *Mikrocytos mackini* when *basic biosecurity conditions* have been continuously met in the *zone* or *compartment* for at least the past 2 years.

OR

2. In a country of unknown status for *Mikrocytos mackini*, a *zone* or *compartment* where any *susceptible species* referred to in Article 2.2.5.2. are present but there has never been any observed occurrence of the *disease* for at least the past 10 years despite conditions – in all areas where the species are present – that are conducive to its clinical expression, as described in Chapter 2.2.5. of the *Aquatic Manual*, may be declared free from *Mikrocytos mackini* when *basic biosecurity conditions* have been continuously met in the *zone* or *compartment* for at least the past 2 years and infection with *Mikrocytos mackini* is not known to be established in wild populations.

OR

3. A *zone* or *compartment* where the last known clinical occurrence was within the past 10 years, or where the *infection* status prior to *targeted surveillance* was unknown, for example (e.g. because of the absence of conditions conducive to clinical expression, as described in Chapter 2.2.5. of the *Aquatic Manual*), may be declared free from *Mikrocytos mackini* when:
 - a) *basic biosecurity conditions* have been continuously met for at least the past 2 years; and
 - b) *targeted surveillance*, as described in Chapters 1.1.4. and 2.2.5. of the *Aquatic Manual*, has been in place for at least the past 2 years without detection of *Mikrocytos mackini*.

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OR

4. A *zone* previously declared free from *Mikrocytos mackini* but in which the *disease* is subsequently detected may **not** be declared free from *Mikrocytos mackini* again **until when** the following conditions have been met:
- a) on detection of the *disease*, the affected area was declared an *infected zone* and a *buffer 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 (see *Aquatic Manual*) have been completed; and
 - c) *targeted surveillance*, as described in Chapters 1.1.4. and 2.2.5. of the *Aquatic Manual*, has been in place for at least the past 2 years without detection of *Mikrocytos mackini*; **and**
 - d) previously existing basic biosecurity conditions have been reviewed and modified as necessary and have continuously been in place for at least the past 2 years.**

Article 2.2.5.6.

Maintenance of free status

A country, *zone* or *compartment* that is declared free from *Mikrocytos mackini* following the provisions of points 1 or 2 of Articles 2.2.5.4. or 2.2.5.5. (as relevant) may maintain its status as *Mikrocytos mackini* free provided that *basic biosecurity conditions* are continuously maintained.

A country, *zone* or *compartment* that is declared free from *Mikrocytos mackini* following the provisions of point 3 of Articles 2.2.5.4. or 2.2.5.5. (as relevant) may discontinue *targeted surveillance* and maintain its status as *Mikrocytos mackini* free provided that conditions that are conducive to clinical expression of infection with *Mikrocytos mackini*, as described in Chapter 2.2.5. 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 *Mikrocytos mackini*, *targeted surveillance* needs to be continued at a level determined by the *Competent Authority* on the basis of the likelihood of *infection*.

Article 2.2.5.7.

Importation of live aquatic animals from a country, zone or compartment declared free from *Mikrocytos mackini*

When importing live *aquatic animals* of species referred to in Article 2.2.5.2. from a country, *zone* or *compartment* declared free from *Mikrocytos mackini*, the *Competent Authority* of the *importing country* should require an *international aquatic animal health certificate* issued by the *Competent Authority* of the *exporting country* or a *certifying official* approved by the *importing country*.

This *certificate* must certify, on the basis of the procedures described in Articles 2.2.5.4. or 2.2.5.5. (as applicable), whether the place of production of the commodity consignment is a country, *zone* or *compartment* declared free from *Mikrocytos mackini*.

The *certificate* should be in accordance with the Model Certificate in Appendix 4.1.2.

This Article does not apply to *commodities* referred to in point 1 of Article 2.2.5.3.

Article 2.2.5.8.

Importation of live aquatic animals for aquaculture from a country, zone or compartment not declared free from *Mikrocytos mackini*

1. When importing, for *aquaculture*, live *aquatic animals* of species referred to in Article 2.2.5.2. from a country, *zone* or *compartment* not declared free from *Mikrocytos mackini*, the *Competent Authority* of the *importing country* should assess the *risk* and, if justified, apply the following risk mitigation measures such as:
 - a)1. the direct delivery into and lifelong holding of the consignment in biosecure quarantine facilities for;
 2. ~~the~~ continuous isolation ~~of the imported aquatic animals~~ from the local environment; and
 - b)3. the treatment of all effluent and waste material from the processing in a manner that ensures inactivation of *Mikrocytos mackini*.
2. If the intention of the introduction is the establishment of a new stock, international standards, such as 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 followed.
3. For the purposes of the Aquatic Code, the ICES Code may be summarised to the following main points:
 - a) identify stock of interest (cultured or wild) in its current location;
 - b) evaluate stock health/disease history;
 - c) take and test samples for *Mikrocytos mackini*, pests and general health/disease status;
 - d) import and quarantine in a secure facility a founder (F-0) population;
 - e) produce F-1 generation from the F-0 stock in quarantine;
 - f) culture F1 stock and at critical times in its development (life cycle) sample and test for *Mikrocytos mackini* and perform general examinations for pests and general health/disease status;
 - g) if *Mikrocytos mackini* 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 free of infection with *Mikrocytos mackini* or specific pathogen free (SPF) for *Mikrocytos mackini*;
 - h) release SPF F-1 stock from quarantine for aquaculture or stocking purposes in the country, zone or compartment.

This Article does not apply to *commodities* referred to in point 1 of Article 2.2.5.3.

Article 2.2.5.9.

Importation of live aquatic animals for processing for human consumption from a country, zone or compartment not declared free from *Mikrocytos mackini*

When importing, for processing for human consumption, live *aquatic animals* of species referred to in Article 2.2.5.2. from a country, *zone* or *compartment* not declared free from *Mikrocytos mackini*, the *Competent Authority* of the *importing country* should assess the risk and, if justified, require that:

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1. the consignment be delivered directly to and held in *quarantine* facilities until processing and/or consumption; and
2. all effluent and waste material from the processing be treated in a manner that ensures inactivation of *Mikrocytos mackini*.

This Article does not apply to *commodities* referred to in point 1 of Article 2.2.5.3.

Article 2.2.5.10.

Importation of aquatic animal products from a country, zone or compartment declared free from *Mikrocytos mackini*

When importing *aquatic animal products* of species referred to in Article 2.2.5.2. from a country, *zone* or *compartment* declared free from *Mikrocytos mackini*, 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*.

This *certificate* must certify, on the basis of the procedures described in Articles 2.2.5.4. or 2.2.5.5. (as applicable), whether or not the place of production of the consignment is a country, *zone* or *compartment* declared free from *Mikrocytos mackini*.

The *certificate* should be in accordance with the Model Certificate in Appendix X.X.X. (under study).

This Article does not apply to *commodities* referred to in point 1 of Article 2.2.5.3.

Article 2.2.5.11.

Importation of aquatic animal products from a country, zone or compartment not declared free from *Mikrocytos mackini*

When importing *aquatic animal products* of species referred to in Article 2.2.5.2. from a country, *zone* or *compartment* not declared free from *Mikrocytos mackini*, the *Competent Authority* of the *importing country* should assess the risk and apply appropriate risk mitigation measures.

This Article does not apply to *commodities* referred to in point 1 of Article 2.2.5.3.

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CHAPTER 2.2.8.

INFECTION WITH *XENOHALLOTIS CALIFORNIENSIS*

Article 2.2.8.1.

For the purposes of the *Aquatic Code*, infection with *Xenohaliotis californiensis* means *infection* only with *Xenohaliotis californiensis*.

Methods for conducting surveillance, diagnosis and confirmatory identification of infection with *Xenohaliotis californiensis* are provided in the *Aquatic Manual*.

Article 2.2.8.2.

Scope

The recommendations in this Chapter apply to: black abalone (*Haliotis cracherodii*), white abalone (*H. sorenseni*), red abalone (*H. rufescens*), pink abalone (*H. corrugata*), green abalone (*H. tuberculata* and *H. fulgens*), flat abalone (*H. wallalensis*) and Japanese abalone (*H. discus-hanna*). These recommendations also apply to any other *susceptible species* referred to in the *Aquatic Manual* when traded internationally.

Article 2.2.8.3.

Commodities

1. When authorising the importation or transit of the following *commodities*, the *Competent Authorities* should not require any *Xenohaliotis californiensis* related conditions, regardless of the *Xenohaliotis californiensis* status of the *exporting country, zone or compartment*:
 - a) For the species referred to in Article 2.2.8.2. being used for any purpose:
 - i) commodities treated in a manner that kills the host (and thereby inactivates the disease agent) e.g. commercially sterile canned or pasteurised products or other heat treated products;
 - ii) gametes, eggs and larvae;
 - iii) shells;
 - iv) biological samples preserved for diagnostic applications in such a manner as to inactivate the disease agent.
 - b) The following *commodities* destined for human consumption from the species referred to in Article 2.2.8.2. which have been prepared and packaged for direct retail trade in such a way as to minimise the likelihood of alternative uses:
 - i) chemically preserved products (e.g. smoked, salted, pickled, marinated, etc.);
 - ii) non commercially sterile products (e.g. ready prepared meals) that have been heat treated in a manner to ensure the inactivation of the bacterium parasite;
 - iii) off the shell, eviscerated abalone (chilled or frozen) packaged for direct retail trade.

For the *commodities* referred to in point 1b), Member Countries should consider introducing internal measures to prevent the *commodity* being used for any purpose other than for human consumption.

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2. When authorising the importation or transit of *commodities* of a species referred to in Article 2.2.8.2., other than *commodities* referred to in point 1 of Article 2.2.8.3., the *Competent Authorities* should require the conditions prescribed in Articles 2.2.8.7. to 2.2.8.11. relevant to the *Xenohaliotis californiensis* status of the *exporting country, zone or compartment*.
3. When considering the importation/transit from an *exporting country, zone or compartment* not declared free of infection with *Xenohaliotis californiensis* of a *commodity* from mollusc species not covered in Article 2.2.8.2. (especially those of the genus *Haliotis*) but which could reasonably be expected to be a potential *Xenohaliotis californiensis* vector, the *Competent Authorities* should conduct a risk analysis in accordance with the recommendations in the *Aquatic Code* of the risk of introduction, establishment and spread of *Haplosporidium nelsoni*, and the potential consequences, associated with the importation of the *commodity* prior to a decision. The *exporting country* should be informed of the outcome of this assessment.

Article 2.2.8.4.

***Xenohaliotis californiensis* free country**

A country may make a *self-declaration of freedom* from *Xenohaliotis californiensis* if it meets the conditions in points 1, 2, 3 or 4 below.

If a country shares a *zone* with one or more other countries, it can only make a *self-declaration of freedom* from *Xenohaliotis californiensis* if all the areas covered by the shared water are declared *Xenohaliotis californiensis* free zones (see Article 2.2.8.5.).

1. A country where none of the *susceptible species* referred to in Article 2.2.8.2. is present may make a *self-declaration of freedom* from *Xenohaliotis californiensis* when *basic biosecurity conditions* have been continuously met in the country for at least the past 3 ~~2~~ years.

OR

2. A country where any *susceptible species* referred to in Article 2.2.8.2. are present but there has never been any observed occurrence of the *disease* for at least the past 10 years despite conditions – in all areas where the species are present – that are conducive to its clinical expression, as described in Chapter 2.2.8. of the *Aquatic Manual*, may make a *self-declaration of freedom* from *Xenohaliotis californiensis* when *basic biosecurity conditions* have been continuously met in the country for at least the past 3 ~~2~~ years and infection with *Xenohaliotis californiensis* is not known to be established in wild populations.

OR

3. A country where the last known clinical occurrence was within the past 10 years, or where the *infection* status prior to *targeted surveillance* was unknown, for example (e.g.) because of the absence of conditions conducive to clinical expression, as described in Chapter 2.2.8. of the *Aquatic Manual*, may make a *self-declaration of freedom* from *Xenohaliotis californiensis* when:

- a) *basic biosecurity conditions* have been continuously met for at least the past 3 ~~2~~ years; and
- b) *targeted surveillance*, as described in Chapters 1.1.4. and 2.2.8. of the *Aquatic Manual*, has been in place for at least the past 2 years without detection of *Xenohaliotis californiensis*.

OR

4. A country that has previously made a *self-declaration of freedom* from *Xenohaliotis californiensis* but in which the *disease* is subsequently detected may **not** make a *self-declaration of freedom* from *Xenohaliotis californiensis* again **until when** the following conditions have been met:

- a) on detection of the *disease*, the affected area was declared an *infected zone* and a *buffer 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 (see *Aquatic Manual*) have been completed; and
- c) *targeted surveillance*, as described in Chapters 1.1.4. and 2.2.8. of the *Aquatic Manual*, has been in place for at least the past 2 years without detection of *Xenohaliotis californiensis* and

d) previously existing basic biosecurity conditions have been reviewed and modified as necessary and have continuously been in place for at least the past 3 years.

In the meantime, part of the non-affected area may be declared a free zone provided that it such part meets the conditions in point 3 of Article 2.2.8.5.

Article 2.2.8.5.

***Xenohaliotis californiensis* free zone or free compartment**

A *zone* or *compartment* free from *Xenohaliotis californiensis* may be established within the *territory* of one or more countries of infected or unknown status for infection with *Xenohaliotis californiensis* and declared free by the *Competent Authority(ies)* of the country(ies) concerned if the *zone* or *compartment* meets the conditions referred to in points 1, 2, 3 or 4 below.

If a *zone* or *compartment* extends over more than one country, it can only be declared a *Xenohaliotis californiensis* free *zone* or *compartment* if the conditions outlined below apply to all areas of the *zone* or *compartment*.

1. In a country of unknown status for *Xenohaliotis californiensis*, a *zone* or *compartment* where none of the *susceptible species* referred to in Article 2.2.8.2. is present may be declared free from *Xenohaliotis californiensis* when *basic biosecurity conditions* have been continuously met in the *zone* or *compartment* for at least the past 3 ~~2~~ years.

OR

2. In a country of unknown status for *Xenohaliotis californiensis*, a *zone* or *compartment* where any *susceptible species* referred to in Article 2.2.8.2. are present but there has never been any observed occurrence of the *disease* for at least the past 10 years despite conditions – in all areas where the species are present – that are conducive to its clinical expression, as described in Chapter 2.2.8. of the *Aquatic Manual*, may be declared free from *Xenohaliotis californiensis* when *basic biosecurity conditions* have been continuously met in the *zone* or *compartment* for at least the past 3 ~~2~~ years and infection with *Xenohaliotis californiensis* is not known to be established in wild populations.

OR

3. A *zone* or *compartment* where the last known clinical occurrence was within the past 10 years, or where the *infection* status prior to *targeted surveillance* was unknown, for example (e.g. because of the absence of conditions conducive to clinical expression, as described in Chapter 2.2.8. of the *Aquatic Manual*,), may be declared free from *Xenohaliotis californiensis* when:
 - a) *basic biosecurity conditions* have been continuously met for at least the past 3 ~~2~~ years; and
 - b) *targeted surveillance*, as described in Chapters 1.1.4. and 2.2.8. of the *Aquatic Manual*, has been in place for at least the past 2 years without detection of *Xenohaliotis californiensis*.

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OR

4. A *zone* previously declared free from *Xenohaliotis californiensis* but in which the *disease* is subsequently detected may **not** be declared free from *Xenohaliotis californiensis* again **until** **when** the following conditions have been met:
- a) on detection of the *disease*, the affected area was declared an *infected zone* and a *buffer 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 (see *Aquatic Manual*) have been completed; and
 - c) *targeted surveillance*, as described in Chapters 1.1.4. and 2.2.8. of the *Aquatic Manual*, has been in place for at least the past 2 years without detection of *Xenohaliotis californiensis*; **and**
 - d) previously existing basic biosecurity conditions have been reviewed and modified as necessary and have continuously been in place for at least the past 3 years.**

Article 2.2.8.6.

Maintenance of free status

A country, *zone* or *compartment* that is declared free from *Xenohaliotis californiensis* following the provisions of points 1 or 2 of Articles 2.2.8.4. or 2.2.8.5. (as relevant) may maintain its status as *Xenohaliotis californiensis* free provided that *basic biosecurity conditions* are continuously maintained.

A country, *zone* or *compartment* that is declared free from *Xenohaliotis californiensis* following the provisions of point 3 of Articles 2.2.8.4. or 2.2.8.5. (as relevant) may discontinue *targeted surveillance* and maintain its status as *Xenohaliotis californiensis* free provided that conditions that are conducive to clinical expression of infection with *Xenohaliotis californiensis*, as described in Chapter 2.2.8. 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 *Xenohaliotis californiensis*, *targeted surveillance* needs to be continued at a level determined by the *Competent Authority* on the basis of the likelihood of *infection*.

Article 2.2.8.7.

Importation of live aquatic animals from a country, zone or compartment declared free from *Xenohaliotis californiensis*

When importing live *aquatic animals* of species referred to in Article 2.2.8.2. from a country, *zone* or *compartment* declared free from *Xenohaliotis californiensis*, the *Competent Authority* of the *importing country* should require an *international aquatic animal health certificate* issued by the *Competent Authority* of the *exporting country* or a *certifying official* approved by the *importing country*.

This *certificate* must certify, on the basis of the procedures described in Articles 2.2.8.4. or 2.2.8.5. (as applicable), whether the place of production of the commodity consignment is a country, *zone* or *compartment* declared free from *Xenohaliotis californiensis*.

The *certificate* should be in accordance with the Model Certificate in Appendix 4.1.2.

This Article does not apply to *commodities* referred to in point 1 of Article 2.2.8.3.

Article 2.2.8.8.

Importation of live aquatic animals for aquaculture from a country, zone or compartment not declared free from *Xenohaliotis californiensis*

1. When importing, for *aquaculture*, live *aquatic animals* of species referred to in Article 2.2.8.2. from a country, *zone* or *compartment* not declared free from *Xenohaliotis californiensis*, the *Competent Authority* of the *importing country* should assess the *risk* and, if justified, apply the following risk mitigation measures such as:
 - a)1. the direct delivery into and lifelong holding of the consignment in biosecure quarantine facilities for;
 2. the continuous isolation of the imported aquatic animals from the local environment; and
 - b)3. the treatment of all effluent and waste material from the processing in a manner that ensures inactivation of *Xenohaliotis californiensis*.
2. If the intention of the introduction is the establishment of a new stock, international standards, such as 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 followed.
3. For the purposes of the Aquatic Code, the ICES Code may be summarised to the following main points:
 - a) identify stock of interest (cultured or wild) in its current location;
 - b) evaluate stock health/disease history;
 - c) take and test samples for *Xenohaliotis californiensis*, pests and general health/disease status;
 - d) import and quarantine in a secure facility a founder (F-0) population;
 - e) produce F-1 generation from the F-0 stock in quarantine;
 - f) culture F1 stock and at critical times in its development (life cycle) sample and test for *Xenohaliotis californiensis* and perform general examinations for pests and general health/disease status;
 - g) if *Xenohaliotis californiensis* 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 free of infection with *Xenohaliotis californiensis* or specific pathogen free (SPF) for *Xenohaliotis californiensis*;
 - h) release SPF F-1 stock from quarantine for aquaculture or stocking purposes in the country, zone or compartment.

This Article does not apply to *commodities* referred to in point 1 of Article 2.2.8.3.

Article 2.2.8.9.

Importation of live aquatic animals for processing for human consumption from a country, zone or compartment not declared free from *Xenohaliotis californiensis*

When importing, for processing for human consumption, live *aquatic animals* of species referred to in Article 2.2.8.2. from a country, *zone* or *compartment* not declared free from *Xenohaliotis californiensis*, the *Competent Authority* of the *importing country* should assess the risk and, if justified, require that:

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1. the consignment be delivered directly to and held in *quarantine* facilities until processing and/or consumption; and
2. all effluent and waste material from the processing be treated in a manner that ensures inactivation of *Xenohaliotis californiensis*.

This Article does not apply to *commodities* referred to in point 1 of Article 2.2.8.3.

Article 2.2.8.10.

Importation of aquatic animal products from a country, zone or compartment declared free from *Xenohaliotis californiensis*

When importing *aquatic animal products* of species referred to in Article 2.2.8.2. from a country, *zone* or *compartment* declared free from *Xenohaliotis californiensis*, 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*.

This *certificate* must certify, on the basis of the procedures described in Articles 2.2.8.4. or 2.2.8.5. (as applicable), whether or not the place of production of the consignment is a country, *zone* or *compartment* declared free from *Xenohaliotis californiensis*.

The *certificate* should be in accordance with the Model Certificate in Appendix X.X.X. (under study).

This Article does not apply to *commodities* referred to in point 1 of Article 2.2.8.3.

Article 2.2.8.11.

Importation of aquatic animal products from a country, zone or compartment not declared free from *Xenohaliotis californiensis*

When importing *aquatic animal products* of species referred to in Article 2.2.8.2. from a country, *zone* or *compartment* not declared free from *Xenohaliotis californiensis*, the *Competent Authority* of the *importing country* should assess the risk and apply appropriate risk mitigation measures.

This Article does not apply to *commodities* referred to in point 1 of Article 2.2.8.3.

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CHAPTER 1.5.1.

RECOMMENDATIONS FOR TRANSPORT

Article 1.5.1.1.

General arrangements

1. These arrangements should be compulsory in all countries either by legislative or regulatory texts and methods of application should be described in a manual available to all concerned.
2. *Vehicles (or containers) used for the transport of aquatic animals shall be designed, constructed and fitted in such a way as to withstand the weight of the aquatic animals and water and to ensure their safety and welfare during transportation. Vehicles shall be thoroughly cleansed and disinfected before use according to the guidelines given in the Aquatic Code.*
3. *Vehicles (or containers) in which aquatic animals are confined during transport by sea or by air shall be secured to maintain optimal conditions for the aquatic animals during transport, and to allow easy access by the attendant.*

Article 1.5.1.2.

Particular arrangements for containers

1. The construction of *containers* intended for *transportation of aquatic animals* shall be such that the **accidental** release of water, etc., is prevented during *transport*.
2. In the case of the *transportation of aquatic animals*, provision shall be made to enable preliminary observation of the contents of *containers*.
3. *Containers* in transit in which there are *aquatic animal products* shall not be opened unless the *Competent Authorities* of the *transit country* consider it necessary. If this is the case, *containers* shall be subject to precautions **taken to avoid any risk of prevent** contamination.
4. *Containers* shall be loaded only with one kind of product or, at least, with products not susceptible to contamination by one another.
5. It rests with each country to decide on the facilities it requires for the *transport* and importation of *aquatic animals* and *aquatic animal products* in *containers*.

Article 1.5.1.3.

Particular arrangements for the transport of aquatic animals by air

1. The stocking densities for the *transport of aquatic animals in aircraft* or *containers* should be determined by taking the following into consideration **when transporting by air**:
 - a) the total **cubic metres volume** of available space for each type of *aquatic animal*;
 - b) the oxygenation capacity **of the equipment attached to the aircraft and available to supply the containers** while on the ground and during all stages of the flight.

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With regard to fish, molluscs and crustaceans, the space reserved for each *aquatic animal* species in ~~the aircraft or~~ *containers* that have been fitted for the separate *transportation* of several *aquatic animals* or for the *transportation* of groups of *aquatic animals* should comply with acceptable densities specified for the species in question.

2. The OIE-approved International Air Transport Association (IATA) Regulations for live animals ~~(which are approved by the OIE)~~ may be adopted if they do not conflict with national legislative arrangements. (Copies of these Regulations are obtainable from the International Air Transport Association, 800 Place Victoria, P.O. Box 113, Montreal, Quebec H4Z 1M1, Canada.)

Article 1.5.1.4.

Disinfection and other sanitary measures

1. *Disinfection* and all zoo-sanitary work should be carried out in order to:
 - a) avoid all unjustified inconvenience and to prevent damage or injury to the health of people and *aquatic animals*;
 - b) avoid damage to the structure of the *vehicle* or its appliances;
 - c) prevent, as far as possible, any damage to *aquatic animal products*, ~~fish eggs~~ as well as mollusc and crustacean larvae.
2. On request, the *Competent Authority* shall issue the transporters with a certificate indicating the measures that have been applied to all *vehicles*, the parts of the *vehicle* that have been treated, the methods used and the reasons that led to the application of the measures.

In the case of aircraft, the certificate may be replaced, on request, by an entry in the General Declaration of the aircraft.

3. Likewise, the *Competent Authority* shall issue on request:
 - a) a certificate showing the date of arrival and departure of the *aquatic animals*;
 - b) a certificate to the shipper or exporter, the consignee and transporter or their representatives, indicating the measures applied.

Article 1.5.1.5.**Transportation water**

Water to be used for transportation of aquatic animals should be appropriately treated in order to minimise the risk of transferring pathogens. The specific recommendations are provided in the Chapter on "Disinfection" of the Aquatic Code.

~~Article 1.5.1.5.6~~**Treatment of transportation water**

Water to be used for transportation of aquatic animals should be appropriately treated after transport and/or before discharge in order to minimise the risk of transferring pathogens. The specific recommendations are provided in the Chapter of the Aquatic Code on Disinfection.

During transportation of aquatic animals, the transporter should not be permitted to evacuate and replace the water in the transport tanks except on specifically designated sites in the national territory. The waste and rinsing water should not be emptied into a drainage system that is directly connected to an aquatic environment where aquatic animals are present. The water from the tanks should therefore either be disinfected by a recognised process (for example, 50 mg iodine or chlorine/litre for one hour), or sprayed over land that does not directly drain into waters containing aquatic animals. Each country shall designate the sites in their national territories where these operations can be carried out.

Article 1.5.1.6.

Discharge of infected material

The Competent Authority shall take all practical measures to prevent the discharge of any infective material, including transport water, into internal or territorial waters.

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CHAPTER 2.1.17.

KOI HERPESVIRUS DISEASE

Article 2.1.17.1.

For the purposes of the *Aquatic Code*, koi herpesvirus disease (KHVD) means *infection* with the viral species koi herpesvirus (KHV) tentatively placed in the sub-family *Cyprinid herpesvirus* of the family Herpesviridae.

Methods for conducting surveillance and diagnosis of koi herpesvirus disease are provided in the *Aquatic Manual*.

Article 2.1.17.2.

Scope

The recommendations in this Chapter apply to: common carp (*Cyprinus carpio carpio*), ghost carp (*Cyprinus carpio goi*), koi carp (*Cyprinus carpio koi*) and common carp hybrids (e.g. *Cyprinus carpio* x *Carassius auratus*). These recommendations also apply to any other *susceptible species* referred to in the *Aquatic Manual* when traded internationally.

Article 2.1.17.3.

Commodities

1. When authorising the importation or transit of the following *commodities*, the *Competent Authorities* should not require any KHVD related conditions, regardless of the KHVD status of the *exporting country, zone* or *compartment*:
 - a) For the species referred to in Article 2.1.17.2. being used for any purpose:
 - i) commodities treated in a manner that kills the host and inactivates the disease agent e.g. leather made from fish skin, pasteurised products and ready to eat meals; and fish oil and fish meal intended for use in animal feeds commercially sterile canned fish;
 - ii) leather made from fish skin biological samples preserved for diagnostic applications in such a manner as to inactivate the disease agent.
 - b) The following *commodities* destined for human consumption from the species referred to in Article 2.1.17.2. which have been prepared and packaged for direct retail trade in such a way as to minimise the likelihood of alternative uses:
 - i) chemically preserved products (e.g. smoked, salted, pickled, marinated, etc.);
 - ii) products (e.g. ready prepared meals and fish oil) that have been heat treated in a manner to ensure the inactivation of the pathogen;
 - iii) eviscerated fish (chilled or frozen) packaged for direct retail trade;
 - iv) fillets or cutlets (chilled or frozen);

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iii) dried *eviscerated fish* (including air dried, flame dried and sun dried).

For the *commodities* referred to in point 1b), Member Countries should consider introducing internal measures to prevent the *commodity* being used for any purpose other than for human consumption.

2. When authorising the importation or transit of the *commodities* of a species referred to in Article 2.1.17.2., other than those referred to in point 1 of Article 2.1.17.3., the *Competent Authorities* should require the conditions prescribed in Articles 2.1.17.7. to 2.1.17.12. relevant to the KHVD status of the *exporting country, zone or compartment*.
3. When considering the importation/transit from an *exporting country, zone or compartment* not declared free of KHVD of any live *commodity* of a species not covered in Article 2.1.17.2. **but which could reasonably be expected to be a potential KHV vector**, the *Competent Authorities* should conduct an **risk analysis in accordance with the recommendations in the Aquatic Code** ~~of the risk of introduction, establishment and spread of KHVD, and the potential consequences, associated with the importation of the commodity prior to a decision~~. The *exporting country* should be informed of the outcome of this assessment.

Article 2.1.17.4.

Koi herpesvirus disease free country

A country may make a *self-declaration of freedom* from KHVD if it meets the conditions in points 1, 2, 3 or 4 below.

If a country shares a *zone* with one or more other countries, it can only make a *self-declaration of freedom* from KHVD if all the areas covered by the shared water are declared KHVD free countries or *zones* (see Article 2.1.17.5.).

1. A country where none of the *susceptible species* referred to in Article 2.1.17.2. is present may make a *self-declaration of freedom* from KHVD when basic biosecurity conditions have been continuously met in the country for at least the past 2 years.

OR

2. A country where the *susceptible species* referred to in Article 2.1.17.2. are present but there has never been any observed occurrence of the *disease* for at least the past 25 years despite conditions that are conducive to its clinical expression, as described in Chapter 2.1.17. of the *Aquatic Manual*, may make a *self-declaration of freedom* from KHVD when *basic biosecurity conditions* have been continuously met in the country for at least the past 10 years.

OR

3. A country where the last observed occurrence of the *disease* was within the past 25 years, or where the *infection status* prior to *targeted surveillance* was unknown, **for example because of (e.g.) the absence of conditions conducive to its clinical expression**, as described in Chapter 2.1.17. of the *Aquatic Manual*, may make a *self-declaration of freedom* from KHVD when:
 - a) *basic biosecurity conditions* have been continuously met for at least the past 2 years; and
 - b) *targeted surveillance*, as described in Chapters 1.1.4. and 2.1.17. of the *Aquatic Manual*, has been in place for at least the last 2 years without detection of KHV.

OR

4. A country that has previously made a *self-declaration of freedom* from KHVD but in which the *disease* is subsequently detected may **not** make a *self-declaration of freedom* from KHVD again **until when** the following conditions have been met:
- a) on detection of the *disease*, the affected area was declared an *infected zone* and a *buffer 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 (see *Aquatic Manual*) have been completed; and
 - c) *targeted surveillance*, as described in Chapters 1.1.4. and 2.1.17. of the *Aquatic Manual*, has been in place for at least the last 2 years without detection of KHV; **and**
 - d) previously existing basic biosecurity conditions have been reviewed and modified as necessary and have continuously been in place for at least the past 2 years.**

In the meantime, part of the non-affected area may be declared a free *zone* provided that it **such part** meets the conditions in point 3 of Article 2.1.17.5.

Article 2.1.17.5.

Koi herpesvirus disease free zone or free compartment

A *zone* or *compartment* within the *territory* of one or more countries not declared free from KHVD may be declared free by the *Competent Authority(ies)* of the country(ies) concerned if the *zone* or *compartment* meets the conditions referred to in points 1, 2, 3 or 4 below.

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

1. A *zone* or *compartment* where none of the *susceptible species* referred to in Article 2.1.17.2. is present may be declared free from KHVD when *basic biosecurity conditions* have been continuously met in the *zone* or *compartment* for at least the past 2 years.

OR

2. A *zone* or *compartment* where the *susceptible species* referred to in Article 2.1.17.2. are present but there has never been any observed occurrence of the *disease* for at least the past 25 years despite conditions that are *conducive* to its clinical expression, as described in Chapter 2.1.17. of the *Aquatic Manual*, may be declared free from KHVD when *basic biosecurity conditions* have been continuously met in the *zone* or *compartment* for at least the past 10 years.

OR

3. A *zone* or *compartment* where the last observed occurrence of the *disease* was within the past 25 years, or where the *infection status* prior to *targeted surveillance* was unknown, **for example (e.g.)** because of the absence of conditions conducive to its clinical expression, as described in Chapter 2.1.17. of the *Aquatic Manual*, may be declared free from KHVD when:

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- a) *basic biosecurity conditions* have been continuously met for at least the past 2 years; and
- b) *targeted surveillance*, as described in Chapters 1.1.4. and 2.1.17. of the *Aquatic Manual*, has been in place for at least the last 2 years without detection of KHV ~~koi herpesvirus detection~~.

OR

- 4. A *zone* previously declared free from KHVD but in which the *disease* is subsequently detected may **not** be declared free from KHVD again **until when** the following conditions have been met:
 - a) on detection of the *disease*, the affected area was declared an *infected zone* and a *buffer 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 (see *Aquatic Manual*) have been completed; and
 - c) *targeted surveillance*, as described in Chapters 1.1.4. and 2.1.17. of the *Aquatic Manual*, has been in place for at least the last 2 years without detection of KHV ~~koi herpesvirus detection~~; and
 - d) previously existing *basic biosecurity conditions* have been reviewed and modified as necessary and have continuously been in place for at least the past 2 years.

Article 2.1.17.6.

Maintenance of free status

A country, *zone* or *compartment* that is declared free from KHVD following the provisions of points 1 or 2 of Articles 2.1.17.4. or 2.1.17.5. (as relevant) may maintain its status as KHVD free provided that *basic biosecurity conditions* are continuously maintained.

A country, *zone* or *compartment* that is declared free from KHVD following the provisions of point 3 of Articles 2.1.17.4. or 2.1.17.5. (as relevant) may discontinue *targeted surveillance* and maintain its status as KHVD free provided that conditions that are conducive to clinical expression of KHVD, as described in Chapter 2.1.17. 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 KHVD, *targeted surveillance* needs to be continued at a level determined by the *Competent Authority* on the basis of the likelihood of *infection*.

Article 2.1.17.7.

Importation of live aquatic animals from a country, zone or compartment declared free from koi herpesvirus disease

When importing live *aquatic animals* of species referred to in Article 2.1.17.2. from a country, *zone* or *compartment* declared free from KHVD, the *Competent Authority* of the *importing country* should require an *international aquatic animal health certificate* issued by the *Competent Authority* of the *exporting country* or a *certifying official* approved by the *importing country* attesting that, on the basis of the procedures described in Articles 2.1.17.4. or 2.1.17.5. (as applicable), the place of production of the *commodity* is a country, *zone* or *compartment* declared free from KHVD.

The *certificate* should be in accordance with the Model Certificate in Appendix 4.1.1.

This Article does not apply to *commodities* referred to in point 1 of Article 2.1.17.3.

Article 2.1.17.8.

Importation of live aquatic animals for aquaculture from a country, zone or compartment not declared free from koi herpesvirus disease

- 1.** When importing, for *aquaculture*, live *aquatic animals* of species referred to in Article 2.1.17.2. from a country, *zone* or *compartment* not declared free from KHVD, the *Competent Authority* of the *importing country* should assess the *risk* and, if justified, apply the following risk mitigation measures such as:
 - a)1. the direct delivery into and lifelong holding of the consignment in biosecure quarantine facilities for;
 2. the continuous isolation of the imported aquatic animals and their first generation progeny from the local environment; and
 - b)3. the treatment of all effluent and waste materials in a manner that ensures inactivation of koi herpesvirus.
2. If the intention of the introduction is the establishment of a new stock, international standards, such as 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 followed.
3. For the purposes of the Aquatic Code, the ICES Code may be summarised to the following main points:
 - a) identify stock of interest (cultured or wild) in its current location;
 - b) evaluate stock health/disease history;
 - c) take and test samples for KHV, pests and general health/disease status;
 - d) import and quarantine in a secure facility a founder (F-0) population;
 - 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 KHV and perform general examinations for pests and general health/disease status;
 - g) if KHV 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 KHVD free or specific pathogen free (SPF) for KHV;
 - h) release SPF F-1 stock from quarantine for aquaculture or stocking purposes in the country, zone or compartment.

This Article does not apply to *commodities* referred to in point 1 of Article 2.1.17.3.

Article 2.1.17.9.

Importation of live aquatic animals for processing for human consumption from a country, zone or compartment not declared free from koi herpesvirus disease

When importing, for processing for human consumption, live *aquatic animals* of species referred to in Article 2.1.17.2. from a country, *zone* or *compartment* not declared free from KHVD, the *Competent Authority* of the *importing country* should assess the risk and, if justified, require that:

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1. the consignment be delivered directly to and held in *quarantine* facilities for slaughter and processing to one of the products referred to in point 1 of Article 2.1.17.3. or other products authorised by the *Competent Authority*, and
2. all effluent and waste materials from the processing be treated in a manner that ensures inactivation of koi herpesvirus.

This Article does not apply to *commodities* referred to in point 1 of Article 2.1.17.3.

Article 2.1.17.10.

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 koi herpesvirus disease

When importing, for use in animal feed, or for agricultural, industrial or pharmaceutical use, live *aquatic animals* of species referred to in Article 2.1.17.2. from a country, *zone* or *compartment* not declared free from KHVD, the *Competent Authority* of the *importing country* should require that:

1. the consignment be delivered directly to and held in *quarantine* facilities for slaughter and processing to products authorised by the *Competent Authority*, and
2. all effluent and waste materials from the processing be treated in a manner that ensures inactivation of koi herpesvirus.

This Article does not apply to *commodities* referred to in point 1 of Article 2.1.17.3.

Article 2.1.17.11.

Importation of aquatic animal products from a country, zone or compartment declared free from koi herpesvirus disease

When importing *aquatic animal products* of species referred to in Article 2.1.17.2. from a country, *zone* or *compartment* declared free from KHVD, the *Competent Authority* of the *importing country* should require an *international aquatic animal health certificate* issued by the *Competent Authority* of the *exporting country* or a *certifying official* approved by the *importing country* attesting that, on the basis of the procedures described in Articles 2.1.17.4. or 2.1.17.5. (as applicable), the place of production of the consignment is a country, *zone* or *compartment* declared free from KHVD.

The *certificate* should be in accordance with the Model Certificate in Appendix 4.2.1.

This Article does not apply to *commodities* referred to in point 1 of Article 2.1.17.3.

Article 2.1.17.12.

Importation of aquatic animal products from a country, zone or compartment not declared free from koi herpesvirus disease

When importing *aquatic animal products* of species referred to in Article 2.1.17.2. from a country, *zone* or *compartment* not declared free from KHVD, the *Competent Authority* of the *importing country* should assess the risk and apply appropriate risk mitigation measures.

In the case of dead *aquatic animals*, whether *eviscerated* or *uneviscerated*, such risk mitigation measures may include:

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1. the direct delivery into and holding of the consignment in biosecure/*quarantine* facilities for processing to one of the products referred to in point 1 of Article 2.1.17.3. or other products authorised by the *Competent Authority*;
2. the treatment of all effluent and waste materials in a manner that ensures inactivation of koi herpesvirus.

This Article does not apply to *commodities* referred to in point 1 of Article 2.1.17.3.

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CHAPTER 2.3.1.

TAURA SYNDROME

Article 2.3.1.1.

For the purposes of the *Aquatic Code*, Taura syndrome (TS) means *infection* with Taura syndrome virus (TSV). *Taura syndrome virus* is classified as a species in the family *Dicistroviridae*. Common synonyms are listed in Chapter 4.1.1. of the *Aquatic Manual*.

Methods for conducting surveillance and diagnosis of TS are provided in the *Aquatic Manual*.

Article 2.3.1.2.

Scope

The recommendations in this Chapter apply to: Pacific white shrimp or whiteleg shrimp (*Penaeus vannamei*), blue shrimp (*P. stylirostris*), northern white shrimp (*P. setiferus*), southern white shrimp (*P. schmitti*), greasyback prawn (*Metapenaeus ensis*) and giant tiger prawn (*P. monodon*). These recommendations also apply to any other susceptible species referred to in the *Aquatic Manual* when traded internationally.

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

Article 2.3.1.3.

Commodities

1. When authorising the importation or transit of the following *commodities*, the *Competent Authorities* should not require any TS related conditions, regardless of the TS status of the *exporting country, zone or compartment*.
 - a) For the species referred to in Article 2.3.1.2. being used for any purpose:
 - i) commodities treated in a manner that inactivates the disease agent e.g. boiled, canned or pasteurised products and ready to eat meals; and crustacean oil and crustacean meal intended for use in animal feeds commercially sterile canned products;
 - ii) boiled products (e.g. boiled whole shrimp or tails, lobsters, crabs);
 - iii) chemically extracted chitin;
 - iv) crustacean meals or by products made non infectious by heating or drying (e.g. flame dried or sun dried);
 - iiiv) crustacean products made non-infectious through processing as dry feeds (e.g. pelleted or extruded feeds);
 - ivv) biological samples preserved for diagnostic applications in such a manner as to inactivate the disease agent TSV (e.g. formalin or alcohol preserved samples).

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- b) The following products destined for human consumption from species referred to in Article 2.3.1.2. which have been prepared and packaged for direct retail trade in such a way as to minimise the likelihood of alternative uses:
- i) chemically preserved products (e.g. salted, pickled, marinated, pastes, etc.);
 - ii) products that have been heat treated or dried (e.g. ready prepared meals) in a manner to ensure the inactivation of the pathogen.

For the *commodities* listed in point 1b), Member Countries should consider introducing internal measures to prevent the *commodity* being used for any purpose other than for human consumption.

2. When authorising the importation or transit of the *commodities* of a species referred to in Article 2.3.1.2., other than those listed in point 1 of Article 2.3.1.3., the *Competent Authorities* should require the conditions prescribed in Articles 2.3.1.7. to 2.3.1.11. relevant to the TS status of the *exporting country, zone* or *compartment*.
3. When considering the importation/transit from an *exporting country, zone* or *compartment* not declared free of TS of any other commodity of a species not covered in Article 2.3.1.2. but which could reasonably be expected to be a potential TSV carrier vector, the *Competent Authorities of the importing country* should conduct a risk analysis in accordance with the recommendations in the Aquatic Code of the risk of introduction, establishment and spread of TSV, and the potential consequences, associated with the importation of the commodity prior to a decision. The *exporting country* should be informed of the outcome of this assessment.

Article 2.3.1.4.

Taura syndrome free country

A country may make a *self-declaration of freedom* from TS if it meets the conditions in points 1, 2, 3 or 4 below.

If a country shares a *zone* with one or more other countries, it can only make a *self-declaration of freedom* from TS if all the areas covered by the shared water are declared TS free countries or *zones* (see Article 2.3.1.5.).

1. A country where none of the *susceptible species* referred to in Article 2.3.1.2. is present may make a *self-declaration of freedom* from TS when *basic biosecurity conditions* have been continuously met in the country for at least the past 2 years.

OR

2. A country where the *susceptible species* referred to in Article 2.3.1.2. are present but there has never been any observed occurrence of the *disease* for at least the past 10 years despite conditions that are conducive to its clinical expression, as described in Chapter X.X.X. of the *Aquatic Manual*, may make a *self-declaration of freedom* from TS when *basic biosecurity conditions* have been continuously met in the country for at least the past 2 years.

OR

3. A country where the last observed occurrence of the *disease* was within the past 10 years, or where the *infection* status prior to *targeted surveillance* was unknown, for example (e.g.) because of the absence of conditions conducive to its clinical expression, as described in Chapter X.X.X. of the *Aquatic Manual*, may make a *self-declaration of freedom* from TS when:

- a) *basic biosecurity* conditions have been continuously met for at least the past 2 years; and
- b) *targeted surveillance*, as described in Chapters 1.1.4. and X.X.X. of the *Aquatic Manual*, has been in place for at least the last 2 years without detection of TSV.

OR

4. A country that has previously made a *self-declaration of freedom* from TS but in which the *disease* is subsequently detected may **not** make a *self-declaration of freedom* from TS again **until when** the following conditions have been met:
 - a) on detection of the *disease*, the affected area was declared an *infected zone* and a *buffer 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 (see *Aquatic Manual*) have been completed; and
 - c) *targeted surveillance*, as described in Chapters 1.1.4. and X.X.X. of the *Aquatic Manual*, has been in place for at least the past 2 years without detection of TSV; **and**
 - d) previously existing *basic biosecurity conditions* have been reviewed and modified as necessary and have continuously been in place for at least the past 2 years.**

In the meantime, part of the non-affected area may be declared a free *zone* provided that **they such part** meets the conditions in point 3 of Article 2.3.1.5.

Article 2.3.1.5.

Taura syndrome free zone or free compartment

A *zone* or *compartment* within the *territory* of one or more countries not declared free from TS may be declared free by the *Competent Authority(ies)* of the country(ies) concerned if the *zone* or *compartment* meets the conditions referred to in points 1, 2, 3 or 4 below.

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

1. A *zone* or *compartment* where none of the *susceptible species* referred to in Article 2.3.1.2. is present may be declared free from TS when *basic biosecurity conditions* have been continuously met in the *zone* or *compartment* for at least the past 2 years.

OR

2. A *zone* or *compartment* where the *susceptible species* referred to in Article 2.3.1.2. are present but in which there has not been any observed occurrence of the *disease* for at least the past 10 years despite conditions that are conducive to its clinical expression, as described in Chapter X.X.X. of the *Aquatic Manual*, may be declared free from TS when *basic biosecurity conditions* have been continuously met in the *zone* or *compartment* for at least the past 2 years.

OR

3. A *zone* or *compartment* where the last observed occurrence of the *disease* was within the past 10 years, **or** where the *infection* status prior to *targeted surveillance* was unknown, **for example (e.g.)** because of the absence of conditions conducive to its clinical expression, **as described in Chapter X.X.X. of the *Aquatic Manual***, may be declared free from TS when:
 - a) *basic biosecurity conditions* have been continuously met for at least the past 2 years; and
 - b) *targeted surveillance*, as described in Chapters 1.1.4. and X.X.X. of the *Aquatic Manual*, has been in place, through the *zone* or *compartment*, for at least the past 2 years without detection of TSV.

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OR

4. A *zone* previously declared free from TS but in which the *disease* is subsequently detected may **not** be declared free from TS again **until when** the following conditions have been met:
- a) on detection of the *disease*, the affected area was declared an *infected zone* and a *buffer 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 (see *Aquatic Manual*) have been completed; and
 - c) *targeted surveillance*, as described in Chapters 1.1.4. and X.X.X. of the *Aquatic Manual*, has been in place for at least the past 2 years without detection of TSV; **and**
 - d) previously existing basic biosecurity conditions have been reviewed and modified as necessary and have continuously been in place for at least the past 2 years.**

Article 2.3.1.6.

Maintenance of free status

A country, *zone* or *compartment* that is declared free from TS following the provisions of points 1 or 2 of Articles 2.3.1.4. or 2.3.1.5. (as relevant) may maintain its status as TS free provided that *basic biosecurity conditions* are continuously maintained.

A country, *zone* or *compartment* that is declared free from TS following the provisions of point 3 of Articles 2.3.1.4. or 2.3.1.5. (as relevant) may discontinue *targeted surveillance* and maintain its status as TS free provided that conditions that are conducive to clinical expression of TS, as described in Chapter X.X.X. 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 TS, *targeted surveillance* needs to be continued at a level determined by the *Competent Authority* on the basis of the likelihood of *infection*.

Article 2.3.1.7.

Importation of live aquatic animals from a country, zone or compartment declared free from Taura syndrome

When importing live *aquatic animals* of species referred to in Article 2.3.1.2. from a country, *zone* or *compartment* declared free from TS, the *Competent Authority* of the *importing country* should require an *international aquatic animal health certificate* issued by the *Competent Authority* of the *exporting country* or a *certifying official* approved by the *importing country* attesting that, on the basis of the procedures described in Articles 2.3.1.4. or 2.3.1.5. (as applicable), the place of production of the commodity consignment is a country, *zone* or *compartment* declared free from TS.

The *certificate* should be in accordance with the Model Certificate in Appendix 4.1.3.

This Article does not apply to *commodities* listed in point 1 of Article 2.3.1.3.

Article 2.3.1.8.

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

1. When importing, for *aquaculture*, live *aquatic animals* of species referred to in Article 2.3.1.2. from a country, *zone* or *compartment* not declared free from TS, the *Competent Authority* of the *importing country* should assess the *risk* and if justified, apply the following risk mitigation measures such as:
 - a) the direct delivery into and lifelong holding of the consignment in biosecure quarantine facilities for;
 - ~~b) the continuous isolation of the imported live aquatic animals and their first generation progeny from the local environment; and~~
 - ~~e)b) the treatment of all effluent and waste materials from the processing in a manner that ensures inactivation of TSV.~~
2. If the intention of the introduction is the establishment of a new stock genetic lines, international standards, such as the Guidelines Code of Practice on the Introductions and Transfers of Marine Organisms of the International Council for the Exploration of the Seas (ICES), should be followed.
3. For the purposes of the *Aquatic Code*, the ICES Guidelines Code may be summarised to the following main points:
 - a) identify stock of interest (cultured or wild) in its current location;
 - b) evaluate stock health/disease history;
 - c) take and test samples for TSV, pests and general health/disease status;
 - d) import and quarantine in a secure facility a founder (F-0) population;
 - 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 TSV and perform general examinations for pests and general health/disease status;
 - g) if TSV 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 TS free or specific pathogen free (SPF) for TSV;
 - h) release SPF F-1 stock from *quarantine* for *aquaculture* or stocking purposes in the country, *zone* or *compartment*.

This Article does not apply to *commodities* listed in point 1 of Article 2.3.1.3.

Article 2.3.1.9.

Importation of live aquatic animals for human consumption from a country, zone or compartment not declared free from Taura syndrome

When importing, for human consumption, live *aquatic animals* of species referred to in Article 2.3.1.2. from a country, *zone* or *compartment* not declared free from TS, the *Competent Authority* of the *importing country* should assess the risk and, if justified, require that:

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1. the consignment be delivered directly to and held in isolation until consumption; and
2. all effluent, dead *aquatic animals* and waste materials from the processing be treated in a manner that ensures inactivation of TSV.

Member Countries should consider introducing internal measures to prevent such *commodities* being used for any purpose other than for human consumption.

This Article does not apply to *commodities* listed in point 1 of Article 2.3.1.3.

Article 2.3.1.10.

Importation of aquatic animal products from a country, zone or compartment declared free from Taura syndrome

When importing *aquatic animal products* of species referred to in Article 2.3.1.2. from a country, *zone* or *compartment* declared free from TS, the *Competent Authority* of the *importing country* should require an *international aquatic animal health certificate* issued by the *Competent Authority* of the *exporting country* or a *certifying official* approved by the *importing country* attesting that, on the basis of the procedures described in Articles 2.3.1.4. or 2.3.1.5. (as applicable), the place of production of the consignment is a country, *zone* or *compartment* declared free from TS.

The *certificate* should be in accordance with the Model Certificate in Appendix 4.2.2.

This Article does not apply to *commodities* listed in point 1 of Article 2.3.1.3.

Article 2.3.1.11.

Importation of aquatic animal products from a country, zone or compartment not declared free from Taura syndrome

When importing *aquatic animal products* of species referred to in Article 2.3.1.2. from a country, *zone* or *compartment* not declared free from TS, the *Competent Authority* of the *importing country* should assess the risk and apply appropriate risk mitigation measures.

This Article does not apply to *commodities* listed in point 1 of Article 2.3.1.3.

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CHAPTER 2.3.2.

WHITE SPOT DISEASE

Article 2.3.2.1.

For the purposes of the *Aquatic Code*, white spot disease (WSD) means *infection* with white spot syndrome virus (WSSV). *White spot syndrome virus 1* is classified as a species in the genus *Whispovirus* of the family *Nimaviridae*. Common synonyms are listed in Chapter 4.1.2. of the *Aquatic Manual*.

Methods for conducting surveillance and diagnosis of WSD are provided in the *Aquatic Manual*.

Article 2.3.2.2.

Scope

The recommendations in this Chapter apply to all decapod (order *Decapoda*) crustaceans from marine, brackish and freshwater sources. These recommendations also apply to any other *susceptible species* referred to in the *Aquatic Manual* when traded internationally.

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

Article 2.3.2.3.

Commodities

1. When authorising the importation or transit of the following *commodities*, the *Competent Authorities* should not require any WSD related conditions, regardless of the WSD status of the *exporting country, zone* or *compartment*.
 - a) For the species referred to in Article 2.3.2.2. being used for any purpose:
 - i) commodities treated in a manner that inactivates the disease agent e.g. boiled, canned or pasteurised products and ready to eat meals; and crustacean oil and crustacean meal intended for use in animal feeds ~~commercially sterile canned products;~~
 - ii) boiled products (e.g. boiled whole shrimp or tails, lobsters, crabs);
 - iii) chemically extracted chitin;
 - iv) crustacean meals or by products made non infectious by heating or drying (e.g. flame dried or sun dried);
 - iiiiv) crustacean products made non-infectious through processing as dry feeds (e.g. pelleted or extruded feeds);
 - ivi) biological samples preserved for diagnostic applications in such a manner as to inactivate the disease agent WSSV (e.g. formalin or alcohol preserved samples).
 - b) The following products destined for human consumption from species referred to in Article 2.3.2.2. which have been prepared and packaged for direct retail trade in such a way as to minimise the likelihood of alternative uses;

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- i) chemically preserved products (e.g. salted, pickled, marinated, pastes, etc.);
- ii) ~~products that have been heat treated or dried (e.g. ready prepared meals) in a manner to ensure the inactivation of the pathogen.~~

For the *commodities* listed in point 1b), Member Countries should consider introducing internal measures to prevent the *commodity* being used for any purpose other than for human consumption.

2. When authorising the importation or transit of the *commodities* of a species referred to in Article 2.3.2.2., other than those listed in point 1 of Article 2.3.2.3., the *Competent Authorities* should require the conditions prescribed in Articles 2.3.2.7. to 2.3.2.11. relevant to the WSD status of the *exporting country, zone or compartment*.
3. When considering the importation/transit from an *exporting country, zone or compartment* not declared free of WSD of ~~any other commodity~~ of a species not covered in Article 2.3.2.2. but which could reasonably be expected to be a potential WSSV **carrier vector**, the *Competent Authorities* should conduct a **risk analysis in accordance with the recommendations in the Aquatic Code of the risk of introduction, establishment and spread of WSSV, and the potential consequences, associated with the importation of the commodity prior to a decision.** The *exporting country* should be informed of the outcome of this assessment.

Article 2.3.2.4.

White spot disease free country

A country may make a *self-declaration of freedom* from WSD if it meets the conditions in points 1, 2, 3 or 4 below.

If a country shares a *zone* with one or more other countries, it can only make a *self-declaration of freedom* from WSD if all the areas covered by the shared water are declared WSD free countries or *zones* (see Article 2.3.2.5.).

1. A country where none of the *susceptible species* referred to in Article 2.3.2.2. is present may make a *self-declaration of freedom* from WSD when *basic biosecurity conditions* have been continuously met in the country for at least the past 2 years.

OR

2. A country where the *susceptible species* referred to in Article 2.3.2.2. are present but there has never been any observed occurrence of the *disease* for at least the past 10 years despite conditions that are conducive to its clinical expression, as described in Chapter X.X.X. of the *Aquatic Manual*, may make a *self-declaration of freedom* from WSD when *basic biosecurity conditions* have been continuously met in the country for at least the past 2 years.

OR

3. A country where the last observed occurrence of the *disease* was within the past 10 years, or where the *infection* status prior to *targeted surveillance* was unknown, **for example** because of the absence of conditions conducive to its clinical expression, as described in Chapter X.X.X. of the *Aquatic Manual*, may make a *self-declaration of freedom* from WSD when:

- a) *basic biosecurity conditions* have been continuously met for at least the past 2 years; and
- b) *targeted surveillance*, as described in Chapters 1.1.4. and X.X.X. of the *Aquatic Manual*, has been in place for at least the last 2 years without detection of WSSV.

OR

4. A country that has previously made a *self-declaration of freedom* from WSD but in which the *disease* is subsequently detected may **not** make a *self-declaration of freedom* from WSD again **until when** the following conditions have been met:
- a) on detection of the *disease*, the affected area was declared an *infected zone* and a *buffer 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 (see *Aquatic Manual*) have been completed; and
 - c) *targeted surveillance*, as described in Chapters 1.1.4. and X.X.X. of the *Aquatic Manual*, has been in place for at least the past 2 years without detection of WSSV; **and**
 - d) previously existing basic biosecurity conditions have been reviewed and modified as necessary and have continuously been in place for at least the past 2 years.**

In the meantime, part of the non-affected area may be declared a free *zone* provided that **they such part** meets the conditions in point 3 of Article 2.3.2.5.

Article 2.3.2.5.

White spot disease free zone or free compartment

A *zone* or *compartment* within the *territory* of one or more countries not declared free from WSD may be declared free by the *Competent Authority(ies)* of the country(ies) concerned if the *zone* or *compartment* meets the conditions referred to in points 1, 2, 3 or 4 below.

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

1. A *zone* or *compartment* where none of the *susceptible species* referred to in Article 2.3.2.2. is present may be declared free from WSD when *basic biosecurity conditions* have been continuously met in the *zone* or *compartment* for at least the past 2 years.

OR

2. A *zone* or *compartment* where the *susceptible species* referred to in Article 2.3.2.2. are present but in which there has not been any observed occurrence of the *disease* for at least the past 10 years despite conditions that are conducive to its clinical expression, as described in Chapter X.X.X. of the *Aquatic Manual*, may be declared free from WSD when *basic biosecurity conditions* have been continuously met in the *zone* or *compartment* for at least the past 2 years.

OR

3. A *zone* or *compartment* where the last observed occurrence of the *disease* was within the past 10 years, or where the *infection* status prior to *targeted surveillance* was unknown; **for example** because of the absence of conditions conducive to its clinical expression; as described in Chapter X.X.X. of the *Aquatic Manual*, may be declared free from WSD when:
 - a) *basic biosecurity conditions* have been continuously met for at least the past 2 years; and
 - b) *targeted surveillance*, as described in Chapters 1.1.4. and X.X.X. of the *Aquatic Manual*, has been in place, through the *zone* or *compartment*, for at least the past 2 years without detection of WSSV.

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OR

4. A *zone* previously declared free from WSD but in which the *disease* is subsequently detected may **not** be declared free from WSD again **until when** the following conditions have been met:
- a) on detection of the *disease*, the affected area was declared an *infected zone* and a *buffer 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 (see *Aquatic Manual*) have been completed; and
 - c) *targeted surveillance*, as described in Chapters 1.1.4. and X.X.X. of the *Aquatic Manual*, has been in place for at least the past 2 years without detection of WSSV; **and**
 - d) previously existing basic biosecurity conditions have been reviewed and modified as necessary and have continuously been in place for at least the past 2 years.**

Article 2.3.2.6.

Maintenance of free status

A country, *zone* or *compartment* that is declared free from WSD following the provisions of points 1 or 2 of Articles 2.3.2.4. or 2.3.2.5. (as relevant) may maintain its status as WSD free provided that *basic biosecurity conditions* are continuously maintained.

A country, *zone* or *compartment* that is declared free from WSD following the provisions of point 3 of Articles 2.3.2.4. or 2.3.2.5. (as relevant) may discontinue *targeted surveillance* and maintain its status as WSD free provided that conditions that are conducive to clinical expression of WSD, as described in Chapter X.X.X. 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 WSD, *targeted surveillance* needs to be continued at a level determined by the *Competent Authority* on the basis of the likelihood of *infection*.

Article 2.3.2.7.

Importation of live aquatic animals from a country, zone or compartment declared free from white spot disease

When importing live *aquatic animals* of species referred to in Article 2.3.2.2. from a country, *zone* or *compartment* declared free from WSD, the *Competent Authority* of the *importing country* should require an *international aquatic animal health certificate* issued by the *Competent Authority* of the *exporting country* or a *certifying official* approved by the *importing country* attesting that, on the basis of the procedures described in Articles 2.3.2.4. or 2.3.2.5. (as applicable), the place of production of the commodity consignment is a country, *zone* or *compartment* declared free from WSD.

The *certificate* should be in accordance with the Model Certificate in Appendix 4.1.3.

This Article does not apply to *commodities* listed in point 1 of Article 2.3.2.3.

Article 2.3.2.8.

Importation of live aquatic animals for aquaculture from a country, zone or compartment not declared free from white spot disease

1. When importing, for *aquaculture*, live *aquatic animals* of species referred to in Article 2.3.2.2. from a country, *zone* or *compartment* not declared free from WSD, the *Competent Authority* of the *importing country* should assess the *risk* and, if justified, apply the following risk mitigation measures such as:
 - a) the direct delivery into and lifelong holding of the consignment in biosecure quarantine facilities for;
 - ~~b) the continuous isolation of the imported live aquatic animals and their first generation progeny from the local environment; and~~
 - ~~e)b) the treatment of all effluent and waste materials from the processing in a manner that ensures inactivation of WSSV.~~
2. If the intention of the introduction is the establishment of a new stock genetic lines, international standards, such as the Guidelines Code of Practice on the Introductions and Transfers of Marine Organisms of the International Council for the Exploration of the Seas (ICES), should be followed.
3. For the purposes of the *Aquatic Code*, the ICES Guidelines Code may be summarised to the following main points:
 - a) identify stock of interest (cultured or wild) in its current location;
 - b) evaluate stock health/ *disease* history;
 - c) take and test samples for WSSV, pests and general health/ *disease* status;
 - d) import and quarantine in a secure facility a founder (F-0) population;
 - 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 WSSV and perform general examinations for pests and general health/ *disease* status;
 - g) if WSSV 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 WSD free or specific pathogen free (SPF) for WSSV;
 - h) release SPF F-1 stock from *quarantine* for *aquaculture* or stocking purposes in the country, *zone* or *compartment*.

This Article does not apply to *commodities* listed in point 1 of Article 2.3.2.3.

Article 2.3.2.9.

Importation of live aquatic animals for human consumption from a country, zone or compartment not declared free from white spot disease

When importing, for human consumption, live *aquatic animals* of species referred to in Article 2.3.2.2. from a country, *zone* or *compartment* not declared free from WSD, the *Competent Authority* of the *importing country* should assess the risk and, if justified, require that:

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1. the consignment be delivered directly to and held in isolation until consumption; and
2. all effluent, dead *aquatic animals* and waste materials from the processing be treated in a manner that ensures inactivation of WSSV.

Member Countries should consider introducing internal measures to prevent such *commodities* being used for any purpose other than for human consumption.

This Article does not apply to *commodities* listed in point 1 of Article 2.3.2.3.

Article 2.3.2.10.

Importation of aquatic animal products from a country, zone or compartment declared free from white spot disease

When importing *aquatic animal products* of species referred to in Article 2.3.2.2. from a country, *zone* or *compartment* declared free from WSD, the *Competent Authority* of the *importing country* should require an *international aquatic animal health certificate* issued by the *Competent Authority* of the *exporting country* or a *certifying official* approved by the *importing country* attesting that, on the basis of the procedures described in Articles 2.3.2.4. or 2.3.2.5. (as applicable), the place of production of the consignment is a country, *zone* or *compartment* declared free from WSD.

The *certificate* should be in accordance with the Model Certificate in Appendix 4.2.2.

This Article does not apply to *commodities* listed in point 1 of Article 2.3.2.3.

Article 2.3.2.11.

Importation of aquatic animal products from a country, zone or compartment not declared free from white spot disease

When importing *aquatic animal products* of species referred to in Article 2.3.2.2. from a country, *zone* or *compartment* not declared free from WSD, the *Competent Authority* of the *importing country* should assess the risk and apply appropriate risk mitigation measures.

This Article does not apply to *commodities* listed in point 1 of Article 2.3.2.3.

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CHAPTER 2.3.3.

YELLOWHEAD DISEASE

Article 2.3.3.1.

For the purposes of the *Aquatic Code*, yellowhead disease (YHD) means *infection* with yellow head virus (YHV). YHV and the related *Gill-associated virus* are classified as a species in the genus *Okavirus*, family *Roniviridae* and order *Nidovirales*. Common synonyms are listed in Chapter 4.1.3. of the *Aquatic Manual*.

Methods for conducting surveillance and diagnosis of yellowhead disease are provided in the *Aquatic Manual*.

Article 2.3.3.2.

Scope

The recommendations in this Chapter apply to: 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.

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

Article 2.3.3.3.

Commodities

1. When authorising the importation or transit of the following *commodities*, the *Competent Authorities* should not require any YHD related conditions, regardless of the YHD status of the *exporting country, zone or compartment*.
 - a) For the species referred to in Article 2.3.3.2. being used for any purpose:
 - i) **commodities treated in a manner that inactivates the disease agent e.g. boiled, canned or pasteurised products and ready to eat meals; and crustacean oil and crustacean meal intended for use in animal feeds commercially sterile canned products;**
 - ii) **boiled products (e.g. boiled whole shrimp or tails, lobsters, crabs);**
 - iii) chemically extracted chitin;
 - iv) **crustacean meals or by products made non infectious by heating or drying (e.g. flame dried or sun dried);**
 - v) **crustacean products made non-infectious through processing as dry feeds (e.g. pelleted or extruded feeds);**
 - vi) biological samples preserved for diagnostic applications in such a manner as to inactivate the **disease agent YHV** (e.g. formalin or alcohol preserved samples).
 - b) The following products destined for human consumption from species referred to in Article 2.3.3.2. which have been prepared **and packaged for direct retail trade in such a way as to minimise the likelihood of alternative uses.**

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- i) chemically preserved products (e.g. salted, pickled, marinated, pastes, etc.);
- ii) products that have been heat treated or dried (e.g. ready prepared meals) in a manner to ensure the inactivation of the pathogen.

For the *commodities* listed in point 1b), Member Countries should consider introducing internal measures to prevent the *commodity* being used for any purpose other than for human consumption.

2. When authorising the importation or transit of the *commodities* of a species referred to in Article 2.3.3.2., other than those listed in point 1 of Article 2.3.3.3., the *Competent Authorities* should require the conditions prescribed in Articles 2.3.3.7. to 2.3.3.11. relevant to the YHD status of the *exporting country, zone* or *compartment*.
3. When considering the importation/transit from an *exporting country, zone* or *compartment* not declared free of YHD of any other *commodity* of a species not covered in Article 2.3.3.2. but which could reasonably be expected to be a potential YHV carrier vector, the *Competent Authorities* should conduct a risk analysis in accordance with the recommendations in the *Aquatic Code* of the risk of introduction, establishment and spread of YHV, and the potential consequences, associated with the importation of the *commodity* prior to a decision. The *exporting country* should be informed of the outcome of this assessment.

Article 2.3.3.4.

Yellowhead disease free country

A country may make a *self-declaration of freedom* from YHD if it meets the conditions in points 1, 2, 3 or 4 below.

If a country shares a *zone* with one or more other countries, it can only make a *self-declaration of freedom* from YHD if all the areas covered by the shared water are declared YHD free countries or *zones* (see Article 2.3.3.5.).

1. A country where none of the *susceptible species* referred to in Article 2.3.3.2. is present may make a *self-declaration of freedom* from YHD when *basic biosecurity conditions* have been continuously met in the country for at least the past 2 years.

OR

2. A country where the *susceptible species* referred to in Article 2.3.3.2. are present but there has never been any observed occurrence of the *disease* for at least the past 10 years despite conditions that are conducive to its clinical expression, as described in Chapter X.X.X. of the *Aquatic Manual*, may make a *self-declaration of freedom* from YHD when *basic biosecurity conditions* have been continuously met in the country for at least the past 2 years.

OR

3. A country where the last observed occurrence of the *disease* was within the past 10 years, or where the *infection status* prior to *targeted surveillance* was unknown, for example (e.g.) because of the absence of conditions conducive to its clinical expression, as described in Chapter X.X.X. of the *Aquatic Manual*, may make a *self-declaration of freedom* from YHD when:
 - a) *basic biosecurity conditions* have been continuously met for at least the past 2 years; and
 - b) *targeted surveillance*, as described in Chapters 1.1.4. and X.X.X. of the *Aquatic Manual*, has been in place for at least the last 2 years without detection of YHV.

OR

4. A country that has previously made a *self-declaration of freedom* from YHD but in which the *disease* is subsequently detected may **not** make a *self-declaration of freedom* from YHD again **until when** the following conditions have been met:
- a) on detection of the *disease*, the affected area was declared an *infected zone* and a *buffer 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 (see *Aquatic Manual*) have been completed; and
 - c) *targeted surveillance*, as described in Chapters 1.1.4. and X.X.X. of the *Aquatic Manual*, has been in place for at least the past 2 years without detection of YHV; **and**
 - d) previously existing basic biosecurity conditions have been reviewed and modified as necessary and have continuously been in place for at least the past 2 years.**

In the meantime, part of the non-affected area may be declared a free *zone* provided that **they such part** meets the conditions in point 3 of Article 2.3.3.5.

Article 2.3.3.5.

Yellowhead disease free zone or free compartment

A *zone* or *compartment* within the *territory* of one or more countries not declared free from YHD may be declared free by the *Competent Authority(ies)* of the country(ies) concerned if the *zone* or *compartment* meets the conditions referred to in points 1, 2, 3 or 4 below.

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

1. A *zone* or *compartment* where none of the *susceptible species* referred to in Article 2.3.3.2. is present may be declared free from YHD when *basic biosecurity conditions* have been continuously met in the *zone* or *compartment* for at least the past 2 years.

OR

2. A *zone* or *compartment* where the *susceptible species* referred to in Article 2.3.3.2. are present but in which there has not been any observed occurrence of the *disease* for at least the past 10 years despite conditions that are conducive to its clinical expression, as described in Chapter X.X.X. of the *Aquatic Manual*, may be declared free from YHD when *basic biosecurity conditions* have been continuously met in the *zone* or *compartment* for at least the past 2 years.

OR

3. A *zone* or *compartment* where the last observed occurrence of the *disease* was within the past 10 years, or where the *infection* status prior to *targeted surveillance* was unknown, **for example (e.g.)** because of the absence of conditions conducive to its clinical expression, as described in Chapter X.X.X. of the *Aquatic Manual*, may be declared free from YHD when:
 - a) *basic biosecurity conditions* have been continuously met for at least the past 2 years; and
 - b) *targeted surveillance*, as described in Chapters 1.1.4. and X.X.X. of the *Aquatic Manual*, has been in place, through the *zone* or *compartment*, for at least the past 2 years without detection of YHV.

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OR

4. A *zone* previously declared free from YHD but in which the *disease* is subsequently detected may **not** be declared free from YHD again **until when** the following conditions have been met:
- a) on detection of the *disease*, the affected area was declared an *infected zone* and a *buffer 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 (see *Aquatic Manual*) have been completed; and
 - c) *targeted surveillance*, as described in Chapters 1.1.4. and X.X.X. of the *Aquatic Manual*, has been in place for at least the past 2 years without detection of YHV; **and**
 - d) previously existing basic biosecurity conditions have been reviewed and modified as necessary and have continuously been in place for at least the past 2 years.**

Article 2.3.3.6.

Maintenance of free status

A country, *zone* or *compartment* that is declared free from YHD following the provisions of points 1 or 2 of Articles 2.3.3.4. or 2.3.3.5. (as relevant) may maintain its status as YHD free provided that *basic biosecurity conditions* are continuously maintained.

A country, *zone* or *compartment* that is declared free from YHD following the provisions of point 3 of Articles 2.3.3.4. or 2.3.3.5. (as relevant) may discontinue *targeted surveillance* and maintain its status as YHD free provided that conditions that are conducive to clinical expression of YHD, as described in Chapter X.X.X. 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 YHD, *targeted surveillance* needs to be continued at a level determined by the *Competent Authority* on the basis of the likelihood of *infection*.

Article 2.3.3.7.

Importation of live aquatic animals from a country, zone or compartment declared free from yellowhead disease

When importing live *aquatic animals* of species referred to in Article 2.3.3.2. from a country, *zone* or *compartment* declared free from YHD, the *Competent Authority* of the *importing country* should require an *international aquatic animal health certificate* issued by the *Competent Authority* of the *exporting country* or a *certifying official* approved by the *importing country* attesting that, on the basis of the procedures described in Articles 2.3.3.4. or 2.3.3.5. (as applicable), the place of production of the commodity consignment is a country, *zone* or *compartment* declared free from YHD.

The *certificate* should be in accordance with the Model Certificate in Appendix 4.1.3.

This Article does not apply to *commodities* listed in point 1 of Article 2.3.3.3.

Article 2.3.3.8.

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

1. When importing, for *aquaculture*, live *aquatic animals* of species referred to in Article 2.3.3.2. from a country, *zone* or *compartment* not declared free from YHD, the *Competent Authority* of the *importing country* should assess the *risk* and, if justified, apply the following risk mitigation measures such as:
 - a) the direct delivery into and lifelong holding of the consignment in biosecure quarantine facilities for;
 - ~~b) the continuous isolation of the imported live aquatic animals and their first generation progeny from the local environment; and~~
 - ~~e)b) the treatment of all effluent and waste materials from the processing in a manner that ensures inactivation of YHV.~~
2. If the intention of the introduction is the establishment of a new stock genetic lines, international standards, such as the Guidelines Code of Practice on the Introductions and Transfers of Marine Organisms of the International Council for the Exploration of the Seas (ICES), should be followed.
3. For the purposes of the *Aquatic Code*, the ICES Guidelines Code may be summarised to the following main points:
 - a) identify stock of interest (cultured or wild) in its current location;
 - b) evaluate stock health/ *disease* history;
 - c) take and test samples for YHV, pests and general health/ *disease* status;
 - d) import and quarantine in a secure facility a founder (F-0) population;
 - 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 YHV and perform general examinations for pests and general health/ *disease* status;
 - g) if YHV 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 YHD free or specific pathogen free (SPF) for YHV;
 - h) release SPF F-1 stock from *quarantine* for *aquaculture* or stocking purposes in the country, *zone* or *compartment*.

This Article does not apply to *commodities* listed in point 1 of Article 2.3.3.3.

Article 2.3.3.9.

Importation of live aquatic animals for human consumption from a country, zone or compartment not declared free from yellowhead disease

When importing, for human consumption, live *aquatic animals* of species referred to in Article 2.3.3.2. from a country, *zone* or *compartment* not declared free from YHD, the *Competent Authority* of the *importing country* should assess the risk and, if justified, require that:

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1. the consignment be delivered directly to and held in isolation until consumption; and
2. all effluent, dead *aquatic animals* and waste materials from the processing be treated in a manner that ensures inactivation of YHV.

Member Countries should consider introducing internal measures to prevent such *commodities* being used for any purpose other than for human consumption.

This Article does not apply to *commodities* listed in point 1 of Article 2.3.3.3.

Article 2.3.3.10.

Importation of aquatic animal products from a country, zone or compartment declared free from yellowhead disease

When importing *aquatic animal products* of species referred to in Article 2.3.3.2. from a country, *zone* or *compartment* declared free from YHD, the *Competent Authority* of the *importing country* should require an *international aquatic animal health certificate* issued by the *Competent Authority* of the *exporting country* or a *certifying official* approved by the *importing country* attesting that, on the basis of the procedures described in Articles 2.3.3.4. or 2.3.3.5. (as applicable), the place of production of the consignment is a country, *zone* or *compartment* declared free from YHD.

The *certificate* should be in accordance with the Model Certificate in Appendix 4.2.2.

This Article does not apply to *commodities* listed in point 1 of Article 2.3.3.3.

Article 2.3.3.11.

Importation of aquatic animal products from a country, zone or compartment not declared free from yellowhead disease

When importing *aquatic animal products* of species referred to in Article 2.3.3.2. from a country, *zone* or *compartment* not declared free from YHD, the *Competent Authority* of the *importing country* should assess the risk and apply appropriate risk mitigation measures.

This Article does not apply to *commodities* listed in point 1 of Article 2.3.3.3.

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CHAPTER 2.3.4.

TETRAHEDRAL BACULOVIROSIS

Article 2.3.4.1.

For the purposes of the *Aquatic Code*, tetrahedral baculovirus means *infection with Baculovirus penaei* (BPV). This virus is closely related to *Penaeus monodon baculovirus* (Chapter 4.1.5.) which has been classified as a tentative species in the genus *Nucleopolyhedrovirus*. Common synonyms are listed in Chapter 4.1.4. of the *Aquatic Manual*.

Methods for conducting surveillance and diagnosis of tetrahedral baculovirus are provided in the *Aquatic Manual*.

Article 2.3.4.2.

Scope

The recommendations in this Chapter apply to the following genera: *Penaeus*, *Trachypenaeus* and *Protrachypene*. These recommendations also apply to any other *susceptible species* referred to in the *Aquatic Manual* when traded internationally.

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

Article 2.3.4.3.

Commodities

1. When authorising the importation or transit of the following *commodities*, the *Competent Authorities* should not require any tetrahedral baculovirus related conditions, regardless of the tetrahedral baculovirus status of the *exporting country, zone or compartment*.
 - a) For the species referred to in Article 2.3.4.2. being used for any purpose:
 - i) commodities treated in a manner that inactivates the disease agent e.g. boiled, canned or pasteurised products and ready to eat meals; and crustacean oil and crustacean meal intended for use in animal feeds ~~commercially sterile canned products;~~
 - ii) boiled products (e.g. boiled whole shrimp or tails, lobsters, crabs);
 - iii) chemically extracted chitin;
 - iv) crustacean meals or by products made non infectious by heating or drying (e.g. flame dried or sun dried);
 - iiiv) crustacean products made non-infectious through processing as dry feeds (e.g. pelleted or extruded feeds);
 - ivv) biological samples preserved for diagnostic applications in such a manner as to inactivate the disease agent BPV (e.g. formalin or alcohol preserved samples).

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- b) The following products destined for human consumption from species referred to in Article 2.3.4.2. which have been prepared and packaged for direct retail trade in such a way as to minimise the likelihood of alternative uses:
- i) chemically preserved products (e.g. salted, pickled, marinated, pastes, etc.);
 - ii) ~~products that have been heat treated or dried (e.g. ready prepared meals) in a manner to ensure the inactivation of the pathogen;~~
 - iii) ~~de-headed and de-veined~~ “de-veined” (intestine removed) shrimp tails.

For the *commodities* listed in point 1b), Member Countries should consider introducing internal measures to prevent the *commodity* being used for any purpose other than for human consumption.

2. When authorising the importation or transit of the *commodities* of a species referred to in Article 2.3.4.2., other than those listed in point 1 of Article 2.3.4.3., the *Competent Authorities* should require the conditions prescribed in Articles 2.3.4.7. to 2.3.4.11., relevant to the tetrahedral baculovirus status of the *exporting country, zone or compartment*.
3. When considering the importation/transit from an *exporting country, zone or compartment* not declared free of tetrahedral baculovirus of ~~any other~~ *commodity* of a species not covered in Article 2.3.4.2. but which could reasonably be expected to be a potential BPV carrier vector, the *Competent Authorities* should conduct a risk analysis in accordance with the recommendations in the Aquatic Code of the risk of introduction, establishment and spread of BPV, and the potential consequences, associated with the importation of the commodity prior to a decision. The *exporting country* should be informed of the outcome of this assessment.

Article 2.3.4.4.

Tetrahedral baculovirus free country

A country may make a *self-declaration of freedom* from tetrahedral baculovirus if it meets the conditions in points 1, 2, 3 or 4 below.

If a country shares a *zone* with one or more other countries, it can only make a *self-declaration of freedom* from tetrahedral baculovirus if all the areas covered by the shared water are declared tetrahedral baculovirus free countries or *zones* (see Article 2.3.4.5.).

1. A country where none of the *susceptible species* referred to in Article 2.3.4.2. is present may make a *self-declaration of freedom* from tetrahedral baculovirus when *basic biosecurity conditions* have been continuously met in the country for at least the past 2 years.

OR

2. A country where the *susceptible species* referred to in Article 2.3.4.2. are present but there has never been any observed occurrence of the *disease* for at least the past 10 years despite conditions that are conducive to its clinical expression, as described in Chapter X.X.X. of the *Aquatic Manual*, may make a *self-declaration of freedom* from tetrahedral baculovirus when *basic biosecurity conditions* have been continuously met in the country for at least the past 2 years.

OR

3. A country where the last observed occurrence of the *disease* was within the past 10 years, or where the infection status prior to targeted surveillance was unknown, for example (e.g.) because of the absence of conditions conducive to its clinical expression, as described in Chapter X.X.X. of the Aquatic Manual, may make a *self-declaration of freedom* from tetrahedral baculovirus when:
 - a) *basic biosecurity conditions* have been continuously met for at least the past 2 years; and

- b) *targeted surveillance*, as described in Chapters 1.1.4. and X.X.X. of the *Aquatic Manual*, has been in place for at least the last 2 years without detection of BPV.

OR

4. A country that has previously made a *self-declaration of freedom* from tetrahedral baculovirus but in which the *disease* is subsequently detected may **not** make a *self-declaration of freedom* from tetrahedral baculovirus again **until when** the following conditions have been met:
- on detection of the *disease*, the affected area was declared an *infected zone* and a *buffer zone* was established; and
 - 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 (see *Aquatic Manual*) have been completed; and
 - targeted surveillance*, as described in Chapters 1.1.4. and X.X.X. of the *Aquatic Manual*, has been in place for at least the past 2 years without detection of BPV **and**
 - previously existing basic biosecurity conditions have been reviewed and modified as necessary and have continuously been in place for at least the past 2 years.**

In the meantime, part of the non-affected area may be declared a free *zone* provided that **they such part** meets the conditions in point 3 of Article 2.3.4.5.

Article 2.3.4.5.

Tetrahedral baculovirus free zone or free compartment

A *zone* or *compartment* within the *territory* of one or more countries not declared free from tetrahedral baculovirus may be declared free by the *Competent Authority(ies)* of the country(ies) concerned if the *zone* or *compartment* meets the conditions referred to in points 1, 2, 3 or 4 below.

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

- A *zone* or *compartment* where none of the *susceptible species* referred to in Article 2.3.4.2. is present may be declared free from tetrahedral baculovirus when *basic biosecurity conditions* have been continuously met in the *zone* or *compartment* for at least the past 2 years.

OR

- A *zone* or *compartment* where the *susceptible species* referred to in Article 2.3.4.2. are present but in which there has not been any observed occurrence of the *disease* for at least the past 10 years despite conditions that are conducive to its clinical expression, as described in Chapter X.X.X. of the *Aquatic Manual*, may be declared free from tetrahedral baculovirus when *basic biosecurity conditions* have been continuously met in the *zone* or *compartment* for at least the past 2 years.

OR

- A *zone* or *compartment* where the last observed occurrence of the *disease* was within the past 10 years, or where the *infection* status prior to *targeted surveillance* was unknown, **for example (e.g.)** because of the absence of conditions conducive to its clinical expression, as described in Chapter X.X.X. of the *Aquatic Manual*, may be declared free from tetrahedral baculovirus when:

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- a) *basic biosecurity conditions* have been continuously met for at least the past 2 years; and
- b) *targeted surveillance*, as described in Chapters 1.1.4. and X.X.X. of the *Aquatic Manual*, has been in place, through the *zone* or *compartment*, for at least the past 2 years without detection of BPV.

OR

4. A *zone* previously declared free from tetrahedral baculovirus but in which the *disease* is subsequently detected may **not** be declared free from tetrahedral baculovirus again **until** **when** the following conditions have been met:
 - a) on detection of the *disease*, the affected area was declared an *infected zone* and a *buffer 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 (see *Aquatic Manual*) have been completed; and
 - c) *targeted surveillance*, as described in Chapters 1.1.4. and X.X.X. of the *Aquatic Manual*, has been in place for at least the past 2 years without detection of BPV; **and**
 - d) previously existing basic biosecurity conditions have been reviewed and modified as necessary and have continuously been in place for at least the past 2 years.**

Article 2.3.4.6.

Maintenance of free status

A country, *zone* or *compartment* that is declared free from tetrahedral baculovirus following the provisions of points 1 or 2 of Articles 2.3.4.4. or 2.3.4.5. (as relevant) may maintain its status as tetrahedral baculovirus free provided that *basic biosecurity conditions* are continuously maintained.

A country, *zone* or *compartment* that is declared free from tetrahedral baculovirus following the provisions of point 3 of Articles 2.3.4.4. or 2.3.4.5. (as relevant) may discontinue *targeted surveillance* and maintain its status as tetrahedral baculovirus free provided that conditions that are conducive to clinical expression of tetrahedral baculovirus, as described in Chapter X.X.X. 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 tetrahedral baculovirus, *targeted surveillance* needs to be continued at a level determined by the *Competent Authority* on the basis of the likelihood of *infection*.

Article 2.3.4.7.

Importation of live aquatic animals from a country, zone or compartment declared free from tetrahedral baculovirus

When importing live *aquatic animals* of species referred to in Article 2.3.4.2. from a country, *zone* or *compartment* declared free from tetrahedral baculovirus, the *Competent Authority* of the *importing country* should require an *international aquatic animal health certificate* issued by the *Competent Authority* of the *exporting country* or a *certifying official* approved by the *importing country* attesting that, on the basis of the procedures described in Articles 2.3.4.4. or 2.3.4.5. (as applicable), the place of production of the commodity consignment is a country, *zone* or *compartment* declared free from tetrahedral baculovirus.

The *certificate* should be in accordance with the Model Certificate in Appendix 4.1.3.

This Article does not apply to *commodities* listed in point 1 of Article 2.3.4.3.

Article 2.3.4.8.

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

1. When importing, for *aquaculture*, live *aquatic animals* of species referred to in Article 2.3.4.2. from a country, *zone* or *compartment* not declared free from tetrahedral baculovirus, the *Competent Authority* of the *importing country* should assess the *risk* and, if justified, apply the following risk mitigation measures such as:
 - a) the direct delivery into and lifelong holding of the consignment in biosecure quarantine facilities for;
 - ~~b) the continuous isolation of the imported live aquatic animals and their first generation progeny from the local environment; and~~
 - ~~e)b) the treatment of all effluent and waste materials from the processing in a manner that ensures inactivation of BPV.~~
2. If the intention of the introduction is the establishment of a new stock genetic lines, international standards, such as the Guidelines Code of Practice on the Introductions and Transfers of Marine Organisms of the International Council for the Exploration of the Seas (ICES), should be followed.
3. For the purposes of the *Aquatic Code*, the ICES Guidelines Code may be summarised to the following main points:
 - a) identify stock of interest (cultured or wild) in its current location;
 - b) evaluate stock health/ *disease* history;
 - c) take and test samples for BPV, pests and general health/ *disease* status;
 - d) import and quarantine in a secure facility a founder (F-0) population;
 - 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 BPV and perform general examinations for pests and general health/ *disease* status;
 - g) if BPV 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 tetrahedral baculovirus free or specific pathogen free (SPF) for BPV;
 - h) release SPF F-1 stock from *quarantine* for *aquaculture* or stocking purposes in the country, *zone* or *compartment*.

This Article does not apply to *commodities* listed in point 1 of Article 2.3.4.3.

Appendix XVIII (contd)

Article 2.3.4.9.

Importation of live aquatic animals for human consumption from a country, zone or compartment not declared free from tetrahedral baculovirus

When importing, for human consumption, live *aquatic animals* of species referred to in Article 2.3.4.2. from a country, *zone* or *compartment* not declared free from tetrahedral baculovirus, the *Competent Authority* of the *importing country* should **assess the risk and, if justified**, require that:

1. the consignment be delivered directly to and held in isolation until consumption; and
2. all effluent, dead *aquatic animals* and waste materials from the processing be treated in a manner that ensures inactivation of BPV.

Member Countries should consider introducing internal measures to prevent such *commodities* being used for any purpose other than for human consumption.

This Article does not apply to *commodities* listed in point 1 of Article 2.3.4.3.

Article 2.3.4.10.

Importation of aquatic animal products from a country, zone or compartment declared free from tetrahedral baculovirus

When importing *aquatic animal products* of species referred to in Article 2.3.4.2. from a country, *zone* or *compartment* declared free from tetrahedral baculovirus, the *Competent Authority* of the *importing country* should require an *international aquatic animal health certificate* issued by the *Competent Authority* of the *exporting country* or a *certifying official* approved by the *importing country* attesting that, on the basis of the procedures described in Articles 2.3.4.4. or 2.3.4.5. (as applicable), the place of production of the consignment is a country, *zone* or *compartment* declared free from tetrahedral baculovirus.

The *certificate* should be in accordance with the Model Certificate in Appendix 4.2.2.

This Article does not apply to *commodities* listed in point 1 of Article 2.3.4.3.

Article 2.3.4.11.

Importation of aquatic animal products from a country, zone or compartment not declared free from tetrahedral baculovirus

When importing *aquatic animal products* of species referred to in Article 2.3.4.2. from a country, *zone* or *compartment* not declared free from tetrahedral baculovirus, the *Competent Authority* of the *importing country* should assess the risk and apply appropriate risk mitigation measures.

This Article does not apply to *commodities* listed in point 1 of Article 2.3.4.3.

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CHAPTER 2.3.5.

SPHERICAL BACULOVIRUS

Article 2.3.5.1.

For the purposes of the *Aquatic Code*, spherical baculovirus means *infection* with *Penaeus monodon* baculovirus (MBV). *Penaeus monodon baculovirus* is classified as a tentative species in the genus *Nucleopolyhedrovirus*. Common synonyms are listed in Chapter 4.1.5. of the *Aquatic Manual*.

Methods for conducting surveillance and diagnosis of spherical baculovirus are provided in the *Aquatic Manual*.

Article 2.3.5.2.

Scope

The recommendations in this Chapter apply to the following genera: *Penaeus* and *Metapenaeus*. These recommendations also apply to any other *susceptible species* referred to in the *Aquatic Manual* when traded internationally.

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

Article 2.3.5.3.

Commodities

1. When authorising the importation or transit of the following *commodities*, the *Competent Authorities* should not require any spherical baculovirus related conditions, regardless of the spherical baculovirus status of the *exporting country, zone* or *compartment*.
 - a) For the species referred to in Article 2.3.5.2. being used for any purpose:
 - i) commodities treated in a manner that inactivates the disease agent e.g. boiled, canned or pasteurised products and ready to eat meals; and crustacean oil and crustacean meal intended for use in animal feeds ~~commercially sterile canned products;~~
 - ii) boiled products (e.g. boiled whole shrimp or tails, lobsters, crabs);
 - iii) chemically extracted chitin;
 - iv) crustacean meals or by products made non infectious by heating or drying (e.g. flame dried or sun dried);
 - ~~iii)~~ crustacean products made non-infectious through processing as dry feeds (e.g. pelleted or extruded feeds);
 - iv) biological samples preserved for diagnostic applications in such a manner as to inactivate the disease agent MBV (e.g. formalin or alcohol preserved samples).

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- b) The following products destined for human consumption from species referred to in Article 2.3.5.2. which have been prepared and packaged for direct retail trade in such a way as to minimise the likelihood of alternative uses:
- i) chemically preserved products (e.g. salted, pickled, marinated, pastes, etc.);
 - ii) ~~products that have been heat treated or dried (e.g. ready prepared meals) in a manner to ensure the inactivation of the pathogen;~~
 - iii) ~~de-headed and de-veined~~ “de-veined” (intestine removed) shrimp tails.

For the *commodities* listed in point 1b), Member Countries should consider introducing internal measures to prevent the *commodity* being used for any purpose other than for human consumption.

2. When authorising the importation or transit of the *commodities* of a species referred to in Article 2.3.5.2., other than those listed in point 1 of Article 2.3.5.3., the *Competent Authorities* should require the conditions prescribed in Articles 2.3.5.7. to 2.3.5.11. relevant to the spherical baculovirus status of the *exporting country, zone or compartment*.
3. When considering the importation/transit from an *exporting country, zone or compartment* not declared free of spherical baculovirus of any other commodity of a species not covered in Article 2.3.5.2. but which could reasonably be expected to be a potential MBV carrier vector, the *Competent Authorities* should conduct a risk analysis in accordance with the recommendations in the Aquatic Code of the risk of introduction, establishment and spread of MBV, and the potential consequences, associated with the importation of the commodity, prior to a decision. The *exporting country* should be informed of the outcome of this assessment.

Article 2.3.5.4.

Spherical baculovirus free country

A country may make a *self-declaration of freedom* from spherical baculovirus if it meets the conditions in points 1, 2, 3 or 4 below.

If a country shares a *zone* with one or more other countries, it can only make a *self-declaration of freedom* from spherical baculovirus if all the areas covered by the shared water are declared spherical baculovirus free countries or *zones* (see Article 2.3.5.5.).

1. A country where none of the *susceptible species* referred to in Article 2.3.5.2. is present may make a *self-declaration of freedom* from spherical baculovirus when *basic biosecurity conditions* have been continuously met in the country for at least the past 2 years.

OR

2. A country where the *susceptible species* referred to in Article 2.3.5.2. are present but there has never been any observed occurrence of the *disease* for at least the past 10 years despite conditions that are conducive to its clinical expression, as described in Chapter X.X.X. of the *Aquatic Manual*, may make a *self-declaration of freedom* from spherical baculovirus when *basic biosecurity conditions* have been continuously met in the country for at least the past 2 years.

OR

3. A country where the last observed occurrence of the *disease* was within the past 10 years, or where the *infection status* prior to *targeted surveillance* was unknown, ~~for example (e.g.)~~ because of the absence of conditions conducive to its clinical expression, as described in Chapter X.X.X. of the *Aquatic Manual*, may make a *self-declaration of freedom* from spherical baculovirus when:
 - a) *basic biosecurity conditions* have been continuously met for at least the past 2 years; and

- b) *targeted surveillance*, as described in Chapters 1.1.4. and X.X.X. of the *Aquatic Manual*, has been in place for at least the last 2 years without detection of MBV.

OR

4. A country that has previously made a *self-declaration of freedom* from spherical baculovirus but in which the *disease* is subsequently detected may **not** make a *self-declaration of freedom* from spherical baculovirus again **until when** the following conditions have been met:
- on detection of the *disease*, the affected area was declared an *infected zone* and a *buffer zone* was established; and
 - 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 (see *Aquatic Manual*) have been completed; and
 - targeted surveillance*, as described in Chapters 1.1.4. and X.X.X. of the *Aquatic Manual*, has been in place for at least the past 2 years without detection of MBV; **and**
 - previously existing basic biosecurity conditions have been reviewed and modified as necessary and have continuously been in place for at least the past 2 years.**

In the meantime, part of the non-affected area may be declared a free *zone* provided that they meet the conditions in point 3 of Article 2.3.5.5.

Article 2.3.5.5.

Spherical baculovirus free zone or free compartment

A *zone* or *compartment* within the *territory* of one or more countries not declared free from spherical baculovirus may be declared free by the *Competent Authority(ies)* of the country(ies) concerned if the *zone* or *compartment* meets the conditions referred to in points 1, 2, 3 or 4 below.

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

- A *zone* or *compartment* where none of the *susceptible species* referred to in Article 2.3.5.2. is present may be declared free from spherical baculovirus when *basic biosecurity conditions* have been continuously met in the *zone* or *compartment* for at least the past 2 years.

OR

- A *zone* or *compartment* where the *susceptible species* referred to in Article 2.3.5.2. are present but in which there has not been any observed occurrence of the *disease* for at least the past 10 years despite conditions that are conducive to its clinical expression, as described in Chapter X.X.X. of the *Aquatic Manual*, may be declared free from spherical baculovirus when *basic biosecurity conditions* have been continuously met in the *zone* or *compartment* for at least the past 2 years.

OR

- A *zone* or *compartment* where the last observed occurrence of the *disease* was within the past 10 years, or where the *infection status* prior to *targeted surveillance* was unknown, **for example (e.g.)** because of the absence of conditions conducive to its clinical expression, as described in Chapter X.X.X. of the *Aquatic Manual*, may be declared free from spherical baculovirus when:

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- a) *basic biosecurity conditions* have been continuously met for at least the past 2 years; and
- b) *targeted surveillance*, as described in Chapters 1.1.4. and X.X.X. of the *Aquatic Manual*, has been in place, through the *zone* or *compartment*, for at least the past 2 years without detection of MBV.

OR

4. A *zone* previously declared free from spherical baculovirus but in which the *disease* is detected may **not** be declared free from spherical baculovirus again **until when** the following conditions have been met:
- a) on detection of the *disease*, the affected area was declared an *infected zone* and a *buffer 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 (see *Aquatic Manual*) have been completed; and
 - c) *targeted surveillance*, as described in Chapters 1.1.4. and X.X.X. of the *Aquatic Manual*, has been in place for at least the past 2 years without detection of MBV; **and**
 - d) previously existing *basic biosecurity conditions* have been reviewed and modified as necessary and have continuously been in place for at least the past 2 years.**

Article 2.3.5.6.

Maintenance of free status

A country, *zone* or *compartment* that is declared free from spherical baculovirus following the provisions of points 1 or 2 of Articles 2.3.5.4. or 2.3.5.5. (as relevant) may maintain its status as spherical baculovirus free provided that *basic biosecurity conditions* are continuously maintained.

A country, *zone* or *compartment* that is declared free from spherical baculovirus following the provisions of point 3 of Articles 2.3.5.4. or 2.3.5.5. (as relevant) may discontinue *targeted surveillance* and maintain its status as spherical baculovirus free provided that conditions that are conducive to clinical expression of spherical baculovirus, as described in Chapter X.X.X. 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 spherical baculovirus, *targeted surveillance* needs to be continued at a level determined by the *Competent Authority* on the basis of the likelihood of *infection*.

Article 2.3.5.7.

Importation of live aquatic animals from a country, zone or compartment declared free from spherical baculovirus

When importing live *aquatic animals* of species referred to in Article 2.3.5.2. from a country, *zone* or *compartment* declared free from spherical baculovirus, the *Competent Authority* of the *importing country* should require an *international aquatic animal health certificate* issued by the *Competent Authority* of the *exporting country* or a *certifying official* approved by the *importing country* attesting that, on the basis of the procedures described in Articles 2.3.5.4. or 2.3.5.5. (as applicable), the place of production of the commodity consignment is a country, *zone* or *compartment* declared free from spherical baculovirus.

The *certificate* should be in accordance with the Model Certificate in Appendix 4.1.3.

This Article does not apply to *commodities* listed in point 1 of Article 2.3.5.3.

Article 2.3.5.8.

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

1. When importing, for *aquaculture*, live *aquatic animals* of species referred to in Article 2.3.5.2. from a country, *zone* or *compartment* not declared free from spherical baculovirus, the *Competent Authority* of the *importing country* should assess the *risk* and, if justified, apply the following risk mitigation measures such as:
 - a) the direct delivery into and lifelong holding of the consignment in biosecure quarantine facilities for;
 - b) ~~the continuous isolation of the imported live aquatic animals and their first generation progeny from the local environment; and~~
 - e)b) the treatment of all effluent and waste materials from the processing in a manner that ensures inactivation of MBV.
2. If the intention of the introduction is the establishment of a new stock genetic lines, international standards, such as the Guidelines Code of Practice on the Introductions and Transfers of Marine Organisms of the International Council for the Exploration of the Seas (ICES), should be followed.
3. For the purposes of the *Aquatic Code*, the ICES Guidelines Code may be summarised to the following main points:
 - a) identify stock of interest (cultured or wild) in its current location;
 - b) evaluate stock health/ *disease* history;
 - c) take and test samples for MBV, pests and general health/ *disease* status;
 - d) import and quarantine in a secure facility a founder (F-0) population;
 - 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 MBV and perform general examinations for pests and general health/ *disease* status;
 - g) if MBV 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 spherical baculovirus free or specific pathogen free (SPF) for MBV;
 - h) release SPF F-1 stock from *quarantine* for *aquaculture* or stocking purposes in the country, *zone* or *compartment*.

This Article does not apply to *commodities* listed in point 1 of Article 2.3.5.3.

Article 2.3.5.9.

Importation of live aquatic animals for human consumption from a country, zone or compartment not declared free from spherical baculovirus

When importing, for human consumption, live *aquatic animals* of species referred to in Article 2.3.5.2. from a country, *zone* or *compartment* not declared free from spherical baculovirus, the *Competent Authority* of the *importing country* should assess the risk and, if justified, require that:

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1. the consignment be delivered directly to and held in isolation until consumption; and
2. all effluent, dead *aquatic animals* and waste materials from the processing be treated in a manner that ensures inactivation of MBV.

Member Countries should consider introducing internal measures to prevent such *commodities* being used for any purpose other than for human consumption.

This Article does not apply to *commodities* listed in point 1 of Article 2.3.5.3.

Article 2.3.5.10.

Importation of aquatic animal products from a country, zone or compartment declared free from spherical baculovirus

When importing *aquatic animal products* of species referred to in Article 2.3.5.2. from a country, *zone* or *compartment* declared free from spherical baculovirus, the *Competent Authority* of the *importing country* should require an *international aquatic animal health certificate* issued by the *Competent Authority* of the *exporting country* or a *certifying official* approved by the *importing country* attesting that, on the basis of the procedures described in Articles 2.3.5.4. or 2.3.5.5. (as applicable), the place of production of the consignment is a country, *zone* or *compartment* declared free from spherical baculovirus.

The *certificate* should be in accordance with the Model Certificate in Appendix 4.2.2.

This Article does not apply to *commodities* listed in point 1 of Article 2.3.5.3.

Article 2.3.5.11.

Importation of aquatic animal products from a country, zone or compartment not declared free from spherical baculovirus

When importing *aquatic animal products* of species referred to in Article 2.3.5.2. from a country, *zone* or *compartment* not declared free from spherical baculovirus, the *Competent Authority* of the *importing country* should assess the risk and apply appropriate risk mitigation measures.

This Article does not apply to *commodities* listed in point 1 of Article 2.3.5.3.

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CHAPTER 2.3.6.

INFECTIOUS HYPODERMAL AND HAEMATOPOIETIC NECROSIS

Article 2.3.6.1.

For the purposes of the *Aquatic Code*, infectious hypodermal and haematopoietic necrosis (IHHN) means infection with infectious hypodermal and haematopoietic necrosis virus (IHHNV). IHHNV is classified as the species *Penaeus stylirostris densovirus* in the genus *Brevidensovirus* in the family *Parvoviridae*.

Methods for conducting surveillance and diagnosis of IHHN are provided in the *Aquatic Manual*.

Article 2.3.6.2.

Scope

The recommendations in this Chapter apply to: giant tiger prawn (*Penaeus monodon*), Pacific white shrimp (*P. vannamei*) and blue shrimp (*P. stylirostris*). These recommendations also apply to any other *susceptible species* referred to in the *Aquatic Manual* when traded internationally.

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

Article 2.3.6.3.

Commodities

1. When authorising the importation or transit of the following *commodities*, the *Competent Authorities* should not require any IHHN related conditions, regardless of the IHHN status of the *exporting country, zone or compartment*.
 - a) For the species referred to in Article 2.3.6.2. being used for any purpose:
 - i) commodities treated in a manner that inactivates the disease agent e.g. boiled, canned or pasteurised products and ready to eat meals; and crustacean oil and crustacean meal intended for use in animal feeds commercially sterile canned products;
 - ii) boiled products (e.g. boiled whole shrimp or tails, lobsters, crabs);
 - iii) chemically extracted chitin;
 - iv) crustacean meals or by products made non infectious by heating or drying (e.g. flame dried or sun dried);
 - iii) crustacean products made non-infectious through processing as dry feeds (e.g. pelleted or extruded feeds);
 - iv) biological samples preserved for diagnostic applications in such a manner as to inactivate the disease agent IHHNV (e.g. formalin or alcohol preserved samples).
 - b) The following products destined for human consumption from species referred to in Article 2.3.6.2 which have been prepared and packaged for direct retail trade in such a way as to minimise the likelihood of alternative uses:

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- i) chemically preserved products (e.g. salted, pickled, marinated, pastes, etc.);
- ii) products that have been heat treated or dried (e.g. ready prepared meals) in a manner to ensure the inactivation of the pathogen.

For the *commodities* listed in point 1b), Member Countries should consider introducing internal measures to prevent the *commodity* being used for any purpose other than for human consumption.

2. When authorising the importation or transit of the *commodities* of a species referred to in Article 2.3.6.2., other than those listed in point 1 of Article 2.3.6.3., the *Competent Authorities* should require the conditions prescribed in Articles 2.3.6.7. to 2.3.6.11. relevant to the IHHN status of the *exporting country, zone* or *compartment*.
3. When considering the importation/transit from an *exporting country, zone* or *compartment* not declared free of IHHN of any other *commodity* of a species not covered in Article 2.3.6.2. but which could reasonably be expected to be a potential IHHNV *carrier vector*, the *Competent Authorities* should conduct a *risk analysis in accordance with the recommendations in the Aquatic Code of the risk of introduction, establishment and spread of IHHNV, and the potential consequences, associated with the importation of the commodity prior to a decision*. The *exporting country* should be informed of the outcome of this assessment.

Article 2.3.6.4.

Infectious hypodermal and haematopoietic necrosis free country

A country may make a *self-declaration of freedom* from IHHN if it meets the conditions in points 1, 2, 3 or 4 below.

If a country shares a *zone* with one or more other countries, it can only make a *self-declaration of freedom* from IHHN if all the areas covered by the shared water are declared IHHN free countries or *zones* (see Article 2.3.6.5.).

1. A country where none of the *susceptible species* referred to in Article 2.3.6.2. is present may make a *self-declaration of freedom* from IHHN when *basic biosecurity conditions* have been continuously met in the country for at least the past 2 years.

OR

2. A country where the *susceptible species* referred to in Article 2.3.6.2. are present but there has never been any observed occurrence of the *disease* for at least the past 10 years despite conditions that are conducive to its clinical expression, as described in Chapter X.X.X. of the *Aquatic Manual*, may make a *self-declaration of freedom* from IHHN when *basic biosecurity conditions* have been continuously met in the country for at least the past 2 years.

OR

3. A country where the last observed occurrence of the *disease* was within the past 10 years, or where the *infection status* prior to *targeted surveillance* was unknown, for example (e.g.) because of the absence of conditions conducive to its clinical expression, as described in Chapter X.X.X. of the *Aquatic Manual*, may make a *self-declaration of freedom* from IHHN when:
 - a) *basic biosecurity conditions* have been continuously met for at least the past 2 years; and
 - b) *targeted surveillance*, as described in Chapters 1.1.4. and X.X.X. of the *Aquatic Manual*, has been in place for at least the last 2 years without detection of IHHNV.

OR

4. A country that has previously made a *self-declaration of freedom* from IHHN but in which the *disease* is subsequently detected may **not** make a *self-declaration of freedom* from IHHN again **until when** the following conditions have been met:
 - a) on detection of the *disease*, the affected area was declared an *infected zone* and a *buffer 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 (see *Aquatic Manual*) have been completed; and
 - c) *targeted surveillance*, as described in Chapters 1.1.4. and X.X.X. of the *Aquatic Manual*, has been in place for at least the past 2 years without detection of IHHNV; **and**
 - d) previously existing basic biosecurity conditions have been reviewed and modified as necessary and have continuously been in place for at least the past 2 years.**

In the meantime, part of the non-affected area may be declared a free *zone* provided that **they such part** meets the conditions in point 3 of Article 2.3.6.5.

Article 2.3.6.5.

Infectious hypodermal and haematopoietic necrosis free zone or free compartment

A *zone* or *compartment* within the *territory* of one or more countries not declared free from IHHN may be declared free by the *Competent Authority(ies)* of the country(ies) concerned if the *zone* or *compartment* meets the conditions referred to in points 1, 2, 3 or 4 below.

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

1. A *zone* or *compartment* where none of the *susceptible species* referred to in Article 2.3.6.2. is present may be declared free from IHHN when *basic biosecurity conditions* have been continuously met in the *zone* or *compartment* for at least the past 2 years.

OR

2. A *zone* or *compartment* where the *susceptible species* referred to in Article 2.3.6.2. are present but in which there has not been any observed occurrence of the *disease* for at least the past 10 years despite conditions that are conducive to its clinical expression, as described in Chapter X.X.X. of the *Aquatic Manual*, may be declared free from IHHN when *basic biosecurity conditions* have been continuously met in the *zone* or *compartment* for at least the past 2 years.

OR

3. A *zone* or *compartment* where the last observed occurrence of the *disease* was within the past 10 years, or where the *infection* status prior to *targeted surveillance* was unknown, **for example (e.g.)** because of the absence of conditions conducive to its clinical expression, as described in Chapter X.X.X. of the *Aquatic Manual*, may be declared free from IHHN when:
 - a) *basic biosecurity conditions* have been continuously met for at least the past 2 years; and
 - b) *targeted surveillance*, as described in Chapters 1.1.4. and X.X.X. of the *Aquatic Manual*, has been in place, through the *zone* or *compartment*, for at least the past 2 years without detection of IHHNV.

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OR

4. A *zone* previously declared free from IHHN but in which the *disease* is subsequently detected may **not** be declared free from IHHN again **until when** the following conditions have been met:
- a) on detection of the *disease*, the affected area was declared an *infected zone* and a *buffer 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 (see *Aquatic Manual*) have been completed; and
 - c) *targeted surveillance*, as described in Chapters 1.1.4. and X.X.X. of the *Aquatic Manual*, has been in place for at least the past 2 years without detection of IHHNV; and
 - d) previously existing *basic biosecurity conditions* have been reviewed and modified as necessary and have continuously been in place for at least the past 2 years.**

Article 2.3.6.6.

Maintenance of free status

A country, *zone* or *compartment* that is declared free from IHHN following the provisions of points 1 or 2 of Articles 2.3.6.4. or 2.3.6.5. (as relevant) may maintain its status as IHHN free provided that *basic biosecurity conditions* are continuously maintained.

A country, *zone* or *compartment* that is declared free from IHHN following the provisions of point 3 of Articles 2.3.6.4. or 2.3.6.5. (as relevant) may discontinue *targeted surveillance* and maintain its status as IHHN free provided that conditions that are conducive to clinical expression of IHHN, as described in Chapter X.X.X. 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 IHHN, *targeted surveillance* needs to be continued at a level determined by the *Competent Authority* on the basis of the likelihood of *infection*.

Article 2.3.6.7.

Importation of live aquatic animals from a country, zone or compartment declared free from infectious hypodermal and haematopoietic necrosis

When importing live *aquatic animals* of species referred to in Article 2.3.6.2. from a country, *zone* or *compartment* declared free from IHHN, the *Competent Authority* of the *importing country* should require an *international aquatic animal health certificate* issued by the *Competent Authority* of the *exporting country* or a *certifying official* approved by the *importing country* attesting that, on the basis of the procedures described in Articles 2.3.6.4. or 2.3.6.5. (as applicable), the place of production of the commodity consignment is a country, *zone* or *compartment* declared free from IHHN.

The *certificate* should be in accordance with the Model Certificate in Appendix 4.1.3.

This Article does not apply to *commodities* listed in point 1 of Article 2.3.6.3.

Article 2.3.6.8.

Importation of live aquatic animals for aquaculture from a country, zone or compartment not declared free from infectious hypodermal and haematopoietic necrosis

1. When importing, for *aquaculture*, live *aquatic animals* of species referred to in Article 2.3.6.2. from a country, zone or compartment not declared free from IHHN, the *Competent Authority* of the *importing country* should assess the *risk* and, if justified, apply the following risk mitigation measures such as:
 - a) the direct delivery into and lifelong holding of the consignment in biosecure quarantine facilities for;
 - ~~b) the continuous isolation of the imported live aquatic animals and their first generation progeny from the local environment; and~~
 - ~~e)b) the treatment of all effluent and waste materials from the processing in a manner that ensures inactivation of IHHNV.~~
2. If the intention of the introduction is the establishment of a new stock genetic lines, international standards, such as the Guidelines Code of Practice on the Introductions and Transfers of Marine Organisms of the International Council for the Exploration of the Seas (ICES), should be followed.
3. For the purposes of the *Aquatic Code*, the ICES Guidelines Code may be summarised to the following main points:
 - a) identify stock of interest (cultured or wild) in its current location;
 - b) evaluate stock health/ *disease* history;
 - c) take and test samples for IHHNV, pests and general health/ *disease* status;
 - d) import and quarantine in a secure facility a founder (F-0) population;
 - 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 IHHNV and perform general examinations for pests and general health/ *disease* status;
 - g) if IHHNV 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 IHHN free or specific pathogen free (SPF) for IHHNV;
 - h) release SPF F-1 stock from *quarantine* for *aquaculture* or stocking purposes in the country, zone or compartment.

This Article does not apply to *commodities* listed in point 1 of Article 2.3.6.3.

Article 2.3.6.9.

Importation of live aquatic animals for human consumption from a country, zone or compartment not declared free from infectious hypodermal and haematopoietic necrosis

When importing, for human consumption, live *aquatic animals* of species referred to in Article 2.3.6.2. from a country, zone or compartment not declared free from IHHN, the *Competent Authority* of the *importing country* should assess the risk and, if justified, require that:

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1. the consignment be delivered directly to and held in isolation until consumption; and
2. all effluent, dead *aquatic animals* and waste materials from the processing be treated in a manner that ensures inactivation of IHNV.

Member Countries should consider introducing internal measures to prevent such *commodities* being used for any purpose other than for human consumption.

This Article does not apply to *commodities* listed in point 1 of Article 2.3.6.3.

Article 2.3.6.10.

Importation of aquatic animal products from a country, zone or compartment declared free from infectious hypodermal and haematopoietic necrosis

When importing *aquatic animal products* of species referred to in Article 2.3.6.2. from a country, *zone* or *compartment* declared free from IHNV, the *Competent Authority* of the *importing country* should require an *international aquatic animal health certificate* issued by the *Competent Authority* of the *exporting country* or a *certifying official* approved by the *importing country* attesting that, on the basis of the procedures described in Articles 2.3.6.4. or 2.3.6.5. (as applicable), the place of production of the consignment is a country, *zone* or *compartment* declared free from IHNV.

The *certificate* should be in accordance with the Model Certificate in Appendix 4.2.2.

This Article does not apply to *commodities* listed in point 1 of Article 2.3.6.3.

Article 2.3.6.11.

Importation of aquatic animal products from a country, zone or compartment not declared free from infectious hypodermal and haematopoietic necrosis

When importing *aquatic animal products* of species referred to in Article 2.3.6.2. from a country, *zone* or *compartment* not declared free from IHNV, the *Competent Authority* of the *importing country* should assess the risk and apply appropriate risk mitigation measures.

This Article does not apply to *commodities* listed in point 1 of Article 2.3.6.3.

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CHAPTER 2.3.7.

CRAYFISH PLAGUE

Article 2.3.7.1.

For the purposes of the *Aquatic Code*, crayfish plague means *infection* with *Aphanomyces astaci* Schikora. This organism is a member of a group commonly known as the water moulds (the Oomycetida). Common synonyms are listed in Chapter 4.1.7. of the *Aquatic Manual*.

Methods for conducting surveillance and diagnosis of crayfish plague are provided in the *Aquatic Manual*.

Article 2.3.7.2.

Scope

The recommendations in this Chapter apply to all species of crayfish in all three crayfish families (*Cambaridae*, *Astacidae*, and *Parastacidae*). These recommendations also apply to any other *susceptible species* referred to in the *Aquatic Manual* when traded internationally.

Crayfish plague is most severe in European crayfish species including the noble crayfish (*Astacus astacus*), the white claw crayfish (*Austropotamobius pallipes*), stone crayfish (*Austropotamobius torrentium*), and the Turkish crayfish (*Astacus leptodactylus*). In general, the Parastacidae and the Astacidae (except N. American genera such as *Pacifastacus*) are highly susceptible, while the *Cambaridae* are resistant to *disease*, but are potential carriers.

There is some evidence of transfer by movement of fish (and their transport water) from waters containing infected crayfish.

Article 2.3.7.3.

Commodities

1. When authorising the importation or transit of the following *commodities*, the *Competent Authorities* should not require any crayfish plague related conditions, regardless of the crayfish plague status of the *exporting country, zone* or *compartment*.
 - a) For the species referred to in Article 2.3.7.2. being used for any purpose:
 - i) commodities treated in a manner that inactivates the disease agent e.g. cooked (for >2 minutes at 60°), canned or pasteurised products and ready to eat meals; and crustacean oil and crustacean meal intended for use in animal feeds commercially-sterile canned products;
 - ii) boiled products (e.g. cooked whole shrimp or tails, lobsters, crabs);
 - iii) chemically extracted chitin;
 - iv) crustacean meals or by products made non-infectious by heating (>60°C for >5 minutes) or drying by product (e.g. flame dried or sun dried);
 - iii) crustacean products made non-infectious during processing as dry feeds (e.g. pelleted or extruded feeds);
 - iv) biological samples preserved for diagnostic applications in such a manner as to inactivate the disease agent *A. astaci* (e.g. formalin or alcohol preserved samples);
 - v) frozen products that have been subjected to -1020°C or lower temperatures for at least 24 72 hours.

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- b) The following products destined for human consumption from species referred to in Article 2.3.7.2. which have been prepared and packaged for direct retail trade in such a way as to minimise the likelihood of alternative uses:
- i) chemically preserved products (e.g. salted, pickled, marinated, pastes, etc.);
 - ii) ~~products that have been heat treated or dried (e.g. ready prepared meals) in a manner to ensure the inactivation of the pathogen.~~

For the *commodities* listed in point 1b), Member Countries should consider introducing internal measures to prevent the *commodity* being used for any purpose other than for human consumption.

2. When authorising the importation or transit of the *commodities* of a species referred to in Article 2.3.7.2., other than those listed in point 1 of Article 2.3.7.3., the *Competent Authorities* should require the conditions prescribed in Articles 2.3.7.7. to 2.3.7.11. relevant to the crayfish plague status of the *exporting country, zone or compartment*.
3. When considering the importation/transit from an *exporting country, zone or compartment* not declared free of crayfish plague of ~~any other commodity~~ of a species not covered in Article 2.3.7.2. but which could reasonably be expected to be a potential *A. astaci* carrier vector, the *Competent Authorities* should conduct a risk analysis in accordance with the recommendations in the Aquatic Code of the risk of introduction, establishment and spread of A. astaci, and the potential consequences, associated with the importation of the commodity prior to a decision. The *exporting country* should be informed of the outcome of this assessment.

Article 2.3.7.4.

Crayfish plague free country

A country may make a *self-declaration of freedom* from crayfish plague if it meets the conditions in points 1, 2, 3 or 4 below.

If a country shares a *water catchment* or with one or more other countries, it can only make a *self-declaration of freedom* from crayfish plague if all the areas covered by the shared water are declared crayfish plague free countries or *zones* (see Article 2.3.7.5.).

1. A country where none neither of the *susceptible species* or potential carrier species referred to in Article 2.3.7.2. is-are present may make a *self-declaration of freedom* from crayfish plague when *basic biosecurity conditions* have been continuously met in the country for at least the past 2 years.

OR

2. ~~A country where the *susceptible species* referred to in Article 4.1.7.2. are present but there has never been any observed occurrence of the *disease* for at least the past 25 years despite conditions that are conducive to its clinical expression, as described in Chapter X.X.X. of the *Aquatic Manual*, may make a *self-declaration of freedom* from crayfish plague when *basic biosecurity conditions* have been met continuously in the country for at least the past 2 years.~~

OR

3. ~~A country where the last observed occurrence of the *disease* was within the past 25 years or where the *infection status* prior to *targeted surveillance* was unknown, for example because of the absence of conditions conducive to its clinical expression, as described in Chapter X.X.X. of the *Aquatic Manual*, may make a *self-declaration of freedom* from crayfish plague when:~~

- a) *basic biosecurity conditions* have been met continuously for at least the past 2 years; and
- b) *targeted surveillance*, as described in Chapters 1.1.4. and X.X.X. of the *Aquatic Manual*, has been in place for at least the last 5 years without detection of *A. astaci*.

OR

4. A country that has previously made a *self declaration of freedom* from crayfish plague but in which the *disease* is subsequently detected may not make a *self declaration of freedom* from crayfish plague again until the following conditions have been met:

- a) on detection of the *disease*, the affected area was declared an *infected zone* and a *buffer 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 (see *Aquatic Manual*) have been completed; and
- e) *targeted surveillance*, as described in Chapters 1.1.4. and X.X.X. of the *Aquatic Manual*, has been in place for at least the past 5 years without detection of *A. astaci*.

In the meantime, part of the non affected area may be declared a *free zone* provided that they meet the conditions in point 3 of Article 4.1.7.5.

Article 2.3.7.5.

Crayfish plague free zone or free compartment

A *zone* or *compartment* within the *territory* of one or more countries not declared free from crayfish plague may be declared free by the *Competent Authority(ies)* of the country(ies) concerned if the *zone* or *compartment* meets the conditions referred to in points 1, 2, 3 or 4 below.

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

1. A *zone* or *compartment* where ~~none~~ neither of the *susceptible species* or potential carrier species referred to in Article 2.3.7.2. ~~is-are~~ present may be declared free from crayfish plague when *basic biosecurity conditions* have been continuously met in the *zone* or *compartment* for at least the past 2 years.

OR

2. A *zone* or *compartment* where the *susceptible species* referred to in Article 4.1.7.2. are present but in which there has not been any observed occurrence of the *disease* for at least the past 10 years despite conditions that are conducive to its clinical expression, as described in Chapter X.X.X. of the *Aquatic Manual*, may be declared free from crayfish plague when *basic biosecurity conditions* have been met continuously in the *zone* or *compartment* for at least the past 2 years.

OR

3. A *zone* or *compartment* where the last observed occurrence of the *disease* was within the past 10 years or where the *infection* status prior to *targeted surveillance* was unknown, for example because of the absence of conditions conducive to its clinical expression, as described in Chapter X.X.X. of the *Aquatic Manual*, may be declared free from crayfish plague when:

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- a) *basic biosecurity conditions* have been met continuously for at least the past 2 years; and
- b) *targeted surveillance*, as described in Chapters 1.1.4. and X.X.X. of the *Aquatic Manual*, has been in place, through the *zone* or *compartment*, for at least the past 2 years without detection of *A. astaci*.

OR

- 4. A *zone* previously declared free from crayfish plague but in which the *disease* is detected may not be declared free from crayfish plague again until the following conditions have been met:
 - a) on detection of the *disease*, the affected area was declared an *infected zone* and a *buffer 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 (see *Aquatic Manual*) have been completed; and
 - e) *targeted surveillance*, as described in Chapters 1.1.4. and X.X.X. of the *Aquatic Manual*, has been in place for at least the past 2 years without detection of *A. astaci*.

Article 2.3.7.6.

Maintenance of free status

A country, *zone* or *compartment* that is declared free from crayfish plague following the provisions of points 1 or 2 of Articles 2.3.7.4. or 2.3.7.5. (as relevant) may maintain its status as crayfish plague free provided that *basic biosecurity conditions* are continuously maintained.

A country, *zone* or *compartment* that is declared free from crayfish plague following the provisions of point 3 of Articles 2.3.7.4. or 2.3.7.5. (as relevant) may discontinue *targeted surveillance* and maintain its status as crayfish plague free provided that conditions that are conducive to clinical expression of crayfish plague, as described in Chapter X.X.X. 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 crayfish plague, *targeted surveillance* needs to be continued at a level determined by the *Competent Authority* on the basis of the likelihood of *infection*.

Article 2.3.7.7.

Importation of live aquatic animals from a country, zone or compartment declared free from crayfish plague

When importing live *aquatic animals* of species referred to in Article 2.3.7.2. from a country, *zone* or *compartment* declared free from crayfish plague, the *Competent Authority* of the *importing country* should require an *international aquatic animal health certificate* issued by the *Competent Authority* of the *exporting country* or a *certifying official* approved by the *importing country* attesting that, on the basis of the procedures described in Articles 2.3.7.4. or 2.3.7.5. (as applicable), the place of production of the commodity ~~consignment~~ is a country, *zone* or *compartment* declared free from crayfish plague.

The *certificate* should be in accordance with the Model Certificate in Appendix 4.1.3.

This Article does not apply to *commodities* listed in point 1 of Article 2.3.7.3.

Article 2.3.7.8.

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

1. When importing, for *aquaculture*, live *aquatic animals* of species referred to in Article 2.3.7.2. from a country, *zone* or *compartment* not declared free from crayfish plague, the *Competent Authority* of the *importing country* should assess the *risk* and, if justified, apply the following risk mitigation measures such as:
 - a) the direct delivery into and lifelong holding of the consignment in biosecure quarantine facilities for;
 - ~~b) the continuous isolation of the imported live aquatic animals and their first generation progeny from the local environment; and~~
 - ~~e)b) the treatment of all effluent and waste materials from the processing in a manner that ensures inactivation of *A. astaci*.~~
2. If the intention of the introduction is the establishment of a new stock genetic lines, international standards, such as the Guidelines Code of Practice on the Introductions and Transfers of Marine Organisms of the International Council for the Exploration of the Seas (ICES), should be followed.
3. For the purposes of the *Aquatic Code*, the ICES Guidelines Code may be summarised to the following main points:
 - a) identify stock of interest (cultured or wild) in its current location;
 - b) evaluate stock health/ *disease* history;
 - c) take and test samples for *A. astaci*, pests and general health/ *disease* status;
 - d) import and quarantine in a secure facility a founder (F-0) population;
 - 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 *A. astaci* and perform general examinations for pests and general health/ *disease* status;
 - g) if *A. astaci* 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 crayfish plague free or specific pathogen free (SPF) for *A. astaci*;
 - h) release SPF F-1 stock from *quarantine* for *aquaculture* or stocking purposes in the country, *zone* or *compartment*.

This Article does not apply to *commodities* listed in point 1 of Article 2.3.7.3.

Article 2.3.7.9.

Importation of live aquatic animals for human consumption from a country, zone or compartment not declared free from crayfish plague

When importing, for human consumption, live *aquatic animals* of species referred to in Article 2.3.7.2. from a country, *zone* or *compartment* not declared free from crayfish plague, the *Competent Authority* of the *importing country* should assess the risk and, if justified, require that:

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1. the consignment be delivered directly to and held in isolation until consumption; and
2. all effluent, dead *aquatic animals* and waste materials from the processing be treated in a manner that ensures inactivation of *A. astaci*.

Member Countries should consider introducing internal measures to prevent such *commodities* being used for any purpose other than for human consumption.

This Article does not apply to *commodities* listed in point 1 of Article 2.3.7.3.

Article 2.3.7.9. bis.**Importation of live fish from a country, zone or compartment not declared free from crayfish plague**

Because live fish and their transport water are potential vectors of crayfish plague, the *Competent Authority* of the *importing country* should require appropriate treatment of transport water as indicated in Chapter 1.5.1., when importing live fish from a country, zone or compartment not declared free from crayfish plague.

Article 2.3.7.10.

Importation of aquatic animal products from a country, zone or compartment declared free from crayfish plague

When importing *aquatic animal products* of species referred to in Article 2.3.7.2. from a country, *zone* or *compartment* declared free from crayfish plague, the *Competent Authority* of the *importing country* should require an *international aquatic animal health certificate* issued by the *Competent Authority* of the *exporting country* or a *certifying official* approved by the *importing country* attesting that, on the basis of the procedures described in Articles 2.3.7.4. or 2.3.7.5. (as applicable), the place of production of the consignment is a country, *zone* or *compartment* declared free from crayfish plague.

The *certificate* should be in accordance with the Model Certificate in Appendix 4.2.2.

This Article does not apply to *commodities* listed in point 1 of Article 2.3.7.3.

Article 2.3.7.11.

Importation of aquatic animal products from a country, zone or compartment not declared free from crayfish plague

When importing *aquatic animal products* of species referred to in Article 2.3.7.2. from a country, *zone* or *compartment* not declared free from crayfish plague, the *Competent Authority* of the *importing country* should assess the risk and apply appropriate risk mitigation measures.

This Article does not apply to *commodities* listed in point 1 of Article 2.3.7.3.

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CHAPTER 2.1.17.

KOI HERPESVIRUS DISEASE**1. Case definition**

Koi herpesvirus disease (KHVD) is a herpesvirus infection (17) capable of inducing a contagious and acute viraemia in common carp (*Cyprinus carpio*) and varieties such as koi carp and ghost carp (15).

2. Information for the design of surveillance programmes**a) Agent factors**

The aetiological agent is koi herpesvirus (KHV) in the family Herpesviridae (17, 40) although it has also been given the name carp interstitial nephritis and gill necrosis virus (CNGV) (19, 28). Waltzek *et al.* (39) provided evidence to support the classification of the virus as a herpesvirus, and named it cyprinid herpesvirus 3 (CyHV-3) following the nomenclature of other cyprinid herpesviruses: CyHV-1 (carp pox virus, fish papilloma) and CyHV-2 (goldfish haematopoietic necrosis virus). Estimates of the genome size of KHV vary from at least 150 kbp (11) to 277 kbp (19) to 295 kbp (39). Four genes coding for a helicase, an intercapsomeric triplex protein, DNA polymerase, and major capsid protein have been identified, and sequence analysis of these genes has shown that KHV is closely related to CyHV-1 and CyHV-2, and distantly related to channel catfish virus virus (Ictalurid herpesvirus: IchV-1) (39). Estimates of virion size also vary. Nucleocapsids of negative stained virus have been measured at 103–112 nm diameter surrounded by an envelope (17, 19, 37). The nucleocapsids of thin sectioned virus have been measured at 80–110 and 110–120 nm in diameter (4, 17, 26).

Serum from koi carp containing antibodies to KHV have been shown to cross-react with CyHV-1, a further indication that these viruses are closely related. Evidence of cross reacting antibodies was demonstrated in reciprocal enzyme-linked immunosorbent assay (ELISA) and western blot analyses of serum from koi infected with CyHV-1 or KHV (1).

Comparisons of the genomes of KHV isolates from different geographical areas by restriction enzyme analysis (9, 15) or nucleotide sequence analysis (13, 20, 29) have shown them to be practically identical. Likewise, the polypeptides of KHV isolates from different geographic areas were similar, although one isolate from Israel had two additional polypeptides (7, 9).

The virus is inactivated by UV radiation and temperatures above 50°C for 1 minute. The following disinfectants are also effective for inactivation: iodophore at 200 mg/litre for 20 minutes, benzalkonium chloride at 60 mg/litre for 20 minutes, ethyl alcohol at 30% for 20 minutes and sodium hypochlorite at 200 mg/litre for 30 seconds, all at 15°C (21).

b) Host factors

Naturally occurring KHV infections have only been recorded from common carp (*Cyprinus carpio carpio*), koi carp (*Cyprinus carpio koi*) and ghost carp (*Cyprinus carpio goi*) and hybrids of these varieties. All age groups of fish appear to be susceptible to KHVD (4, 29, 36), but under experimental conditions, 2.5–6 g fish were more susceptible than 230 g fish (26). Differential resistance to KHVD has been shown among different common carp strains (32) and other studies have suggested an age-related resistance (26). Morbidity of affected populations can be 100%, and mortality 70–80% (4, 38), but the latter can be as high as 90 or 100% (4, 37).

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Carp are often raised in polyculture with other fish species, but no signs of disease or mortalities have been observed in those other fish, during KHVD outbreaks, under normal polyculture conditions. Refractory species include goldfish (*Carrassius auratus*), grass carp (*Ctenopharyngodon idellus*), silver carp (*Hypophthalmichthys molitrix*), tench (*Tinca tinca*), sturgeon (*Acipenser* sp.) Nile tilapia (*Oreochromis niloticus*), silver perch (*Bidyanus bidyanus*) and channel catfish (*Ictalurus punctatus*) (4, 17, 26, 35).

The disease is temperature dependent, occurring between 16–25°C (6, 17, 26, 29, 36, 37). Under experimental conditions the disease has caused high mortality at 28°C (10) but not at 29 or 30°C (19, 25), nor at 13°C (10). However, viral DNA was detected in the fish by PCR at 13°C, and it is possible that infected fish surviving at low temperatures may be reservoirs of the virus (10). The disease course can be rapid. The disease manifested itself in 3 days following the addition of naïve fish to a pond containing diseased fish (38), but usually under those circumstances it takes 8–21 days for the disease to be observed in the naïve fish (4, 17). It is not known whether under natural conditions survivors of KHVD are persistently infected with virus, and if so, whether they shed the virus or for how long the fish retain the virus. Some of these aspects have been investigated in experimentally infected fish where it was shown that virus could persist in common carp infected at a permissive temperature and subsequently maintained at a lower than permissive temperature (33).

Common carp (*Cyprinus carpio*) strains are currently the only reported host of KHVD and therefore considered to be most susceptible to KHV infection. Goldfish x common carp hybrids, produced by hybridising male goldfish with female carp, have been reported to show some susceptibility to KHV infection. Approximately 50% of these hybrids examined at 25 days after intraperitoneal injection with a high dose of KHV possessed viral genomic DNA, as detected by PCR (18). In contrast to findings elsewhere, recent experimental data from Germany suggests a susceptibility of goldfish and grass carp to KHV but further confirmation of these findings are needed (14, 18). When sampling during surveillance programmes for KHV, common carp or strains such as koi or ghost (koi × common) carp should be preferentially selected followed by any common carp hybrids present on the site such as goldfish × common carp. Cyprinid species are commonly mixed together in polyculture systems and the risk of transmission of virus between species, during disease outbreaks, is high. If the findings from Germany were confirmed then, for disease surveillance purposes, all cyprinid species would need to be considered as potential covert carriers of KHV.

The reservoirs of KHVD are clinically infected fish and covert virus carriers among cultured, feral or wild fish. Virulent virus is shed via faeces, urine, gill and skin mucus. However, gill, kidney, and spleen are the organs in which KHV is most abundant during the course of overt infection (10).

The mode of transmission of KHV is horizontal but 'egg-associated' transmission (usually called 'vertical' transmission) cannot currently be ruled out. Horizontal transmission may be direct (fish to fish) or vectorial, water being the major abiotic vector. However, animate vectors (e.g. parasitic invertebrates and piscivorous birds and mammals) and fomites may also be involved in transmission.

c) **Disease pattern**

Disease patterns are influenced by water temperature, virulence of the virus, age and condition of the fish, population density and stress factors. The immune status of the fish will also be an important factor with both non-specific (interferon) and specific immunity (serum antibodies, cellular immunity) having important roles in herpesvirus infections. Clinical disease dominates at water temperatures above 18°C when the host immune response is at its optimum. Infected carp produce antibodies against the virus, which have been detected by ELISA methods at high serum dilution. Antibody has been detected in the serum at 3 weeks after experimental infection and in survivors after 1 year following a natural infection (1, 28, 33). Secondary and concomitant bacterial and/or parasitic infections are commonly seen in diseased carp and may affect the mortality rate and display of signs (15).

Following the first reports of KHVD in Israel and Germany (4, 16, 26) the geographical range of the disease has become extensive. The disease has been spread to many countries world-wide, predominantly through the trade in Koi carp before the current knowledge of the disease and means to detect it were available. It is now known to occur in, or has been recorded in fish imported into at least 21 different countries. In Europe this includes Austria, Belgium, Denmark, France, Italy, Luxembourg, The Netherlands, Poland, Switzerland and the United Kingdom (3, 6, 15, 30). In Asia, China (Hong Kong) (15), Indonesia (35), Japan (29), Malaysia (15, 22, 23), Singapore (in fish imported from Malaysia), Taipei China (37) and Thailand (in fish imported into Germany, 15). Elsewhere, South Africa (15) and the United States of America (11, 16, 36) have reported occurrence of KHVD. It is likely that the virus is present in many more countries, but has not yet been identified there or reported.

d) Control and prevention

Methods to control KHVD should mainly rely on avoiding exposure to the virus coupled with good hygiene and biosecurity practices. This is feasible on small farms supplied by spring or borehole water and a secure system to prevent fish entering the farm via the discharge water. Biosecurity measures should also include ensuring that new introductions of fish are from disease free sources and a quarantine system where new fish are held with sentinel fish at permissive temperatures for KHVD. The fish are then quarantined for a minimum of 4 weeks to 2 months before transfer to the main site and mixing with naïve fish. Hygiene measures on site should be similar to those recommended for SVC and include disinfection of eggs by iodophore treatment (21), regular disinfection of ponds, chemical disinfection of farm equipment, careful handling of fish to avoid stress and safe disposal of dead fish.

In rearing facilities with a controlled environment, elevation of water temperature above 26–28°C can reduce mortalities during KHVD outbreaks (7, 28). Lowering the stocking density, and treating secondary infections may also help reduce the severity of the disease (35). A safe and effective vaccine is not currently widely available. However, attenuated virus has been used to vaccinate carp and protect the fish from virus challenge (25, 28). The vaccine preparation induced antibody against the virus, but the duration of the protection is unknown. The vaccine is currently licensed for use in Israel and has been widely used in carp farms across the country.

3. Diagnostic methods

Diagnosis of KHVD in clinically affected fish can be achieved by virus isolation. However, the virus is isolated in only a limited number of cell lines and these cells can be difficult to handle. Also, cell culture isolation is not as sensitive as the published PCR-based methods to detect KHV DNA and is not considered to be a reliable diagnostic method for KHVD (15). Immunodiagnostic methods, similar to those used for diagnosis of SVC (e.g. immunofluorescence [IF] tests or ELISAs), may be suitable for rapid identification and diagnosis of KHVD but have not been extensively reported, compared or validated. Until such time as validated tests are available, diagnosis of KHVD should not rely on just one test but a combination of two or three tests (15).

KHV infection produces a detectable antibody response in carp and enzyme immunoassays that reliably detect these antibodies have been published (1, 28). These methods can be used as rapid presumptive tests during the acute disease, however various parameters, such as antibody sensitivity and specificity and sample preparation, can influence the results and therefore a negative result should be viewed with caution.

Detection of antibodies may prove to be a valuable method of establishing previous exposure to KHV in apparently healthy fish, and until PCR-based methods have been developed that are able to reliably detect persistent virus in exposed fish, antibody assays may be the only surveillance tools available. However, due to insufficient knowledge of the serological responses of fish to virus infections, the detection of fish antibodies to viruses has not thus far been accepted as a routine screening method for assessing the viral status of fish populations. Validation of some serological techniques for certain fish virus infections could arise in the near future, rendering the use of fish serology more widely acceptable for health screening purposes.

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Fish material suitable for virological examination is:

- **Asymptomatic fish** (apparently healthy fish): Gill, kidney, spleen, and encephalon (any size fish).
- **Clinically affected fish:** Gill, kidney, spleen, gut and encephalon (any size fish).

a) Field diagnostic methods

During a KHVD outbreak there will be a noticeable increase in mortality in the population. All age groups of fish appear to be susceptible to KHVD, although, generally, younger fish up to 1 year are more susceptible to clinical disease. Fish become lethargic, separate from the shoal and gather at the water inlet or sides of a pond and gasp at the surface of the water. Some fish may experience loss of equilibrium and disorientation but they may also show signs of hyperactivity. On closer examination of individual fish, typical clinical signs include pale discolouration or reddening of the skin, which may also have a rough texture, focal or total loss of epidermis, over- or under-production of mucus on the skin and gills. Other gross signs include enophthalmia (sunken eyes) and haemorrhages on the skin and base of the fins and fin erosion.

b) Clinical methods

There are no pathognomic gross lesions. Final diagnosis must await direct detection of viral DNA or antigen in tissues or virus isolation and identification. However, the most consistent gross pathology is seen in the gills and this can vary in extent from pale necrotic patches to extensive discolouration, severe necrosis and inflammation. Further examination can reveal erosion of primary lamellae, fusion of secondary lamellae, and swelling at the tips of the primary and secondary lamella. Other internal lesions are variable in occurrence and often absent in cases of sudden mortality. Other gross pathologies that have been reported include adhesions in the abdominal cavity with or without abnormal colouration of internal organs (lighter or darker). The kidney or liver may be enlarged, and they may also exhibit petechial or focal haemorrhages.

Presence of gross pathologies may also be complicated because diseased fish, particularly common carp, are also infested with ectoparasites such as *Argulus* sp., *Chilodonella* sp., *Cryptobia* sp., *Dactylogyrus* sp., *Gyrodactylus* sp., *Ichthyobodo* sp., *Ichthyophthirius* sp., *Trichodina* sp. and gill monogeneans, as well as numerous species of bacteria.

The histopathology of the disease can be non-specific and variable, but inflammation and necrosis of gill tissues is a consistent feature. Gills also exhibit hyperplasia and hypertrophy of branchial epithelium, and fusion of secondary lamellae and adhesion of gill filaments can be seen. Necrosis, ranging from small areas of necrotic epithelial cells of secondary lamellae to complete loss of the lamellae is observed. Branchial epithelial cells and leucocytes may have prominent nuclear swelling, margination of chromatin to give a "signet ring" appearance and pale diffuse eosinophilic intranuclear inclusions have been observed. Inflammation, necrosis and nuclear inclusions have been observed (individually or together) in other organs, particularly the kidney, but also in the spleen, pancreas, liver, brain, gut and oral epithelium.

c) Agent detection and identification methods

Detailed methods are not presented here because there have not been extensive comparison and validation of detection and identification methods for KHV. However, a short description of available published methods is provided. Method recommendations will rely on further testing and validation and further data being obtained from laboratories that have developed the methods to decide if they are 'fit-for-purpose'.

- **Direct detection methods**

- i) Isolation of KHV in cell culture**

The virus can be isolated in a limited number of cell cultures, but cell culture isolation is not as sensitive as PCR and is not considered to be a reliable diagnostic method for KHVD (15).

The virus replicates in koi fin cells (KF-1) (17), carp fin (CaF-2) and carp brain (CCB) cells (24), and in primary cells from fins of common or koi carp (19, 26, 28). Other cell lines used routinely for isolation of fish pathogenic viruses such as EPC, FHM, BF-2, CHSE-214 and RTG-2 cells are refractory to the virus (4, 19, 24, 37). The virus is most abundant in gill, kidney, and spleen tissues during the course of overt infection (10) and it is recommended to sample these tissues for virus isolation. The optimum incubation temperature for virus isolation in KF-1 or CCB cells is 20°C but 8–12 days' incubation may be required before a cytopathic effect (CPE) is observed (7).

- ii) Identification of virus isolated in cell culture**

Viruses isolated in cell culture must be definitively identified, as a number of different viruses have been isolated from carp exhibiting clinical signs resembling those of KHVD (5, 15).

Rapid presumptive methods

Immunodiagnostic methods, similar to those used for presumptive identification of SVC (e.g. IF tests or ELISAs), may well be suitable for rapid identification and diagnosis of KHVD (27, 32).

Confirmatory identification methods

The most reliable method for confirmatory identification is by PCR, or one of its variants, which have also been used to identify KHV DNA directly in fish tissues (2, 8–11, 13, 19, 20, 27, 40).

A PCR based on the thymidine kinase (TK) gene of KHV was reported to be more sensitive than PCR methods described by Gilad *et al.* (9) and Gray *et al.* (11), and could detect 10 fg of KHV DNA (2); the PCR of Ishioka *et al.* (20), based on the DNA polymerase gene, detected 100 fg of KHV DNA. The loop-mediated isothermal amplification (LAMP) method (13) was also based on the KHV TK gene, and was as sensitive as a PCR method developed by the same authors, but was more rapid than the PCR. The PCR described by Gray *et al.* (11) was improved by Yuasa *et al.* (40), and has been incorporated in the official Japanese guidelines for the detection of KHV.

New improved diagnostic PCR tests will continue to be developed and it is hoped that they will be validated as recommended in Chapter 1.1.3 of this *Aquatic Manual*.

The DNA extraction and PCR protocols detailed below for direct detection of KHV in fish tissues are also suitable for confirmatory identification of infected cell culture supernatants.

- iii) Diagnostic methods for clinically diseased fish**

Direct detection in fish tissues

KHV has been identified in touch imprints of liver, kidney and brain of infected fish by IF. Highest levels of positive immunofluorescence was seen in the kidney and the virus could be detected by IF on a kidney imprint 1 day post-infection (27, 32). Virus antigen has also been detected in infected tissues by an immunoperoxidase staining method. The virus antigen was detected by 2 days post infection in the kidney, and was also observed in the gills and liver (27). However, the detection of KHV by immunostaining must be interpreted with care, as positive staining cells could result from cross-reaction with serologically related virus (e.g. CyHV-1) or a non-viral protein (27).

ELISA-based methods for direct detection of KHV antigen in infected tissues are under development in a number of laboratories worldwide but no methods have been published.

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The most commonly used method for detection of KHV directly in fish tissues is using PCR assays specific for KHV (see above, under confirmatory identification).

In studies carried out at the Cefas Weymouth laboratory, published primer sets were compared using a standard PCR protocol for detection of KHV DNA in carp tissues (K. Way, unpublished data). The primer set targeting the TK gene (2) was the most sensitive with a detection limit three log greater than Gilad primers. CNGV primers (27) and modified Gray SpH primers that target short regions of the genome (109 bp and 151 bp, respectively) also performed well, particularly on decomposed tissues. The TK primer set later performed well in a method ring-trial carried out in 21 laboratories in 19 countries around the world (K. Way, unpublished data).

The same study at Cefas and the method ring-trial also compared commercial DNA extraction kits for their ability to provide KHV DNA of sufficient quality for the PCR. Of the commercial kits tested at Cefas, EasyDNA (Invitrogen), DNeasy (Qiagen) and DNAzol reagent (Invitrogen) all extracted DNA of suitable quality. In the ring-trial, the High Pure PCR template preparation kit (Roche), QIAamp DNA blood minikit (Qiagen) and the Puregene DNA purification kit, all performed well. However, some laboratories found the DNAzol reagent not to be as reliable.

The sample preparation protocol detailed below uses the DNAzol reagent for extraction of KHV DNA. This is an easy to use, short duration protocol that is also relatively inexpensive compared to some kits. Laboratories that are not familiar with DNAzol extraction may find the method less reliable in their hands. However, a number of DNA extraction kits are available commercially (including those listed above) that will produce high quality DNA suitable for use with the PCR protocol detailed.

The PCR protocol detailed below uses the TK primer set developed by Bercovier and colleagues at the Hebrew University-Hadassah Medical School in Israel (2). Of the published single-round-PCR methods, this is currently considered to be the most sensitive for detection of KHV DNA in fresh tissue samples from clinically diseased carp. This protocol may also allow detection of subclinical levels of virus. If the tissue shows evidence of decomposition then primer sets (see above) targeting shorter regions of the genome may need to be used in place of the TK primer set.

General notes

PCR is prone to false-positive and false-negative results. Therefore each assay and tissue extraction should include a negative control to rule out contamination. To further minimise the risk of contamination, aerosol-preventing pipette tips should be used for all sample and PCR reaction preparation steps.

Sample preparation

- i) Virus extraction from organ tissues should be carried out using the procedure described in Chapter I.1 (Section B.3.2).
- ii) Add 100 µl of tissue homogenate (1/10 [w/v]) or virus culture supernatant to a 1.5 ml microcentrifuge tube containing 1 ml DNAZOL[®] reagent.
- iii) Mix gently by inverting the tube five times and stand at room temperature for 5 minutes then centrifuge at 10,000 rpm for 10 minutes using a microcentrifuge.
- iv) Remove 1 ml of the supernatant to a new 1.5 ml microcentrifuge tube containing 0.5 ml of ethanol.

- v) Mix gently by inverting the tube five times and stand at room temperature for 5 minutes, then centrifuge at 13,000 rpm for 30 minutes using a microcentrifuge.
- vi) Remove the supernatant and rinse the pellet with 250 µl of 70% ethanol in molecular biology grade water.
- vii) Spin samples for 5 minutes at 13,000 rpm.
- viii) Remove the ethanol using a pipette and air-dry the pellet by leaving the tubes open on the bench for 5 minutes.
- ix) Resuspend the pellet in 50 µl molecular biology grade water, prewarmed at 60°C, and incubate at 60°C for 5 minutes. Samples can be stored at -20°C until required.

PCR

All PCR reactions are prepared in a clean area that is separate from the area where the amplifications are performed. This will minimise the risk of contamination.

- i) For each sample prepare a master mix containing:

For Go Taq Polymerase:

10 µl	Reaction buffer (×10 conc.)
5 µl	MgCl ₂ (25 mM stock)
0.5 µl	dNTPs (25 mM mix) [Promega Cat.no.U1240]
0.5 µl	Forward primer (100 pmol/µl stock)
0.5 µl	Reverse primer (100 pmol/µl stock)
0.25 µl	Go Taq polymerase 500 µ (5 µ/µl) [Promega Cat.no.M8305]
30.75 µl	Molecular biology grade water

Bercovier TK primers:

Forward = 5'-GGG-TTA-CCT-GTA-CGA-G-3'

Reverse = 5'-CAC-CCA-GTA-GAT-TAT-GC-3'

Product size = 409 bp

For each sample dispense 47.5 µl into a 0.5 ml thin walled microcentrifuge tube. Overlay with two drops of mineral oil.

- ii) Add 2.5 µl of the DNA extracted DNAzol®. Store the remainder of the DNA at -20°C.
- iii) Place tubes in a thermal cycler and perform programme:
 - 1 cycle of: 5 minutes at 94°C
 - 40 cycles of: 1 minute at 95°C
 - 1 minute at 55°C
 - 1 minute at 72°C
 Followed by a final extension step of 10 minutes at 72°C.

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- iv) Electrophorese 20 µl volumes of PCR product on a 2% ethidium bromide stained agarose gel (4% when separating smaller amplification products of <300 bp) at 120 V for 20 minutes and visualise under UV light. An appropriate molecular weight ladder should be included on the gel to determine the size of the product.
- v) Products of the correct size should be confirmed as KHV in origin by sequence analysis.

4. Rating of tests against purpose of use

The methods currently available for surveillance, detection and diagnosis of KHVD are listed in Table 1. The designations used in the table indicate: A = the method is currently the recommended method for reasons of availability, utility and diagnostic sensitivity and specificity; B = the method is a standard method with good diagnostic sensitivity and specificity; C = the method has application in some situations, but cost, accuracy or other factors severely limits its application; D = the method is currently not recommended for this purpose. Although not all of the tests listed as category A or B have undergone formal standardisation and validation (at least stages 1 and 2 of figure 1 of Chapter 1.1.2), their routine nature and the fact that they have been used widely without dubious results makes them acceptable.

Table 1. KHVD surveillance, detection and diagnostic methods

Method	Surveillance to declare freedom from infection	Presumptive diagnosis of infection or disease	Confirmatory diagnosis of infection or disease
Gross signs	D	B	D
Histopathology of tissues and organs	D	B	C
Isolation of in cell culture	D	C	D
Antibody-based assays to detect KHV antigen (IFAT, ELISA)	D	B	C
Transmission EM of tissues	D	B	C
PCR of tissue extracts*	C	A	A
PCR – sequence analysis	NA	C	A
Detection of KHV antibodies in exposed fish (ELISA)**	C	C	D

IFAT = Indirect fluorescent antibody test; ELISA = enzyme-linked immunosorbent assay;
EM = electron microscopy; PCR = polymerase chain reaction.

*Diagnostic virologists should be aware that fish recently vaccinated against KHV may test positive by PCR. No information is currently available to indicate any genome sequence differences between the attenuated vaccine strain and wild-type (w.t.) KHV. Until this sequence information is provided, diagnostic laboratories will not be able to distinguish between w.t. and vaccine strain of KHV and this could lead to a false diagnosis.

**Diagnostic virologists should be aware that fish recently vaccinated against KHV may test positive by ELISA. There may also be a low-level cross reaction with antibodies to CyHV-1.

NOTE: Many diagnostic laboratories may encounter difficulties in obtaining antibodies against KHV that are suitable for use in immunodiagnostic tests. However, a limited number of monoclonal and polyclonal antibodies may be very soon available from commercial sources. It is quite likely that diagnostic kits will also soon be available from the same sources.

5. Corroborative diagnostic criteria

a) Definition of suspect case

A suspect case of KHVD is defined as the presence of typical clinical signs of the disease in a population of susceptible fish OR presentation of typical histopathology in tissue sections OR typical CPE in cell cultures without identification of the causative agent OR a single positive result from one of the diagnostic assays described above.

b) Definition of confirmed case

A confirmed case is defined as a suspect case with subsequent identification of the causative agent by one of the serological or molecular assays described above OR a second positive result from a separate and different diagnostic assay described above.

6. Diagnostic/detection methods to declare freedom

There are no currently recommended methods for surveillance of susceptible fish populations for declaration of freedom from KHV. However, many laboratories are investigating further development of molecular-based methods to increase sensitivity (e.g. real-time and nested PCR) or to reliably detect low levels of persistent virus DNA. These assays may well prove suitable for surveillance programmes.

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NB: There are OIE Reference Laboratories for Koi herpesvirus disease (see Table at the end of this *Aquatic Manual* or consult the OIE Web site for the most up-to-date list: www.oie.int).

CHAPTER 2.3.9.

INFECTIOUS MYONECROSIS

Article 2.3.9.1.

For the purposes of the *Aquatic Code*, infectious myonecrosis (IMN) means *infection* with infectious myonecrosis virus (IMNV). This virus is similar to members of the family *Totiviridae*.

Methods for conducting surveillance and diagnosis of IMN are provided in the *Aquatic Manual*.

Article 2.3.9.2.

Scope

The recommendations in this Chapter apply to: Pacific white shrimp (*Penaeus vannamei*). These recommendations also apply to any other *susceptible species* referred to in the *Aquatic Manual* when traded internationally.

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

Article 2.3.9.3.

Commodities

1. When authorising importation or transit of the following *commodities*, the *Competent Authorities* should not require any IMN related conditions, regardless of the IMN status of the *exporting country, zone or compartment*.
 - a) For the species referred to in Article 2.3.9.2. being used for any purpose:
 - i) commodities treated in a manner that inactivates the disease agent e.g. boiled, canned or pasteurised products and ready to eat meals; and crustacean oil and crustacean meal intended for use in animal feeds ~~commercially sterile canned products;~~
 - ii) boiled products (e.g. boiled whole shrimp or tails, lobsters, crabs);
 - iii) chemically extracted chitin;
 - iv) crustacean meals or by products made non infectious by heating or drying (e.g. flame dried or sun dried);
 - iiiiv) crustacean products made non-infectious through processing as dry feeds (e.g. pelleted or extruded feeds);
 - ivi) biological samples preserved for diagnostic applications in such a manner as to inactivate the disease agent IMNV (e.g. formalin or alcohol preserved samples).
 - b) The following products destined for human consumption from species referred to in Article 2.3.9.2. which have been prepared and packaged for direct retail trade ~~in such a way as to minimise the likelihood of alternative uses;~~

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- i) chemically preserved products (e.g. salted, pickled, marinated, pastes, etc.);
- ii) products that have been heat treated or dried (e.g. ready prepared meals) in a manner to ensure the inactivation of the pathogen.

For the *commodities* listed in point 1b), Member Countries should consider introducing internal measures to prevent the *commodity* being used for any purpose other than for human consumption.

2. When authorising the importation or transit of the *commodities* of a species referred to in Article 2.3.9.2., other than those listed in point 1 of Article 2.3.9.3., the *Competent Authorities* should require the conditions prescribed in Articles 2.3.9.7. to 2.3.9.11. relevant to the IMN status of the *exporting country, zone* or *compartment*.
3. When considering the importation/transit from an *exporting country, zone* or *compartment* not declared free of IMN of any other *commodity* of a species not covered in Article 2.3.9.2. but which could reasonably be expected to be a potential IMNV *carrier vector*, the *Competent Authorities* should conduct a *risk analysis in accordance with the recommendations in the Aquatic Code of the risk of introduction, establishment and spread of IMNV, and the potential consequences, associated with the importation of the commodity prior to a decision*. The *exporting country* should be informed of the outcome of this assessment.

Article 2.3.9.4.

Infectious myonecrosis free country

A country may make a *self-declaration of freedom* from IMN if it meets the conditions in points 1, 2, 3 or 4 below.

If a country shares a *zone* with one or more other countries, it can only make a *self-declaration of freedom* from IMN if all the areas covered by the shared water are declared IMN free countries or *zones* (see Article 2.3.9.5.).

1. A country where none of the *susceptible species* referred to in Article 2.3.9.2. is present may make a *self-declaration of freedom* from IMN when *basic biosecurity conditions* have been continuously met in the country for at least the past 2 years.

OR

2. A country where the *susceptible species* referred to in Article 2.3.9.2. are present but there has never been any observed occurrence of the *disease* for at least the past 10 years despite conditions that are conducive to its clinical expression, as described in Chapter X.X.X. of the *Aquatic Manual*, may make a *self-declaration of freedom* from IMN when *basic biosecurity conditions* have been continuously met in the country for at least the past 2 years.

OR

3. A country where the last observed occurrence of the *disease* was within the past 10 years, or where the *infection* status prior to *targeted surveillance* was unknown, for example (e.g. because of the absence of conditions conducive to its clinical expression, as described in Chapter X.X.X. of the *Aquatic Manual*), may make a *self-declaration of freedom* from IMN when:
 - a) *basic biosecurity conditions* have been continuously met for at least the past 2 years; and
 - b) *targeted surveillance*, as described in Chapters 1.1.4. and X.X.X. of the *Aquatic Manual*, has been in place for at least the last 2 years without detection of IMNV.

OR

4. A country that has previously made a *self-declaration of freedom* from IMN but in which the *disease* is subsequently detected may **not** make a *self-declaration of freedom* from IMN again **until when** the following conditions have been met:
- a) on detection of the *disease*, the affected area was declared an *infected zone* and a *buffer 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 (see *Aquatic Manual*) have been completed; and
 - c) *targeted surveillance*, as described in Chapters 1.1.4. and X.X.X. of the *Aquatic Manual*, has been in place for at least the past 2 years without detection of IMNV; and
 - d) previously existing basic biosecurity conditions have been reviewed and modified as necessary and have continuously been in place for at least the past 2 years.**

In the meantime, part of the non-affected area may be declared a free *zone* provided that **they such part** meets the conditions in point 3 of Article 2.3.9.5.

Article 2.3.9.5.

Infectious myonecrosis free zone or free compartment

A *zone* or *compartment* within the *territory* of one or more countries not declared free from IMN may be declared free by the *Competent Authority(ies)* of the country(ies) concerned if the *zone* or *compartment* meets the conditions referred to in points 1, 2, 3 or 4 below.

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

1. A *zone* or *compartment* where none of the *susceptible species* referred to in Article 2.3.9.2. is present may be declared free from IMN when *basic biosecurity conditions* have been continuously met in the *zone* or *compartment* for at least the past 2 years.

OR

2. A *zone* or *compartment* where the *susceptible species* referred to in Article 2.3.9.2. are present but in which there has not been any observed occurrence of the *disease* for at least the past 10 years despite conditions that are conducive to its clinical expression, as described in Chapter X.X.X. of the *Aquatic Manual*, may be declared free from IMN when *basic biosecurity conditions* have been continuously met in the *zone* or *compartment* for at least the past 2 years.

OR

3. A *zone* or *compartment* where the last observed occurrence of the *disease* was within the past 10 years, or where the *infection* status prior to *targeted surveillance* was unknown; **for example** because of the absence of conditions conducive to its clinical expression; as described in Chapter X.X.X. of the *Aquatic Manual*, may be declared free from IMN when:
 - a) *basic biosecurity conditions* have been continuously met for at least the past 2 years; and
 - b) *targeted surveillance*, as described in Chapters 1.1.4. and X.X.X. of the *Aquatic Manual*, has been in place, through the *zone* or *compartment*, for at least the past 2 years without detection of IMNV.

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OR

4. A *zone* previously declared free from IMN but in which the *disease* is subsequently detected may **not** be declared free from IMN again **until when** the following conditions have been met:
- a) on detection of the *disease*, the affected area was declared an *infected zone* and a *buffer 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 (see *Aquatic Manual*) have been completed; and
 - c) *targeted surveillance*, as described in Chapters 1.1.4. and X.X.X. of the *Aquatic Manual*, has been in place for at least the past 2 years without detection of IMNV; **and**
 - d) previously existing basic biosecurity conditions have been reviewed and modified as necessary and have continuously been in place for at least the past 2 years.**

Article 2.3.9.6.

Maintenance of free status

A country, *zone* or *compartment* that is declared free from IMN following the provisions of points 1 or 2 of Articles 2.3.9.4. or 2.3.9.5. (as relevant) may maintain its status as IMN free provided that *basic biosecurity conditions* are continuously maintained.

A country, *zone* or *compartment* that is declared free from IMN following the provisions of point 3 of Articles 2.3.9.4. or 2.3.9.5. (as relevant) may discontinue *targeted surveillance* and maintain its status as IMN free provided that conditions that are conducive to clinical expression of IMN, as described in Chapter X.X.X. 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 IMN, *targeted surveillance* needs to be continued at a level determined by the *Competent Authority* on the basis of the likelihood of *infection*.

Article 2.3.9.7.

Importation of live aquatic animals from a country, zone or compartment declared free from infectious myonecrosis

When importing live *aquatic animals* of species referred to in Article 2.3.9.2. from a country, *zone* or *compartment* declared free from IMN, the *Competent Authority* of the *importing country* should require an *international aquatic animal health certificate* issued by the *Competent Authority* of the *exporting country* or a *certifying official* approved by the *importing country* attesting that, on the basis of the procedures described in Articles 2.3.9.4. or 2.3.9.5. (as applicable), the place of production of the commodity ~~consignment~~ is a country, *zone* or *compartment* declared free from IMN.

The *certificate* should be in accordance with the Model Certificate in Appendix 4.1.3.

This Article does not apply to *commodities* listed in point 1 of Article 2.3.9.3.

Article 2.3.9.8.

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

1. When importing, for *aquaculture*, live *aquatic animals* of species referred to in Article 2.3.9.2. from a country, *zone* or *compartment* not declared free from IMN, the *Competent Authority* of the *importing country* should assess the *risk* and, if justified, apply the following risk mitigation measures such as:
 - a) the direct delivery into and lifelong holding of the consignment in biosecure quarantine facilities for;
 - ~~b) the continuous isolation of the imported live aquatic animals and their first generation progeny from the local environment; and~~
 - ~~e)b) the treatment of all effluent and waste materials from the processing in a manner that ensures inactivation of IMNV.~~
2. If the intention of the introduction is the establishment of a new stock genetic lines, international standards, such as the Guidelines Code of Practice on the Introductions and Transfers of Marine Organisms of the International Council for the Exploration of the Seas (ICES), should be followed.
3. For the purposes of the *Aquatic Code*, the ICES Guidelines Code may be summarised to the following main points:
 - a) identify stock of interest (cultured or wild) in its current location;
 - b) evaluate stock health/ *disease* history;
 - c) take and test samples for IMNV, pests and general health/ *disease* status;
 - d) import and quarantine in a secure facility a founder (F-0) population;
 - 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 IMNV and perform general examinations for pests and general health/ *disease* status;
 - g) if IMNV 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 IMN free or specific pathogen free (SPF) for IMNV;
 - h) release SPF F-1 stock from *quarantine* for *aquaculture* or stocking purposes in the country, *zone* or *compartment*.

This Article does not apply to *commodities* listed in point 1 of Article 2.3.9.3.

Article 2.3.9.9.

Importation of live aquatic animals for human consumption from a country, zone or compartment not declared free from infectious myonecrosis

When importing, for human consumption, live *aquatic animals* of species referred to in Article 2.3.9.2. from a country, *zone* or *compartment* not declared free from IMN, the *Competent Authority* of the *importing country* should assess the risk and, if justified, require that:

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1. the consignment be delivered directly to and held in isolation until consumption; and
2. all effluent, dead *aquatic animals* and waste materials from the processing be treated in a manner that ensures inactivation of IMNV.

Member Countries should consider introducing internal measures to prevent such *commodities* being used for any purpose other than for human consumption.

This Article does not apply to *commodities* listed in point 1 of Article 2.3.9.3.

Article 2.3.9.10.

Importation of aquatic animal products from a country, zone or compartment declared free from infectious myonecrosis

When importing *aquatic animal products* of species referred to in Article 2.3.9.2. from a country, *zone* or *compartment* declared free from IMN, the *Competent Authority* of the *importing country* should require an *international aquatic animal health certificate* issued by the *Competent Authority* of the *exporting country* or a *certifying official* approved by the *importing country* attesting that, on the basis of the procedures described in Articles 2.3.9.4. or 2.3.9.5. (as applicable), the place of production of the consignment is a country, *zone* or *compartment* declared free from IMN.

The *certificate* should be in accordance with the Model Certificate in Appendix 4.2.2.

This Article does not apply to *commodities* listed in point 1 of Article 2.3.9.3.

Article 2.3.9.11.

Importation of aquatic animal products from a country, zone or compartment not declared free from infectious myonecrosis

When importing *aquatic animal products* of species referred to in Article 2.3.9.2. from a country, *zone* or *compartment* not declared free from IMN, the *Competent Authority* of the *importing country* should assess the risk and apply appropriate risk mitigation measures.

This Article does not apply to *commodities* listed in point 1 of Article 2.3.9.3.

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CHAPTER 2.3.10.

NECROTISING HEPATOPANCREATITIS

Article 2.3.10.1.

For the purposes of the *Aquatic Code*, necrotising hepatopancreatitis (NHP) means *infection* with necrotising hepatopancreatitis bacteria (NHP-B). This obligate intracellular bacterium is a member of the order *α-Proteobacteria*.

Methods for conducting surveillance and diagnosis of NHP are provided in the *Aquatic Manual*.

Article 2.3.10.2.

Scope

The recommendations in this Chapter apply to: Pacific white shrimp (*Penaeus vannamei*), blue shrimp (*P. stylirostris*), northern white shrimp (*P. setiferus*) and northern brown shrimp (*P. aztecus*). These recommendations also apply to any other *susceptible species* referred to in the *Aquatic Manual* when traded internationally.

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

Article 2.3.10.3.

Commodities

1. When authorising the importation or transit of the following *commodities*, the *Competent Authorities* should not require any NHP related conditions, regardless of the NHP status of the *exporting country, zone* or *compartment*.
 - a) For the species referred to in Article 2.3.10.2. being used for any purpose:
 - i) commodities treated in a manner that inactivates the disease agent e.g. boiled, canned or pasteurised products and ready to eat meals; and crustacean oil and crustacean meal intended for use in animal feeds commercially sterile canned products;
 - ii) boiled products (e.g. boiled whole shrimp or tails, lobsters, crabs);
 - iii) chemically extracted chitin;
 - iv) crustacean meals or by products made non infectious by heating or drying (e.g. flame dried or sun dried);
 - iii) crustacean products made non-infectious through processing as dry feeds (e.g. pelleted or extruded feeds);
 - iv) biological samples preserved for diagnostic applications in such a manner as to inactivate the disease agent NHP-B (e.g. formalin or alcohol preserved samples);
 - vii) frozen products.

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- b) The following products destined for human consumption from species referred to in Article 2.3.10.2. which have been prepared and packaged for direct retail trade in such a way as to minimise the likelihood of alternative uses:
- i) chemically preserved products (e.g. salted, pickled, marinated, pastes, etc.);
 - ii) products that have been heat treated or dried (e.g. ready prepared meals) in a manner to ensure the inactivation of the pathogen;
 - iii) de-headed and de-veined “de-veined” (intestine removed) shrimp tails.

For the *commodities* listed in point 1b), Member Countries should consider introducing internal measures to prevent the *commodity* being used for any purpose other than for human consumption.

2. When authorising the importation or transit of the *commodities* of a species referred to in Article 2.3.10.2., other than those listed in point 1 of Article 2.3.10.3., the *Competent Authorities* should require the conditions prescribed in Articles 2.3.10.7. to 2.3.10.11. relevant to the NHP status of the *exporting country, zone or compartment*.
3. When considering the importation/transit from an *exporting country, zone or compartment* not declared free of NHP of any other commodity of a species not covered in Article 2.3.10.2. but which could reasonably be expected to be a potential NHP-B carrier vector, the *Competent Authorities* should conduct a risk analysis in accordance with the recommendations in the Aquatic Code of the risk of introduction, establishment and spread of NHP B, and the potential consequences, associated with the importation of the commodity prior to a decision. The *exporting country* should be informed of the outcome of this assessment.

Article 2.3.10.4.

Necrotising hepatopancreatitis free country

A country may make a *self-declaration of freedom* from NHP if it meets the conditions in points 1, 2, 3 or 4 below.

If a country shares a *zone* with one or more other countries, it can only make a *self-declaration of freedom* from NHP if all the areas covered by the shared water are declared NHP free countries or zones (see Article 2.3.10.5.).

1. A country where none of the *susceptible species* referred to in Article 2.3.10.2. is present may make a *self-declaration of freedom* from NHP when *basic biosecurity conditions* have been continuously met in the country for at least the past 2 years.

OR

2. A country where the *susceptible species* referred to in Article 2.3.10.2. are present but there has never been any observed occurrence of the *disease* for at least the past 10 years despite conditions that are conducive to its clinical expression, as described in Chapter X.X.X. of the *Aquatic Manual*, may make a *self-declaration of freedom* from NHP when *basic biosecurity conditions* have been continuously met in the country for at least the past 2 years.

OR

3. A country where the last observed occurrence of the *disease* was within the past 10 years, or where the infection status prior to targeted surveillance was unknown, for example (e.g.) because of the absence of conditions conducive to its clinical expression, as described in Chapter X.X.X. of the Aquatic Manual, may make a *self-declaration of freedom* from NHP when:

- a) *basic biosecurity conditions* have been continuously met for at least the past 2 years; and

- b) *targeted surveillance*, as described in Chapters 1.1.4. and X.X.X. of the *Aquatic Manual*, has been in place for at least the last 2 years without detection of NHP-B.

OR

4. A country that has previously made a *self-declaration of freedom* from NHP but in which the *disease* is subsequently detected may **not** make a *self-declaration of freedom* from NHP again **until when** the following conditions have been met:
- a) on detection of the *disease*, the affected area was declared an *infected zone* and a *buffer 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 (see *Aquatic Manual*) have been completed; and
 - c) *targeted surveillance*, as described in Chapters 1.1.4. and X.X.X. of the *Aquatic Manual*, has been in place for at least the past 2 years without detection of NHP-B; and
 - d) previously existing basic biosecurity conditions have been reviewed and modified as necessary and have continuously been in place for at least the past 2 years.**

In the meantime, part of the non-affected area may be declared a free *zone* provided that **they such part** meets the conditions in point 3 of Article 2.3.10.5.

Article 2.3.10.5.

Necrotising hepatopancreatitis free zone or free compartment

A *zone* or *compartment* within the *territory* of one or more countries not declared free from NHP may be declared free by the *Competent Authority(ies)* of the country(ies) concerned if the *zone* or *compartment* meets the conditions referred to in points 1, 2, 3 or 4 below.

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

1. A *zone* or *compartment* where none of the *susceptible species* referred to in Article 2.3.10.2. is present may be declared free from NHP when *basic biosecurity conditions* have been continuously met in the *zone* or *compartment* for at least the past 2 years.

OR

2. A *zone* or *compartment* where the *susceptible species* referred to in Article 2.3.10.2. are present but in which there has not been any observed occurrence of the *disease* for at least the past 10 years despite conditions that are conducive to its clinical expression, as described in Chapter X.X.X. of the *Aquatic Manual*, may be declared free from NHP when *basic biosecurity conditions* have been continuously met in the *zone* or *compartment* for at least the past 2 years.

OR

3. A *zone* or *compartment* where the last observed occurrence of the *disease* was within the past 10 years, or where the *infection* status prior to *targeted surveillance* was unknown, **for example (e.g.)** because of the absence of conditions conducive to its clinical expression, as described in Chapter X.X.X. of the *Aquatic Manual*, may be declared free from NHP when:
 - a) *basic biosecurity conditions* have been continuously met for at least the past 2 years; and

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- b) *targeted surveillance*, as described in Chapters 1.1.4. and X.X.X. of the *Aquatic Manual*, has been in place, through the *zone* or *compartment*, for at least the past 2 years without detection of NHP-B.

OR

4. A *zone* previously declared free from NHP but in which the *disease* is subsequently detected may **not** be declared free from NHP again **until when** the following conditions have been met:
- a) on detection of the *disease*, the affected area was declared an *infected zone* and a *buffer 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 (see *Aquatic Manual*) have been completed; and
 - c) *targeted surveillance*, as described in Chapters 1.1.4. and X.X.X. of the *Aquatic Manual*, has been in place for at least the past 2 years without detection of NHP-B; **and**
 - d) previously existing basic biosecurity conditions have been reviewed and modified as necessary and have continuously been in place for at least the past 2 years.**

Article 2.3.10.6.

Maintenance of free status

A country, *zone* or *compartment* that is declared free from NHP following the provisions of points 1 or 2 of Articles 2.3.10.4. or 2.3.10.5. (as relevant) may maintain its status as NHP free provided that *basic biosecurity conditions* are continuously maintained.

A country, *zone* or *compartment* that is declared free from NHP following the provisions of point 3 of Articles 2.3.10.4. or 2.3.10.5. (as relevant) may discontinue *targeted surveillance* and maintain its status as NHP free provided that conditions that are conducive to clinical expression of NHP, as described in Chapter X.X.X. 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 NHP, *targeted surveillance* needs to be continued at a level determined by the *Competent Authority* on the basis of the likelihood of *infection*.

Article 2.3.10.7.

Importation of live aquatic animals from a country, zone or compartment declared free from necrotising hepatopancreatitis

When importing live *aquatic animals* of species referred to in Article 2.3.10.2. from a country, *zone* or *compartment* declared free from NHP, the *Competent Authority* of the *importing country* should require an *international aquatic animal health certificate* issued by the *Competent Authority* of the *exporting country* or a *certifying official* approved by the *importing country* attesting that, on the basis of the procedures described in Articles 2.3.10.4. or 2.3.10.5. (as applicable), the place of production of the commodity consignment is a country, *zone* or *compartment* declared free from NHP.

The *certificate* should be in accordance with the Model Certificate in Appendix 4.1.3.

This Article does not apply to *commodities* listed in point 1 of Article 2.3.10.3.

Article 2.3.10.8.

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

1. When importing, for *aquaculture*, live *aquatic animals* of species referred to in Article 2.3.10.2. from a country, *zone* or *compartment* not declared free from NHP, the *Competent Authority* of the *importing country* should assess the *risk* and, if justified, apply the following risk mitigation measures such as:
 - a) the direct delivery into and lifelong holding of the consignment in biosecure quarantine facilities for;
 - ~~b) the continuous isolation of the imported live aquatic animals and their first generation progeny from the local environment; and~~
 - ~~e)b) the treatment of all effluent and waste materials from the processing in a manner that ensures inactivation of NHP-B.~~
2. If the intention of the introduction is the establishment of a new stock genetic lines, international standards, such as the Guidelines Code of Practice on the Introductions and Transfers of Marine Organisms of the International Council for the Exploration of the Seas (ICES), should be followed.
3. For the purposes of the *Aquatic Code*, the ICES Guidelines Code may be summarised to the following main points:
 - a) identify stock of interest (cultured or wild) in its current location;
 - b) evaluate stock health/ *disease* history;
 - c) take and test samples for NHP-B, pests and general health/ *disease* status;
 - d) import and quarantine in a secure facility a founder (F-0) population;
 - 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 NHP-B and perform general examinations for pests and general health/ *disease* status;
 - g) if NHP-B 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 NHP free or specific pathogen free (SPF) for NHP-B;
 - h) release SPF F-1 stock from *quarantine* for *aquaculture* or stocking purposes in the country, *zone* or *compartment*.

This Article does not apply to *commodities* listed in point 1 of Article 2.3.10.3.

Article 2.3.10.9.

Importation of live aquatic animals for human consumption from a country, zone or compartment not declared free from necrotising hepatopancreatitis

When importing, for human consumption, live *aquatic animals* of species referred to in Article 2.3.10.2. from a country, *zone* or *compartment* not declared free from NHP, the *Competent Authority* of the *importing country* should assess the risk and, if justified require that:

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1. the consignment be delivered directly to and held in isolation until consumption; and
2. all effluent, dead *aquatic animals* and waste materials from the processing be treated in a manner that ensures inactivation of NHP-B.

Member Countries should consider introducing internal measures to prevent such *commodities* being used for any purpose other than for human consumption.

This Article does not apply to *commodities* listed in point 1 of Article 2.3.10.3.

Article 2.3.10.10.

Importation of aquatic animal products from a country, zone or compartment declared free from necrotising hepatopancreatitis

When importing *aquatic animal products* of species referred to in Article 2.3.10.2. from a country, *zone* or *compartment* declared free from NHP, the *Competent Authority* of the *importing country* should require an *international aquatic animal health certificate* issued by the *Competent Authority* of the *exporting country* or a *certifying official* approved by the *importing country* attesting that, on the basis of the procedures described in Articles 2.3.10.4. or 2.3.10.5. (as applicable), the place of production of the consignment is a country, *zone* or *compartment* declared free from NHP.

The *certificate* should be in accordance with the Model Certificate in Appendix 4.2.2.

This Article does not apply to *commodities* listed in point 1 of Article 2.3.10.3.

Article 2.3.10.11.

Importation of aquatic animal products from a country, zone or compartment not declared free from necrotising hepatopancreatitis

When importing *aquatic animal products* of species referred to in Article 2.3.10.2. from a country, *zone* or *compartment* not declared free from NHP, the *Competent Authority* of the *importing country* should assess the risk and apply appropriate risk mitigation measures.

This Article does not apply to *commodities* listed in point 1 of Article 2.3.10.3.

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CHAPTER 2.3.11.

WHITE TAIL DISEASE

Article 2.3.11.1.

For the purposes of the *Aquatic Code*, white tail disease (WTD) means *infection* with macrobrachium nodavirus (MrNV). This virus has yet to be formally classified.

Methods for conducting surveillance and diagnosis of WTD are provided in the *Aquatic Manual*.

Article 2.3.11.2.

Scope

The recommendations in this Chapter apply to: the giant fresh water prawn (*Macrobrachium rosenbergii*). Other common names are listed in the *Aquatic Manual*. These recommendations also apply to any other *susceptible species* referred to in the *Aquatic Manual* when traded internationally.

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

Article 2.3.11.3.

Commodities

1. When authorising the importation or transit of the following *commodities*, the *Competent Authorities* should not require any WTD related conditions, regardless of the WTD status of the *exporting country, zone or compartment*.
 - a) For the species referred to in Article 2.3.11.2. being used for any purpose:
 - i) commodities treated in a manner that inactivates the disease agent e.g. boiled, canned or pasteurised products and ready to eat meals; and crustacean oil and crustacean meal intended for use in animal feeds commercially sterile canned products;
 - ii) boiled products (e.g. boiled whole shrimp or tails, lobsters, crabs);
 - iii) chemically extracted chitin;
 - iv) crustacean meals or by products made non infectious by heating or drying (e.g. flame dried or sun dried);
 - iiiv) crustacean products made non-infectious through processing as dry feeds (e.g. pelleted or extruded feeds);
 - iv) biological samples preserved for diagnostic applications in such a manner as to inactivate the disease agent MrNV (e.g. formalin or alcohol preserved samples).
 - b) The following products destined for human consumption from species referred to in Article 2.3.11.2. which have been prepared and packaged for direct retail trade in such a way as to minimise the likelihood of alternative uses:

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- i) chemically preserved products (e.g. salted, pickled, marinated, pastes, etc.);
- ii) products that have been heat treated or dried (e.g. ready prepared meals) in a manner to ensure the inactivation of the pathogen.

For the *commodities* listed in point 1b), Member Countries should consider introducing internal measures to prevent the *commodity* being used for any purpose other than for human consumption.

2. When authorising the importation or transit of the *commodities* of a species referred to in Article 2.3.11.2., other than those listed in point 1 of Article 2.3.11.3., the *Competent Authorities* should require the conditions prescribed in Articles 2.3.11.7. to 2.3.11.11. relevant to the WTD status of the *exporting country, zone* or *compartment*.
3. When considering the importation/transit from an *exporting country, zone* or *compartment* not declared free of WTD of any other *commodity* of a species not covered in Article 2.3.11.2. but which could reasonably be expected to be a potential MrNV carrier vector, the *Competent Authorities* should conduct a risk analysis in accordance with the recommendations in the *Aquatic Code* of the risk of introduction, establishment and spread of MrNV, and the potential consequences, associated with the importation of the *commodity* prior to a decision. The *exporting country* should be informed of the outcome of this assessment.

Article 2.3.11.4.

White tail disease free country

A country may make a *self-declaration of freedom* from WTD if it meets the conditions in points 1, 2, 3 or 4 below.

If a country shares a *zone* with one or more other countries, it can only make a *self-declaration of freedom* from WTD if all the areas covered by the shared water are declared WTD free countries or *zones* (see Article 2.3.11.5.).

1. A country where none of the *susceptible species* referred to in Article 2.3.11.2. is present may make a *self-declaration of freedom* from WTD when *basic biosecurity conditions* have been continuously met in the country for at least the past 2 years.

OR

2. A country where the *susceptible species* referred to in Article 2.3.11.2. are present but there has never been any observed occurrence of the *disease* for at least the past 10 years despite conditions that are conducive to its clinical expression, as described in Chapter X.X.X. of the *Aquatic Manual*, may make a *self-declaration of freedom* from WTD when *basic biosecurity conditions* have been continuously met in the country for at least the past 2 years.

OR

3. A country where the last observed occurrence of the *disease* was within the past 10 years, or where the *infection status* prior to *targeted surveillance* was unknown, for example because of the absence of conditions conducive to its clinical expression, as described in Chapter X.X.X. of the *Aquatic Manual*, may make a *self-declaration of freedom* from WTD when:
 - a) *basic biosecurity conditions* have been continuously met for at least the past 2 years; and
 - b) *targeted surveillance*, as described in Chapters 1.1.4. and X.X.X. of the *Aquatic Manual*, has been in place for at least the last 2 years without detection of MrNV.

OR

4. A country that has previously made a *self-declaration of freedom* from WTD but in which the *disease* is subsequently detected may **not** make a *self-declaration of freedom* from WTD again **until when** the following conditions have been met:
- a) on detection of the *disease*, the affected area was declared an *infected zone* and a *buffer 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 (see *Aquatic Manual*) have been completed; and
 - c) *targeted surveillance*, as described in Chapters 1.1.4. and X.X.X. of the *Aquatic Manual*, has been in place for at least the past 2 years without detection of MrNV; and
 - d) previously existing basic biosecurity conditions have been reviewed and modified as necessary and have continuously been in place for at least the past 2 years.**

In the meantime, part of the non-affected area may be declared a free *zone* provided that **they such part** meets the conditions in point 3 of Article 2.3.11.5.

Article 2.3.11.5.

White tail disease free zone or free compartment

A *zone* or *compartment* within the *territory* of one or more countries not declared free from WTD may be declared free by the *Competent Authority(ies)* of the country(ies) concerned if the *zone* or *compartment* meets the conditions referred to in points 1, 2, 3 or 4 below.

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

1. A *zone* or *compartment* where none of the *susceptible species* referred to in Article 2.3.11.2. is present may be declared free from WTD when *basic biosecurity conditions* have been continuously met in the *zone* or *compartment* for at least the past 2 years.

OR

2. A *zone* or *compartment* where the *susceptible species* referred to in Article 2.3.11.2. are present but in which there has not been any observed occurrence of the *disease* for at least the past 10 years despite conditions that are conducive to its clinical expression, as described in Chapter X.X.X. of the *Aquatic Manual*, may be declared free from WTD when *basic biosecurity conditions* have been continuously met in the *zone* or *compartment* for at least the past 2 years.

OR

3. A *zone* or *compartment* where the last observed occurrence of the *disease* was within the past 10 years, or where the *infection* status prior to *targeted surveillance* was unknown, **for example (e.g.)** because of the absence of conditions conducive to its clinical expression, as described in Chapter X.X.X. of the *Aquatic Manual*, may be declared free from WTD when:
 - a) *basic biosecurity conditions* have been continuously met for at least the past 2 years; and
 - b) *targeted surveillance*, as described in Chapters 1.1.4. and X.X.X. of the *Aquatic Manual*, has been in place, through the *zone* or *compartment*, for at least the past 2 years without detection of MrNV.

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OR

4. A *zone* previously declared free from WTD but in which the *disease* is subsequently detected may **not** be declared free from WTD again **until when** the following conditions have been met:
- a) on detection of the *disease*, the affected area was declared an *infected zone* and a *buffer 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 (see *Aquatic Manual*) have been completed; and
 - c) *targeted surveillance*, as described in Chapters 1.1.4. and X.X.X. of the *Aquatic Manual*, has been in place for at least the past 2 years without detection of MrNV: **and**
 - d) previously existing basic biosecurity conditions have been reviewed and modified as necessary and have continuously been in place for at least the past 2 years.**

Article 2.3.11.6.

Maintenance of free status

A country, *zone* or *compartment* that is declared free from WTD following the provisions of points 1 or 2 of Articles 2.3.11.4. or 2.3.11.5. (as relevant) may maintain its status as WTD free provided that *basic biosecurity conditions* are continuously maintained.

A country, *zone* or *compartment* that is declared free from WTD following the provisions of point 3 of Articles 2.3.11.4. or 2.3.11.5. (as relevant) may discontinue *targeted surveillance* and maintain its status as WTD free provided that conditions that are conducive to clinical expression of WTD, as described in Chapter X.X.X. 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 WTD, *targeted surveillance* needs to be continued at a level determined by the *Competent Authority* on the basis of the likelihood of *infection*.

Article 2.3.11.7.

Importation of live aquatic animals from a country, zone or compartment declared free from white tail disease

When importing live *aquatic animals* of species referred to in Article 2.3.11.2. from a country, *zone* or *compartment* declared free from WTD, the *Competent Authority* of the *importing country* should require an *international aquatic animal health certificate* issued by the *Competent Authority* of the *exporting country* or a *certifying official* approved by the *importing country* attesting that, on the basis of the procedures described in Articles 2.3.11.4. or 2.3.11.5. (as applicable), the place of production of the *commodity* is a country, *zone* or *compartment* declared free from WTD.

The *certificate* should be in accordance with the Model Certificate in Appendix 4.1.3.

This Article does not apply to *commodities* listed in point 1 of Article 2.3.11.3.

Article 2.3.11.8.

Importation of live aquatic animals for aquaculture from a country, zone or compartment not declared free from white tail disease

1. When importing, for *aquaculture*, live *aquatic animals* of species referred to in Article 2.3.11.2. from a country, *zone* or *compartment* not declared free from WTD, the *Competent Authority* of the *importing country* should assess the *risk* and, if justified, apply the following risk mitigation measures such as:
 - a) the direct delivery into and lifelong holding of the consignment in biosecure quarantine facilities for;
 - ~~b) the continuous isolation of the imported live aquatic animals and their first generation progeny from the local environment; and~~
 - ~~e)b) the treatment of all effluent and waste materials from the processing in a manner that ensures inactivation of MrNV.~~
2. If the intention of the introduction is the establishment of a new stock genetic lines, international standards, such as the Guidelines Code of Practice on the Introductions and Transfers of Marine Organisms of the International Council for the Exploration of the Seas (ICES), should be followed.
3. For the purposes of the *Aquatic Code*, the ICES Guidelines Code may be summarised to the following main points:
 - a) identify stock of interest (cultured or wild) in its current location;
 - b) evaluate stock's health/ *disease* history;
 - c) take and test samples for MrNV, pests and general health/ *disease* status;
 - d) import and quarantine in a secure facility a founder (F-0) population;
 - 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 MrNV and perform general examinations for pests and general health/ *disease* status;
 - g) if MrNV 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 WTD free or specific pathogen free (SPF) for MrNV;
 - h) release SPF F-1 stock from *quarantine* for *aquaculture* or stocking purposes in the country, *zone* or *compartment*.

This Article does not apply to *commodities* listed in point 1 of Article 2.3.11.3.

Article 2.3.11.9.

Importation of live aquatic animals for human consumption from a country, zone or compartment not declared free from white tail disease

When importing, for human consumption, live *aquatic animals* of species referred to in Article 2.3.11.2. from a country, *zone* or *compartment* not declared free from WTD, the *Competent Authority* of the *importing country* should assess the risk and, if justified require that:

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1. the consignment be delivered directly to and held in isolation until consumption; and
2. all effluent, dead *aquatic animals* and waste materials from the processing be treated in a manner that ensures inactivation of MrNV.

Member Countries should consider introducing internal measures to prevent such *commodities* being used for any purpose other than for human consumption.

This Article does not apply to *commodities* listed in point 1 of Article 2.3.11.3.

Article 2.3.11.10.

Importation of aquatic animal products from a country, zone or compartment declared free from white tail disease

When importing *aquatic animal products* of species referred to in Article 2.3.11.2. from a country, *zone* or *compartment* declared free from WTD, the *Competent Authority* of the *importing country* should require an *international aquatic animal health certificate* issued by the *Competent Authority* of the *exporting country* or a *certifying official* approved by the *importing country* attesting that, on the basis of the procedures described in Articles 2.3.11.4. or 2.3.11.5. (as applicable), the place of production of the consignment is a country, *zone* or *compartment* declared free from WTD.

The *certificate* should be in accordance with the Model Certificate in Appendix 4.2.2.

This Article does not apply to *commodities* listed in point 1 of Article 2.3.11.3.

Article 2.3.11.11.

Importation of aquatic animal products from a country, zone or compartment not declared free from white tail disease

When importing *aquatic animal products* of species referred to in Article 2.3.11.2. from a country, *zone* or *compartment* not declared free from WTD, the *Competent Authority* of the *importing country* should assess the risk and apply appropriate risk mitigation measures.

This Article does not apply to *commodities* listed in point 1 of Article 2.3.11.3.

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CHAPTER 2.3.12.

HEPATOPANCREATIC PARVOVIRUS DISEASE

Article 2.3.12.1.

For the purposes of the *Aquatic Code*, hepatopancreatic parvovirus disease (HPVD) means *infection* with hepatopancreatic parvovirus (HPV). It is considered to be a member of the subfamily of the *Densovirinae* in the family *Parvoviridae*.

Methods for conducting surveillance and diagnosis of HPVD are provided in the *Aquatic Manual*.

Article 2.3.12.2.

Scope

The recommendations in this Chapter apply to: Indian white shrimp (*Penaeus indicus*), black tiger shrimp (*Penaeus monodon*), Pacific white shrimp (*Penaeus vannamei*) and Pacific blue shrimp (*P. stylirostris*). These recommendations also apply to any other *susceptible species* referred to in the *Aquatic Manual* when traded internationally.

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

Article 2.3.12.3.

Commodities

1. When authorising the importation or transit of the following *commodities*, the *Competent Authorities* should not require any HPVD related conditions, regardless of the HPVD status of the *exporting country, zone or compartment*.
 - a) For the species referred to in Article 2.3.12.2. being used for any purpose:
 - i) commodities treated in a manner that inactivates the disease agent e.g. boiled, canned or pasteurised products and ready to eat meals; and crustacean oil and crustacean meal intended for use in animal feeds commercially sterile canned products;
 - ii) boiled products (e.g. boiled whole shrimp or tails, lobsters, crabs);
 - iii) chemically extracted chitin;
 - iv) crustacean meals or by products made non infectious by heating or drying (e.g. flame dried or sun dried);
 - iiiv) crustacean products made non-infectious through processing as dry feeds (e.g. pelleted or extruded feeds);
 - ivi) biological samples preserved for diagnostic applications in such a manner as to inactivate the disease agent HPV (e.g. formalin or alcohol preserved samples).
 - b) The following products destined for human consumption from species referred to in Article 2.3.12.2. which have been prepared and packaged for direct retail trade in such a way as to minimise the likelihood of alternative uses:
 - i) chemically preserved products (e.g. salted, pickled, marinated, pastes, etc.);

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- ii) ~~products that have been heat treated or dried (e.g. ready prepared meals) in a manner to ensure the inactivation of the pathogen;~~
- iii) de-headed and “de-veined” (intestine removed) shrimp tails.

For the *commodities* listed in point 1b), Member Countries should consider introducing internal measures to prevent the *commodity* being used for any purpose other than for human consumption.

2. When authorising the importation or transit of the *commodities* of a species referred to in Article 2.3.12.2., other than those listed in point 1 of Article 2.3.12.3., the *Competent Authorities* should require the conditions prescribed in Articles 2.3.12.7. to 2.3.12.11. relevant to the HPVD status of the *exporting country, zone* or *compartment*.
3. When considering the importation/transit from an *exporting country, zone* or *compartment* not declared free of HPVD of ~~any other commodity~~ of a species not referred to in Article 2.3.12.2. but which could reasonably be expected to be a potential HPV ~~carrier vector~~, the *Competent Authorities* should conduct a ~~risk analysis in accordance with the recommendations in the Aquatic Code of the risk of introduction, establishment and spread of HPV, and the potential consequences, associated with the importation of the commodity prior to a decision.~~ The *exporting country* should be informed of the outcome of this assessment.

Article 2.3.12.4.

Hepatopancreatic parvovirus disease free country

A country may make a *self-declaration of freedom* from HPVD if it meets the conditions in points 1, 2, 3 or 4 below.

If a country shares a *zone* with one or more other countries, it can only make a *self-declaration of freedom* from HPVD if all the areas covered by the shared water are declared HPVD free countries or *zones* (see Article 2.3.12.5.).

1. A country where none of the *susceptible species* referred to in Article 2.3.12.2. is present may make a *self-declaration of freedom* from HPVD when *basic biosecurity conditions* have been continuously met in the country for at least the past 2 years.

OR

2. A country where the *susceptible species* referred to in Article 2.3.12.2. are present but there has never been any observed occurrence of the *disease* for at least the past 10 years despite conditions that are conducive to its clinical expression, as described in Chapter X.X.X. of the *Aquatic Manual*, may make a *self-declaration of freedom* from HPVD when *basic biosecurity conditions* have been continuously met in the country for at least the past 2 years.

OR

3. A country where the last observed occurrence of the *disease* was within the past 10 years, or where the *infection status* prior to *targeted surveillance* was unknown, ~~for example (e.g.)~~ because of the absence of conditions conducive to its clinical expression, as described in Chapter X.X.X. of the *Aquatic Manual*, may make a *self-declaration of freedom* from HPVD when:
 - a) *basic biosecurity conditions* have been continuously met for at least the past 2 years; and
 - b) *targeted surveillance*, as described in Chapters 1.1.4. and X.X.X. of the *Aquatic Manual*, has been in place for at least the last 2 years without detection of HPV.

OR

4. A country that has previously made a *self-declaration of freedom* from HPVD but in which the *disease* is subsequently detected may **not** make a *self-declaration of freedom* from HPVD again **until** **when** the following conditions have been met:
 - a) on detection of the *disease*, the affected area was declared an *infected zone* and a *buffer 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 (see *Aquatic Manual*) have been completed; and
 - c) *targeted surveillance*, as described in Chapters 1.1.4. and X.X.X. of the *Aquatic Manual*, has been in place for at least the past 2 years without detection of HPV; **and**
 - d) previously existing basic biosecurity conditions have been reviewed and modified as necessary and have continuously been in place for at least the past 2 years.**

In the meantime, part of the non-affected area may be declared a free *zone* provided that **they** **such** **part** **meets** the conditions in point 3 of Article 2.3.12.5.

Article 2.3.12.5.

Hepatopancreatic parvovirus disease free zone or free compartment

A *zone* or *compartment* within the *territory* of one or more countries not declared free from HPVD may be declared free by the *Competent Authority(ies)* of the country(ies) concerned if the *zone* or *compartment* meets the conditions referred to in points 1, 2, 3 or 4 below.

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

1. A *zone* or *compartment* where none of the *susceptible species* referred to in Article 2.3.12.2. is present may be declared free from HPVD when *basic biosecurity conditions* have been continuously met in the *zone* or *compartment* for at least the past 2 years.

OR

2. A *zone* or *compartment* where the *susceptible species* referred to in Article 2.3.12.2. are present but in which there has not been any observed occurrence of the *disease* for at least the past 10 years despite conditions that are conducive to its clinical expression, as described in Chapter X.X.X. of the *Aquatic Manual*, may be declared free from HPVD when *basic biosecurity conditions* have been continuously met in the *zone* or *compartment* for at least the past 2 years.

OR

3. A *zone* or *compartment* where the last observed occurrence of the *disease* was within the past 10 years, or where the *infection* status prior to *targeted surveillance* was unknown, for example **(e.g.** because of the absence of conditions conducive to its clinical expression, as described in Chapter X.X.X. of the *Aquatic Manual*, may be declared free from HPVD when:
 - a) *basic biosecurity conditions* have been continuously met for at least the past 2 years; and
 - b) *targeted surveillance*, as described in Chapters 1.1.4. and X.X.X. of the *Aquatic Manual*, has been in place, through the *zone* or *compartment*, for at least the past 2 years without detection of HPV.

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OR

4. A *zone* previously declared free from HPVD but in which the *disease* is subsequently detected may **not** be declared free from HPVD again **until when** the following conditions have been met:
- a) on detection of the *disease*, the affected area was declared an *infected zone* and a *buffer 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 (see *Aquatic Manual*) have been completed; and
 - c) *targeted surveillance*, as described in Chapters 1.1.4. and X.X.X. of the *Aquatic Manual*, has been in place for at least the past 2 years without detection of HPV; **and**
 - d) previously existing basic biosecurity conditions have been reviewed and modified as necessary and have continuously been in place for at least the past 2 years.**

Article 2.3.12.6.

Maintenance of free status

A country, *zone* or *compartment* that is declared free from HPVD following the provisions of points 1 or 2 of Articles 2.3.12.4. or 2.3.12.5. (as relevant) may maintain its status as HPVD free provided that *basic biosecurity conditions* are continuously maintained.

A country, *zone* or *compartment* that is declared free from HPVD following the provisions of point 3 of Articles 2.3.12.4. or 2.3.12.5. (as relevant) may discontinue *targeted surveillance* and maintain its status as HPVD free provided that conditions that are conducive to clinical expression of HPVD, as described in Chapter X.X.X. 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 HPVD, *targeted surveillance* needs to be continued at a level determined by the *Competent Authority* on the basis of the likelihood of *infection*.

Article 2.3.12.7.

Importation of live aquatic animals from a country, zone or compartment declared free from hepatopancreatic parvovirus disease

When importing live *aquatic animals* of species referred to in Article 2.3.12.2. from a country, *zone* or *compartment* declared free from HPVD, the *Competent Authority* of the *importing country* should require an *international aquatic animal health certificate* issued by the *Competent Authority* of the *exporting country* or a *certifying official* approved by the *importing country* attesting that, on the basis of the procedures described in Articles 2.3.12.4. or 2.3.12.5. (as applicable), the place of production of the *commodity* is a country, *zone* or *compartment* declared free from HPVD.

The *certificate* should be in accordance with the Model Certificate in Appendix 4.1.3.

This Article does not apply to *commodities* listed in point 1 of Article 2.3.12.3.

Article 2.3.12.8.

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

1. When importing, for *aquaculture*, live *aquatic animals* of species referred to in Article 2.3.12.2. from a country, zone or compartment not declared free from HPVD, the *Competent Authority* of the *importing country* should assess the *risk* and, if justified, apply the following risk mitigation measures such as:
 - a) the direct delivery into and lifelong holding of the consignment in biosecure quarantine facilities for;
 - b) the continuous isolation of the imported live aquatic animals and their first generation progeny from the local environment; and
 - e)b) the treatment of all effluent and waste materials from the processing in a manner that ensures inactivation of HPV.
2. If the intention of the introduction is the establishment of a new stock genetic lines, international standards, such as the Guidelines Code of Practice on the Introductions and Transfers of Marine Organisms of the International Council for the Exploration of the Seas (ICES), should be followed.
3. For the purposes of the *Aquatic Code*, the ICES Guidelines Code may be summarised to the following main points:
 - a) identify stock of interest (cultured or wild) in its current location;
 - b) evaluate stock health/ *disease* history;
 - c) take and test samples for HPV, pests and general health/ *disease* status;
 - d) import and quarantine in a secure facility a founder (F-0) population;
 - 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 HPV and perform general examinations for pests and general health/ *disease* status;
 - g) if HPV 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 HPVD free or specific pathogen free (SPF) for HPV;
 - h) release SPF F-1 stock from *quarantine* for *aquaculture* or stocking purposes in the country, zone or compartment.

This Article does not apply to *commodities* listed in point 1 of Article 2.3.12.3.

Article 2.3.12.9.

Importation of live aquatic animals for human consumption from a country, zone or compartment not declared free from hepatopancreatic parvovirus disease

When importing, for human consumption, live *aquatic animals* of species referred to in Article 2.3.12.2. from a country, zone or compartment not declared free from HPVD, the *Competent Authority* of the *importing country* should assess the risk and, if justified, require that:

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1. the consignment be delivered directly to and held in isolation until consumption; and
2. all effluent, dead *aquatic animals* and waste materials from the processing be treated in a manner that ensures inactivation of HPV.

Member Countries should consider introducing internal measures to prevent such *commodities* being used for any purpose other than for human consumption.

This Article does not apply to *commodities* listed in point 1 of Article 2.3.12.3.

Article 2.3.12.10.

Importation of aquatic animal products from a country, zone or compartment declared free from hepatopancreatic parvovirus disease

When importing *aquatic animal products* of species referred to in Article 2.3.12.2. from a country, *zone* or *compartment* declared free from HPVD, the *Competent Authority* of the *importing country* should require an *international aquatic animal health certificate* issued by the *Competent Authority* of the *exporting country* or a *certifying official* approved by the *importing country* attesting that, on the basis of the procedures described in Articles 2.3.12.4. or 2.3.12.5. (as applicable), the place of production of the consignment is a country, *zone* or *compartment* declared free from HPVD.

The *certificate* should be in accordance with the Model Certificate in Appendix 4.2.2.

This Article does not apply to *commodities* listed in point 1 of Article 2.3.12.3.

Article 2.3.12.11.

Importation of aquatic animal products from a country, zone or compartment not declared free from hepatopancreatic parvovirus disease

When importing *aquatic animal products* of species referred to in Article 2.3.12.2. from a country, *zone* or *compartment* not declared free from HPVD, the *Competent Authority* of the *importing country* should assess the risk and apply appropriate risk mitigation measures.

This Article does not apply to *commodities* listed in point 1 of Article 2.3.12.3.

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CHAPTER 2.3.13.

MOURILYAN VIRUS DISEASE

Article 2.3.13.1.

For the purposes of the *Aquatic Code*, Mourilyan virus disease (MoVD) means *infection* with Mourilyan virus (MoV). This virus is similar to members of the *Bunyaviridae*, but has yet to be formally classified.

Methods for conducting surveillance and diagnosis of MoVD are provided in the *Aquatic Manual*.

Article 2.3.13.2.

Scope

The recommendations in this Chapter apply to: black tiger shrimp (*Penaeus monodon*) and kuruma shrimp (*Penaeus japonicus*). These recommendations also apply to any other *susceptible species* referred to in the *Aquatic Manual* when traded internationally.

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

Article 2.3.13.3.

Commodities

1. When authorising the importation or transit of the following *commodities*, the *Competent Authorities* should not require any MoVD related conditions, regardless of the MoVD status of the *exporting country, zone or compartment*.
 - a) For the species referred to in Article 2.3.13.2. being used for any purpose:
 - i) commodities treated in a manner that inactivates the disease agent e.g. boiled, canned or pasteurised products and ready to eat meals; and crustacean oil and crustacean meal intended for use in animal feeds commercially sterile canned products;
 - ii) boiled products (e.g. boiled whole shrimp or tails, lobsters, crabs);
 - iii) chemically extracted chitin;
 - iv) crustacean meals or by products made non infectious by heating or drying (e.g. flame dried or sun dried);
 - iiiv) crustacean products made non-infectious through processing as dry feeds (e.g. pelleted or extruded feeds);
 - ivi) biological samples preserved for diagnostic applications in such a manner as to inactivate the disease agent MoV (e.g. formalin or alcohol preserved samples).
 - b) The following products destined for human consumption from species referred to in Article 2.3.13.2. which have been prepared and packaged for direct retail trade in such a way as to minimise the likelihood of alternative uses:
 - i) chemically preserved products (e.g. salted, pickled, marinated, pastes, etc.);

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- ii) ~~products that have been heat treated or dried (e.g. ready prepared meals) in a manner to ensure the inactivation of the pathogen.~~

For the *commodities* listed in point 1b), Member Countries should consider introducing internal measures to prevent the *commodity* being used for any purpose other than for human consumption.

2. When authorising the importation or transit of the *commodities* of a species referred to in Article 2.3.13.2., other than those listed in point 1 of Article 2.3.13.3., the *Competent Authorities* should require the conditions prescribed in Articles 2.3.13.7. to 2.3.13.11. relevant to the MoVD status of the *exporting country, zone* or *compartment*.
3. When considering the importation/transit from an *exporting country, zone* or *compartment* not declared free of MoVD of ~~any other commodity~~ of a species not covered in Article 2.3.13.2. but which could reasonably be expected to be a potential MoV ~~carrier vector~~, the *Competent Authorities* should conduct a ~~risk analysis in accordance with the recommendations in the Aquatic Code of the risk of introduction, establishment and spread of MoV, and the potential consequences, associated with the importation of the commodity prior to a decision.~~ The *exporting country* should be informed of the outcome of this assessment.

Article 2.3.13.4.

Mourilyan virus disease free country

A country may make a *self-declaration of freedom* from MoVD if it meets the conditions in points 1, 2, 3 or 4 below.

If a country shares a *zone* with one or more other countries, it can only make a *self-declaration of freedom* from MoVD if all the areas covered by the shared water are declared MoVD free countries or *zones* (see Article 2.3.13.5.).

1. A country where none of the *susceptible species* referred to in Article 2.3.13.2. is present may make a *self-declaration of freedom* from MoVD when *basic biosecurity conditions* have been continuously met in the country for at least the past 2 years.

OR

2. A country where the *susceptible species* referred to in Article 2.3.13.2. are present but there has never been any observed occurrence of the *disease* for at least the past 10 years despite conditions that are conducive to its clinical expression, as described in Chapter X.X.X. of the *Aquatic Manual*, may make a *self-declaration of freedom* from MoVD when *basic biosecurity conditions* have been continuously met in the country for at least the past 2 years.

OR

3. A country where the last observed occurrence of the *disease* was within the past 10 years, or where the *infection status* prior to *targeted surveillance* was unknown, ~~for example (e.g.)~~ because of the absence of conditions conducive to its clinical expression, as described in Chapter X.X.X. of the *Aquatic Manual*, may make a *self-declaration of freedom* from MoVD when:
 - a) *basic biosecurity conditions* have been continuously met for at least the past 2 years; and
 - b) *targeted surveillance*, as described in Chapters 1.1.4. and X.X.X. of the *Aquatic Manual*, has been in place for at least the last 2 years without detection of MoV.

OR

4. A country that has previously made a *self-declaration of freedom* from MoVD but in which the *disease* is subsequently detected may **not** make a *self-declaration of freedom* from MoVD again **until** **when** the following conditions have been met:
 - a) on detection of the *disease*, the affected area was declared an *infected zone* and a *buffer 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 (see *Aquatic Manual*) have been completed; and
 - c) *targeted surveillance*, as described in Chapters 1.1.4. and X.X.X. of the *Aquatic Manual*, has been in place for at least the past 2 years without detection of MoV; **and**
 - d) previously existing basic biosecurity conditions have been reviewed and modified as necessary and have continuously been in place for at least the past 2 years.**

In the meantime, part of the non-affected area may be declared a free *zone* provided that **they** **such** **part** **meets** the conditions in point 3 of Article 2.3.13.5.

Article 2.3.13.5.

Mourilyan virus disease free zone or free compartment

A *zone* or *compartment* within the *territory* of one or more countries not declared free from MoVD may be declared free by the *Competent Authority(ies)* of the country(ies) concerned if the *zone* or *compartment* meets the conditions referred to in points 1, 2, 3 or 4 below.

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

1. A *zone* or *compartment* where none of the *susceptible species* referred to in Article 2.3.13.2. is present may be declared free from MoVD when *basic biosecurity conditions* have been continuously met in the *zone* or *compartment* for at least the past 2 years.

OR

2. A *zone* or *compartment* where the *susceptible species* referred to in Article 2.3.13.2. are present but in which there has not been any observed occurrence of the *disease* for at least the past 10 years despite conditions that are conducive to its clinical expression, as described in Chapter X.X.X. of the *Aquatic Manual*, may be declared free from MoVD when *basic biosecurity conditions* have been continuously met in the *zone* or *compartment* for at least the past 2 years.

OR

3. A *zone* or *compartment* where the last observed occurrence of the *disease* was within the past 10 years, **or** where the *infection* status prior to *targeted surveillance* was unknown, **for example** **(e.g.)** because of the absence of conditions conducive to its clinical expression, **as described in Chapter X.X.X. of the Aquatic Manual,** may be declared free from MoVD when:
 - a) *basic biosecurity conditions* have been continuously met for at least the past 2 years; and
 - b) *targeted surveillance*, as described in Chapters 1.1.4. and X.X.X. of the *Aquatic Manual*, has been in place, through the *zone* or *compartment*, for at least the past 2 years without detection of MoV.

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OR

4. A *zone* previously declared free from MoVD but in which the *disease* is subsequently detected may **not** be declared free from MoVD again **until when** the following conditions have been met:
- a) on detection of the *disease*, the affected area was declared an *infected zone* and a *buffer 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 (see *Aquatic Manual*) have been completed; and
 - c) *targeted surveillance*, as described in Chapters 1.1.4. and X.X.X. of the *Aquatic Manual*, has been in place for at least the past 2 years without detection of MoV; **and**
 - d) previously existing basic biosecurity conditions have been reviewed and modified as necessary and have continuously been in place for at least the past 2 years.**

Article 2.3.13.6.

Maintenance of free status

A country, *zone* or *compartment* that is declared free from MoVD following the provisions of points 1 or 2 of Articles 2.3.13.4. or 2.3.13.5. (as relevant) may maintain its status as MoVD free provided that *basic biosecurity conditions* are continuously maintained.

A country, *zone* or *compartment* that is declared free from MoVD following the provisions of point 3 of Articles 2.3.13.4. or 2.3.13.5. (as relevant) may discontinue *targeted surveillance* and maintain its status as MoVD free provided that conditions that are conducive to clinical expression of MoVD, as described in Chapter X.X.X. 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 MoVD, *targeted surveillance* needs to be continued at a level determined by the *Competent Authority* on the basis of the likelihood of *infection*.

Article 2.3.13.7.

Importation of live aquatic animals from a country, zone or compartment declared free from Mourilyan virus disease

When importing live *aquatic animals* of species referred to in Article 2.3.13.2. from a country, *zone* or *compartment* declared free from MoVD, the *Competent Authority* of the *importing country* should require an *international aquatic animal health certificate* issued by the *Competent Authority* of the *exporting country* or a *certifying official* approved by the *importing country* attesting that, on the basis of the procedures described in Articles 2.3.13.4. or 2.3.13.5. (as applicable), the place of production of the *commodity* is a country, *zone* or *compartment* declared free from MoVD.

The *certificate* should be in accordance with the Model Certificate in Appendix 4.1.3.

This Article does not apply to *commodities* listed in point 1 of Article 2.3.13.3.

Article 2.3.13.8.

Importation of live aquatic animals for aquaculture from a country, zone or compartment not declared free from Mourilyan virus disease

1. When importing, for *aquaculture*, live *aquatic animals* of species referred to in Article 2.3.13.2. from a country, *zone* or *compartment* not declared free from MoVD, the *Competent Authority* of the *importing country* should assess the *risk* and, if justified, apply the following risk mitigation measures such as:
 - a) the direct delivery into and lifelong holding of the consignment in biosecure quarantine facilities for;
 - ~~b) the continuous isolation of the imported live aquatic animals and their first generation progeny from the local environment; and~~
 - ~~e)b) the treatment of all effluent and waste materials from the processing in a manner that ensures inactivation of MoV.~~
2. If the intention of the introduction is the establishment of a new stock genetic lines, international standards, such as the Guidelines Code of Practice on the Introductions and Transfers of Marine Organisms of the International Council for the Exploration of the Seas (ICES), should be followed.
3. For the purposes of the *Aquatic Code*, the ICES Guidelines Code may be summarised to the following main points:
 - a) identify stock of interest (cultured or wild) in its current location;
 - b) evaluate stock health/ *disease* history;
 - c) take and test samples for MoV, pests and general health/ *disease* status;
 - d) import and quarantine in a secure facility a founder (F-0) population;
 - 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 MoV and perform general examinations for pests and general health/ *disease* status;
 - g) if MoV 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 MoVD free or specific pathogen free (SPF) for MoV;
 - h) release SPF F-1 stock from *quarantine* for *aquaculture* or stocking purposes in the country, *zone* or *compartment*.

This Article does not apply to *commodities* listed in point 1 of Article 2.3.13.3.

Article 2.3.13.9.

Importation of live aquatic animals for human consumption from a country, zone or compartment not declared free from Mourilyan virus disease

When importing, for human consumption, live *aquatic animals* of species referred to in Article 2.3.13.2. from a country, *zone* or *compartment* not declared free from MoVD, the *Competent Authority* of the *importing country* should assess the risk and, if justified require that:

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1. the consignment be delivered directly to and held in isolation until consumption; and
2. all effluent, dead *aquatic animals* and waste materials from the processing be treated in a manner that ensures inactivation of MoV.

Member Countries should consider introducing internal measures to prevent such *commodities* being used for any purpose other than for human consumption.

This Article does not apply to *commodities* listed in point 1 of Article 2.3.13.3.

Article 2.3.13.10.

Importation of aquatic animal products from a country, zone or compartment declared free from Mourilyan virus disease

When importing *aquatic animal products* of species referred to in Article 2.3.13.2. from a country, *zone* or *compartment* declared free from MoVD, the *Competent Authority* of the *importing country* should require an *international aquatic animal health certificate* issued by the *Competent Authority* of the *exporting country* or a *certifying official* approved by the *importing country* attesting that, on the basis of the procedures described in Articles 2.3.13.4. or 2.3.13.5. (as applicable), the place of production of the consignment is a country, *zone* or *compartment* declared free from MoVD.

The *certificate* should be in accordance with the Model Certificate in Appendix 4.2.2.

This Article does not apply to *commodities* listed in point 1 of Article 2.3.13.3.

Article 2.3.13.11.

Importation of aquatic animal products from a country, zone or compartment not declared free from Mourilyan virus disease

When importing *aquatic animal products* of species referred to in Article 2.3.13.2. from a country, *zone* or *compartment* not declared free from MoVD, the *Competent Authority* of the *importing country* should assess the risk and apply appropriate risk mitigation measures.

This Article does not apply to *commodities* listed in point 1 of Article 2.3.13.3.

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DRAFT GUIDELINES FOR THE CONTROL OF AQUATIC ANIMAL HEALTH HAZARDS IN AQUATIC ANIMAL FEEDS

1. INTRODUCTION

One of the key objectives of the OIE *Aquatic Animal Health Code* (hereafter referred to as the *Aquatic Code*) is to help Member Countries trade safely in *aquatic animals* and their products by developing relevant aquatic animal health measures. These Guidelines address aquatic animal health *hazards* in aquatic animal *feeds*. It does not address food safety issues as this is not within the mandate of the OIE Aquatic Animal Health Standards Commission (hereafter referred to as the Aquatic Animals Commission). These Guidelines should be read in conjunction with relevant recommendations of the OIE *Terrestrial Animal Health Code* (hereafter referred to as the *Terrestrial Code*) (Appendix containing recommendations on animal *feed*). The Food and Agriculture Organization of the United Nations (FAO) has also published recommendations¹ relevant to terrestrial and aquatic animal *feed*.

Key considerations relevant to aquatic animal *feeds* are as follows:

- Intensive rearing in *aquaculture establishments* causes a concentration of fish, *feed* and faecal matter in time and space and this heightens the risk of *disease* transmission, whether the pathogen enters the culture system via *feed* or other means.
- For many aquatic species, predation (including cannibalism) is their natural way of feeding in their natural habitat.
- Historically, animal proteins used in *feeds* were mainly sourced from the maine environment, due to the nutritional needs of *aquatic animals* and for reasons of economy. This practice increases the *disease* risks, especially when animals are fed with live or whole fish of the same or related species. There are many examples of this type of practice, e.g. early stage crustaceans fed on *Artemia* species and aquaculture tuna fed on whole wild caught fish.
- The usage of *feed* in moist, semi-moist and dry form implies different levels of risk due to the processing applied to the *feed*.
- With the increasing number of species being farmed (especially marine finfish), the use of live and *moist feed* has increased. It is likely that these industries will shift in future to formulate *feeds* as appropriate formulations are developed.
- *Hazards* may be transmitted from *feed* to *aquatic animals* via direct or indirect means. Direct transmission occurs when the cultured species consumes *feed* containing a pathogenic agent (e.g. shrimp larvae consuming rotifer infected with white spot syndrome virus) while indirect transmission refers to pathogens in *feed* entering the aquatic environment or infecting non target species, and thereby establishing a mechanism for indirect infection of the species of commercial interest. Pathogens that are less host-specific (e.g. white spot syndrome virus, *Vibrio* species) present a greater risk of indirect transmission as they can establish reservoirs of infection in multiple species.

¹ Technical guidelines for responsible fisheries – aquaculture development: 1. Good aquaculture feed manufacturing practice. FAO 2001.
Draft good practices for the animal feed industry – implementing the Codex Alimentarius' Code of practice on good animal feeding, IFIF/FAO (*In preparation*).

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- As new species become the subject of *aquaculture*, new pathogens emerge in association with these hosts. The expression of *disease* may be facilitated by culturing species under intensive and novel conditions. Also, it is necessary to conduct research and develop new *feeds* (and *feed ingredients*) that are appropriate to the species and its culture system. As more and more aquatic species are being cultured, it is difficult to make recommendations for all significant *disease agent*/host species combinations.

2. PURPOSE AND SCOPE

To document risk mitigation measures, including traceability and certification, to deal with aquatic animal health risks through trade in aquatic animal *feeds* and *feed ingredients*. *Hazards* include *diseases* of interest i.e. *OIE-listed diseases* and any others considered to be important to aquatic animal health.

This guideline recommends the control of aquatic animal health *hazards* through adherence to recommended practices during the production (procurement, handling, storage, processing and distribution) and use of both commercial and on-farm produced *feed* (and *feed ingredients*) for *aquatic animals*. While *aquatic animals* grown for food are the main focus, the same principles apply to *feed* for aquarium species.

3. DEFINITIONS***Cross contamination***

Means contamination of a material or product with another material or product containing a *hazard*.

Dry feed

Means *feed* that has a dry matter content = or > than 90%.

Feed

Means any material (single or multiple), whether processed, semi-processed or raw that is intended to be fed directly to food-producing animals.

Feed additives

Means any ingredient intentionally added in micro-amounts not normally consumed as *feed* by itself, whether or not it has nutritional value, which affects the characteristics of *feed* or animal products. Micro-organisms, enzymes, acidity regulators, trace elements, vitamins, attractants, pigments, synthetic binders, synthetic amino acids, antioxidants and other products fall within the scope of this definition, depending on the purpose of use and method of administration. This excludes veterinary drugs.

Feed ingredient

Means a component, part or constituent of any combination or mixture making up a *feed*, including *feed additives*, whether or not it has a nutritional value in the animal's diet. Ingredients may be of plant, animal or aquatic origin and may be organic or inorganic substances.

Hazard

Means a biological, chemical or physical agent in, or a condition of, *feed* or a *feed ingredient* with the potential to cause an adverse effect on animal or public health.

Intra/inter species feeding

Means feeding *aquatic animals* on products made from animals of the same species, or products made from species that are susceptible to the same pathogens as the animals receiving the *feed*.

Live feed

Means live farmed or wild caught animals used as *feed* for *aquatic animals*. *Live feed* is often fed to aquatic species at an early life-stage (e.g. Artemia cysts, rotifers, copepods) and to aquatic species that have been cultured for a relatively short time.

Medicated feed

Means any *feed* which contains a veterinary drug administered to food producing animals, for therapeutic or prophylactic purposes or for modification of physiological functions.

Moist (or wet) feed

Means *feed* that has a dry matter content = or < than 30% (e.g. frozen adult Artemia, whole fish or fish *offal*, molluscs, crustaceans, polychaetes for feed purposes).

Semi-moist feed

Means *feed* that has a dry matter content between 30 and 90%.

Fish solubles

Means a by-product of the fish oil production system, comprising the product remaining when water is drawn off (evaporated) from the residual aqueous phase.

Undesirable substance

Means a contaminant or other substance that is present in and/or on *feed* or *feed ingredients* and that constitutes a risk to animal or public health.

4. GENERAL PRINCIPLES**a) Roles and responsibilities**

The *Competent Authority* has the legal power to set and enforce regulatory requirements related to animal *feeds*, and has final responsibility for verifying that these requirements are met. The *Competent Authority* may establish regulatory requirements for relevant parties, including requirements to provide information and assistance.

It is a particular responsibility of the *Competent Authority* to set and enforce the regulatory requirements pertaining to the use of veterinary drugs, animal disease control and the food safety aspects that relate to the management of live animals on farm.

Those involved in the production and use of animal *feed* and *feed ingredients* have the responsibility to ensure that these products meet regulatory requirements². All personnel involved in the procurement, manufacture, storage and handling of *feed* and *feed ingredients* should be adequately trained and aware of their role and responsibility in preventing the spread of *hazards* of animal health and public health significance. Appropriate contingency plans should be developed in case of a *feed-borne disease* outbreak. Equipment for producing, storing and transporting *feed* should be kept clean and maintained in good working order.

² If at the national level, there are specific food-safety or animal health regulations related to genetically modified organisms, these should be taken into account in relation to feed and feed ingredients as these products form an important part of the food chain.

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Private *veterinarians* and others (e.g. laboratories) providing specialist services to producers and to the feed industry may be required to meet specific regulatory requirements pertaining to the services they provide (e.g. disease reporting, quality standards, transparency).

b) Regulatory standards for feed safety

All *feed* and *feed ingredients* should meet regulatory standards for feed safety. In defining limits and tolerances for *hazards*, scientific evidence, including the sensitivity of analytical methods and on the characterisation of risks, should be taken into account.

c) Risk analysis

Internationally accepted principles and practices on risk analysis (see Section 1.4. of the *Aquatic Code* and relevant Codex texts) should be used in developing and applying the regulatory framework.

A generic *risk analysis* framework should be applied to provide a systematic and consistent process for managing disease risks and the risk of contamination with *undesirable substances*.

d) Good practices

Where national guidelines exist, good *aquaculture* practices and good manufacturing practices (including good hygienic practices) should be followed. Countries without such guidelines are encouraged to develop them.

Where appropriate, Hazard Analysis and Critical Control Point³ (HACCP) principles should be followed to control *hazards* that may occur in *feed*.

e) Relationship between terrestrial animal disease agents and aquatic species

Scientific knowledge is lacking on the relationship between certain terrestrial animal *disease agents*, notably prions, and aquatic species. There is no evidence to suggest that the use of terrestrial animal by-products as ingredients in aquatic animal *feeds* gives rise to risks in respect of prion *diseases*. More scientific information is desirable to enable *aquaculture* industries to utilise more terrestrial animal by-products and plant matter as a means of reducing dependency on aquatic protein and lipid sources.

f) Bioaccumulation

Heavy metals and polychlorinated biphenyls (PCB) persist in fatty tissues and therefore tend to accumulate through the food chain.

g) Geographic and environmental considerations

Aquatic and terrestrial harvest areas for *feed ingredients* should not be located in proximity to sources of animal health or food safety *hazards*. Where this cannot be avoided, preventive measures should be applied to control risk. The same recommendations apply for the processing of *feed ingredients*, the manufacture of *feed* and the location of *aquaculture* operations.

³ Hazard Analysis and Critical Control Point, as defined in the Annex to the Recommended International Code of Practice on General Principles of Food Hygiene (CAC/RCP 1-1969).

Appendix XXVIII (contd)

Aquatic animal health considerations include factors such as *disease* status, location of quarantined premises, existence of processing plants without proper biosecurity measures and the existence of *zones/compartments* of specified health status.

Public health considerations include factors such as industrial operations and waste treatment plants that generate pollutants and other hazardous products. The potential accumulation of pollutants in the food chain through *feed ingredients* needs to be considered.

h) Zoning and compartmentalisation

Feed and *feed ingredients* are important components of biosecurity and need to be considered when defining a *compartment* or *zone* in accordance with Chapter 1.4.4. of the *Aquatic Code*.

i) Sampling and analysis

Sampling and analytical protocols should be based on scientifically recognized principles and procedures and OIE standards, where applicable.

j) Labelling

Labelling should be clear and informative on how the *feed* and *feed ingredients* should be handled, stored and used and should comply with regulatory requirements. Labelling should provide for trace-back.

See Section 4.2. of Codex Code of practice on good animal feeding (CAC/RCP 54-2004).

k) Design and management of inspection programmes

In meeting animal and public health objectives prescribed in national legislation or required by *importing countries*, *Competent Authorities* contribute through the direct performance of some tasks or through the auditing of animal and public health activities conducted by other agencies or the private sector.

Operators in the *feed* and *feed ingredients* business and other relevant industries should implement procedures to ensure compliance with regulatory standards for procurement, handling, storage, processing, distribution and use of *feed* and *feed ingredients*. Operators have the primary responsibility for implementing systems for process control. Where such systems are applied, the *Competent Authority* should verify that they achieve all regulatory requirements.

l) Assurance and certification

Competent Authorities are responsible for providing assurances domestically and to trading partners that regulatory requirements have been met.

m) Hazards associated with animal feed

Biological hazards

Biological hazards that may occur in *feed* and *feed ingredients* include agents such as bacteria, viruses, prions, fungi and parasites.

Appendix XXVIII (contd)Chemical hazards

Chemical hazards that may occur in *feed* and *feed ingredients* include naturally occurring chemicals (such as mycotoxins, gossypol and free radicals), industrial and environmental contaminants (such as heavy metals, dioxins and PCBs), residues of veterinary drugs and pesticides and radionuclides.

Physical hazards

Physical hazards that may occur in *feed* and *feed ingredients* include foreign objects (such as pieces of glass, metal, plastic or wood).

n) Cross contamination

It is important to avoid cross-contamination during the manufacture, storage, distribution (including transport) and use of *feed* and *feed ingredients*. Appropriate provisions should be included in the regulatory framework. Scientific evidence, including the sensitivity of analytical methods and on the characterisation of risks, should be drawn upon in developing this framework.

Procedures such as flushing, sequencing and physical clean-out should be used to avoid cross-contamination between batches of *feed* or *feed ingredients*. National regulations should be followed in order to avoid the use of unauthorised *feed ingredients* with a risk of cross-contamination.

o) Antimicrobial resistance

Concerning the use of antimicrobials in animal *feed* refer to Section X.X.X. of the *Aquatic Code*.

p) Management of information

The *Competent Authority* should establish requirements for the provision of information by the private sector on regulatory requirements.

Records should be maintained in a readily accessible form on the production, distribution and use of *feed* and *feed ingredients*. These records are required to facilitate the prompt trace-back of *feed* and *feed ingredients* to the immediate previous source, and trace-forward to the next/subsequent recipients, to address animal health or public health concerns.

Animal identification (in the case of *aquatic animals* this will normally be on a group basis) and traceability are tools for addressing animal health and food safety risks arising from animal *feed* (see Section 3.5. of the *Terrestrial Code*; Section 4.3 of CAC/RCP 54-2004).

5. HAZARDSBiological

This document addresses the following biological hazards:

- a) bacteria, virus, parasites, fungi affecting *aquatic animals*. These hazards include the *OIE-listed diseases* (Chapter 1.2.3. of the *Aquatic Code*) and other important *diseases* (including IPN and IMNV);
- b) prions.

Chemical

[under study]

Physical

[under study]

6. **PATHOGENS IN FEED**

- a) Pathogens in *feed* can be introduced at two points:
- i) at source: via the harvest of infected *aquatic animals*;
 - ii) during storage, processing and transport.

Contamination may occur at the manufacturing facility via poor hygienic practices and/or the presence of pests.

Feed and *feed ingredients* may be exposed to contamination during storage, manufacturing or transport, due to residues of previous batches of *feed* remaining in processing lines, containers or transport vehicles.

- b) Exposure pathways include:

- i) Direct exposure

The use of raw unprocessed *feed* or *feed ingredients* derived from *aquatic animals* to feed aquatic species presents a risk of exposure to *hazards* of infectious nature. There are risks associated with feeding whole *aquatic animals* and unprocessed products of *aquatic animals* to species that are susceptible to the same *diseases* as the 'fed animal' e.g. feeding salmonid *offal* to salmonids or feeding rotifers or *Artemia* species to crustaceans.

- ii) Indirect exposure

Feed and *feed ingredients* containing pathogenic agents may be transmitted to *aquatic animals* in *aquaculture* and wild fish via contamination of the environment, including infection/contamination on non-target species.

7. **RECOMMENDED APPROACHES TO RISK MITIGATION**

The following measures are relevant to *exporting countries*:

- a) **Source of raw materials**

Raw materials/ingredients should not be sourced from areas/*populations* known to be infected with significant pathogens. It may be appropriate to adopt routine testing procedures to verify that pathogens are not present at unacceptable levels; or

When using *feed* and *feed ingredients* originating from areas known to be affected by a significant pathogen:

- i) *feed* and *feed ingredients* should be delivered directly to feed manufacturing plants for processing under conditions approved by the *Competent Authority*; and
- ii) effluent and other wastes from the feed manufacturing plants should be treated under conditions approved by the *Competent Authority* before discharge into the aquatic environment; or
- iii) *feed* and *feed ingredients* known or suspected to be infected with significant pathogens should only be used and/or processed in a *zone* or *compartment* that does not contain species susceptible to the pathogen in question.

Appendix XXVIII (contd)b) Feed production

To prevent contamination by pathogens during production, storage and transport of *feed* and *feed ingredients*:

- i) flushing, sequencing or physical clean-out of manufacturing lines and storage facilities should be performed between as appropriate;
- ii) buildings and equipment for processing and transporting *feed* and *feed ingredients* should be constructed in a manner that facilitates operation, maintenance and cleaning and prevents *feed* contamination;
- iii) in particular, *feed* manufacturing plants should be designed to avoid cross-contamination between batches;
- iv) processed *feed* and *feed ingredients* should be stored separately from unprocessed *feed ingredients*, under appropriate packaging conditions;
- v) *feed* and *feed ingredients*, manufacturing equipment, storage facilities and their immediate surroundings should be kept clean and pest control programmes should be implemented;
- vi) measures to inactivate pathogens, such as heat treatment or the addition of authorised chemicals, should be used where appropriate. Where such measures are used, the efficacy of treatments should be monitored at appropriate stages in the manufacturing process;
- vii) labelling should provide for the identification of *feed* and *feed ingredients* as to the batch/lot and place/date of production. To assist in tracing *feed* and *feed ingredients* as may be required to deal with animal disease incidents, labelling should provide for identification by batch/lot and date/place of production.

c) The following measures are relevant to *importing countries*:

- i) imported *feed* and *feed ingredients* should be delivered directly to feed manufacturing plants or *aquaculture* facilities for processing/use under conditions approved by the *Competent Authority*;
- ii) effluent and waste material from feed manufacturing plants and *aquaculture* facilities should be managed under conditions approved by the *Competent Authority*, including, where appropriate, treatment before discharge into the aquatic environment;
- iii) *feed* that is known to contain significant pathogens should only be used in a *zone* or *compartment* that does not contain species susceptible to the *disease* in question;
- iv) the importation of raw unprocessed *feed* or *feed ingredients* derived from *aquatic animals* to feed aquatic species should be avoided where possible.

8. CERTIFICATION PROCEDURES FOR AQUATIC FEEDS

- a) The following products represent a negligible risk because of the extensive processing used to produce them:

- i) fish oil;
- ii) crustacean oil;
- iii) *fish solubles*;
- iv) fish meal;
- v) crustacean meal;
- vi) squid meal and squid liver-meal;
- vii) bivalve meal;
- viii) finished *feed* (e.g. flake, pelleted and extruded *feeds*).

For these products, *Competent Authorities* should not require conditions in relation to aquatic animal diseases, regardless of the aquatic health status of the *exporting country, zone or compartment*⁴.

- b) Other products

The following risk mitigation measures should be considered:

- i) sourcing *feed* and *feed ingredients* from a *disease free area*; or
- ii) confirmation (e.g. by testing) that pathogens are not present in the product; or
- iii) treatment (e.g. by heat or acidification) of product to inactivate pathogens.

- c) Importing country measures

When importing *feed* and *feed ingredients* of aquatic origin, 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*).

This certificate should certify:

- i) that *feed* and *feed ingredients* of aquatic origin were imported from a country, *zone* or *compartment* that is free from relevant aquatic animal diseases⁵; or

⁴ In relation to the risk associated with contamination after harvest/processing, point 4 (below) applies.

⁵ Conditions agreed between the Competent Authorities of the importing and exporting countries in accordance with the recommendations of the OIE *Aquatic Animal Health Code*.

Appendix XXVIII (contd)

- ii) that *feed* and *feed ingredients* of aquatic origin were tested for relevant aquatic animal *diseases*⁶ and shown to be free of these *diseases*; or
- iii) that *feed* and *feed ingredients* of aquatic origin have been processed to ensure that they are free of relevant aquatic animal *diseases*.

9. **RISK CHART OF PATHOGEN TRANSMISSION AND CONTAMINATION THROUGH HARVEST OF FEED INGREDIENTS AND MANUFACTURE OF AQUATIC FEEDS**

Some ingredients used in *aquaculture*, in particular of aquatic origin (e.g., krill, shrimp, fish, crab, Artemia) can be a source of pathogen contamination to cultured aquatic species. These ingredients can carry live pathogens (virus, bacteria, and parasites) and reach the aquaculture operation through different types of *feeds* (live, moist, semi-moist or dry feeds).

In aquaculture farms, there are two routes of pathogen contamination through aquatic animal feeding: transmission of pathogens and contamination. **Transmission of pathogens** can take place when the *feed* itself is already infected with a pathogen. This type of contamination is more common with *live feeds* and *moist feeds*. Ingredients that constitute their composition are either kept in a raw state in the final product (e.g., feeding tuna with wild caught fish) or at times require little treatment(s) prior to feeding aquatic organisms.

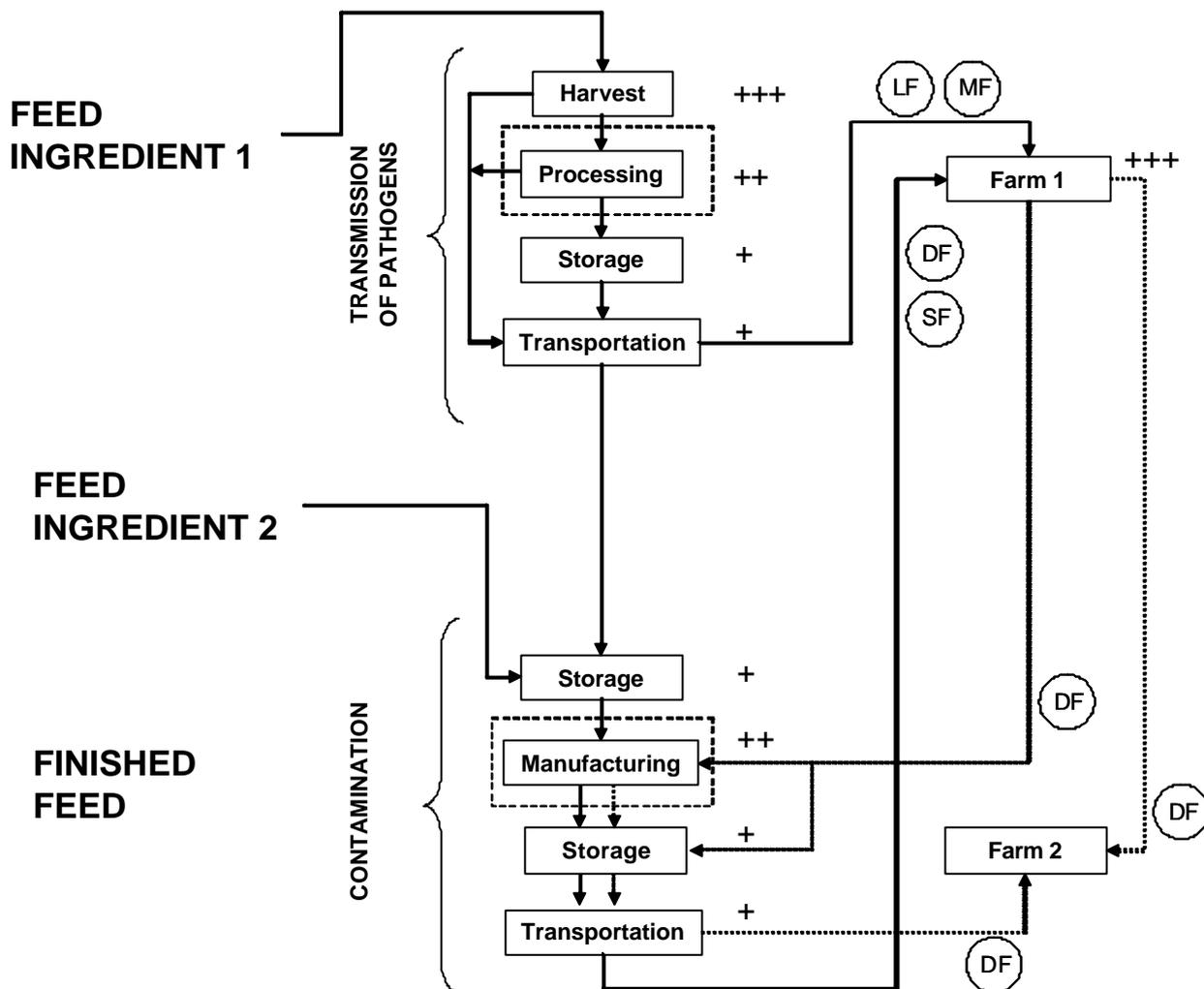
Harvest of aquatic ingredient sources from infected areas has a high *risk* of pathogen contamination, especially if these are transported to an *aquaculture* operation without any prior treatment. Processing of these ingredients places a moderate risk of contamination, and it should actually be taken as a possibility to reduce the risk of pathogen transmission (e.g., through heat, chemical treatments). Storage and transportation of these ingredients has a low risk of contamination, but should also be considered as a direct route of pathogen contamination. For example, when two or more batches of ingredients of different sanitary status are handled, stored and/or transported together without any biosecurity measure there is a risk of direct contamination to the farmed animal.

Contamination occurs when the pathogen is introduced in a feed manufacturing facility, both through infected ingredients or finished feeds and later to the aquaculture facility. Contamination occurs with the use of *semi-moist feeds* and *dry feeds*. With these feed types, contamination can take place in the manufacturing plant during:

- a) Storage of ingredients: it has a low risk of contamination, but it can take place when ingredients of different sanitary status are handled or placed together.
- b) Feed manufacturing: during feed processing, ingredients are commonly subjected to heat treatment which can eliminate certain pathogens. However, use of manufacturing lines with remains of contaminated ingredients from a previous batch of feed can result in cross contamination of feeds.
- c) Storage and transportation of finished feeds: it has a low risk of contamination, but when finished feeds are stored or transported together with unprocessed ingredients or with feeds of different sanitary status it can result in pathogen contamination.

⁶ Conditions agreed between the Competent Authorities of the importing and exporting countries in accordance with the recommendations of the OIE *Aquatic Animal Health Code*.

An aquaculture facility can also be a source of pathogen contamination in aquatic feeds. At this level, contamination can take place when a finished feed is delivered to a farm located in an infected area. Transmission of pathogens can occur when feed is withdrawn from the aquaculture and is returned to the manufacturing facility for reprocessing or transferred to another farm.



LF: Live feed MF: Moist feed SF: Semi-moist feed DF: Dry feed	[Dashed box] Possibility for risk reduction
+++: High risk of pathogen contamination ++: Moderate risk of p. c. +: Low risk of p. c.	[Dotted arrow] Redistribution or recycling of finished feed

APPENDIX X.X.X.

GENERAL GUIDELINES FOR AQUATIC ANIMAL
HEALTH SURVEILLANCE

Article 3.8.1.1.

Introduction and objectives

1. Surveillance is aimed at:
 - demonstrating the absence of *disease* or *infection*,
 - identifying events requiring notification as listed in Article 1.2.1.3. of the *Aquatic Code*
 - determining the occurrence or distribution of endemic *disease* or *infection*, including changes to their incidence or prevalence (or its contributing factors), in order to:
 - provide information for domestic *disease* control programmes,
 - provide relevant *disease* occurrence information to be used by trading partners for qualitative and quantitative risk assessment.

The type of surveillance applied depends on the desired outputs needed to support decision-making. Surveillance data determine the quality of *disease* status reports and should satisfy information requirements for accurate risk analysis both for *international trade* as well as for national decision-making.

2. Essential prerequisites to enable a Member Country to provide information for the evaluation of its animal health status are:
 - a) that the particular Member Country complies with the provisions of Chapter 1.4.3. of the *Aquatic Code* on the quality and evaluation of the *Competent Authorities*;
 - b) that, where possible, surveillance data be complemented by other sources of information (e.g. scientific publications, research data, documented field observations and other non-survey data);
 - c) that transparency in the planning and execution of surveillance activities and the analysis and availability of data and information, be maintained at all times, in accordance with Chapter 1.2.1. of the *Aquatic Code*

The following guidelines may be applied to all *diseases*, their agents and susceptible species as listed in the *Aquatic Manual*, and are designed to assist with the development of surveillance methodologies. Where possible, the development of surveillance systems using these guidelines should be based on the relevant information in the individual *disease* chapters.

Article 3.8.1.2.

Definitions

The following definitions apply for the purposes of this Appendix:

Bias: A tendency of an estimate to differ from the true value of a population parameter.

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Case definition: A case definition is a set of criteria used to distinguish a case animal or *epidemiological unit* from a non-case.

Early detection system: an efficient system for ensuring the rapid recognition of signs that are suspicious of a *listed disease*, or an *emerging disease* situation, or unexplained mortality, in *aquatic animals* in an *aquaculture establishment* or in the wild, and the rapid communication of the event to the *Competent Authority*, with the aim of activating diagnostic investigation with minimal delay. Such a system will include the following characteristics:

- a) broad awareness, e.g. among the personnel employed at *aquaculture establishments* or involved in *processing*, of the characteristic signs of the *listed diseases* and *emerging diseases*;
- b) veterinarians or *aquatic animal* health specialists trained in recognising and reporting suspicious *disease* occurrence;
- c) ability of the *Competent Authority* to undertake rapid and effective *disease* investigation;
- d) access by the *Competent Authority* to laboratories with the facilities for diagnosing and differentiating *listed* and *emerging diseases*.

Outbreak: An outbreak is a substantial increase in the occurrence of *disease* above the expected level at a given time in a given population.

Probability sampling: A sampling strategy in which every unit has a known non-zero probability of inclusion in the sample.

Sample: The group of elements (sampling units) drawn from a population, on which tests are performed or parameters measured to provide surveillance information.

Sampling unit: The unit that is sampled. This may be an individual animal or a group of animals (e.g. a pond). A list of all the sampling units comprises the sampling frame.

Sensitivity: The proportion of truly positive units that are correctly identified as positive by a test.

Specificity: The proportion of truly negative units that are correctly identified as negative by a test.

Study population: The population from which surveillance data are derived. This may be the same as the target population or a subset of it.

Surveillance: The systematic ongoing collection, collation, and analysis of data, and the timely dissemination of information to those who need to know so that action can be taken.

Survey: An investigation about a defined population in which information is systematically collected within a defined time period.

Target population: The population about which conclusions from analysing data are to be inferred.

Test: A procedure used to classify a unit as either positive, negative or suspect with respect to an *infection* or *disease*.

Article 3.8.1.3.

Principles of surveillance1. Types of surveillance

- a) Surveillance may be based on many different data sources and can be classified in a number of ways, including:
 - i) the means by which data are collected (targeted versus non-targeted);
 - ii) the *disease* focus (pathogen-specific versus general surveillance); and
 - iii) the way in which units for observation are selected (structured surveys versus non-random data sources).
- b) Surveillance activities include:
 - i) structured population-based surveys, such as:
 - systematic sampling at slaughter;
 - random surveys;
 - ii) structured non-random surveillance activities, such as:
 - *disease* reporting or notifications;
 - control programmes/health schemes;
 - targeted testing/screening;
 - ante-mortem and post-mortem inspections;
 - laboratory investigation records;
 - biological specimen banks;
 - sentinel units;
 - field observations;
 - farm production records.
- c) In addition, surveillance data should be supported by related information, such as:
 - i) data on the epidemiology of the *infection*, including environmental, and host and wild reservoir population distributions;
 - ii) data on farmed and wild animal movements and trading patterns for aquatic animals and aquatic animal products, including potential for exposure to wild aquatic animal populations, water sources or other contacts;
 - iii) national animal health regulations, including information on compliance with them and their effectiveness;

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- iv) history of imports of potentially infected material; and
 - v) biosecurity measures in place.
- d) The sources of evidence should be fully described. In the case of a structured survey, this should include a description of the sampling strategy used for the selection of units for testing. For structured non-random data sources, a full description of the system is required including the source(s) of the data, when the data were collected, and a consideration of any biases that may be inherent in the system.

2. Critical elements

In assessing the quality of a surveillance system, the following critical elements need to be addressed over and above quality of *Competent Authority* (Chapter 1.4.3.).

a) Populations

Ideally, surveillance should be carried out in such a way as to take into account all animal species susceptible to the *infection* in a country, *zone* or *compartment*. The surveillance activity may cover all individuals in the population or part of them. Estimates of total population at risk for each species are required. When surveillance is conducted only on a *subpopulation*, care should be taken regarding the inferences made from the results.

Definitions of appropriate populations should be based on the specific recommendations of the *disease* chapters of the *Aquatic Manual*.

b) Epidemiological unit

The relevant *epidemiological unit* for the surveillance system should be defined and documented to ensure that it is representative of the population or targeted *subpopulations* that would generate the most useful inferences about *disease* patterns. Therefore, it should be chosen taking into account factors such as carriers, reservoirs, vectors, immune status, genetic resistance and age, sex, and other host criteria.

c) Clustering

Infection in a country, *zone* or *compartment* usually clusters rather than being uniformly or randomly distributed through a population. Clustering may occur at a number of different levels (e.g. tank, pond, farm, or *compartment*). Clustering should be taken into account in the design of surveillance activities and the statistical analysis of surveillance data, at least at what is judged to be the most significant level of clustering for the particular animal population and *infection*.

d) Case and outbreak definitions

Clear and unambiguous case and outbreak definitions should be developed and documented for each *disease* under surveillance, using, where they exist, the standards in this Appendix and the *Aquatic Manual*.

e) Analytical methodologies

Surveillance data should be analysed using appropriate methodologies, and at the appropriate organisational levels to facilitate effective decision making, whether it be planning interventions or demonstrating status.

Methodologies for the analysis of surveillance data should be flexible to deal with the complexity of real life situations. No single method is applicable in all cases. Different methodologies may be needed to accommodate the relevant pathogens, varying production and surveillance systems, and types and amounts of data and information available.

The methodology used should be based on the best available information that is in accord with current scientific thinking. The methodology should be in accordance with this Appendix and fully documented, and supported by reference to the scientific literature and other sources, including expert opinion. Sophisticated mathematical or statistical analyses should only be carried out when justified by the proper amount and quality of field data.

Consistency in the application of different methodologies should be encouraged and transparency is essential in order to ensure fairness and rationality, consistency in decision making and ease of understanding. The uncertainties, assumptions made, and the effect of these on the final conclusions should be documented.

f) Testing

Surveillance involves the detection of *disease* or *infection* by the use of appropriate case definitions based on the results of one or more tests for evidence of *infection* status. In this context, a test may range from detailed laboratory examinations to field observations and the analysis of production records. The performance of a test at the population level (including field observations) may be described in terms of its sensitivity and specificity and predictive values. Imperfect sensitivity and/or specificity will have an impact on the conclusions from surveillance. Therefore, these parameters should be taken into account in the design of surveillance systems and analysis of surveillance data as described in the *Aquatic Manual*.

Although not determined for many aquatic *diseases*, sensitivity and specificity should be estimated as best as possible for a specific testing situation. Alternatively, where values for sensitivity and/or specificity for a particular test and testing situation are estimated in the *Aquatic Manual*, these values may be used as a guide.

Samples from a number of animals or units may be pooled and subjected to a testing protocol. The results should be interpreted using sensitivity and specificity values that have been determined or estimated for that particular pool size and testing procedure.

g) Quality assurance

Surveillance systems should incorporate the principles of quality assurance and be subjected to periodic auditing to ensure that all components of the system function and provide verifiable documentation of procedures and basic checks to detect significant deviations of procedures from those documented in the design.

h) Validation

Results from animal health surveillance systems are subject to one or more potential biases. When assessing the results, care should be taken to identify potential biases that can inadvertently lead to an over-estimate or an under-estimate of the parameters of interest.

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i) Data collection and management

The success of a surveillance system is dependent on a reliable process for data collection and management. The process may be based on paper records or computerised. Even where data are collected for non-survey purposes (e.g. during *disease* control interventions, inspections for movement control or during *disease* eradication schemes), the consistency and quality of data collection and event reporting in a format that facilitates analysis, is critical. Factors influencing the quality of collected data include:

- the distribution of, and communication between, those involved in generating and transferring data from the field to a centralised location;
- motivation of the people involved in the surveillance system;
- the ability of the data processing system to detect missing, inconsistent or inaccurate data, and to address these problems;
- maintenance of disaggregated data rather than the compilation of summary data;
- minimisation of transcription errors during data processing and communication.

Article 3.8.1.4.

Structured population-based surveys

In addition to the principles for surveillance discussed above, the following guidelines should be used when planning, implementing and analysing surveys.

1. Types of surveys

Surveys may be conducted on the entire target population (i.e. a census) or on a sample. Periodic or repeated surveys conducted in order to document *disease* freedom should be done using probability based sampling methods (simple random selection, cluster sampling, stratified sampling, systematic sampling) so that data from the study population can be extrapolated to the target population in a statistically valid manner. Non-probability based sampling methods (convenience, expert choice, quota) can also be used. Recognising the inherent impracticalities in sampling from some aquatic populations, non-probability based sampling could be used when biases are recognised and used to optimise detection.

The sources of information should be fully described and should include a detailed description of the sampling strategy used for the selection of units for testing. Also, consideration should be made of any biases that may be inherent in the survey design.

2. Survey design

The population of *epidemiological units* should first be clearly defined; hereafter sampling units appropriate for each stage, depending on the design of the survey, should be defined.

The design of the survey will depend on the size and structure of the population being studied, the epidemiology of the *infection* and the resources available.

3. Sampling

The objective of sampling from a population is to select a subset of units from the population that is representative of the population with respect to the object of the study such as the presence or absence of *infection*. Sampling should be carried out in such a way as to provide the best likelihood that the sample will be representative of the population, within the practical constraints imposed by different environments and production systems. In order to detect the presence of an *infection* in a population of unknown *disease* status, targeted sampling methods that optimise the detection of *infection* can be used. In such cases, care should be taken regarding the inferences made from the results.

4. Sampling methods

When selecting *epidemiological units* from within a population the objectives of the surveillance system should be considered. In general, probability sampling (e.g. simple random selection) is preferable. When this is not possible, sampling should provide the best practical chance of generating optimal inferences about *disease* patterns in the target population.

In any case, the sampling method used at all stages should be fully documented and justified.

5. Sample size

In general, surveys are conducted either to demonstrate the presence or absence of a factor (e.g. *infection*) or to estimate a parameter (e.g. the prevalence of *infection*). The method used to calculate sample size for surveys depends on the purpose of the survey, the expected prevalence, the level of confidence desired of the survey results and the performance of the tests used.

Article 3.8.1.5.

Structured non-random surveillance

Surveillance systems routinely use structured non-random data, either alone or in combination with surveys.

1. Common non-random surveillance data sources

A wide variety of non-random surveillance data sources may be available. These vary in their primary purpose and the type of surveillance information they are able to provide. Some surveillance systems are primarily established as *early detection systems*, but may also provide valuable information to demonstrate freedom from *infection*. Other systems provide cross-sectional information suitable for prevalence estimation, either once or repeatedly, while yet others provide continuous information, suitable for the estimate of incidence data (e.g. *disease* reporting systems, sentinel sites, testing schemes).

a) Disease reporting or notification systems

Data derived from *disease* reporting systems can be used in combination with other data sources to substantiate claims of animal health status, to generate data for risk analysis, or for early detection. The first step of a *disease* reporting or notification system is often based on the observation of abnormalities (e.g. clinical signs, reduced growth, elevated mortality rates, behavioural changes, etc.), which can provide important information about the occurrence of endemic, exotic or new *diseases*. Effective laboratory support is, however, an important component of most reporting systems. Reporting systems relying on laboratory confirmation of suspect clinical cases should use tests that have a high specificity. Reports should be released by the laboratory in a timely manner, with the amount of time from *disease* detection to report generation minimised.

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b) Control programmes/health schemes

Animal *disease* control programmes or health schemes, while focusing on the control or eradication of specific *diseases*, should be planned and structured in such a manner as to generate data that are scientifically verifiable and contribute to structured surveillance.

c) Targeted testing/screening

This may involve testing targeted to selected sections of the population (subpopulations), in which *disease* is more likely to be introduced or found. Examples include testing culled and dead animals, animals exhibiting clinical signs, animals located in a defined geographical area and specific age or commodity group.

d) Post-harvest inspections

Inspections of aquatic animal slaughter premises or processing plants may provide valuable surveillance data provided diseased aquatic animals survive to slaughter. Post-harvest inspections are likely to provide good coverage only for particular age groups and geographical areas. Post-harvest surveillance data are subject to obvious biases in relation to target and study populations (e.g. only animals of a particular class and age may be slaughtered for human consumption in significant numbers). Such biases need to be recognised when analysing surveillance data.

Both for traceback in the event of detection of *disease* and for analysis of spatial and population-level coverage, there should be, if possible, an effective identification system that relates each animal in the slaughter premises/processing plant to its locality of origin.

e) Laboratory investigation records

Analysis of laboratory investigation records may provide useful surveillance information. The coverage of the system will be increased if analysis is able to incorporate records from national, accredited, university and private sector laboratories. Valid analysis of data from different laboratories depends on the existence of standardised diagnostic procedures and standardised methods for interpretation and data recording. If available, the method listed in the *Aquatic Manual* in relation to the purpose of testing should be used. As with post-harvest inspections, there needs to be a mechanism to relate specimens to the farm of origin. It must be recognised that laboratory submissions may not accurately reflect the *infection* or *disease* situation on the farm.

f) Biological specimen banks

Specimen banks consist of stored specimens, gathered either through representative sampling or opportunistic collection or both. Specimen banks may contribute to retrospective studies, including providing support for claims of historical freedom from *infection*, and may allow certain studies to be conducted more quickly and at lower cost than alternative approaches.

g) Sentinel units

Sentinel units/sites involve the identification and regular testing of one or more of animals of known health/exposure status in a specified geographical location to detect the occurrence of *disease*. They are particularly useful for surveillance of *diseases* with a strong spatial component, such as vector-borne *diseases*. Sentinel units provide the opportunity to target surveillance depending on the likelihood of *infection* (related to vector habitats and host population distribution), cost and other practical constraints. Sentinel units may provide evidence of freedom from *infection*, or provide data on prevalence and incidence as well as the distribution of *disease*. Cohabitation with a susceptible population should be considered for testing *infection* or *disease* in populations of valuable animals, the lethal sampling of which may be unacceptable (e.g. ornamental fish).

h) Field observations

Clinical observations of epidemiological units in the field are an important source of surveillance data. The sensitivity and/or specificity of field observations may be relatively low, but these can be more easily determined and controlled if a clear, unambiguous and easy to apply standardised case definition is applied. Education of potential field observers in application of the case definition and reporting is an important component. Ideally, both the number of positive observations and the total number of observations should be recorded.

i) Farm production records

Systematic analysis of farm production records may be used as an indicator of the presence or absence of *disease* at the population level. If production records are accurate and consistently maintained, the sensitivity of this approach may be quite high (depending on the *disease*), but the specificity is often quite low.

2. Critical elements for structured non-random surveillance

There is a number of critical factors that should be taken into account when using structured non-random surveillance data such as coverage of the population, duplication of data, and sensitivity and specificity of tests that may give rise to difficulties in the interpretation of data. Surveillance data from non-random data sources may increase the level of confidence or be able to detect a lower level of prevalence with the same level of confidence compared to structured surveys.

3. Analytical methodologies

Different scientifically valid methodologies may be used for the analysis of non-random surveillance data. This most often requires information on parameters of importance to the surveillance system, such as sensitivity and specificity. Where no such data are available, estimates based on expert opinions, gathered and combined using a formal, documented and scientifically valid methodology may be used.

4. Combination of multiple sources of data

The methodology used to combine the evidence from multiple data sources should be scientifically valid, and fully documented including references to published material.

Appendix XXIX (contd)

Surveillance information gathered from the same country, *zone* or *compartment* at different times (e.g. repeated annual surveys) may provide cumulative evidence of animal health status. Such evidence gathered over time may be combined to provide an overall level of confidence. However, a single larger survey, or the combination of data collected during the same time period from multiple random or non-random sources, may be able to achieve the same level of confidence in a shorter period of time.

Analysis of surveillance information gathered intermittently or continuously over time should, where possible, incorporate the time of collection of the information to take into account the decreased value of older information. The sensitivity, specificity and completeness of data from each source should also be taken into account for the final overall confidence level estimation.

Article 3.8.1.6.

Surveillance to demonstrate freedom from disease/infection

1. Demonstration of freedom from infection

A surveillance system to demonstrate freedom from *infection* should meet the following requirements in addition to the general requirements for surveillance outlined in Article 3.8.1.3 of this Appendix.

Freedom from *infection* implies the absence of the pathogenic agent in the country, *zone* or *compartment*. Scientific methods cannot provide absolute certainty of the absence of *infection*. Demonstrating freedom from *infection* involves providing sufficient evidence to demonstrate (to a level of confidence acceptable to Member Countries) that *infection* with a specified pathogen is not present in a population. In practice, it is not possible to prove (i.e. be 100% confident) that a population is free from *infection*. Instead, the aim is to provide adequate evidence (to an acceptable level of confidence), that *infection*, if present, is present in less than a specified proportion of the population.

However, apparent *infection* at any level in the target population automatically invalidates any freedom from *infection* claim unless the positive test results are accepted as false positives based on specificity values described in the relevant *disease* chapter.

2. Requirements to declare a country, zone or compartment free from disease/infection without pathogen specific surveillance

This Article provides general principles for declaring a country, *zone* or *compartment* free from *disease/infection* in relation to the time of last occurrence and in particular for the recognition of historical freedom.

The provisions of this Article are based on the principles described in Article 3.8.1.3. of this Appendix and the following premises:

- in the absence of *disease* and vaccination, the farmed and wild animal populations would become susceptible over a period of time;
- the *disease* agents to which these provisions apply are likely to produce identifiable clinical signs in observable susceptible animals;
- competent and effective *Competent Authority* will be able to investigate, diagnose and report *disease*, if present;

- the absence of *disease/infection* over a long period of time in a susceptible population can be substantiated by effective *disease* investigation and reporting by a Member Country.

a) Absence of susceptible species

Unless otherwise specified in the relevant *disease* chapter, a country, *zone* or *compartment* may be recognised as being free from *infection* without applying *targeted surveillance* if there are no susceptible species (as listed in the relevant chapter of this *Aquatic Manual*, or in the scientific literature) present in that country, *zone* or *compartment*.

b) Historically free

Unless otherwise specified in the relevant *disease* chapter, a country, *zone* or *compartment* may be recognised free from *infection* without formally applying a pathogen-specific surveillance programme when:

- i) there has never been a substantiated occurrence of *disease* reported officially or in the scientific literature (peer reviewed), or
- ii) eradication has been achieved or the *disease/infection* has ceased to occur for at least 25 years,

provided that for at least the past 10 years:

- iii) the *basic biosecurity conditions* are in place and effectively enforced;
- iv) no vaccination against the *disease* has been carried out unless otherwise allowed for in the *Aquatic Code*;
- v) *infection* is not known to be established in wild aquatic animals within the country or *zone* intended to be declared free. (A country or *zone* cannot apply for historical freedom if there is any evidence of *infection* in wild aquatic animals. However, specific surveillance in wild aquatic animals is not necessary.)

A country, *zone* or *compartment* that was self-declared free on the basis of the absence of susceptible species, but subsequently introduces any of the susceptible species as listed in the *Aquatic Manual*, may be considered historically free from the *disease* provided that:

- the country, *zone* or *compartment* of origin was declared free of the *disease* at the time of introduction,
- *basic biosecurity conditions* were introduced prior to the introduction,
- no vaccination against the *disease* has been carried out unless otherwise allowed for in the *disease* specific chapter of this *Aquatic Code*.

c) Last occurrence within the previous 25 years

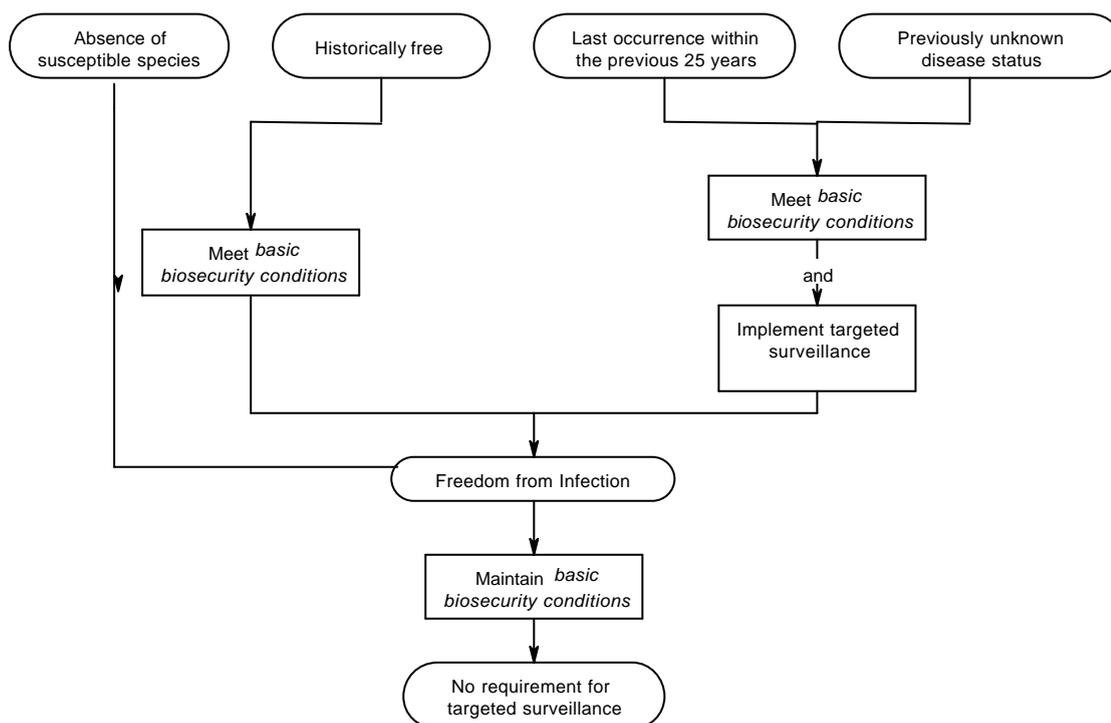
Countries, *zones* or *compartments* that have achieved eradication (or in which the *disease/infection* has ceased to occur) within the previous 25 years, should follow the pathogen-specific surveillance requirements in the *Aquatic Manual* if they exist. In the absence of *disease* specific information to aid the development of a surveillance system, declaration of *disease* freedom should follow at least 2 surveys per year (for at least 2 consecutive years) to be conducted 3 or

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more months apart, at the appropriate life stage and at times of the year when temperature and season offer the best opportunity to detect the pathogen. Surveys should be designed to provide an overall 95% confidence and with a design prevalence at the animal and higher (i.e. pond, farm, village, etc.) levels being 2% or lower (this value may be different for different *diseases* and may be provided in the specific *disease* chapter in the *Aquatic Manual*). Such surveys should not be based on voluntary submission and should be developed following the guidelines provided in the *Aquatic Manual*. Survey results will provide sufficient evidence of *disease* freedom provided that for at least the past 10 years these additional criteria are met:

- i) the *basic biosecurity conditions* are in place and effectively enforced;
- ii) no vaccination against the *disease* has been carried out unless otherwise provided in the *Aquatic Code*;
- iii) *infection* is not known to be established in wild aquatic animals within the country or *zone* intended to be declared free. (A country or *zone* cannot apply for freedom if there is any evidence of *infection* in wild aquatic animals. Specific surveillance in wild aquatic animals of susceptible species is necessary to confirm absence.)

The different paths to recognition of freedom from *infection* are summarised in the diagram below.



2. Guidelines for the discontinuation of pathogen-specific surveillance after recognition of freedom from infection

A country or *zone* that has been recognised as free from *infection* following the provisions of the *Aquatic Code* may discontinue pathogen-specific surveillance while maintaining the *infection*-free status provided that:

- a) the *basic biosecurity conditions* are in place and effectively enforced;
- b) vaccination against the *disease* is not applied;
- c) Surveillance has demonstrated that *infection* is not present in wild aquatic animal populations of susceptible species.

A special case can be made for a *compartment* located in a country or *zone* that is not proven to be free from *infection* if surveillance is maintained and exposure to potential sources of *infection* is prevented.

3. International recognition of disease/infection free status

For *diseases* for which procedures exist whereby the OIE can officially recognise the existence of a *disease/infection* free country, *zone* or *compartment*, a Member Country wishing to apply for recognition of this status shall, via its Permanent Delegate, send to the OIE all the relevant documentation relating to the country, *zone* or *compartment* concerned. Such documentation should be presented according to guidelines prescribed by the OIE for the appropriate animal *diseases*.

Article 3.8.1.7.

Surveillance for distribution and occurrence of infection

Surveillance to determine distribution and occurrence of *infection* or of other relevant health related events is widely used to assess the prevalence and incidence of selected *disease/infection* as an aid to decision making, for example implementation of control and eradication programmes. It also has relevance for the international movement of animals and products when movement occurs among infected countries.

In contrast to surveillance to demonstrate freedom from *infection*, surveillance for the distribution and occurrence of *infection* is usually designed to collect data about a number of variables of animal health relevance, for example:

- a) prevalence or incidence of *infection* in wild or cultured animals;
- b) morbidity and mortality rates;
- c) frequency of *disease/infection* risk factors and their quantification;
- d) frequency distribution of variables in *epidemiological units*;
- e) frequency distribution of the number of days elapsing between suspicion of *infection* and laboratory confirmation of the diagnosis and/or to the adoption of control measures;
- f) farm production records, etc.

CHAPTER 1.1.4.

GUIDELINES FOR AQUATIC ANIMAL HEALTH SURVEILLANCE [REQUIREMENTS FOR SURVEILLANCE FOR INTERNATIONAL RECOGNITION OF FREEDOM FROM INFECTION]

[PART 1]**INTERNATIONAL RECOGNITION OF FREEDOM FROM INFECTION****1. General principles**

General principles are provided below for declaring a country, zone or aquaculture establishment free from infection in relation to the time of last occurrence, and in particular for the recognition of historical freedom.

An essential prerequisite to provide the guarantees required for the recognition of freedom from infection is that the particular Member Country complies with the requirements of Chapter 1.4.3 of the *Aquatic Code* for the evaluation of the Competent Authorities.

The general principles are:

- ▲ in the absence of infection or vaccination, the animal *population* would be susceptible to clinical disease, or infection, over a period of time;
- ▲ the disease agents to which these provisions apply are likely to produce identifiable clinical or pathological signs in susceptible animals;
- ▲ an animal *population* may be free from some specified pathogens but not from others;
- ▲ there are competent and effective personnel of the Competent Authority able to investigate, diagnose and report disease or infection, if present;
- ▲ the absence of infection over a long period of time in susceptible *populations* can be substantiated by effective disease investigation and reporting by the Competent Authority of the Member Country.

2. Requirements to declare a country, zone or aquaculture establishment free from infection with a specified pathogen

The requirements to declare a country, zone or aquaculture establishment free from infection differ depending on the previous infection status of the country, zone or aquaculture establishment, namely:

- ▲ Absence of susceptible species;
- ▲ Historically free;
- ▲ Last known occurrence within the previous 25 years;
- ▲ Previously unknown infection status.

2.1. Absence of susceptible species

Unless otherwise specified in the relevant disease chapter, a country, zone or aquaculture establishment may be recognised as being free from infection without applying *targeted surveillance* if there are no susceptible species (as listed in the relevant chapter of the *Aquatic Code*, or in the scientific literature) present in that country, zone or aquaculture establishment, provided that the *prescribed biosecurity conditions* have been in place continuously in the country, zone or aquaculture establishment for at least the previous 10 years.

2.2. Historically free

Unless otherwise specified in the relevant disease chapter, a country, zone or aquaculture establishment may be recognised as being free from infection without formally applying *targeted surveillance* when:

- ▲ there has never been any observed occurrence of disease;

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or

- eradication has been achieved or the disease has ceased to occur for at least 25 years,

provided that the *prescribed biosecurity conditions* have been in place continuously in the country, zone or aquaculture establishment for at least the previous 10 years.

2.3. Last known occurrence within the previous 25 years

For countries or zones that have achieved eradication (or in which the disease has ceased to occur) within the previous 25 years, in addition to the *prescribed biosecurity conditions*, appropriate *targeted surveillance* must have been applied to demonstrate the absence of the infection, consistent with the provisions of Section B of this chapter.

2.4. Previously unknown infection status

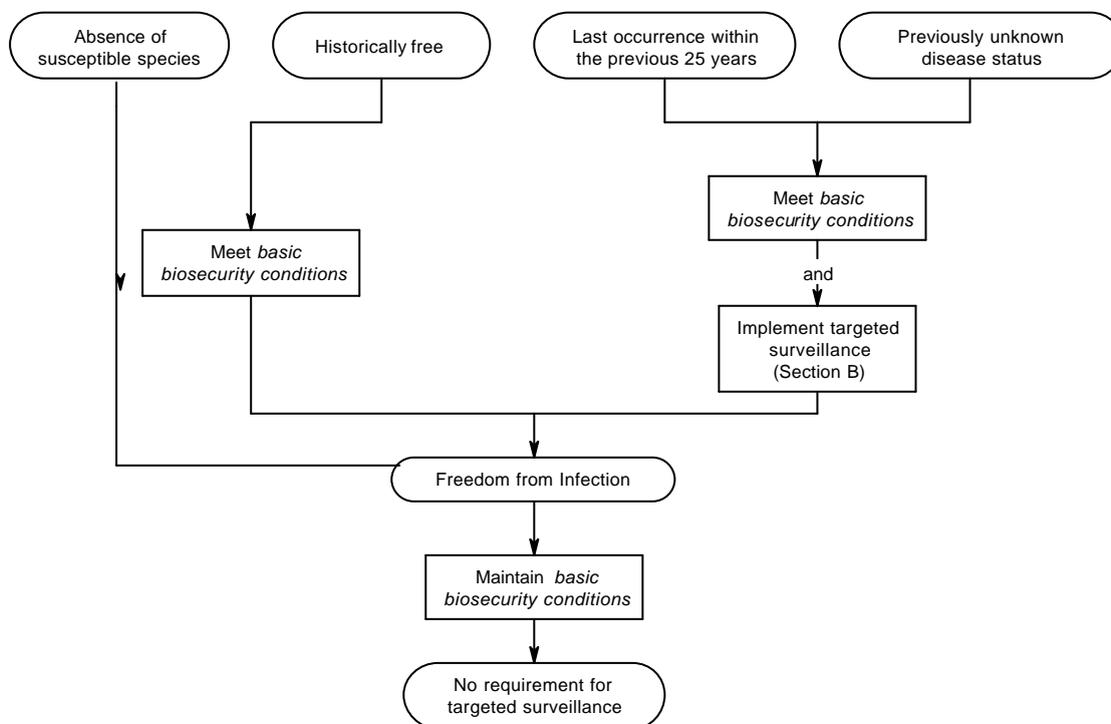
For countries or zones with previously unknown infection status, or which have not previously met the requirements of the Sections A.2.1, A.2.2 or A.2.3 above, the *prescribed biosecurity conditions* must be introduced in addition to *targeted surveillance* consistent with the provisions of Section B of this chapter.

3. Guidelines for the maintenance of continued recognition of freedom from infection

A country, zone or aquaculture establishment that has been recognised free from infection following the provisions of Sections A.2.1 or A.2.2, may maintain its official status as infection free provided that the *prescribed biosecurity conditions* are continuously maintained.

A country, zone or aquaculture establishment that has been recognised free from infection following the provisions of Sections A.2.3 or A.2.4, may discontinue *targeted surveillance* and maintain its official status as infection free provided that the *prescribed biosecurity conditions* are continuously maintained.

The different paths to recognition of freedom from infection are summarised in the diagram below.]



B. GUIDELINES FOR AQUATIC ANIMAL HEALTH SURVEILLANCE

1. Introduction

1. [This section provides standards to be applied when demonstrating country, zone or aquaculture establishment freedom from infection, in accordance with the principles of Section A. Standards described in this section] Surveillance is aimed at:

- demonstrating the absence of *disease* or *infection*.
- identifying events requiring notification as listed in Article 1.2.1.3 of the *Aquatic Code*.
- determining the occurrence or distribution of endemic *disease* or *infection*, including changes to their incidence or prevalence (or its contributing factors), in order to:
 - provide information for domestic disease control programmes.
 - provide relevant disease occurrence information to be used by trading partners for qualitative and quantitative risk assessment.

The type of surveillance applied depends on the desired outputs needed to support decision-making. Surveillance data determine the quality of disease status reports and should satisfy information requirements for accurate risk analysis both for *international trade* as well as for national decision-making.

The following guidelines may be applied to all *diseases*, their agents and susceptible species as listed in the *Aquatic Manual*, and are designed to assist with the development of surveillance methodologies. Where possible, the development of surveillance systems using these guidelines should be based on the relevant information in the individual disease chapters.

There is sometimes a perception that surveillance can only be conducted using sophisticated methodologies. However, an effective surveillance system can also be developed by making use of gross observations and already available resources.

Surveillance of endemic diseases provides valuable information for day-to-day health management and can act as the foundation for detecting outbreaks of exotic disease and demonstrating specific disease freedom.

Surveillance may address both infectious and non-infectious diseases of concern to the country.

Section B provides standards to be applied when: (a) demonstrating country, zone or compartment freedom from infection, in accordance with the principles of Section A and (b) assessing the occurrence and distribution of a specific *infection/disease* or syndrome

Standards described in this section may be applied to all *diseases*, their agents and susceptible species as listed in the *Aquatic Code*, and are designed to assist with the development of surveillance methodologies. Nevertheless surveillance may include also non listed diseases

It would be impractical to try to develop a surveillance system for all the known aquatic animal diseases for which a country has susceptible species. Therefore prioritising the diseases to be included in a surveillance system should be conducted considering:

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- the needs to provide assurance of disease status for trade purposes
- the resources of the country
- the financial impact or threat posed by the different diseases
- the importance of an industry-wide disease control programme within a country or region

The concept of risk encompasses both the probability of the disease occurring and the severity of its consequences

More detailed information in each disease chapter (where it exists) of this *Aquatic Manual* may be used to further refine the general approaches described in this chapter. Where detailed *disease/infection*-specific information is not available, surveillance can also be conducted following the guidelines in this chapter. Access to epidemiological expertise would be invaluable for the design, implementation of the system and interpretation of results derived from a surveillance system.

2. General principles

~~[Demonstrating freedom from infection involves providing sufficient evidence to demonstrate that infection with a specified agent is not present in a specified population. In practice, it is not possible to definitively prove that a population is free from infection (unless every member of the population is examined simultaneously with a perfect test with both sensitivity and specificity equal to 100%). Instead, the aim is to provide adequate evidence (to an acceptable level of confidence), that infection, if present, is present in less than a specified proportion of the population.]~~

~~Methodologies to demonstrate freedom from infection should be]~~ Surveillance methodologies should be flexible to deal with the complexity of real life situations. No single method is applicable in all cases. Methodologies must be able to accommodate the variety of aquatic animal species, the multiple diseases of relevance, varying production ~~[and surveillance]~~ systems, and types and amounts of data and information available.

The methodology used should be based on the best available information that is in accord with current scientific thinking. The methodology should be well documented and supported with references to the scientific literature and other sources, including expert opinion. Efforts should be made to address the information gaps wherever possible.

~~[Consistency in methodologies should be encouraged and transparency is]~~ Methodologies that are consistent and transparent are essential to ensure fairness and rationality, consistency in decision making and ease of understanding by all the interested parties. ~~[Applications for]~~ The presentation of the results generated through surveillance (e.g. recognition of infection-free status or measures of disease frequency) should document the uncertainties, the assumptions made, and the potential effect of these on the final estimate.

3. Surveillance ~~General requirements for demonstration of freedom from~~ disease [infection]

This section describes surveillance to demonstrate freedom from disease.

3.1. Objectives ~~[Population]~~

~~[The target population to which the demonstration of freedom from infection applies is all individuals of all species susceptible to the infection in a country, zone or aquaculture establishment.]~~

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The *study population* may be the same as the *target population* or a subset of it. The *study population* should be (in order of preference):

- ▲ The appropriate *study population* as defined in the relevant disease chapter of the *Aquatic Code* (if such a definition exists);
- ▲ A subset of the *target population* that defines a group of animals which, if infection were present, would be most likely to have a higher prevalence of infection than the *target population*. This subset should be defined in terms of:
 - ▲ species;
 - ▲ time (e.g. season or month of year);
 - ▲ stage of life cycle or growth period;
 - ▲ production system and/or management characteristics;
 - ▲ location;
 - ▲ readily identifiable physical or behavioural characteristics.
- ▲ The same as the *target population*;
- ▲ A subset of the *target population* with the same or lower probability of infection. The nature and impact of any biases on the results of the analysis must be considered, documented and taken into account in the analysis.]

The objective of this kind of surveillance system is to contribute on an on-going basis evidence to demonstrate freedom from disease in a particular country, zone or compartment with a known confidence and reference to a predetermined design prevalence and diagnostic test characteristics. The level of confidence and the design prevalence will depend on the testing situation, disease and host population characteristics and on the resources available.

A single such survey can contribute evidence adding to an on-going collection of health data (see also Section 5. Specific requirements for complex non-survey data sources). However, single surveys in isolation rarely, if ever, provide sufficient evidence that an aquatic animal disease is absent and must be augmented with on-going targeted evidence collection (e.g. ongoing disease sampling or passive detection capabilities) to substantiate claims of freedom from disease.

3.2. Population

The *population* of *epidemiological units* must be clearly defined. The *target population* consists of all individuals of all species susceptible to the disease or infection in a country, zone or compartment to which the surveillance results apply. Sometimes components of the target population are at higher risk of being the point of introduction for an exotic disease. In these cases, it is advisable to focus surveillance efforts on this part of the population, such as farms on a geographical border.

The design of the survey will depend on the size and structure of the *population* being studied. If the *population* is relatively small and can be considered to be homogenous with regards to risk of infection, a single-stage survey can be used.

In larger *populations* where a sampling frame is not available, or when there is a likelihood of clustering of disease, multi-stage sampling is required. In two-stage sampling, at the first stage of sampling, groups of animals (e.g. ponds, farms or villages) are selected. At the second stage, animals are selected for testing from each of the selected groups.

In the case of a complex (e.g. multi-level) population structure, multi-level sampling (ref) may be used and the data analysed accordingly in survey design.

3.3. Sources of evidence

Surveillance data may originate [~~Evidence of freedom from infection may be based on a~~] from a number of different sources, including:

- structured, population-based surveys using one or more *tests* to detect [~~for the presence of~~] the agent;
- other surveillance, including structured non-random surveillance sources, such as:
 - sentinel sites;
 - disease notifications and laboratory investigation records;
 - academic and other scientific studies;
- a knowledge of the biology of the agent, including environmental, host *population* distribution, known geographical distribution, vector distribution and climatic information;
- history of imports of potentially infected material;
- biosecurity measures in place;
- [• ~~evaluation of the official services; or~~]
- any other sources of information that provide contributory evidence regarding disease or [~~that~~] infection is not present in the country, zone or compartment [~~aquaculture establishment~~].

The sources of evidence [~~used to demonstrate freedom from infection~~] must be fully described. In the case of a structured survey, this must include a description of the sampling strategy used for the selection of *units* for testing. For complex *surveillance systems*, a full description of the system is required including consideration of any biases that may be inherent in the system. Evidence to support claims of freedom of disease can use structured non-random sources of information provided any potential error is to detect rather than miss positive cases (i.e. it should be biased towards detection).

3.4. Statistical methodology

Analysis of *test* results from a survey shall be in accordance with the provisions of this chapter and consider the following factors:

- The survey design;
- The sensitivity and specificity of the *test*, or *test system*;
- The design prevalence (or prevalences where a multi-stage design is used);
- The results of the survey.

Analysis of data for evidence of freedom from infection involves estimating the probability (a) that the evidence observed (the results of surveillance) could have been produced under the null hypothesis that infection is present in the *population* at a specified prevalence(s) (the design prevalence[s]). The *confidence* in (or, equivalently, the sensitivity of) the *surveillance system* that produced the evidence is equal to 1-a. If the *confidence* level exceeds a pre-set threshold, the evidence is deemed adequate to demonstrate freedom from infection.

The required level of *confidence* in the *surveillance system* (probability that the system would detect infection if infection were present at the specified level) must be greater than or equal to 95%.

The power (probability that the system would report that no infection is present if infection is truly not present) may be set to any value. By convention, this is often set to 80%, but may be adjusted according to the country's or zone's requirements.

Different statistical methodologies for the calculation of the probability *a*, including both quantitative and qualitative approaches, are acceptable as long as they are based on accepted scientific principles.

The methodology used to calculate the *confidence* in the *surveillance system* must be scientifically based and clearly documented, including references to published work describing the methodology.

3.4. Clustering of infection

Infection in a country, zone or aquaculture establishment usually clusters rather than being uniformly distributed through a *population*. Clustering may occur at a number of different levels (e.g. a cluster of moribund fish in a pond, a cluster of ponds in a farm, or a cluster of farms in a zone). Except when dealing with demonstrably homogenous *populations*, approaches to demonstrating freedom must take this clustering into account in the design and the statistical analysis of the data, at least at what is judged to be the most significant level of clustering for the particular animal *population* and infection.

3.5. Design prevalence

Statistical analysis of surveillance data often requires assumptions about population parameters or test characteristics. These are usually based on expert opinion, previous studies on the same or different populations, expected biology of the agent, and so on. The uncertainty around these assumptions must be quantified and considered in the analysis (e.g. in the form of prior probability distributions in a Bayesian setting).

For surveillance systems used to demonstrate freedom from specific diseases, calculation of the *confidence* of a *surveillance system* is based on the null hypothesis that infection is present in the *population*. The level of infection is specified by the design prevalence. In the simplest case, this is the prevalence of infection in a homogenous *population*. More commonly, in the presence of a complex (e.g. multi-level) population structure ~~disease clustering, two more than one~~ design prevalence value is required, for instance, the animal-level prevalence (proportion of ~~fish~~ animals in an infected farm) and the group-level prevalence (proportion of infected farms in the country, zone or compartment ~~[aquaculture establishment]~~). Further levels of clustering may be considered, requiring further design prevalence values.

The values for design prevalence used in calculations must be those specified in the relevant disease chapter (if present) of this *Aquatic Manual*. If not specified for the particular disease, justification for the selection of design prevalence values must be provided, and should be based on the following guidelines:

- At the individual animal level, the design prevalence is based on the biology of the infection in the *population*. It is equal to the minimum expected prevalence of infection in the *study population*, if the infection had become established in that *population*. It is dependent on the dynamics of infection in the *population* and the definition of the *study population* (which may be defined to maximise the expected prevalence in the presence of infection).

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- A suitable design prevalence value at the animal level (e.g. prevalence of infected animals in a cage) may be:
 - between 1% and 5% for infections that are present in a small part of the population e.g. are transmitted slowly or are at the early stages of an outbreak, etc.; [and]
 - over 5% for highly transmissible infections [more contagious infections].

If reliable information on the expected prevalence in an infected population is not available, a value of 2% should be used for the design prevalence.

- At higher levels (e.g. cage, pond, farm, village, etc.) the design prevalence usually reflects the prevalence of infection that is practically and reasonably able to be detected by a *surveillance system*. Detection of infection at the lowest limit (a single infected *unit* in the *population*) is rarely feasible in large *populations*. The expected behaviour of the infection may also play a role. Infections that have the ability to spread rapidly between farms may have a higher farm-level design prevalence than slow-moving infections.

A suitable design prevalence value for the first level of clustering, (e.g. proportion of infected farms in a zone) may be up to 2%.

When surveillance data are used to estimate incidence and prevalence measures for the purpose of describing disease occurrence in terms of animal unit, time and place. These measures can be calculated for an entire population and specific time period, or for subsets defined by host characteristics (e.g. age-specific incidence). Incidence estimation requires on-going surveillance to detect new cases while prevalence is the estimated proportion of infected individuals in a population at a given time point. The estimation process must consider test sensitivity and specificity.

3.5. Clustering of infection

Infection in a country, zone or compartment [aquaculture establishment] usually clusters rather than being uniformly distributed through a *population*. Clustering may occur at a number of different levels (e.g. a cluster of moribund fish in a pond, a cluster of ponds in a farm, or a cluster of farms in a zone). Except when dealing with demonstrably homogenous *populations*, surveillance must take this clustering into account in the design and the statistical analysis of the data, at least at what is judged to be the most significant level of clustering for the particular animal *population* and infection.

~~[3.5. Expected prevalence]~~

3.6. Test characteristics

All surveillance involves performing one or more *tests* for evidence of the presence of current or past infection, ranging from detailed laboratory examinations to farmer observations. The performance level of a *test* at the *population* level is described in terms of its *sensitivity* and *specificity*. Imperfect sensitivity and/or specificity impact on the interpretation of surveillance results and must be taken into account in the analysis of surveillance data. For example, in the case of a test with imperfect specificity, if the population is free of disease or has a very low prevalence of infection, all or a large proportion of positive tests will be false. Subsequently, samples that test positive can be confirmed or refuted using a highly specific test. Where more than one test is used in a *surveillance system* (sometimes called using tests in series or parallel), the *sensitivity* and *specificity* of the test combination must be calculated [using a scientifically valid method].

All calculations must take the performance level (sensitivity and specificity) of any *tests* used into account. The values of sensitivity and specificity used for calculations must be specified, and the method used to determine or estimate these values must be documented. [Where] Test sensitivity and specificity can be different when applied to different populations and testing scenarios. For example, test sensitivity may be lower when testing carrier animals with low level infections compared to moribund animals with clinical disease. Alternatively, specificity depends on the presence of cross-reacting agents, the distribution of which may be different under different conditions or regions. Ideally, test performance should be assessed under the conditions of use otherwise increased uncertainty exists regarding their performance. In the absence of local assessment of tests, values for sensitivity and/or specificity for a particular test that are specified in this Aquatic Manual may be used but the increased uncertainty associated with these estimates should be incorporated into the analysis of results [these values may be used without justification].

Pooled testing involves the pooling of specimens from multiple individuals and performing a single *test* on the pool. Pooled testing is an acceptable approach in many situations. Where pooled testing is used, the results of testing must be interpreted using sensitivity and specificity values that have been determined or estimated for that particular pooled testing procedure and for the applicable pool sizes being used. Analysis of the results of pooled testing must, where possible, be performed using accepted, statistically based methodologies, which must be fully documented, including published references.

3.7. Multiple sources of information [evidence]

Where multiple different data sources providing evidence of freedom from infection exist [or are generated], each of these data sources may be analysed accordingly [to the provisions of Sections B.3, B.4 (for structured surveys) and B.5 (for complex data sources)]. The resulting estimates of the *confidence* in each data source may be combined to provide an overall level of *confidence* for the combined data sources.

The methodology used to combine the estimates from multiple data sources:

- must be scientifically valid, and fully documented, including references to published material; and
- should, where possible, take into account any lack of statistical independence between different data sources.

Surveillance information gathered from the same country, zone or compartment at different times (e.g. repeated annual surveys) may provide cumulative evidence of animal health status. Such evidence gathered over time may be combined to provide an overall level of confidence. However, a single larger survey, or the combination of data collected during the same time period from multiple random or non-random sources, may be able to achieve the same level of confidence in a shorter period of time.

[Surveillance information gathered from the same country, zone or aquaculture establishment at different times may provide cumulative evidence of freedom from infection. Such evidence gathered over time may be combined into an overall level of confidence. For instance, repeated annual surveys may be analysed to provide a cumulative level of confidence. However, a single (larger) survey may be able to achieve the same level of confidence in just 1 year.]

Analysis of surveillance information gathered intermittently or continuously over time should, where possible, incorporate the time of collection of the information to take into account the decreased value of older information. The sensitivity, specificity and completeness of data from each source should also be taken into account for the final overall confidence level estimation.

3.8. Survey design

The most important unit of diagnosis is the epidemiological unit.]

3.8. Sampling

The objective of sampling from a *population* is to select a subset of *units* from the *population* that is representative of the *population* with respect to the characteristic of interest (in this case, the presence or absence of infection). The survey design may involve sampling at several levels. For sampling at the level of the *epidemiological units* or higher *units*, a formal *probability sampling* (e.g. simple random sampling) method must be used. Sampling should be carried out in such a way as to provide the best likelihood that the sample will be representative of the *population*, within the practical constraints imposed by different environments and production systems.

~~3.9. Sampling methods~~

When sampling below the level of the *epidemiological unit* (e.g. individual animal), the sampling method used should provide the best practical chance of generating a sample that is representative of the *population* of the chosen *epidemiological unit*. Collecting a truly representative sample of individual animals (whether from a pond, cage or fishery) is often very difficult. To maximise the chance of finding infection, the aim should be to bias the sampling towards infected animals, e.g. selecting moribund animals, life stages with a greater chance of active infection, etc.

Biased or targeted sampling in this context involves sampling from a defined *study population* that has a different probability of infection than the *target population* of which it is a subpopulation. Once the *study population* has been identified, the objective is still to select a representative sample from this subpopulation.

The sampling method used at all levels must be fully documented and justified.

3.9. Sample size

The number of *units* to be sampled from a *population* should be calculated using a statistically valid technique that takes at least the following factors into account:

- The sensitivity and specificity of the *diagnostic test*, or *test system*;
- The design prevalence (or prevalences where a multi-stage design is used);
- The level of *confidence* that is desired of the survey results.

Additionally, other factors may be considered in sample size calculations, including (but not limited to):

- The size of the *population* (but it is acceptable to assume that the *population* is infinitely large);
- The desired power of the survey;
- Uncertainty about [or variability in estimates of] sensitivity and specificity.

The specific sampling requirements will need to be tailor-made for each individual disease, taking into account its characteristics and the specificity and sensitivity of the accepted testing methods for detecting the disease agent in host populations.

FreeCalc⁷ is a suitable software for the calculation of sample sizes at varying parameter values. The table below provides examples of sample sizes generated by the software for a type I and type II error of 5% (i.e. 95% confidence and 95% statistical power). However, this does not mean that a type 1 and type 2 error of 0.05 should always be used. For example, using a test with sensitivity and specificity of 99%, 528 units should be sampled. If 9 or less of those units test positive, the population can still be considered free of the disease at a design prevalence of 2% provided that all effort is made to ensure that all presumed false positives are indeed false. This means that there is a 95% confidence that the prevalence is 2% or lower.

In the case in which the values of Se and Sp are not known (e.g. no information is available in the specific disease chapter in the *Aquatic Manual*), they should not automatically be assumed to be 100%. All positive results should be included and discussed in any report regarding that particular survey and all efforts should be made to ensure that all presumed false positives are indeed false.

⁷ FreeCalc – Cameron, AR. Software for the calculation of sample size and analysis of surveys to demonstrate freedom from disease. Available for free download from <http://www.ausvet.com.au>.

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Design prevalence	Sensitivity (%)	Specificity (%)	Sample size	Maximum number of false +ve if the population is free
2	100	100	149	0
2	100	99	524	9
2	100	95	1671	98
2	99	100	150	0
2	99	99	528	9
2	99	95	1707	100
2	95	100	157	0
2	95	99	542	9
2	95	95	1854	108
2	90	100	165	0
2	90	99	607	10
2	90	95	2059	119
2	80	100	186	0
2	80	99	750	12
2	80	95	2599	148
5	100	100	59	0
5	100	99	128	3
5	100	95	330	23
5	99	100	59	0
5	99	99	129	3
5	99	95	331	23
5	95	100	62	0
5	95	99	134	3
5	95	95	351	24
5	90	100	66	0
5	90	99	166	4
5	90	95	398	27
5	80	100	74	0
5	80	99	183	4
5	80	95	486	32
10	100	100	29	0
10	100	99	56	2
10	100	95	105	9
10	99	100	29	0
10	99	99	57	2
10	99	95	106	9
10	95	100	30	0
10	95	99	59	2
10	95	95	109	9
10	90	100	32	0
10	90	99	62	2
10	90	95	123	10
10	80	100	36	0
10	80	99	69	2
10	80	95	152	12

[Detailed guidelines are to be provided in the next (fifth) edition of the *Aquatic Manual*. In the meantime, the sampling procedures given in Chapters I.1, I.2 and I.3 may be applied.]

3.10. Quality assurance

Surveys should include a documented quality assurance system, to ensure that field and other procedures conform to the specified survey design. Acceptable systems may be quite simple, as long as they provide verifiable documentation of procedures and basic checks to detect significant deviations of procedures from those documented in the survey design.

4. Specific requirements for complex non-survey data sources for freedom from disease

Data sources that provide evidence of freedom from infection, but are not based on structured population-based surveys may also be used to demonstrate freedom, either alone or in combination with other data sources. Different methodologies may be used for the analysis of such data sources, but the methodology must comply with the provisions of Section B.3. The approach used should, where possible, also take into account any lack of statistical independence between observations.

Analytical methodologies based on the use of step-wise probability estimates to describe the *surveillance system* may determine the probability of each step either by:

- the analysis of available data, using a scientifically valid methodology; or where no data are available,
- the use of estimates based on expert opinion, gathered and combined using a formal, documented and scientifically valid methodology.

Where there is significant uncertainty and/or variability in estimates used in the analysis, stochastic modelling or other equivalent techniques should be used to assess the impact of this uncertainty and/or variability on the final estimate of confidence.

5. Specific requirements for structured survey design and analysis to assess disease occurrence

This section describes surveillance to estimate parameters of disease occurrence.

5.1. Objectives

The objective of this kind of surveillance system is to contribute on an on-going basis evidence to assess the occurrence and distribution of disease or infection in a particular country, zone or compartment. This will provide information for domestic disease control programmes and relevant disease occurrence information to be used by trading partners for qualitative and quantitative risk assessment.

A single such survey can contribute evidence adding to an on-going collection of health data (see also Section 5. Specific requirements for complex non-survey data sources).

5.2. Population

The population of epidemiological units must be clearly defined. The target population consists of all individuals of all species susceptible to the disease or infection in a country, zone or compartment to which the surveillance results apply. Some local areas within a region may be known to be free of the disease of concern, allowing resources to be concentrated on known positive areas for greater precision of prevalence estimates and only verification of expected 0 prevalence areas.

Appendix XXX (contd)

The design of the survey will depend on the size and structure of the *population* being studied. If the *population* is relatively small and can be considered to be homogenous with regards to risk of infection, a single-stage survey can be used.

In larger *populations* where a sampling frame is not available, or when there is a likelihood of clustering of disease, multi-stage sampling is required. In two-stage sampling, at the first stage of sampling, groups of animals (e.g. ponds, farms or villages) are selected. At the second stage, animals are selected for testing from each of the selected groups.

In the case of a complex (e.g. multi-level) population structure, multi-level sampling (ref) may be used and the data analysed accordingly.

5.3. Sources of evidence

Surveillance data may originate from a number of different sources, including:

- structured, population-based surveys using one or more *tests* to detect the agent:
- other structured non-random sources, such as:
 - sentinel sites:
 - disease notifications and laboratory investigation records:
 - academic and other scientific studies:
- a knowledge of the biology of the agent, including environmental, host *population* distribution, known geographical distribution, vector distribution and climatic information:
- history of imports of potentially infected material:
- biosecurity measures in place:
- any other sources of information that provide contributory evidence regarding disease or infection in the country, zone or compartment.

The sources of evidence must be fully described. In the case of a structured survey, this must include a description of the sampling strategy used for the selection of *units* for testing. For complex *surveillance systems*, a full description of the system is required including consideration of any biases that may be inherent in the system. Evidence to support changes in prevalence/incidence of endemic disease must be based on valid, reliable methods to generate precise estimates with known error.

5.4. Statistical methodology

Analysis of survey data should be in accordance with the provisions of this chapter and should consider the following factors:

- The survey design:
- The sensitivity and specificity of the *test*, or *test system*:
- The results of the survey.

For surveillance systems used to describe disease patterns, the purpose is to estimate prevalence or incidence with confidence intervals or probability intervals. The magnitude of these intervals expresses the precision of the estimates and is related to sample size. Narrow intervals are desirable but will require larger sample sizes and more dedication of resources. The precision of the estimates and the power to detect differences in prevalence between populations or between time points depends not only on sample size, but also on the actual value of the prevalence in the population or the actual difference. For this reason, when designing the surveillance system, a prior estimate/assumption of expected prevalence or expected difference in prevalence must be made.

For the purpose of describing disease occurrence, measures of animal unit, time and place can be calculated for an entire population and specific time period, or for subsets defined by host characteristics (e.g. age-specific incidence). Incidence estimation requires on-going surveillance to detect new cases in a specified time period while prevalence is the estimated proportion of infected individuals in a population at a given time point. The estimation process must consider test sensitivity and specificity.

Statistical analysis of surveillance data often requires assumptions about population parameters or test characteristics. These are usually based on expert opinion, previous studies on the same or different populations, expected biology of the agent, information contained in the specific disease chapter of the *Aquatic Manual*, and so on. The uncertainty around these assumptions must be quantified and considered in the analysis (e.g. in the form of prior probability distributions in a Bayesian setting).

When surveillance objectives are to estimate prevalence/incidence or changes in disease patterns, statistical analysis must account for sampling error. Analytic methods should be thoroughly considered and consultation with biostatistician/quantitative epidemiologist consulted beginning in the planning stages and continued throughout the programme.

5.5. Clustering of infection

Infection in a country, zone or compartment usually clusters rather than being uniformly distributed through a *population*. Clustering may occur at a number of different levels (e.g. a cluster of moribund fish in a pond, a cluster of ponds in a farm, or a cluster of farms in a zone). Except when dealing with demonstrably homogenous *populations*, surveillance must take this clustering into account in the design and the statistical analysis of the data, at least at what is judged to be the most significant level of clustering for the particular animal *population* and infection. For endemic diseases, it is important to identify characteristics of the population which contribute to clustering and thus provide efficiency in disease investigation and control.

5.6. Test characteristics

All surveillance involves performing one or more *tests* for evidence of the presence of current or past infection, ranging from detailed laboratory examinations to farmer observations. The performance level of a *test* at the *population* level is described in terms of its *sensitivity* and *specificity*. Imperfect sensitivity and/or specificity impact on the interpretation of surveillance results and must be taken into account in the analysis of surveillance data. For example, in populations with low prevalence of infection, a large proportion of positive tests may be false unless the tests used have perfect specificity. To ensure detection in such instances, a highly sensitive test is frequently used for initial screening and then confirmed with highly specific tests.

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All calculations must take the performance level (sensitivity and specificity) of any tests used into account. The values of sensitivity and specificity used for calculations must be specified, and the method used to determine or estimate these values must be documented. Test sensitivity and specificity can be different when applied to different populations and testing scenarios. For example, test sensitivity may be lower when testing carrier animals with low level infections compared to moribund animals with clinical disease. Alternatively, specificity depends on the presence of cross-reacting agents, the distribution of which may be different under different conditions or regions. Ideally, test performance should be assessed under the conditions of use otherwise increased uncertainty exists regarding their performance. In the absence of local assessment of tests, values for sensitivity and/or specificity for a particular test that are specified in this *Aquatic Manual* may be used but the increased uncertainty associated with these estimates should be incorporated into the analysis of results.

Pooled testing involves the pooling of specimens from multiple individuals and performing a single test on the pool. Pooled testing is an acceptable approach in many situations. Where pooled testing is used, the results of testing must be interpreted using sensitivity and specificity values that have been determined or estimated for that particular pooled testing procedure and for the applicable pool sizes being used. Analysis of the results of pooled testing must, where possible, be performed using accepted, statistically based methodologies, which must be fully documented, including published references.

Test results from surveillance for endemic disease will provide estimates of apparent prevalence (AP). Using diagnostic sensitivity (DSe) and diagnostic specificity (DSp) as described in chapter 1.1.2 of this *Aquatic Manual*, true prevalence (TP) should be calculated with the following formula:

$$TP = (AP + DSp - 1) / (DSe + DSp - 1)$$

In addition, it should be remembered that different laboratories may obtain conflicting results for various test, host, or procedure-related reasons. Therefore, sensitivity and specificity parameters should be validated for the particular laboratory and process.

5.7. Multiple sources of information

Where multiple different data sources providing information on infection or disease are generated, each of these data sources may be analysed and presented separately.

Surveillance information gathered from the same country, zone or compartment at different times and similar methodology (e.g. repeated annual surveys) may provide cumulative evidence of animal health status and changes. Such evidence gathered over time may be combined (e.g. using Bayesian methodology) to provide more precise estimates and details of disease distribution within a population.

Apparent changes in disease occurrence of endemic diseases may be real or due to other factors influencing detection proficiency.

5.8. Sampling

The objective of sampling from a population is to select a subset of units from the population that is representative of the population with respect to the characteristic of interest (in this case, the presence or absence of infection). The survey design may involve sampling at several levels. For sampling at the level of the epidemiological units or higher units a formal probability sampling (e.g. simple random sampling) method must be used. Sampling should be carried out in such a way as to provide the best likelihood that the sample will be representative of the population, within the practical constraints imposed by different environments and production systems.

When sampling below the level of the *epidemiological unit* (e.g. individual animal), the method used should be probability-based sampling. Collecting a true probability-based sample is often very difficult and care should therefore be taken in the analysis and interpretation of results obtained using any other method, the danger being that inferences could not be made about the sampled *population*.

The sampling method used at all levels must be fully documented and justified.

5.9. Sample size

The number of *units* to be sampled from a *population* should be calculated using a statistically valid technique that takes at least the following factors into account:

- The sensitivity and specificity of the *diagnostic test* (single or in combination):
- Expected prevalence or incidence in the *population* (or prevalences/incidences where a multi-stage design is used):
- The level of *confidence* that is desired of the survey results.
- The *precision* desired (i.e. the width of the *confidence* or *probability intervals*).

Additionally, other factors may be considered in sample size calculations, including (but not limited to):

- The size of the *population* (but it is acceptable to assume that the *population* is infinitely large):
- Uncertainty about sensitivity and specificity.

The specific sampling requirements will need to be tailor-made for each individual disease, taking into account its characteristics and the specificity and sensitivity of the accepted testing methods for detecting the disease agent in host populations.

A number of software packages, e.g. Survey Tool Box, WinPEPI (add links and refs) can be used for the calculation of sample sizes.

In the case in which the values of *Se* and *Sp* are not known (e.g. no information is available in the specific disease chapter in the *Aquatic Manual*), they should not automatically be assumed to be 100%. Assumed values should be produced in consultation with subject-matter experts.

5.10. Quality assurance

Surveys should include a documented quality assurance system, to ensure that field and other procedures conform to the specified survey design. Acceptable systems may be quite simple, as long as they provide verifiable documentation of procedures and basic checks to detect significant deviations of procedures from those documented in the survey design.

PART 2**6. EXAMPLE SURVEILLANCE SYSTEMS FOR FREEDOM FROM DISEASE**

The following examples describe surveillance systems and approaches to the analysis of evidence **for demonstrating freedom from disease** [that are able to meet the requirements of this chapter]. The purpose of these examples is:

- to illustrate the range of approaches that may be acceptable;
- to provide practical guidance and models that may be used for the design of specific surveillance systems; and
- to provide references to available resources that are useful in the development and analysis of surveillance systems.

While these examples demonstrate ways in which freedom from infection may be successfully demonstrated, they are not intended to be prescriptive. Countries are free to use different approaches, as long as they meet the requirements of this chapter.

The examples deal with the use of structured surveys and are designed to illustrate different survey designs, sampling schemes, the calculation of sample size, and analysis of results. It is important to note that alternative approaches to demonstrating freedom using complex non-survey-based data sources are also currently being developed and may soon be published⁸.

Example 1 – one-stage structured survey (farm **certification [accreditation])****Context**

A freshwater aquaculture industry raising fish in tanks has established a farm **certification** [accreditation] scheme. This involves demonstrating farm-level freedom from a particular (hypothetical) disease (Disease X). The disease does not spread very quickly, and is most common during the winter months, with adult fish at the end of the production cycle being most severely affected. Farms consist of a number of grow-out tanks, ranging from 2 to 20, and each tank holds between 1000 and 5000 fish.

Objective

The objective is to implement surveillance that is capable of providing evidence that an individual farm is free from Disease X. (The issue of national or zone freedom, as opposed to farm freedom, is considered in the next example.)

⁸ International EpiLab, Denmark, Research Theme 1: Freedom from disease.
http://www.vetinst.dk/high_uk.asp?page_id=196

Approach

The accreditation scheme establishes a set of standard operating procedures and requirements for recognition of freedom, based on the guidelines given in this chapter. These require farms to undertake a structured survey capable of producing 95% confidence that the disease would be detected if it were present. Once farms have been surveyed without detecting disease, they are recognised as free, as long as they maintain a set of minimum biosecurity standards. These standards are designed to prevent the introduction of Disease X into the farm (through the implementation of controls specific to the method of spread of that disease) and to ensure that the disease would be detected rapidly if it were to enter the farm (based on evidence of adequate health record keeping and the prompt investigation of unusual disease events). The effective implementation of these biosecurity measures is evaluated with annual on-farm audits conducted by independent auditors.

Survey standards

Based on the guidelines given in this chapter, a set of standards are established for the conduct of surveys to demonstrate freedom from infection with causative agent of Disease X. These standards include:

- The level of confidence required of the survey is 95% (i.e. Type I error = 5%).
- The power of the survey is arbitrarily set at 95% (i.e. Type II error = 5%, which means that there is a 5% chance of concluding that a non-diseased farm is infected).
- The target population is all the fish on the farm. Due to the patterns of disease in this production system, in which only fish in the final stages of grow-out, and only in winter are affected, the study population is defined as grow-out fish during the winter months.
- The issue of clustering is considered. As fish are grouped into tanks, this is the logical level at which to consider clustering. However, when a farm is infected, the disease often occurs in multiple tanks, so there is little evidence of strong clustering. Also, the small number of tanks on a single farm means that it is difficult to define a design prevalence at the tank level (i.e. the proportion of infected tanks that the survey should be able to detect on the farm). For these reasons, it is decided to treat the entire grow-out population of each farm as a single homogenous population.
- Stratification is also considered. In order to ensure full representation, it is decided to stratify the sample size by tank, proportional to the population of each tank.
- The design prevalence at the animal level is determined based on the epidemiology of the disease. The disease does not spread quickly, however, in the defined target population, it has been reported to affect at least 10% of fish, if the population is infected. In order to take the most conservative approach, an arbitrarily low design prevalence of 2% is used. A prevalence of 10% may have been used (and would result in a much smaller sample size), but the authorities were not convinced by the thought that the population could still be infected at a level of say 5%, and disease still not be detected.
- The test used involves destructive sampling of the fish, and is based on an antigen-detection enzyme-linked immunosorbent assay (ELISA). Disease X is present in some parts of the country (hence the need for a farm-level accreditation programme). This has provided the opportunity for the sensitivity and the specificity of the ELISA to be evaluated in similar populations to those on farms. A recent study (using a combination of histology and culture as a gold standard) estimated the sensitivity of the ELISA to be 98% (95% confidence interval 96.7–99.2%), and the specificity to be 99.4% (99.2–99.6%). Due to the relatively narrow confidence intervals, it was decided to use the point estimates of the sensitivity and specificity rather than complicate calculations by taking the uncertainty in those estimates into account.

Sample size

The sample size required to meet the objectives of the survey is calculated to take the population size, the test performance, the confidence required and the design prevalence into account. As the population of each farm is relatively large, differences in the total population of each farm have little effect on the calculated sample size. The other parameters for sample size calculation are fixed across all farms. Therefore, a standard sample size (based on the use of this particular ELISA, in this population) is calculated. The sample size calculations are performed using the *FreeCalc* software⁹. Based on the parameters listed above, the sample size required is calculated to be 410 fish per farm. In addition, the program calculates that, given the imperfect specificity, it is still possible for the test to produce up to five false-positive reactors from an uninfected population using this sample size. The authorities are not comfortable with dealing with false-positive reactors, so it is decided to change the test system to include a confirmatory test for any positive reactors. Culture is selected as the most appropriate test, as it has a specificity that is considered to be 100%. However, its sensitivity is only 90% due to the difficulty of growing the organism.

As two tests are now being used, the performance of the test system must be calculated, and the sample size recalculated based on the test system performance.

Using this combination of tests (in which a sample is considered positive only if it tests positive to both tests), the specificity of the combined two tests can be calculated by the formula:

$$Sp_{Combined} = Sp_1 + Sp_2 - (Sp_1 \times Sp_2)$$

which produces a combined specificity of $1 + 0.994 - (1 \times 0.994) = 100\%$

The sensitivity may be calculated by the formula:

$$Se_{Combined} = Se_1 \times Se_2$$

which produces a combined sensitivity of $0.9 \times 0.98 = 88.2\%$

These new values are used to calculate the survey sample size yielding a result of 169 fish. It is worth noting that attempts to improve the performance of a test (in this case increase specificity) generally result in a decrease in the performance of the other aspect of the test performance (sensitivity in this example). However, in this case, the loss of sensitivity is more than compensated for by the decreased sample size due to the improved specificity.

It is also worth noting that, when using a test system with 100% specificity, the effective power of the survey will always be 100%, regardless of the figure used in the design. This is because it is not possible to make a Type II error, and conclude that the farm is infected when it is not.

A check of the impact of population size on the calculated sample size is worthwhile. The calculated sample size is based on an infinitely large population. If the population size is smaller, the impact on sample size is shown in the following table:

⁹ FreeCalc – Cameron, AR. Software for the calculation of sample size and analysis of surveys to demonstrate freedom from disease. Available for free download from <http://www.ausvet.com.au>.

Population size	Sample size
1000	157
2000	163
5000	166
10,000	169

Based on these calculations, it is clear that, for the population sizes under consideration, there is little effect on the sample size. For the sake of simplicity, a standard sample size of 169 is used, regardless of the number of grow-out fish on the farm.

Sampling

The selection of individual fish to include in the sample should be done in such a manner as to give the best chance of the sample being representative of the study population. A fuller description of how this may be achieved under different circumstances is provided in *Survey Toolbox*¹⁰. An example of a single farm will be used to illustrate some of the issues.

One farm has a total of eight tanks, four of which are used for grow-out. At the time of the survey (during winter), the four grow-out tanks have 1850, 4250, 4270 and 4880 fish, respectively, giving a total population of 15,250 grow-out fish.

Simple random sampling from this entire population is likely to produce sample sizes from each tank roughly in proportion to the number of fish in each tank. However, proportional stratified sampling will guarantee that each tank is represented in proportion. This simply involves dividing the sample size between tanks in proportion to their population. The first tank has 1850 fish out of a total of 15,250, representing 12.13%. Therefore 12.13% of the sample (21 fish) should be taken from the first tank. Using a similar approach the sample size for the other three tanks is 47, 47 and 54 fish, respectively.

Once the sample for each tank is determined, the problem remains as to how to select 21 fish from a tank of 1850 so that they are representative of the population. Several options exist.

- If the fish can be handled individually, random systematic sampling may be used. This is likely to be the case if, for example:
 - fish are harvested during winter and samples can be collected at harvest; or
 - routine management activities involving handling the fish (such as grading or vaccination) are conducted during the winter.

If fish are handled, systematic sampling simply involves selecting a fish at regular intervals. For instance, to select 21 from 1850, the sampling interval should be $1850/21 = 88$. This means that every 88th fish from the tank should be sampled. To ensure randomness, it is good practice to use a random number between 1 and 88 (in this case) to select the *first* fish (e.g. using a random number table), and then select every 88th fish after that.

¹⁰ Survey Toolbox for Aquatic Animal Diseases – A Practical Manual and Software Package. Cameron A.R. (2002). Australian Centre for International Agricultural Research (ACIAR), Monograph No. 94, 375 pp. ISBN 1 86320 350 8. Printed version available from ACIAR <http://www.aciar.gov.au> Electronic version available for free download from <http://www.ausvet.com.au>.

Appendix XXX (contd)

- If fish cannot be handled individually (by far the most common, and more difficult, circumstance) then the fish to be sampled must be captured from the tanks. Fish should be captured in the most efficient and practical way possible, however every effort should be made to try to ensure that the sample is representative. In this example, a dip net is the normal method used for capturing fish. Using a dip net, convenience sampling would involve capturing 21 fish by repeatedly dipping at one spot and capturing the easiest fish (perhaps the smaller ones). This approach is strongly discouraged. One method of increasing the representativeness is to sample at different locations in the tank – some at one end, some at either side, some at the other end, some in the middle, some close to the edge. Additionally, if there are differences among the fish, an attempt should be made to capture fish in such a way as to give different groups of fish a chance of being caught (i.e. do not just try to catch the small ones, but include big ones as well).

This method of collecting a sample is far from the ideal of random sampling, but due to the practical difficulties of implementing random sampling of individual fish, this approach is acceptable, as long as the efforts made to increase the representativeness of the sample are both genuine and fully documented.

Testing

Specimens are collected, processed and tested according to standardised procedures developed under the certification ~~(accreditation)~~ programme and designed to meet the requirements of this *Aquatic Manual*. The testing protocol dictates that any specimens that test positive to ELISA be submitted for culture, and that any positive culture results indicate a true positive specimen (i.e. that the farm is not free from disease). It is important that this protocol be adhered to exactly. If a positive culture is found, then it is not acceptable to retest it, unless further testing is specified in the original testing protocol, and the impact of such testing accounted for in the test system sensitivity and specificity estimates (and therefore the sample size).

Analysis

If the calculated sample size of 169 is used, and no positive reactors are found, then the survey will have a confidence of 95%. This can be confirmed by analysing the results using the *FreeCalc* software mentioned above (which reports a confidence level of 95.06%).

It may happen in some cases that the survey is not conducted exactly as planned, and the actual sample size is less than the target sample size. However, the size of the farm may also be smaller. In these cases, it is advisable to analyse the farm data on a farm-by-farm basis. For example, if only 165 specimens were collected from a farm with only 2520 fish, the resulting confidence would still be 95%. If only 160 fish were collected, the confidence is only 94.5%. If a rigid target of 95% confidence is used, then this survey would fail to meet that target and more evidence would be required.

Example 2 – two-stage structured survey (national freedom)**Context**

A country aims to declare freedom from Disease Y of crustaceans. The industry in this country is based largely on small-holder ponds, grouped closely together in and around villages. The disease is reasonably highly contagious, and causes mass mortality mid to late in the production cycle, with affected animals becoming moribund and dying in a matter of days. Affected animals show few characteristic signs, but an infected pond will almost invariably break down with mass mortality unless harvested beforehand. It is more common in late summer, but can occur at any time of year. It also occurs occasionally early in the production cycle. In this country, there are some limitations to the availability of laboratory facilities and the transport infrastructure. However, there is a relatively large government structure, and a comprehensive network of fisheries officers.

Objective

The objective is to establish national freedom from Disease Y. The surveillance system must meet the requirements of [Part 4 of] this chapter, but must also be able to be practically implemented in this small-holder production system.

Approach

The aquaculture authorities decide to use a survey to gather evidence of freedom, using a two-stage survey design (sampling villages at the first level, and ponds at the second). Laboratory testing of specimens from a large number of farms is not considered feasible, so a combined test system is developed to minimise the need for expensive laboratory tests.

The unit of observation and analysis is, in this case, the pond, rather than the individual animal. This means that the diagnosis is being made at the pond level (an infected pond or a non-infected pond) rather than at the animal level.

The survey is therefore a survey to demonstrate that no villages are infected (using a random sample of villages and making a village-level diagnosis). The test used to make a village-level diagnosis is, in fact, another survey, this time to demonstrate that no ponds in the village are affected. A test is then performed at the pond level (farmer observation followed, if necessary, by further laboratory testing).

Survey standards

- The confidence to be achieved by the survey is 95%. The power is set at 95% (but is likely to be virtually 100% if the test system used achieves nearly 100% specificity, as demonstrated in the previous example).
- The target population is all ponds stocked with shrimp in the country during the study period. The study population is the same, except that those remote areas to which access is not possible are excluded. As outbreaks can occur at any time of year, and at any stage of the production cycle, it is decided not to further refine the definition of the population to target a particular time or age.
- Three tests are used. The first is farmer observation, to determine if mass mortality is occurring in a particular pond. If a pond is positive to the first test (i.e. mass mortality is detected), a second test is applied. The second test used is polymerase chain reaction (PCR). Cases positive to PCR are further tested using transmission experiments.
 - Farmer observation can be treated as a test just like any other. In this case, the observation of mass mortality is being used as a test for the presence of Disease Y. As there are a variety of other diseases that are capable of causing mass mortality, the test is not very specific. On the other hand, it is quite unusual for Disease Y to be present, and not result in mass mortality, so the test is quite sensitive. A standard case definition is established for 'mass mortality' (for instance, greater than 20% of the pond's population of shrimp observed dead in the space of less than 1 week). Based on this definition, farmers are able to 'diagnose' each pond as having mass mortality. Some farmers may be over-sensitive and decide that mass mortality is occurring when only a small proportion of shrimp are found dead (false positives, leading to a decrease in specificity) while a small number of others fail to recognise the mortalities, decreasing sensitivity.

In order to quantify the sensitivity and specificity of farmer observation of mass mortalities, as a test for Disease Y, a separate study is carried out. This involves both a retrospective study of the number of mass mortality events in a population that is thought to be free from disease, as well as a study of farmers presented with a series of mortality scenarios, to assess their ability to accurately identify a pond with mass mortality. By combining these results, it is estimated that the sensitivity of farmer-reported mass mortalities as a test for Disease Y is 87% while the specificity is 68%.

Appendix XXX (contd)

- When a farmer detects a pond with mass mortality, specimens are collected from moribund shrimp following a prescribed protocol. Tissue samples from 20 shrimp are collected, and pooled for PCR testing. In the laboratory, the ability of pooled PCR to identify a single infected animal in a pool of 20 has been studied, and the sensitivity of the procedure is 98.6%. A similar study of negative specimens has shown that positive results have occasionally occurred, probably due to laboratory contamination, but maybe also because of the presence of non-viable genetic material from another source (shrimp-based feed stuffs are suspected). The specificity is therefore estimated at 99%.
- Published studies in other countries have shown that the sensitivity of transmission tests, the third type of test to be used, is 95%, partly due to variability in the load of the agent in inoculated material. The specificity is agreed to be 100%.
- Based on these figures, the combined test system sensitivity and specificity are calculated using the formulae presented in Example 1, first with the first two tests, and then with the combined effect of the first two tests and the third test. The result is a sensitivity of 81.5% and a specificity of 100%.
- The design prevalence must be calculated at two levels. First, the pond-level design prevalence (the proportion of ponds in a village that would be infected if disease were present) is determined. In neighbouring infected countries, experience has shown that ponds in close contact with each other are quickly infected. It is unusual to observe an infected village with fewer than 20% of ponds infected. Conservatively, a design prevalence of 5% is used. The second value for design prevalence applies at the village level, or the proportion of infected villages that could be identified by the survey. As it is conceivable that the infection may persist in a local area without rapid spread to other parts of the country, a value of 1% is used. This is considered to be the lowest design prevalence value for which a survey can be practically designed.
- The population of villages in the country is 65,302, according to official government records. Those with shrimp ponds number 12,890, based on records maintained by the aquaculture authorities. These are generated through a five-yearly agricultural census, and updated annually based on reports of fisheries officers. There are no records available of the number of ponds in each of these villages.

Sample size

Sample size is calculated for the two levels of sampling, first the number of villages to be sampled and then the number of ponds to be sampled. The number of villages to be sampled depends on the sensitivity and the specificity of the test used to classify villages as infected or not infected. As the 'test' used in each village is really just another survey, the sensitivity is equal to the confidence and the specificity is equal to the power of the village-level survey. It is possible to adjust both confidence and power by changing the sample size in the village survey (number of ponds examined), which means that we can determine, within certain limits, what sensitivity and specificity we achieve.

This allows a flexible approach to sample size calculation. If a smaller first-stage sample size is desired (a small number of villages), a high sensitivity and specificity are needed, which means that the number of ponds in each village that need to be examined is larger. A smaller number of ponds will result in lower sensitivity and specificity, requiring a larger number of villages. The approach to determining the optimal (least cost) combination of first- and second-stage sample sizes is described in *Survey Toolbox*.

A further complication is presented by the fact that each village has a different number of ponds. In order to achieve the same (or similar) confidence and power (sensitivity and specificity) for each village, a different sample size may be required. The authorities choose to produce a table of sample sizes for the number of ponds to sample in each village, based on the total ponds in each village.

An example of one possible approach to determining the sample size follows:

The target sensitivity (confidence) achieved by each village-level survey is 95%. The target specificity is 100%. Using the *FreeCalc* software, with a design prevalence of 1% (the survey is able to detect disease if 1% or more villages are infected), the first-stage sample size is calculated as 314 villages. Within each village, the test used is the combined test system described above with a sensitivity of 81.5% and a specificity of 100%. Based on these figures the following table is developed, listing the number of ponds that need to be sampled in order to achieve 95% sensitivity.

Population	Sample size
30	29
40	39
60	47
80	52
100	55
120	57
140	59
160	61
180	62
200	63
220	64
240	64
260	65
280	65
300	66
320	66
340	67
360	67
380	67
400	67
420	68
440	68
460	68
480	68
500	68
1000	70

Sampling

First-stage sampling (selection of villages) is done using random numbers and a sampling frame based on the fisheries authorities list of villages with shrimp ponds. The villages are listed on a spreadsheet with each village numbered from 1 to 12,890. A random number table (such as that included in *Survey Toolbox*) or software designed for the generation of random numbers (such as EpiCalc¹¹) is used.

The second stage of sampling involves random selection of ponds within each village. This requires a sampling frame, or list of each pond in the village. The fisheries authorities use trained local fisheries officers to coordinate the survey. For each selected village, the officer visits the village and convenes a meeting of all shrimp farmers. At the meeting, they are asked how many ponds they have and a list of farmers' names and the number of ponds is compiled. A simple random sample of the appropriate number of ponds (between 29 and 70, from the table above, depending on the number of ponds in the village) is selected from this list. This is done either using software (such as Survey Toolbox's *RandomAnimal* program), or manually with a random number table or decimal dice for random number selection. Details of this process are described in *Survey Toolbox*. This selection process identifies a particular pond in terms of the name of the owner, and the sequence number amongst the ponds owned (e.g. Mr Smith's 3^d pond). Identification of the actual pond is based on the owners own numbering system for the ponds.

Testing

Once ponds have been identified, the actual survey consists of 'testing those ponds'. In practice, this involves the farmers observing the ponds during one complete production cycle. The local fisheries officer makes weekly visits to each farmer to check if any of the selected ponds have suffered mass mortality. If any are observed (i.e. the first test is positive), 20 moribund shrimp are collected for laboratory examination (first PCR, and then, if positive, transmission experiments).

Analysis

Analysis is performed in two stages. First, the results from each village are analysed to ensure that they meet the required level of confidence. If the target sample size is achieved (and only negative results obtained), the confidence should be 95% or greater in each village. At the second stage, the results from each village are analysed to provide a country level of confidence. Again, if the target sample size (number of villages) is achieved, this should exceed 95%.

Example 3 – spatial sampling and the use of tests with imperfect specificity

Context

A country has an oyster culture industry, based primarily on rack culture of oysters in 23 estuaries distributed along the coastline. In similar regions in other countries, Disease Z causes mortalities in late summer/early autumn. During an outbreak a high proportion of oysters are affected, however, it is suspected that the agent may be present at relatively low prevalence in the absence of disease outbreaks.

Objective

The national authorities wish to demonstrate national freedom from Disease Z. If the disease should be detected, a secondary objective of the survey is to collect adequate evidence to support zoning at the estuary level.

¹¹ <http://www.myatt.demon.co.uk/epicalc.htm>

Approach

The authorities conclude that clinical surveillance for disease outbreaks is inadequate because of the possibility of low level subclinical infections. It is therefore decided to base surveillance on a structured two-stage survey, in which sampled oysters are subjected to laboratory testing. The first stage of the survey is the selection of estuaries. However, due to the objective of providing evidence for zoning (should disease be found in any of the estuaries), it is decided to use a census approach and sample every estuary. In essence this means that there will be 23 separate surveys, one for each estuary. A range of options for sampling oysters are considered, including sampling at harvest or marketing, or using farms (oyster leases) as a level of sampling or stratification. However the peak time of activity of the agent does not correspond to the harvest period, and the use of farms would exclude the significant numbers of wild oysters present in the estuaries. It is therefore decided to attempt to simulate simple random sampling from the entire oyster population in the estuary, using a spatial sampling approach.

Survey standards

- The target population is all of the oysters in each of the estuaries. The study population is the oysters present during the peak disease-risk period in late summer early autumn. Wild and cultured oysters are both susceptible to disease, and may have associated with them different (but unknown) risks of infection. They are therefore both included in the study population. As will be described below, sampling is based on mapping. Therefore the study population can more accurately be described as that population falling within those mapped areas identified as oyster habitats.
- A design prevalence value is only required at the oyster level (as a census is being used at the estuary level). While the disease is often recognised with very high prevalence during outbreaks, a low value is used to account for the possibility of persistence of the agent in the absence of clinical signs. A value of 2% is selected.
- The test used is histopathology with immuno-staining techniques. This test is known to produce occasional false-positive results due to nonspecific staining, but is very sensitive. Published studies indicate values of 99.1% for sensitivity and 98.2% for specificity. No other practical tests are available. This means that it is not possible to definitively differentiate false positives from true positives, and that in a survey of any size, a few false positives are expected (i.e. 1.8%).
- The confidence is set at 95% and the power at 80%. In the previous examples, due to the assumed 100% specificity achieved by use of multiple tests, the effective power was 100%. In this case, with imperfect specificity, there will be a risk of falsely concluding that a healthy estuary is infected, so the power is not 100%. The choice of a relatively low figure (80%) means that there is a 1 in 5 chance of falsely calling an estuary infected when it is not infected, but it also dramatically decreases the survey costs, through a lower sample size.

Sample size

Based on the assumption that the sampling procedure will mimic simple random sampling, the sample size (number of oysters to sample per estuary) can be calculated with *FreeCalc*. The population size (number of oysters per estuary) is assumed to be very large. The calculated sample size, using the sensitivity, specificity and design prevalence figures given above, is 450. *FreeCalc* also reports that, based on this sample size and the specificity of the test, it is possible to get 10 or fewer false-positive test results, and still conclude that the population is free from disease. This is because, if the population were infected at 2% or greater, the anticipated number of positive reactors from a sample of 450 would be greater than 10. In fact, we would expect 9 true positives ($450 \times 2\% \times 99.1\%$) and 8 false positives ($450 \times 98\% \times 1.8\%$) or a total of 17 positives if the population were infected at a prevalence of 2%.

Appendix XXX (contd)

This illustrates how probability theory and adequate sample size can help differentiate between true- and false-positive results when there is no alternative but to use a test with imperfect specificity.

Sampling

The aim is to collect a sample of 450 oysters that represent an entire estuary. Simple random sampling depends on creating a sampling frame listing every oyster (not possible) and systematic sampling depends on being able to (at least conceptually) line up all the oysters (again, not possible). The authorities decide to use spatial sampling to approximate simple random sampling. Spatial sampling involves selecting random points (defined by coordinates), and then selecting oysters near the selected points. In order to avoid selecting many points with no oysters nearby, the estuary is first mapped (the fisheries authorities already have digital maps defining oyster leases available). To these maps areas with significant concentrations of wild oysters are also added, based on local expertise. Pairs of random numbers are generated such that the defined point falls within the defined oyster areas. Other schemes are considered (including using a rope marked at regular intervals, laid out on a lease to define a transect, and collecting an oyster adjacent to each mark on the rope) but the random coordinate approach is adopted.

Survey teams then visit each point by boat (using a GPS [Global Positioning System] unit to pinpoint the location). A range of approaches is available for selecting which oyster to select from a densely populated area, but it should involve some effort at randomness. Survey staff opt for a simple approach: when the GPS receiver indicates that the site has been reached, a pebble is tossed in the air and the oyster closest to the point where it lands is selected. Where oysters are arranged vertically (e.g. wild oysters growing up a post), a systematic approach is used to determine the depth of the oyster to select. First, an oyster at the surface, next, an oyster halfway down, and thirdly, an oyster as deep as can be reached from the boat.

This approach runs the risk of bias towards lightly populated areas, so an estimate of the relative density of oysters at each sampling point is used to weight the results (see *Survey Toolbox* for more details).

Testing

Specimens are collected, processed, and analysed following a standardised procedure. The results are classified as definitively positive (showing strong staining in a highly characteristic pattern, possibly with associated signs of tissue damage), probably positive (on the balance of probabilities, but less characteristic staining), and negative.

Analysis

The interpretation of the results when using a test with imperfect specificity is based on the assumption that, in order to conclude that the population is free from infection, any positive result identified is really a false positive. With a sample size of 450, up to 10 false positives may be expected while still concluding that the population is free from disease. However, if there is reasonable evidence that there is even a single *true* positive, then the population cannot be considered free. This is the reason for the classification of positive results into definitive and probable positives. If there are any definitive positives at all, the population in that estuary must be considered infected. The probable positives are consistent with false positives, and therefore up to 10 may be accepted. Using *FreeCalc* the actual confidence achieved based on the number of (presumed) false positives detected can be calculated. For instance, if 8 'probably positive' results were detected from an estuary, the confidence level for the survey would be 98.76%. On the other hand, if 15 'probably positive' results were detected, the confidence is only 61.9%, indicating that the estuary is likely to be infected.

Discussion

Normally, it may be safely assumed that a surveillance system aimed at demonstrating freedom from disease is 100% specific. This is because any suspected occurrence of disease is investigated until a definitive decision can be made. If the conclusion is that the case is truly a case of disease, then there is no issue of declaring freedom – the disease is known to be present. This example presents a different situation where, due to lack of suitable tests, it is not possible for the surveillance system to be 100% specific. This may represent an unusual situation in practice, but illustrates that methods exist for dealing with this sort of problem. In practice, a conclusion that a country (or estuary) is free from infection, in the face of a small (but statistically acceptable) number of positive results, will usually be backed up by further evidence (such as the absence of clinical disease).

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December 2006

REPORT OF THE MEETING OF THE OIE *AD HOC* GROUP ON AQUATIC ANIMAL FEEDS
Paris, 12-14 December 2006

The OIE *ad hoc* Group on Aquatic Animal Feeds (hereafter referred to as the *ad hoc* Group) met at the OIE Headquarters from 12 to 14 December 2006.

The members of the *ad hoc* Group and other participants are listed at [Appendix I](#). The Agenda adopted is given at [Appendix II](#).

On behalf of Dr Bernard Vallat, Director General of the OIE, Dr Sarah Kahn welcomed all members and indicated that on request of OIE Member Countries the OIE intends to develop guidelines to address animal health aspects of aquatic animal feeding. She recalled that the OIE Aquatic Animal Health Standards Commission (hereafter referred to as the Aquatic Animals Commission) asked this *ad hoc* Group to focus on aquatic animal disease that can be transmitted through international trade of feed as clarified by the terms of reference.

Dr Sarah Kahn explained that an OIE *ad hoc* Group on animal feeding for terrestrial animals had been convened, on request of the OIE Terrestrial Animal Health Standards Commission, in October this year to address animal health and zoonotic aspects of animal feeding in order to complement the work done by the Codex Alimentarius Commission (CAC). Although the work accomplished in October did not include aquatic animals in its scope, she suggested the *ad hoc* Group take it into consideration. She introduced Prof. Eli Katunguka-Rwakishaya, Member of the OIE Aquatic Animals Commission, and thanked him for his willingness to chair this meeting.

The Chair illustrated the terms of reference to the members of the *ad hoc* Group by clarifying that the main task of the *ad hoc* Group would be to document risk mitigation measures, including traceability and certification, against risks of transmission of important/listed aquatic animal diseases through trade in aquatic animal feeds and ingredients. The *ad hoc* Group would also address non infectious hazards of animal and public health concerns such as contaminants, residues, mycotoxins and trace elements, if time permitted.

Appendix XXXI (contd)

The *ad hoc* Group prepared “Draft Guidelines for the Control of Hazards of Aquatic Animal Health Importance in Aquatic Animal Feeds” (attached at Appendix III) taking in consideration references shown at Appendix IV.

In developing the specific recommendations of these guidelines, the *ad hoc* Group prepared a flow chart illustrating key points of pathogen contamination and transmission through the production chain of aquatic feed ingredients and feed. The chart and explanatory text are presented at Appendix V.

The *ad hoc* Group considered that the feed manufacturing process was a key point for hazards in feed. It considered that most animal disease agents would be inactivated in the dry feed manufacturing process due to its time and temperature conditioning process. Feed in live, moist, semi-moist and dry form represents different levels of risk.

The *ad hoc* Group addressed the issue of OIE listed crustacean diseases (Article 1.2.3.3. of the *OIE Aquatic Animal Health Code* [hereafter referred to as the *Aquatic Code*]) being transferred through pelleted feeds. It noted that there is no evidence that these viruses have been transferred in feed, despite the large amount of pelleted feed that has been consumed by cultivated shrimp over the past two decades. It also noted the risk factors related to the resistance of the listed crustacean disease agents and had an exchange of views with Prof. Lightner (OIE Reference Laboratory for white spot disease) on the inactivation of these pathogens. The *ad hoc* Group concluded that the heat treatments (>90°C and up to 100°C for at least 30 minutes) associated with pelleted feed manufacturing processes would inactivate all listed crustacean pathogens in the feed ingredients.

The practice of trading internationally fresh or frozen marine products, not for human consumption but intended for use as aquatic feed, without animal health certification is common. This especially applies to feed for aquatic species that have only been cultured for a short time and for which no processed feed has been developed. The *ad hoc* Group noted that this represents a potential risk for importing new and previously unreported diseases into a country.

The *ad hoc* Group recommended that, in case of importing fresh whole fish or crustaceans (*Artemia*) for direct feeding, the importing country evaluate the possibility of 1) obtaining guarantees from the exporting country regarding pathogen inactivation (*Artemia*) and/or 2) harvest from a disease free zone and/or 3) test for pathogens prior to export. Given the impracticability of these measures (other than as mentioned above for *Artemia*), the *ad hoc* Group recommends against the import of fresh aquatic species for direct feeding to species that are, or could be, susceptible to pathogens of the imported aquatic species.

The *ad hoc* Group noted that there are several public health issues potentially arising in relation to aquatic animal feed, including: residues of pesticides used on crops and bioaccumulation of pollutants (e.g. heavy metals and PCBs). These public health issues were not addressed as they were not within the terms of reference.

The *ad hoc* Group noted that although some Member Countries have banned the use of ruminant meals for feeding aquatic animals, there has been (in recent years) a continuous increase in the use of animal by-products in aquatic feeds for economic reasons. Based on the limited information available, the *ad hoc* Group concluded that there is no evidence for the existence of natural transmissible spongiform encephalopathies in fish. The *ad hoc* Group did not formulate recommendations in relation to the risk of transmissible spongiform encephalopathy agents being introduced into the food chain through aquatic animal feeding.

Appendix XXXI (contd)

The *ad hoc* Group considered hazards potentially associated with medicated feed in relation to antimicrobial resistance and noted that an OIE *ad hoc* Group on antimicrobial resistance with expertise in aquatic animals would be convened in the future. It recommended that the Aquatic Animals Commission forward the proposed definition of “medicated feed” to the *ad hoc* Group on antimicrobial resistance in aquatic animals.

The *ad hoc* Group addressed the definition of antimicrobials in the OIE *Terrestrial Animal Health Code* (hereafter referred to as the *Terrestrial Code*) and agreed that acids (e.g. formic acid, citric acid) should not be included in this definition as they are not capable of causing antimicrobial resistance. The *ad hoc* Group recommended that the Aquatic Animals Commission consider this statement in light of the report of the *ad hoc* Group on aquatic antimicrobial resistance.

The *ad hoc* Group addressed risks of contamination by non infectious hazards (including feed additives, trace elements, heavy metals, probiotics, products of lipid oxidation and mycotoxins). While many of the same risk management recommendations would apply (e.g. quality assurance of ingredients and prevention of cross-contamination), the Group was not able to provide specific recommendations in the time available.

.../Appendices

MEETING OF THE OIE AD HOC GROUP ON AQUATIC ANIMAL FEEDS

Paris, 12-14 December 2006

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MEETING OF THE OIE AD HOC GROUP ON AQUATIC ANIMAL FEEDS**Paris, 12-14 December 2006**

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Agenda

1. Adoption of the Agenda
2. Risks of transmission of aquatic animal diseases through trade in aquatic animal feeds
3. Related mitigation measures
4. Draft guideline for the OIE *Aquatic Animal Health Code* on the management of hazards in aquatic feed
5. Other business

DRAFT GUIDELINES FOR THE CONTROL OF AQUATIC ANIMAL HEALTH HAZARDS IN AQUATIC ANIMAL FEEDS

INTRODUCTION

One of the key objectives of the OIE *Aquatic Animal Health Code* (hereafter referred to as the *Aquatic Code*) is to help Member Countries trade safely in aquatic animals and their products by developing relevant aquatic animal health measures. This guideline addresses aquatic animal health hazards in aquatic animal feeds. It does not address food safety issues as this is not within the mandate of the OIE Aquatic Animal Health Commission (hereafter referred to as the Aquatic Animals Commission). These Guidelines should be read in conjunction with relevant recommendations of the OIE *Terrestrial Animal Health Code* (hereafter referred to as the *Terrestrial Code*) (Appendix containing recommendations on animal feed). The Food and Agriculture Organization of the United Nations (FAO) has also published recommendations¹² relevant to terrestrial and aquatic animal feed.

Key considerations relevant to aquatic animal feeds are as follows:

- Intensive rearing in aquaculture establishments causes a concentration of fish, feed and faecal matter in time and space and this heightens the risk of disease transmission, whether the pathogen enters the culture system via feed or other means.
- For many aquatic species, cannibalism is their natural way of feeding.
- Historically, animal proteins used in feeds were mainly sourced from the marine environment, due to the nutritional needs of aquatic animals and for reasons of economy. This practice gives risk to disease risks, especially when animals are fed with live or whole fish of the same or related species. There are many examples of this type of practice, e.g. early stage crustaceans fed on *Artemia* species and aquaculture tuna fed on whole wild caught fish.
- The usage of feed in moist, semi-moist and dry form implies different levels of risk due to the processing applied to the feed.
- With the increasing number of species being farmed (especially marine finfish), the use of live and moist feed has increased. It is likely that these industries will shift in future to formulate feeds as appropriate formulations are developed.
- Hazards may be transmitted from feed to aquatic animals via direct or indirect means. Direct transmission occurs when the cultured species consumes feed containing the hazard (e.g. shrimp larvae consuming rotifer infected with white spot syndrome virus) while indirect transmission refers to hazards in feed entering the aquatic environment or non target species, and thereby establishing a mechanism for indirect infection or contamination of the species of commercial interest. Pathogens that are less host-specific (e.g. white spot syndrome virus, *Vibrio* species) present a greater risk of indirect transmission as they can establish reservoirs of infection in multiple species.

¹² Technical guidelines for responsible fisheries – aquaculture development: 1. Good aquaculture feed manufacturing practice. FAO 2001.
Draft good practices for the animal feed industry – implementing the Codex Alimentarius' Code of practice on good animal feeding, IFIF/FAO (*In preparation*).

Appendix XXXI (contd)Appendix III (contd)

- As new species become the subject of aquaculture, new pathogens emerge in association with these hosts. The expression of disease may be facilitated by culturing species under intensive and novel conditions. Also, it is necessary to conduct research and develop new feeds (and feed ingredients) that are appropriate to the species and its culture system. As more and more aquatic species are being cultured, it is difficult to make recommendations for all significant disease agent/host species combinations.

PURPOSE AND SCOPE

To document risk mitigation measures, including traceability and certification, to deal with aquatic animal health risks through trade in aquatic animal feeds and ingredients. Hazards include diseases of interest i.e. OIE listed diseases and any others considered to be important to aquaculture and non infectious hazards of animal health significance, including contaminants, residues and mycotoxins. Public health considerations are not within the mandate of the Aquatic Animals Commission. However, given the Animal Production Food Safety mandate of the OIE, the *ad hoc* Group decided that it should take some hazards in account of food safety hazards in the course of its work.

This guideline recommends the control of aquatic animal health hazards through adherence to recommended practices during the production (procurement, handling, storage, processing and distribution) and use of both commercial and on-farm produced feed (and feed ingredients) for aquatic animals. While aquatic animals grown for food is the main focus, the same principles apply to feed for aquarium species.

DEFINITIONS***Cross contamination***

Means contamination of a material or product with another material or product containing a hazard.

Dry feed

Means feed that has a dry matter content = or > than 90%.

Feed

Means any material (single or multiple), whether processed, semi-processed or raw that is intended to be fed directly to food-producing animals.

Feed additives

Means any ingredient intentionally added in micro-amounts not normally consumed as feed by itself, whether or not it has nutritional value, which affects the characteristics of feed or animal products. Micro-organisms, enzymes, acidity regulators, trace elements, vitamins, attractants, pigments, synthetic binders, synthetic amino acids, antioxidants and other products fall within the scope of this definition, depending on the purpose of use and method of administration. This excludes veterinary drugs.

Feed ingredient

Means a component, part or constituent of any combination or mixture making up a feed, including feed additives, whether or not it has a nutritional value in the animal's diet. Ingredients may be of plant, animal or aquatic origin and may be organic or inorganic substances.

Hazard

Means a biological, chemical or physical agent in, or a condition of, feed or a feed ingredient with the potential to cause an adverse effect on animal or public health.

Intra specific feeding

Means feeding *aquatic animals* on products made from animals of the same species, or products made from species that are susceptible to the same pathogens as the animals receiving the feed.

Live feed

Means live farmed or wild caught animals used as feed for *aquatic animals*. Live feed is often fed to aquatic species at an early life-stage (e.g. *Artemia* cysts, rotifers, copepods) and to aquatic species that have been cultured for a relatively short time.

Medicated feed

Means any feed which contains a veterinary drug administered to food producing animals, for therapeutic or prophylactic purposes or for modification of physiological functions.

Moist (or wet) feed

Means feed that has a dry matter content = or < than 30% (e.g. frozen adult *Artemia*, whole fish or fish offal, molluscs, crustaceans, polychaetes for feed purposes).

Semi-moist feed

Means feed that has a dry matter content between 30 and 90%.

Fish soluble

Means a by-product of the fish oil production system, comprising the product remaining when water is drawn off (evaporated) from the residual aqueous phase.

Undesirable substance

Means a contaminant or other substance that is present in and/or on feed or feed ingredients and that constitutes a risk to animal or public health.

GENERAL PRINCIPLES**Roles and responsibilities**

The *Competent Authority* has the legal power to set and enforce regulatory requirements related to animal feeds, and has final responsibility for verifying that these requirements are met. The *Competent Authority* may establish regulatory requirements for relevant parties, including requirements to provide information and assistance.

It is a particular responsibility of the Competent Authority to set and enforce the regulatory requirements pertaining to the use of veterinary drugs, animal disease control and the food safety aspects that relate to the management of live animals on farm.

Those involved in the production and use of animal feed and feed ingredients have the responsibility to ensure that these products meet regulatory requirements¹³. All personnel involved in the procurement, manufacture, storage and handling of feed and feed ingredients should be adequately trained and aware of their role and responsibility in preventing the spread of hazards of animal health and public health significance. Appropriate contingency plans should be developed in case of a feed-borne disease outbreak. Equipment for producing, storing and transporting feed should be kept clean and maintained in good working order.

¹³ If at the national level, there are specific food-safety or animal health regulations related to genetically modified organisms, these should be taken into account in relation to feed and feed ingredients as these products form an important part of the food chain.

Appendix XXXI (contd)Appendix III (contd)

Private veterinarians and others (e.g. laboratories) providing specialist services to producers and to the feed industry may be required to meet specific regulatory requirements pertaining to the services they provide (e.g. disease reporting, quality standards, transparency).

Regulatory standards for feed safety

All feed and feed ingredients should meet regulatory standards for feed safety. In defining limits and tolerances for hazards, scientific evidence, including the sensitivity of analytical methods and on the characterisation of risks, should be taken into account.

Risk analysis (risk assessment, risk management and risk communication)

Internationally accepted principles and practices on risk analysis (see Section 1.4. of the *Aquatic Code* and relevant Codex texts) should be used in developing and applying the regulatory framework.

A generic risk analysis framework should be applied to provide a systematic and consistent process for managing disease risks and the risk of contamination with undesirable substances.

Good practices

Where national guidelines exist, good aquaculture practices and good manufacturing practices (including good hygienic practices) should be followed. Countries without such guidelines are encouraged to develop them.

Where appropriate, Hazard Analysis and Critical Control Point¹⁴ (HACCP) principles should be followed to control hazards that may occur in feed.

Relationship between terrestrial animal disease agents and aquatic species

Scientific knowledge is lacking on the relationship between certain terrestrial animal disease agents, notably prions, and aquatic species. There is no evidence to suggest that the use of terrestrial animal by-products as ingredients in aquatic animal feeds gives rise to risks in respect of prion diseases. More scientific information is desirable to enable aquaculture industries to utilise more terrestrial animal by-products as a means of reducing dependency on aquatic protein and lipid sources.

Bioaccumulation

Heavy metals and polychlorinated biphenyls (PCB) persist in fatty tissues and therefore tend to accumulate through the food chain.

Geographic and environmental considerations

Aquatic and terrestrial harvest areas for feed ingredients should not be located in proximity to sources of animal health or food safety hazards. Where this cannot be avoided, preventive measures should be applied to control risk. The same recommendations apply for the processing of feed ingredients, the manufacture of feed and the location of aquaculture operations.

Aquatic animal health considerations include factors such as disease status, location of quarantined premises, existence of processing plants without proper biosecurity measures and the existence of zones/compartments of specified health status.

¹⁴ Hazard Analysis and Critical Control Point, as defined in the Annex to the Recommended International Code of Practice on General Principles of Food Hygiene (CAC/RCP 1-1969).

Appendix XXXI (contd)

Appendix III (contd)

Public health considerations include factors such as industrial operations and waste treatment plants that generate pollutants and other hazardous products. The potential accumulation of pollutants in the food chain through feed ingredients needs to be considered.

Zoning and compartmentalisation

Feed and feed ingredients are important components of biosecurity and need to be considered when defining a compartment or zone in accordance with Chapter X.X.X. of the *Aquatic Code*.

Sampling and analysis

Sampling and analytical protocols should be based on scientifically recognized principles and procedures and OIE standards, where applicable.

Labelling

Labelling should be clear and informative on how the feed and feed ingredients should be handled, stored and used and should comply with regulatory requirements. Labelling should provide for trace-back.

See Section 4.2. of Codex Code of practice on good animal feeding (CAC/RCP 54-2004).

Design and management of inspection programmes

In meeting animal and public health objectives prescribed in national legislation or required by importing countries, Competent Authorities contribute through the direct performance of some tasks or through the auditing of animal and public health activities conducted by other agencies or the private sector.

Operators in the feed and feed ingredients business and other relevant industries should implement procedures to ensure compliance with regulatory standards for procurement, handling, storage, processing, distribution and use of feed and feed ingredients. Operators have the primary responsibility for implementing systems for process control. Where such systems are applied, the Competent Authority should verify that they achieve all regulatory requirements.

Assurance and certification

Competent Authorities are responsible for providing assurances domestically and to trading partners that regulatory requirements have been met.

Hazards associated with animal feed

Biological hazards

Biological hazards that may occur in feed and feed ingredients include agents such as bacteria, viruses, prions, fungi and parasites.

Chemical hazards

Chemical hazards that may occur in feed and feed ingredients include naturally occurring chemicals (such as mycotoxins, gossypol and free radicals), industrial and environmental contaminants (such as heavy metals, dioxins and PCBs), residues of veterinary drugs and pesticides and radionuclides.

Appendix XXXI (contd)Appendix III (contd)Physical hazards

Physical hazards that may occur in feed and feed ingredients include foreign objects (such as pieces of glass, metal, plastic or wood).

Cross contamination

It is important to avoid cross-contamination during the manufacture, storage, distribution (including transport) and use of feed and feed ingredients. Appropriate provisions should be included in the regulatory framework. Scientific evidence, including the sensitivity of analytical methods and on the characterisation of risks, should be drawn upon in developing this framework.

Procedures such as flushing, sequencing and physical clean-out should be used to avoid cross-contamination between batches of feed or feed ingredients. National regulations should be followed in order to avoid the use of unauthorised feed ingredients with a risk of cross-contamination.

Antimicrobial resistance

Concerning the use of antimicrobials in animal feed refer to Section X.X.X. of the *Aquatic Code*.

Management of information

The Competent Authority should establish requirements for the provision of information by the private sector on regulatory requirements.

Records should be maintained in a readily accessible form on the production, distribution and use of feed and feed ingredients. These records are required to facilitate the prompt trace-back of feed and feed ingredients to the immediate previous source, and trace-forward to the next/subsequent recipients, to address animal health or public health concerns.

Animal identification (in the case of aquatic animals this will normally be on a group basis) and traceability are tools for addressing animal health and food safety risks arising from animal feed (see Section 3.5. of the *Terrestrial Code*; Section 4.3 of CAC/RCP 54-2004).

BIOLOGICAL HAZARDS

This document addresses the following biological hazards:

- a) bacteria, virus, parasites, fungi affecting aquatic animals. These hazards include the aquatic animal diseases listed by the OIE (Chapter 1.2.3. of the *Aquatic Code*) and other important diseases (including IPN and IMNV);
- b) prions;
- c) non infectious hazards (e.g. contaminants, residues, mycotoxins).

PATHOGENS OR CONTAMINANTS IN FEED

1. Pathogens or contaminants in feed can be introduced at two points:

- a) at source: via the harvest of infected aquatic animals or animals contaminated with non-infectious hazards;
- b) during storage, processing and transport.

Contamination may occur at the manufacturing facility via poor hygienic practices and/or the presence of pests.

Appendix XXXI (contd)Appendix III (contd)

Feed and feed ingredients may be exposed to contamination during storage, manufacturing or transport, due to residues of previous batches of feed remaining in processing lines, containers or transport vehicles.

2. Exposure pathways include:

a) Direct exposure

The use of raw unprocessed feed or feed ingredients derived from aquatic animals to feed aquatic species presents a risk of exposure to hazards of infectious or non-infectious nature. For diseases, intra-specific feeding presents the greatest risk. This is feeding aquatic animals and their products to species that are susceptible to the same diseases as the 'feed animal' e.g. feeding salmonid offal to salmonids or feeding rotifers or Artemia species to crustaceans.

b) Indirect exposure

Feed and feed ingredients containing infectious or non-infectious hazards may be transmitted to aquatic animals in aquaculture (or wild fish) via contamination of the environment, including infection/contamination on non-target species.

RECOMMENDED APPROACHES TO RISK MITIGATION

The following measures are relevant to exporting countries:

1. Source of raw materials

Raw materials/ingredients should not be sourced from areas/populations known to be infected with significant pathogens or contaminated with animal health/food safety hazards. It may be appropriate to adopt routine testing procedures to verify that contaminants including pathogens are not present at unacceptable levels; or

When using feed and feed ingredients originating from areas known to be affected by a significant pathogen or contaminant:

- a) feed and feed ingredients should be delivered directly to feed manufacturing plants for processing under conditions approved by the Competent Authority; and
- b) effluent and other wastes from the feed manufacturing plants should be treated under conditions approved by the Competent Authority before discharge into the aquatic environment;
- c) feed and feed ingredients known or suspected to be infected with significant pathogens should only be used in a zone or compartment that does not contain species susceptible to the pathogen in question.

2. Feed production

To prevent contamination by pathogens or other hazards during production, storage and transport of feed and feed ingredients:

- a) flushing, sequencing or physical clean-out of manufacturing lines and storage facilities should be performed between as appropriate;
- b) buildings and equipment for processing and transporting feed and feed ingredients should be constructed in a manner that facilitates operation, maintenance and cleaning and prevents feed contamination;

Appendix XXXI (contd)Appendix III (contd)

- c) in particular, feed manufacturing plants should be designed to avoid cross-contamination between batches;
 - d) processed feed and feed ingredients should be stored separately from unprocessed feed ingredients, under appropriate packaging conditions;
 - e) feed and feed ingredients, manufacturing equipment, storage facilities and their immediate surroundings should be kept clean and pest control programmes should be implemented;
 - f) measures to inactivate pathogens, such as heat treatment or the addition of authorised chemicals, should be used where appropriate. Where such measures are used, the efficacy of treatments should be monitored at appropriate stages in the manufacturing process;
 - g) labelling should provide for the identification of feed and feed ingredients as to the batch/lot and place/date of production. To assist in tracing feed and feed ingredients as may be required to deal with animal disease or food safety incidents, labelling should provide for identification by batch/lot and date/place of production.
3. The following measures are relevant to importing countries:
- a) imported feed and feed ingredients should be delivered directly to feed manufacturing plants or aquaculture facilities for processing/use under conditions approved by the Competent Authority;
 - b) effluent and waste material from feed manufacturing plants and aquaculture facilities should be managed under conditions approved by the Competent Authority, including, where appropriate, treatment before discharge into the aquatic environment;
 - c) feed that is known to contain significant pathogens should only be used in a zone or compartment that does not contain species susceptible to the disease in question;
 - d) intra-specific feeding should be discouraged wherever possible.

CERTIFICATION PROCEDURES FOR AQUATIC FEEDS

- 1) The following highly processed products represent a negligible risk:
- a) fish oil;
 - b) crustacean oil;
 - c) fish solubles;
 - d) fish meal;
 - e) crustacean meal (subject to heating to >90°C, =30 m.);
 - f) squid meal and squid liver-meal;
 - g) bivalve meal.

For these products, Competent Authorities should not require conditions in relation to aquatic animal diseases, regardless of the aquatic health status of the exporting country, zone or compartment¹⁵.

¹⁵ In relation to the risk associated with contamination after harvest/processing, point 4 (below) applies.

Appendix XXXI (contd)

Appendix III (contd)

2. Other products

The following risk mitigation measures should be considered:

- a) sourcing feed and feed ingredients from a disease/contaminant-free area; or
- b) confirmation (e.g. by testing) that pathogens and/or contaminants are not present in the product; or
- c) treatment (e.g. by heat or acidification) of product to inactivate pathogens; or
- d) processing to reduce the concentration of contaminants to a level considered to be acceptable.

3) Importing country measures

When importing feed and feed ingredients of aquatic origin, 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).

This certificate should certify:

- a) that feed and feed ingredients of aquatic origin were imported from a country, zone or compartment that is free from relevant aquatic animal diseases¹⁶; or
- b) that feed and feed ingredients of aquatic origin were tested for relevant aquatic animal diseases¹⁷ and shown to be free of these diseases; or
- c) that feed and feed ingredients of aquatic origin have been processed to ensure that they are free of relevant aquatic animal diseases.

¹⁶ Conditions agreed between the Competent Authorities of the importing and exporting countries in accordance with the recommendations of the OIE *Aquatic Animal Health Code*.

¹⁷ Conditions agreed between the Competent Authorities of the importing and exporting countries in accordance with the recommendations of the OIE *Aquatic Animal Health Code*.

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Risk chart of pathogen transmission and contamination through harvest of feed ingredients and manufacture of aquatic feeds

Some ingredients used in aquaculture, in particular of aquatic origin (e.g. krill, shrimp, fish, crab, Artemia) can be a source of pathogen contamination to cultured aquatic species. These ingredients can carry live pathogens (virus, bacteria and parasites) and reach the aquaculture operation through different types of feeds (live, moist, semi-moist or dry feeds).

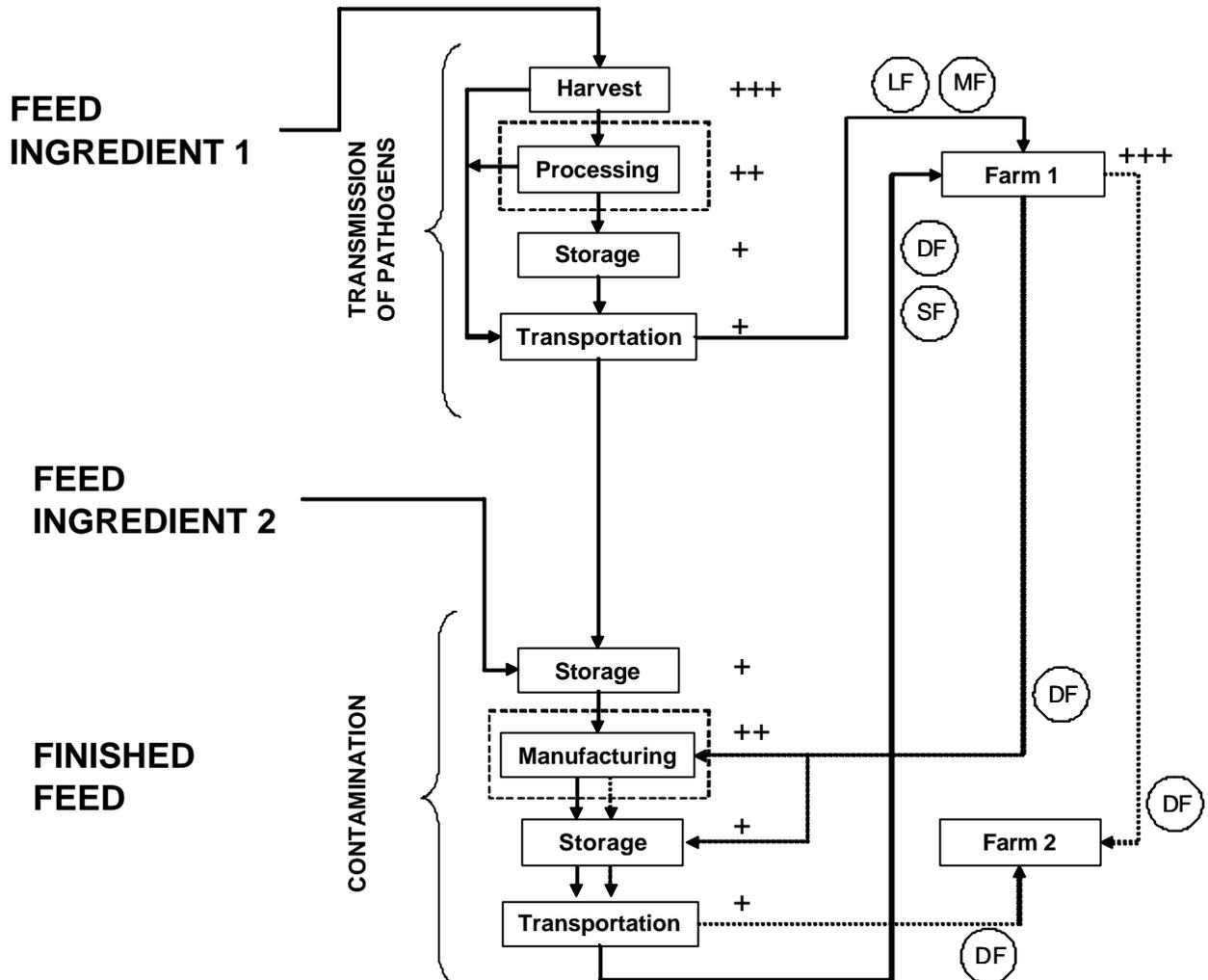
In aquaculture farms, there are two routes of pathogen contamination through aquatic animal feeding: transmission of pathogens and contamination. **Transmission of pathogens** can take place when the feed itself is already infected with a pathogen. This type of contamination is more common with live and moist feeds. Ingredients that constitute their composition are either kept in a raw state in the final product (e.g. feeding tuna with wild caught fish) or at times require little treatment(s) prior to feeding aquatic organisms.

Harvest of aquatic ingredient sources from infected areas has a high risk of pathogen contamination, especially if these are transported to an aquaculture operation without any prior treatment. Processing of these ingredients places a moderate risk of contamination, and it should actually be taken as a possibility to reduce the risk of pathogen transmission (e.g. through heat, chemical treatments). Storage and transportation of these ingredients has a low risk of contamination, but should also be considered as a direct route of pathogen contamination. For example, when two or more batches of ingredients of different sanitary status are handled, stored and/or transported together without any biosecurity measure there is a risk of direct contamination to the farmed animal.

Contamination occurs when the pathogen is introduced in a feed manufacturing facility, both through infected ingredients or finished feeds and later to the aquaculture facility. Contamination occurs with the use of semi-moist and dry feeds. With these feed types, contamination can take place in the manufacturing plant during:

1. Storage of ingredients: it has a low risk of contamination, but it can take place when ingredients of different sanitary status are handled or placed together.
2. Feed manufacturing: during feed processing, ingredients are commonly subjected to heat treatment which can eliminate certain pathogens. However, use of manufacturing lines with remains of contaminated ingredients from a previous batch of feed can result in cross-contamination of feeds.
3. Storage and transportation of finished feeds: it has a low risk of contamination, but when finished feeds are stored or transported together with unprocessed ingredients or with feeds of different sanitary status it can result in pathogen contamination.

An aquaculture facility can also be a source of pathogen contamination in aquatic feeds. At this level, contamination can take place when a finished feed is delivered to a farm located in an infected area. Transmission of pathogens can occur when feed is withdrawn from the aquaculture and is returned to the manufacturing facility for reprocessing or transferred to another farm.



LF: Live feed MF: Moist feed SF: Semi-moist feed DF: Dry feed	Possibility for risk reduction
+++ : High risk of pathogen contamination ++ : Moderate risk of p. c. + : Low risk of p. c.	Redistribution or recycling of finished feed



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January 2007

**REPORT OF THE MEETING OF THE OIE AD HOC GROUP ON
AQUATIC ANIMAL HEALTH SURVEILLANCE
Paris, France, 24–26 July 2006 and 29–31 January 2007**

The OIE *ad hoc* Group on Aquatic Animal Health Surveillance met at the OIE Headquarters in Paris from 24 to 26 July 2006 and from 29 to 31 January 2007.

Dr Gideon Bruckner, Head of the OIE Scientific and Technical Department, welcomed the members on behalf of the OIE Director General, Dr Bernard Vallat.

The meetings were chaired by Prof. Barry Hill. The Agenda, the list of participants and the Terms of Reference for the *ad hoc* Group are presented as Appendices I, II and III, respectively.

1. Aquatic Animal Health Code Appendix on General guidelines for aquatic animal health surveillance

The *ad hoc* Group thoroughly reviewed Appendix 3.8.1 of the OIE *Terrestrial Animal Health Code (Terrestrial Code)* on General guidelines for animal health surveillance and used it as a template to develop new aquatic animal health surveillance guidelines, which included the following major changes:

- a section on the absence of susceptible species as a pathway to self declaration of freedom from disease;
- the need for surveillance of infection in wild populations if the last occurrence of disease was within the previous 25 years.

The Group identified several definitions, such as targeted surveillance and outbreak, in the *Terrestrial Code* Appendix that do not apply in the same way to the *Aquatic Animal Health Code (Aquatic Code)*, i.e. the *Aquatic Code* definition is slightly different (the *Terrestrial Code* may wish to revisit those definitions). In addition, there is a need to include some new definitions such as: design prevalence, type I and type II error, incidence, population at risk, confidence interval, precision, and probability interval.

To be consistent with the *Terrestrial Code* and the current version of the *Manual of Diagnostic Tests for Aquatic Animals*, the Group did not modify the threshold of 25 years and 10 years required to distinguish between pathways to establish disease freedom. However the Group feels that these time periods should be reconsidered taking into account characteristics of the disease, host, and production system.

The proposed guidelines are presented at Appendix IV and are recommended for inclusion in the *Aquatic Code*.

2. *Manual of Diagnostic Tests for Aquatic Animals* Chapter 1.1.4 Requirements for surveillance for international recognition of freedom from infection

The Group reviewed and updated Chapter 1.1.4 of the *Aquatic Manual* on 'Requirements for surveillance for international recognition of freedom from infection'. To reflect a change in scope of the chapter, the Group changed the title of the chapter to 'Guidelines for Aquatic Animal Health Surveillance'.

One major revision made by the Group was to add an entirely new section entitled 'Specific requirements for structured survey design and analysis to assess disease occurrence' on surveillance to determine occurrence and distribution of endemic diseases. This extensive task was completed.

The Group noted that further developments to the chapter could be made, including for example a description of the flow of information and allocation of resources and responsibilities in surveillance systems, and surveillance for diseases in wild fish, etc. These tasks can be addressed should the Group meet to discuss the Member Country comments on the proposed new and revised texts.

The Group discussed the implications of imperfect diagnostic sensitivity and specificity on the design of surveillance systems and the interpretation of the results to substantiate disease freedom and decided to include a table of sample sizes and acceptable number of false positives under varying parameter values. The Group also provided guidance on how to address cases in which supporting information is limited.

In the original version of chapter 1.1.4, both the terms disease and infection were used. For consistency the Group continued the use of both terms. However, the Group recommends that the Aquatic Animal Health Standards Commission (Aquatic Animals Commission) consider clearly distinguishing the two terms in future editions of the *Aquatic Code* and *Aquatic Manual*. In fact, surveillance and control of disease would have different epidemiological implications for a population compared to surveillance and control of infection.

The revised chapter is presented at Appendix V.

3. *Aquatic Manual* chapters I.1, I.2 and I.3 on General information (on diseases of fish, molluscs and crustaceans)

The Group noticed that considerable efforts were required to harmonise the three introductory chapters on general information for diseases of fish, molluscs and crustaceans. Although work on harmonising the chapters was carried out, a number of discrepancies remain among the three documents.

The Group suggests that these chapters be redrafted such that they are strictly limited to surveillance of fish, mollusc and crustacean diseases, respectively and provide a direct link between the new surveillance chapter (1.1.4) and the individual disease chapters. In addition, there was insufficient time to appropriately address revisions to these chapters and provide the Aquatic Animals Commission with a draft showing these changes. The Group will modify the current chapters should the Aquatic Animals Commission agree to this direction.

4. Revise the specific diseases chapter template of the *Aquatic Manual* to ensure that scientific information necessary to develop appropriate surveillance programmes for diseases can be formulated

A number of topic headings were added to the disease chapter template (see Appendix VI). These will be further developed at the next meeting.

The Group identified the need to include in the disease chapters considerable information specifically required for the development of surveillance systems for each disease. The Group realised that generating such information might require a multidisciplinary approach and suggested that the revisions to the chapters would also benefit from having input from epidemiologists. It was noted that the extensive work done recently by the European Union project 'PANDA' (Permanent Advisory Network for Diseases in Aquaculture) on an epidemiology database for aquatic animal diseases provides an excellent source of information, and a template for presenting it in the *Aquatic Manual* chapters, that could be used by the authors of the individual disease chapters in the *Aquatic Manual* when designing surveillance programmes.

5. Develop guidelines for *Aquatic Manual* chapter authors to follow in specifying the surveillance requirements for individual diseases

The Group discussed various options for providing guidance on the design and interpretation of surveillance programmes for specific diseases, but due to time constraints it was not possible to complete the preparation of guidelines for the chapter authors in time for this report. Furthermore, it was felt that it would, in any case, be preferable to first receive expert comments on the proposed new *Aquatic Code* chapter on General Guidelines and the revised *Aquatic Manual* chapter 1.1.4, and to prepare the *Aquatic Manual* general information chapters on diseases of fish, molluscs and crustaceans, before completing the more specific guidelines for the individual disease chapters in the *Aquatic Manual*. These will most likely be prepared at a further meeting of the Group at which it will also take into account the comments received from the Aquatic Animals Commission and OIE member countries.

The Group initiated a description of steps involved in the design of a surveillance system with the intention of including it in the guidelines. This will require further development and harmonisation within the structure of Chapter 1.1.4. The steps are presented at Appendix VII.

6. Concluding remarks

Throughout discussions the Group attempted to balance the scientific validity of the documents with the need to avoid unnecessary burdens of excessive surveillance methodological requirements for Member Countries.

Realising the scale and potential impact of the changes proposed, the Group would welcome feedback from the Aquatic Animals Commission and OIE Member Countries with suggestions for further modifications.

.../Appendices

**REPORT OF THE MEETINGS OF THE OIE AD HOC GROUP ON
AQUATIC ANIMAL HEALTH SURVEILLANCE**

Paris, France, 24–26 July 2006 and 29–31 January 2007

Agenda

1. *Aquatic Animal Health Code* Appendix on General guidelines for aquatic animal health surveillance
 2. *Manual of Diagnostic Tests for Aquatic Animals* Chapter 1.1.4 Requirements for surveillance for international recognition of freedom from infection
 3. *Aquatic Manual* chapters I.1, I.2 and I.3 on General information (on diseases of fish, molluscs and crustaceans)
 4. Revise the specific diseases chapter template of the *Aquatic Manual* to ensure that scientific information necessary to develop appropriate surveillance programmes for diseases can be formulated
 5. Develop guidelines for *Aquatic Manual* chapter authors to follow in specifying the surveillance requirements for individual diseases
 6. Concluding remarks
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**REPORT OF THE MEETING OF THE OIE *AD HOC* GROUP ON
AQUATIC ANIMAL HEALTH SURVEILLANCE**

Paris, France, 24–26 July 2006 and 29–31 January 2007

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**TERMS OF REFERENCE FOR THE OIE *AD HOC* GROUP ON
AQUATIC ANIMAL HEALTH SURVEILLANCE**

1. Review, revise and update Chapter 1.1.4. (Surveillance) of the *Aquatic Manual*, taking into account the general guidelines for surveillance in the *Terrestrial Code*
2. Draft an appendix on general guidelines for aquatic animal health surveillance for inclusion in the *Aquatic Code*
3. Review, revise and update chapters I.1, I.2 and I.3 of the *Aquatic Manual* and ensure consistency and continuity with Chapter 1.1.4 (Surveillance) while including specific provisions related to aquatic animal health surveillance for fish, molluscs and crustaceans
4. Revise the specific diseases chapter template of the *Aquatic Manual* to ensure that scientific information necessary to develop appropriate surveillance programmes for diseases can be formulated
5. Develop guidelines for *Aquatic Manual* chapter authors to follow in specifying the surveillance requirements for individual diseases
6. To submit a report to the OIE Aquatic Animal Health Standards Commission preferably by October 2006.

APPENDIX X.X.X.

GENERAL GUIDELINES FOR AQUATIC ANIMAL HEALTH SURVEILLANCE

Article 3.8.1.1.

Introduction and objectives

1. Surveillance is aimed at:
 - demonstrating the absence of *disease* or *infection*,
 - identifying events requiring notification as listed in Article 1.2.1.3. of the *Aquatic Code*
 - determining the occurrence or distribution of endemic *disease* or *infection*, including changes to their incidence or prevalence (or its contributing factors), in order to:
 - provide information for domestic *disease* control programmes,
 - provide relevant *disease* occurrence information to be used by trading partners for qualitative and quantitative risk assessment.

The type of surveillance applied depends on the desired outputs needed to support decision-making. Surveillance data determine the quality of *disease* status reports and should satisfy information requirements for accurate risk analysis both for *international trade* as well as for national decision-making.

2. Essential prerequisites to enable a Member Country to provide information for the evaluation of its animal health status are:
 - a) that the particular Member Country complies with the provisions of Chapter 1.4.3. of the *Aquatic Code* on the quality and evaluation of the *Competent Authorities*;
 - b) that, where possible, surveillance data be complemented by other sources of information (e.g. scientific publications, research data, documented field observations and other non-survey data);
 - c) that transparency in the planning and execution of surveillance activities and the analysis and availability of data and information, be maintained at all times, in accordance with Chapter 1.2.1. of the *Aquatic Code*

The following guidelines may be applied to all *diseases*, their agents and susceptible species as listed in the *Aquatic Manual*, and are designed to assist with the development of surveillance methodologies. Where possible, the development of surveillance systems using these guidelines should be based on the relevant information in the individual *disease* chapters.

Article 3.8.1.2.

Definitions

The following definitions apply for the purposes of this Appendix:

Bias: A tendency of an estimate to differ from the true value of a population parameter.

Appendix XXXII (contd)

Appendix IV (contd)

Case definition: A case definition is a set of criteria used to distinguish a case animal or *epidemiological unit* from a non-case.

Early detection system: an efficient system for ensuring the rapid recognition of signs that are suspicious of a *listed disease*, or an *emerging disease* situation, or unexplained mortality, in *aquatic animals* in an *aquaculture establishment* or in the wild, and the rapid communication of the event to the *Competent Authority*, with the aim of activating diagnostic investigation with minimal delay. Such a system will include the following characteristics:

- a) broad awareness, e.g. among the personnel employed at *aquaculture establishments* or involved in *processing*, of the characteristic signs of the *listed diseases* and *emerging diseases*;
- b) veterinarians or *aquatic animal* health specialists trained in recognising and reporting suspicious *disease* occurrence;
- c) ability of the *Competent Authority* to undertake rapid and effective *disease* investigation;
- d) access by the *Competent Authority* to laboratories with the facilities for diagnosing and differentiating *listed* and *emerging diseases*.

Outbreak: An outbreak is a substantial increase in the occurrence of *disease* above the expected level at a given time in a given population.

Probability sampling: A sampling strategy in which every unit has a known non-zero probability of inclusion in the sample.

Sample: The group of elements (sampling units) drawn from a population, on which tests are performed or parameters measured to provide surveillance information.

Sampling unit: The unit that is sampled. This may be an individual animal or a group of animals (e.g. a pond). A list of all the sampling units comprises the sampling frame.

Sensitivity: The proportion of truly positive units that are correctly identified as positive by a test.

Specificity: The proportion of truly negative units that are correctly identified as negative by a test.

Study population: The population from which surveillance data are derived. This may be the same as the target population or a subset of it.

Surveillance: The systematic ongoing collection, collation, and analysis of data, and the timely dissemination of information to those who need to know so that action can be taken.

Survey: An investigation about a defined population in which information is systematically collected within a defined time period.

Target population: The population about which conclusions from analysing data are to be inferred.

Test: A procedure used to classify a unit as either positive, negative or suspect with respect to an *infection* or *disease*.

Article 3.8.1.3.

Principles of surveillance1. Types of surveillance

- a) Surveillance may be based on many different data sources and can be classified in a number of ways, including:
 - i) the means by which data are collected (targeted versus non-targeted);
 - ii) the *disease* focus (pathogen-specific versus general surveillance); and
 - iii) the way in which units for observation are selected (structured surveys versus non-random data sources).
- b) Surveillance activities include:
 - i) structured population-based surveys, such as:
 - systematic sampling at slaughter;
 - random surveys;
 - ii) structured non-random surveillance activities, such as:
 - *disease* reporting or notifications;
 - control programmes/health schemes;
 - targeted testing/screening;
 - ante-mortem and post-mortem inspections;
 - laboratory investigation records;
 - biological specimen banks;
 - sentinel units;
 - field observations;
 - farm production records.
- c) In addition, surveillance data should be supported by related information, such as:
 - i) data on the epidemiology of the *infection*, including environmental, and host and wild reservoir population distributions;
 - ii) data on farmed and wild animal movements and trading patterns for aquatic animals and aquatic animal products, including potential for exposure to wild aquatic animal populations, water sources or other contacts;
 - iii) national animal health regulations, including information on compliance with them and their effectiveness;

Appendix XXXII (contd)

Appendix IV (contd)

- iv) history of imports of potentially infected material; and
- v) biosecurity measures in place.
- d) The sources of evidence should be fully described. In the case of a structured survey, this should include a description of the sampling strategy used for the selection of units for testing. For structured non-random data sources, a full description of the system is required including the source(s) of the data, when the data were collected, and a consideration of any biases that may be inherent in the system.

2. Critical elements

In assessing the quality of a surveillance system, the following critical elements need to be addressed over and above quality of *Competent Authority* (Chapter 1.4.3.).

a) Populations

Ideally, surveillance should be carried out in such a way as to take into account all animal species susceptible to the *infection* in a country, *zone* or *compartment*. The surveillance activity may cover all individuals in the population or part of them. Estimates of total population at risk for each species are required. When surveillance is conducted only on a *subpopulation*, care should be taken regarding the inferences made from the results.

Definitions of appropriate populations should be based on the specific recommendations of the *disease* chapters of the *Aquatic Manual*.

b) Epidemiological unit

The relevant *epidemiological unit* for the surveillance system should be defined and documented to ensure that it is representative of the population or targeted *subpopulations* that would generate the most useful inferences about *disease* patterns. Therefore, it should be chosen taking into account factors such as carriers, reservoirs, vectors, immune status, genetic resistance and age, sex, and other host criteria.

c) Clustering

Infection in a country, *zone* or *compartment* usually clusters rather than being uniformly or randomly distributed through a population. Clustering may occur at a number of different levels (e.g. tank, pond, farm, or *compartment*). Clustering should be taken into account in the design of surveillance activities and the statistical analysis of surveillance data, at least at what is judged to be the most significant level of clustering for the particular animal population and *infection*.

d) Case and outbreak definitions

Clear and unambiguous case and outbreak definitions should be developed and documented for each *disease* under surveillance, using, where they exist, the standards in this Appendix and the *Aquatic Manual*.

e) Analytical methodologies

Surveillance data should be analysed using appropriate methodologies, and at the appropriate organisational levels to facilitate effective decision making, whether it be planning interventions or demonstrating status.

Appendix XXXII (contd)Appendix IV (contd)

Methodologies for the analysis of surveillance data should be flexible to deal with the complexity of real life situations. No single method is applicable in all cases. Different methodologies may be needed to accommodate the relevant pathogens, varying production and surveillance systems, and types and amounts of data and information available.

The methodology used should be based on the best available information that is in accord with current scientific thinking. The methodology should be in accordance with this Appendix and fully documented, and supported by reference to the scientific literature and other sources, including expert opinion. Sophisticated mathematical or statistical analyses should only be carried out when justified by the proper amount and quality of field data.

Consistency in the application of different methodologies should be encouraged and transparency is essential in order to ensure fairness and rationality, consistency in decision making and ease of understanding. The uncertainties, assumptions made, and the effect of these on the final conclusions should be documented.

f) Testing

Surveillance involves the detection of *disease* or *infection* by the use of appropriate case definitions based on the results of one or more tests for evidence of *infection* status. In this context, a test may range from detailed laboratory examinations to field observations and the analysis of production records. The performance of a test at the population level (including field observations) may be described in terms of its sensitivity and specificity and predictive values. Imperfect sensitivity and/or specificity will have an impact on the conclusions from surveillance. Therefore, these parameters should be taken into account in the design of surveillance systems and analysis of surveillance data as described in the *Aquatic Manual*.

Although not determined for many aquatic *diseases*, sensitivity and specificity should be estimated as best as possible for a specific testing situation. Alternatively, where values for sensitivity and/or specificity for a particular test and testing situation are estimated in the *Aquatic Manual*, these values may be used as a guide.

Samples from a number of animals or units may be pooled and subjected to a testing protocol. The results should be interpreted using sensitivity and specificity values that have been determined or estimated for that particular pool size and testing procedure.

g) Quality assurance

Surveillance systems should incorporate the principles of quality assurance and be subjected to periodic auditing to ensure that all components of the system function and provide verifiable documentation of procedures and basic checks to detect significant deviations of procedures from those documented in the design.

h) Validation

Results from animal health surveillance systems are subject to one or more potential biases. When assessing the results, care should be taken to identify potential biases that can inadvertently lead to an over-estimate or an under-estimate of the parameters of interest.

i) Data collection and management

The success of a surveillance system is dependent on a reliable process for data collection and management. The process may be based on paper records or computerised. Even where data are collected for non-survey purposes (e.g. during *disease* control interventions, inspections for movement control or during *disease* eradication schemes), the consistency and quality of data collection and event reporting in a format that facilitates analysis, is critical. Factors influencing the quality of collected data include:

- the distribution of, and communication between, those involved in generating and transferring data from the field to a centralised location;
- motivation of the people involved in the surveillance system;
- the ability of the data processing system to detect missing, inconsistent or inaccurate data, and to address these problems;
- maintenance of disaggregated data rather than the compilation of summary data;
- minimisation of transcription errors during data processing and communication.

Article 3.8.1.4.

Structured population-based surveys

In addition to the principles for surveillance discussed above, the following guidelines should be used when planning, implementing and analysing surveys.

1. Types of surveys

Surveys may be conducted on the entire target population (i.e. a census) or on a sample. Periodic or repeated surveys conducted in order to document *disease* freedom should be done using probability based sampling methods (simple random selection, cluster sampling, stratified sampling, systematic sampling) so that data from the study population can be extrapolated to the target population in a statistically valid manner. Non-probability based sampling methods (convenience, expert choice, quota) can also be used. Recognising the inherent impracticalities in sampling from some aquatic populations, non-probability based sampling could be used when biases are recognised and used to optimise detection.

The sources of information should be fully described and should include a detailed description of the sampling strategy used for the selection of units for testing. Also, consideration should be made of any biases that may be inherent in the survey design.

2. Survey design

The population of *epidemiological units* should first be clearly defined; hereafter sampling units appropriate for each stage, depending on the design of the survey, should be defined.

The design of the survey will depend on the size and structure of the population being studied, the epidemiology of the *infection* and the resources available.

3. Sampling

The objective of sampling from a population is to select a subset of units from the population that is representative of the population with respect to the object of the study such as the presence or absence of *infection*. Sampling should be carried out in such a way as to provide the best likelihood that the sample will be representative of the population, within the practical constraints imposed by different environments and production systems. In order to detect the presence of an *infection* in a population of unknown *disease* status, targeted sampling methods that optimise the detection of *infection* can be used. In such cases, care should be taken regarding the inferences made from the results.

4. Sampling methods

When selecting *epidemiological units* from within a population the objectives of the surveillance system should be considered. In general, probability sampling (e.g. simple random selection) is preferable. When this is not possible, sampling should provide the best practical chance of generating optimal inferences about *disease* patterns in the target population.

In any case, the sampling method used at all stages should be fully documented and justified.

5. Sample size

In general, surveys are conducted either to demonstrate the presence or absence of a factor (e.g. *infection*) or to estimate a parameter (e.g. the prevalence of *infection*). The method used to calculate sample size for surveys depends on the purpose of the survey, the expected prevalence, the level of confidence desired of the survey results and the performance of the tests used.

Article 3.8.1.5.

Structured non-random surveillance

Surveillance systems routinely use structured non-random data, either alone or in combination with surveys.

1. Common non-random surveillance data sources

A wide variety of non-random surveillance data sources may be available. These vary in their primary purpose and the type of surveillance information they are able to provide. Some surveillance systems are primarily established as *early detection systems*, but may also provide valuable information to demonstrate freedom from *infection*. Other systems provide cross-sectional information suitable for prevalence estimation, either once or repeatedly, while yet others provide continuous information, suitable for the estimate of incidence data (e.g. *disease* reporting systems, sentinel sites, testing schemes).

a) Disease reporting or notification systems

Data derived from *disease* reporting systems can be used in combination with other data sources to substantiate claims of animal health status, to generate data for risk analysis, or for early detection. The first step of a *disease* reporting or notification system is often based on the observation of abnormalities (e.g. clinical signs, reduced growth, elevated mortality rates, behavioural changes, etc.), which can provide important information about the occurrence of endemic, exotic or new *diseases*. Effective laboratory support is, however, an important component of most reporting systems. Reporting systems relying on laboratory confirmation of suspect clinical cases should use tests that have a high specificity. Reports should be released by the laboratory in a timely manner, with the amount of time from *disease* detection to report generation minimised.

Appendix XXXII (contd)

Appendix IV (contd)

b) Control programmes/health schemes

Animal *disease* control programmes or health schemes, while focusing on the control or eradication of specific *diseases*, should be planned and structured in such a manner as to generate data that are scientifically verifiable and contribute to structured surveillance.

c) Targeted testing/screening

This may involve testing targeted to selected sections of the population (subpopulations), in which *disease* is more likely to be introduced or found. Examples include testing culled and dead animals, animals exhibiting clinical signs, animals located in a defined geographical area and specific age or commodity group.

d) Post-harvest inspections

Inspections of aquatic animal slaughter premises or processing plants may provide valuable surveillance data provided diseased aquatic animals survive to slaughter. Post-harvest inspections are likely to provide good coverage only for particular age groups and geographical areas. Post-harvest surveillance data are subject to obvious biases in relation to target and study populations (e.g. only animals of a particular class and age may be slaughtered for human consumption in significant numbers). Such biases need to be recognised when analysing surveillance data.

Both for traceback in the event of detection of *disease* and for analysis of spatial and population-level coverage, there should be, if possible, an effective identification system that relates each animal in the slaughter premises/processing plant to its locality of origin.

e) Laboratory investigation records

Analysis of laboratory investigation records may provide useful surveillance information. The coverage of the system will be increased if analysis is able to incorporate records from national, accredited, university and private sector laboratories. Valid analysis of data from different laboratories depends on the existence of standardised diagnostic procedures and standardised methods for interpretation and data recording. If available, the method listed in the *Aquatic Manual* in relation to the purpose of testing should be used. As with post-harvest inspections, there needs to be a mechanism to relate specimens to the farm of origin. It must be recognised that laboratory submissions may not accurately reflect the *infection* or *disease* situation on the farm.

f) Biological specimen banks

Specimen banks consist of stored specimens, gathered either through representative sampling or opportunistic collection or both. Specimen banks may contribute to retrospective studies, including providing support for claims of historical freedom from *infection*, and may allow certain studies to be conducted more quickly and at lower cost than alternative approaches.

g) Sentinel units

Sentinel units/sites involve the identification and regular testing of one or more of animals of known health/exposure status in a specified geographical location to detect the occurrence of *disease*. They are particularly useful for surveillance of *diseases* with a strong spatial component, such as vector-borne *diseases*. Sentinel units provide the opportunity to target surveillance depending on the likelihood of *infection* (related to vector habitats and host population distribution), cost and other practical constraints. Sentinel units may provide evidence of freedom from *infection*, or provide data on prevalence and incidence as well as the distribution of *disease*. Cohabitation with a susceptible population should be considered for testing *infection* or *disease* in populations of valuable animals, the lethal sampling of which may be unacceptable (e.g. ornamental fish).

h) Field observations

Clinical observations of epidemiological units in the field are an important source of surveillance data. The sensitivity and/or specificity of field observations may be relatively low, but these can be more easily determined and controlled if a clear, unambiguous and easy to apply standardised case definition is applied. Education of potential field observers in application of the case definition and reporting is an important component. Ideally, both the number of positive observations and the total number of observations should be recorded.

i) Farm production records

Systematic analysis of farm production records may be used as an indicator of the presence or absence of *disease* at the population level. If production records are accurate and consistently maintained, the sensitivity of this approach may be quite high (depending on the *disease*), but the specificity is often quite low.

2. Critical elements for structured non-random surveillance

There is a number of critical factors that should be taken into account when using structured non-random surveillance data such as coverage of the population, duplication of data, and sensitivity and specificity of tests that may give rise to difficulties in the interpretation of data. Surveillance data from non-random data sources may increase the level of confidence or be able to detect a lower level of prevalence with the same level of confidence compared to structured surveys.

3. Analytical methodologies

Different scientifically valid methodologies may be used for the analysis of non-random surveillance data. This most often requires information on parameters of importance to the surveillance system, such as sensitivity and specificity. Where no such data are available, estimates based on expert opinions, gathered and combined using a formal, documented and scientifically valid methodology may be used.

4. Combination of multiple sources of data

The methodology used to combine the evidence from multiple data sources should be scientifically valid, and fully documented including references to published material.

Surveillance information gathered from the same country, *zone* or *compartment* at different times (e.g. repeated annual surveys) may provide cumulative evidence of animal health status. Such evidence gathered over time may be combined to provide an overall level of confidence. However, a single larger survey, or the combination of data collected during the same time period from multiple random or non-random sources, may be able to achieve the same level of confidence in a shorter period of time.

Analysis of surveillance information gathered intermittently or continuously over time should, where possible, incorporate the time of collection of the information to take into account the decreased value of older information. The sensitivity, specificity and completeness of data from each source should also be taken into account for the final overall confidence level estimation.

Article 3.8.1.6.

Surveillance to demonstrate freedom from disease/infection

1. Demonstration of freedom from infection

A surveillance system to demonstrate freedom from *infection* should meet the following requirements in addition to the general requirements for surveillance outlined in Article 3.8.1.3 of this Appendix.

Freedom from *infection* implies the absence of the pathogenic agent in the country, *zone* or *compartment*. Scientific methods cannot provide absolute certainty of the absence of *infection*. Demonstrating freedom from *infection* involves providing sufficient evidence to demonstrate (to a level of confidence acceptable to Member Countries) that *infection* with a specified pathogen is not present in a population. In practice, it is not possible to prove (i.e. be 100% confident) that a population is free from *infection*. Instead, the aim is to provide adequate evidence (to an acceptable level of confidence), that *infection*, if present, is present in less than a specified proportion of the population.

However, apparent *infection* at any level in the target population automatically invalidates any freedom from *infection* claim unless the positive test results are accepted as false positives based on specificity values described in the relevant *disease* chapter.

2. Requirements to declare a country, zone or compartment free from disease/infection without pathogen specific surveillance

This Article provides general principles for declaring a country, *zone* or *compartment* free from *disease/infection* in relation to the time of last occurrence and in particular for the recognition of historical freedom.

The provisions of this Article are based on the principles described in Article 3.8.1.3. of this Appendix and the following premises:

- in the absence of *disease* and vaccination, the farmed and wild animal populations would become susceptible over a period of time;
- the *disease* agents to which these provisions apply are likely to produce identifiable clinical signs in observable susceptible animals;
- competent and effective *Competent Authority* will be able to investigate, diagnose and report *disease*, if present;

Appendix XXXII (contd)Appendix IV (contd)

- o the absence of *disease/infection* over a long period of time in a susceptible population can be substantiated by effective *disease* investigation and reporting by a Member Country.

a) Absence of susceptible species

Unless otherwise specified in the relevant *disease* chapter, a country, *zone* or *compartment* may be recognised as being free from *infection* without applying *targeted surveillance* if there are no susceptible species (as listed in the relevant chapter of this *Aquatic Manual*, or in the scientific literature) present in that country, *zone* or *compartment*.

b) Historically free

Unless otherwise specified in the relevant *disease* chapter, a country, *zone* or *compartment* may be recognised free from *infection* without formally applying a pathogen-specific surveillance programme when:

- i) there has never been a substantiated occurrence of *disease* reported officially or in the scientific literature (peer reviewed), or
- ii) eradication has been achieved or the *disease/infection* has ceased to occur for at least 25 years,

provided that for at least the past 10 years:

- iii) the *basic biosecurity conditions* are in place and effectively enforced;
- iv) no vaccination against the *disease* has been carried out unless otherwise allowed for in the *Aquatic Code*;
- v) *infection* is not known to be established in wild aquatic animals within the country or *zone* intended to be declared free. (A country or *zone* cannot apply for historical freedom if there is any evidence of *infection* in wild aquatic animals. However, specific surveillance in wild aquatic animals is not necessary.)

A country, *zone* or *compartment* that was self-declared free on the basis of the absence of susceptible species, but subsequently introduces any of the susceptible species as listed in the *Aquatic Manual*, may be considered historically free from the *disease* provided that:

- the country, *zone* or *compartment* of origin was declared free of the *disease* at the time of introduction,
- *basic biosecurity conditions* were introduced prior to the introduction,
- no vaccination against the *disease* has been carried out unless otherwise allowed for in the *disease* specific chapter of this *Aquatic Code*.

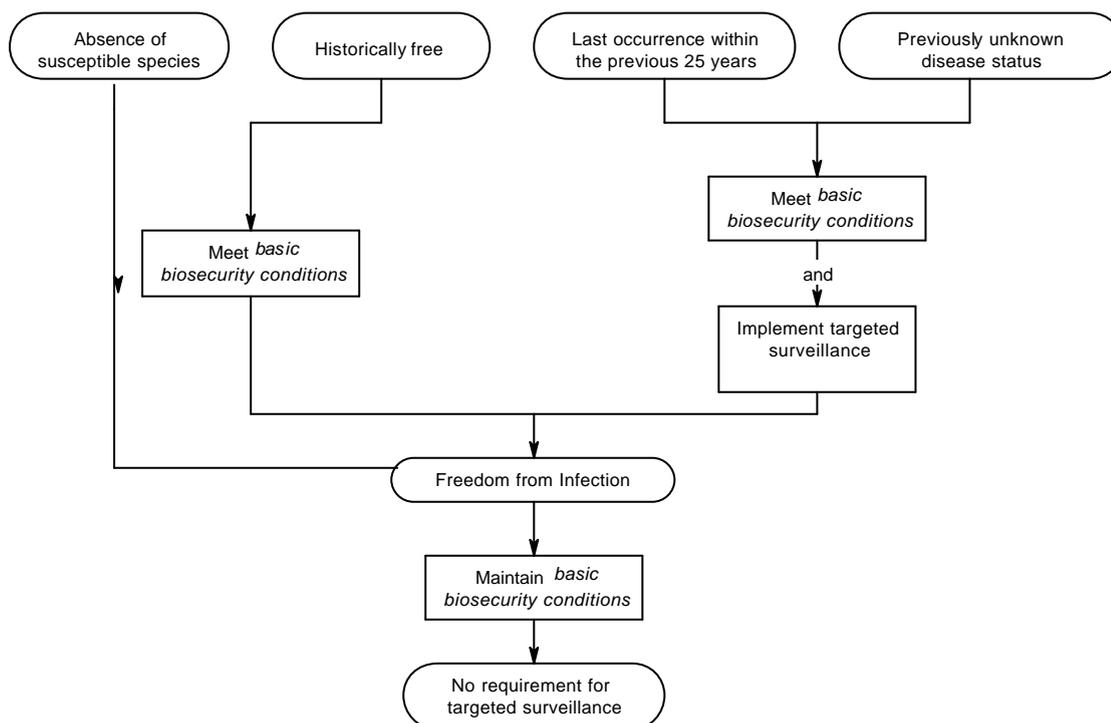
c) Last occurrence within the previous 25 years

Countries, *zones* or *compartments* that have achieved eradication (or in which the *disease/infection* has ceased to occur) within the previous 25 years, should follow the pathogen-specific surveillance requirements in the *Aquatic Manual* if they exist. In the absence of *disease* specific information to aid the development of a surveillance system, declaration of *disease* freedom should follow at least 2 surveys per year (for at least 2 consecutive years) to be conducted 3 or

more months apart, at the appropriate life stage and at times of the year when temperature and season offer the best opportunity to detect the pathogen. Surveys should be designed to provide an overall 95% confidence and with a design prevalence at the animal and higher (i.e. pond, farm, village, etc.) levels being 2% or lower (this value may be different for different *diseases* and may be provided in the specific *disease* chapter in the *Aquatic Manual*). Such surveys should not be based on voluntary submission and should be developed following the guidelines provided in the *Aquatic Manual*. Survey results will provide sufficient evidence of *disease* freedom provided that for at least the past 10 years these additional criteria are met:

- i) the *basic biosecurity conditions* are in place and effectively enforced;
- ii) no vaccination against the *disease* has been carried out unless otherwise provided in the *Aquatic Code*;
- iii) *infection* is not known to be established in wild aquatic animals within the country or *zone* intended to be declared free. (A country or *zone* cannot apply for freedom if there is any evidence of *infection* in wild aquatic animals. Specific surveillance in wild aquatic animals of susceptible species is necessary to confirm absence.)

The different paths to recognition of freedom from *infection* are summarised in the diagram below.



2. Guidelines for the discontinuation of pathogen-specific surveillance after recognition of freedom from infection

A country or *zone* that has been recognised as free from *infection* following the provisions of the *Aquatic Code* may discontinue pathogen-specific surveillance while maintaining the *infection*-free status provided that:

Appendix XXXII (contd)

Appendix IV (contd)

- a) the *basic biosecurity conditions* are in place and effectively enforced;
- b) vaccination against the *disease* is not applied;
- c) Surveillance has demonstrated that *infection* is not present in wild aquatic animal populations of susceptible species.

A special case can be made for a *compartment* located in a country or *zone* that is not proven to be free from *infection* if surveillance is maintained and exposure to potential sources of *infection* is prevented.

3. International recognition of disease/infection free status

For *diseases* for which procedures exist whereby the OIE can officially recognise the existence of a *disease/infection* free country, *zone* or *compartment*, a Member Country wishing to apply for recognition of this status shall, via its Permanent Delegate, send to the OIE all the relevant documentation relating to the country, *zone* or *compartment* concerned. Such documentation should be presented according to guidelines prescribed by the OIE for the appropriate animal *diseases*.

Article 3.8.1.7.

Surveillance for distribution and occurrence of infection

Surveillance to determine distribution and occurrence of *infection* or of other relevant health related events is widely used to assess the prevalence and incidence of selected *disease/infection* as an aid to decision making, for example implementation of control and eradication programmes. It also has relevance for the international movement of animals and products when movement occurs among infected countries.

In contrast to surveillance to demonstrate freedom from *infection*, surveillance for the distribution and occurrence of *infection* is usually designed to collect data about a number of variables of animal health relevance, for example:

- a) prevalence or incidence of *infection* in wild or cultured animals;
- b) morbidity and mortality rates;
- c) frequency of *disease/infection* risk factors and their quantification;
- d) frequency distribution of variables in *epidemiological units*;
- e) frequency distribution of the number of days elapsing between suspicion of *infection* and laboratory confirmation of the diagnosis and/or to the adoption of control measures;
- f) farm production records, etc.

CHAPTER 1.1.4.

**GUIDELINES FOR AQUATIC ANIMAL
HEALTH SURVEILLANCE [REQUIREMENTS FOR
SURVEILLANCE FOR INTERNATIONAL RECOGNITION OF
FREEDOM FROM INFECTION]**

[PART 1

INTERNATIONAL RECOGNITION OF FREEDOM FROM INFECTION

1. General principles

General principles are provided below for declaring a country, zone or aquaculture establishment free from infection in relation to the time of last occurrence, and in particular for the recognition of historical freedom.

An essential prerequisite to provide the guarantees required for the recognition of freedom from infection is that the particular Member Country complies with the requirements of Chapter 1.4.3 of the *Aquatic Code* for the evaluation of the Competent Authorities.

The general principles are:

- in the absence of infection or vaccination, the animal *population* would be susceptible to clinical disease, or infection, over a period of time;
- the disease agents to which these provisions apply are likely to produce identifiable clinical or pathological signs in susceptible animals;
- an animal *population* may be free from some specified pathogens but not from others;
- there are competent and effective personnel of the Competent Authority able to investigate, diagnose and report disease or infection, if present;
- the absence of infection over a long period of time in susceptible *populations* can be substantiated by effective disease investigation and reporting by the Competent Authority of the Member Country.

2. Requirements to declare a country, zone or aquaculture establishment free from infection with a specified pathogen

The requirements to declare a country, zone or aquaculture establishment free from infection differ depending on the previous infection status of the country, zone or aquaculture establishment, namely:

- Absence of susceptible species;
- Historically free;
- Last known occurrence within the previous 25 years;
- Previously unknown infection status.

2.1. Absence of susceptible species

Unless otherwise specified in the relevant disease chapter, a country, zone or aquaculture establishment may be recognised as being free from infection without applying *targeted surveillance* if there are no susceptible species (as listed in the relevant chapter of the *Aquatic Code*, or in the scientific literature) present in that country, zone or aquaculture establishment, provided that the *prescribed biosecurity conditions* have been in place continuously in the country, zone or aquaculture establishment for at least the previous 10 years.

2.2. Historically free

Unless otherwise specified in the relevant disease chapter, a country, zone or aquaculture establishment may be recognised as being free from infection without formally applying *targeted surveillance* when:

- there has never been any observed occurrence of disease;

Appendix XXXII (contd)

Appendix V (contd)

or

- eradication has been achieved or the disease has ceased to occur for at least 25 years,

provided that the *prescribed biosecurity conditions* have been in place continuously in the country, zone or aquaculture establishment for at least the previous 10 years.

2.3. Last known occurrence within the previous 25 years

For countries or zones that have achieved eradication (or in which the disease has ceased to occur) within the previous 25 years, in addition to the *prescribed biosecurity conditions*, appropriate *targeted surveillance* must have been applied to demonstrate the absence of the infection, consistent with the provisions of Section B of this chapter.

2.4. Previously unknown infection status

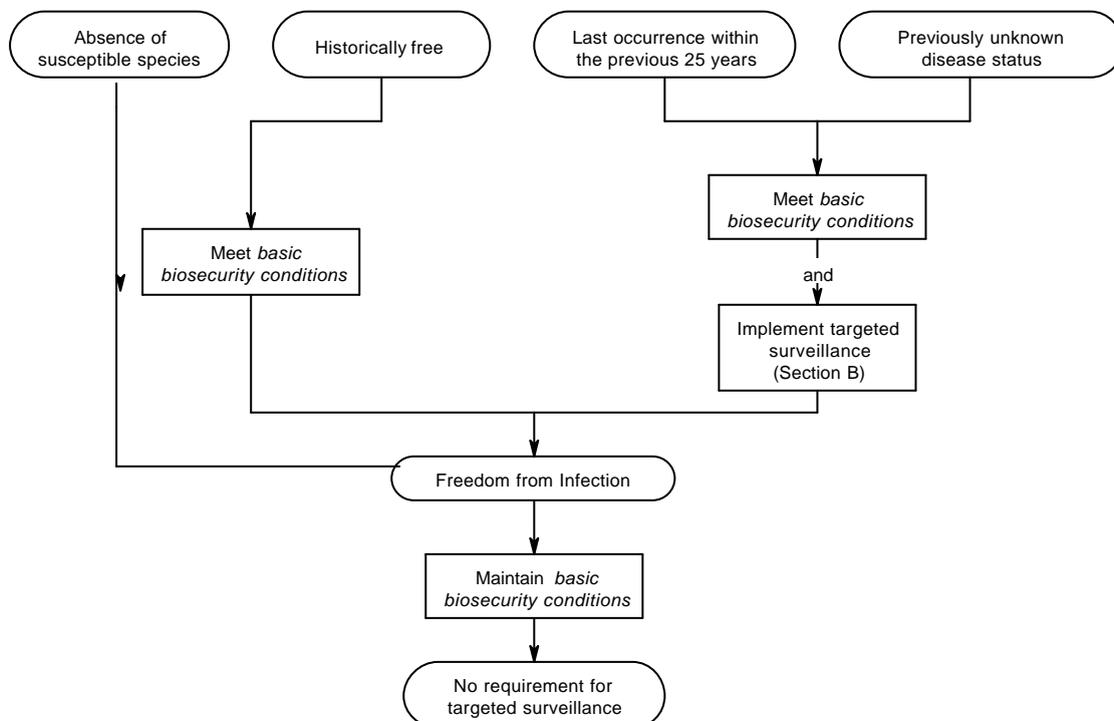
For countries or zones with previously unknown infection status, or which have not previously met the requirements of the Sections A.2.1, A.2.2 or A.2.3 above, the *prescribed biosecurity conditions* must be introduced in addition to *targeted surveillance* consistent with the provisions of Section B of this chapter.

3. Guidelines for the maintenance of continued recognition of freedom from infection

A country, zone or aquaculture establishment that has been recognised free from infection following the provisions of Sections A.2.1 or A.2.2, may maintain its official status as infection free provided that the *prescribed biosecurity conditions* are continuously maintained.

A country, zone or aquaculture establishment that has been recognised free from infection following the provisions of Sections A.2.3 or A.2.4, may discontinue *targeted surveillance* and maintain its official status as infection free provided that the *prescribed biosecurity conditions* are continuously maintained.

The different paths to recognition of freedom from infection are summarised in the diagram below.]



B. GUIDELINES FOR AQUATIC ANIMAL HEALTH SURVEILLANCE

1. Introduction

2. ~~[This section provides standards to be applied when demonstrating country, zone or aquaculture establishment freedom from infection, in accordance with the principles of Section A. Standards described in this section]~~ Surveillance is aimed at:

- = demonstrating the absence of *disease* or *infection*.
- = identifying events requiring notification as listed in Article 1.2.1.3 of the *Aquatic Code*.
- = determining the occurrence or distribution of endemic *disease* or *infection*, including changes to their incidence or prevalence (or its contributing factors), in order to:
 - provide information for domestic disease control programmes.
 - provide relevant disease occurrence information to be used by trading partners for qualitative and quantitative risk assessment.

The type of surveillance applied depends on the desired outputs needed to support decision-making. Surveillance data determine the quality of disease status reports and should satisfy information requirements for accurate risk analysis both for *international trade* as well as for national decision-making.

The following guidelines may be applied to all *diseases*, their agents and susceptible species as listed in the *Aquatic Manual*, and are designed to assist with the development of surveillance methodologies. Where possible, the development of surveillance systems using these guidelines should be based on the relevant information in the individual disease chapters.

There is sometimes a perception that surveillance can only be conducted using sophisticated methodologies. However, an effective surveillance system can also be developed by making use of gross observations and already available resources.

Surveillance of endemic diseases provides valuable information for day-to-day health management and can act as the foundation for detecting outbreaks of exotic disease and demonstrating specific disease freedom.

Surveillance may address both infectious and non-infectious diseases of concern to the country.

Section B provides standards to be applied when: (a) demonstrating country, zone or compartment freedom from infection, in accordance with the principles of Section A and (b) assessing the occurrence and distribution of a specific *infection/disease* or syndrome

Standards described in this section may be applied to all *diseases*, their agents and susceptible species as listed in the *Aquatic Code*, and are designed to assist with the development of surveillance methodologies. Nevertheless surveillance may include also non listed diseases

It would be impractical to try to develop a surveillance system for all the known aquatic animal diseases for which a country has susceptible species. Therefore prioritising the diseases to be included in a surveillance system should be conducted considering:

- the needs to provide assurance of disease status for trade purposes
- the resources of the country
- the financial impact or threat posed by the different diseases
- the importance of an industry-wide disease control programme within a country or region

The concept of risk encompasses both the probability of the disease occurring and the severity of its consequences

More detailed information in each disease chapter (where it exists) of this *Aquatic Manual* may be used to further refine the general approaches described in this chapter. Where detailed *disease/infection*-specific information is not available, surveillance can also be conducted following the guidelines in this chapter. Access to epidemiological expertise would be invaluable for the design, implementation of the system and interpretation of results derived from a surveillance system.

2. General principles

~~[Demonstrating freedom from infection involves providing sufficient evidence to demonstrate that infection with a specified agent is not present in a specified population. In practice, it is not possible to definitively prove that a population is free from infection (unless every member of the population is examined simultaneously with a perfect test with both sensitivity and specificity equal to 100%). Instead, the aim is to provide adequate evidence (to an acceptable level of confidence), that infection, if present, is present in less than a specified proportion of the population.]~~

~~Methodologies to demonstrate freedom from infection should be]~~ Surveillance methodologies should be flexible to deal with the complexity of real life situations. No single method is applicable in all cases. Methodologies must be able to accommodate the variety of aquatic animal species, the multiple diseases of relevance, varying production ~~[and surveillance]~~ systems, and types and amounts of data and information available.

The methodology used should be based on the best available information that is in accord with current scientific thinking. The methodology should be well documented and supported with references to the scientific literature and other sources, including expert opinion. Efforts should be made to address the information gaps wherever possible.

~~[Consistency in methodologies should be encouraged and transparency is]~~ Methodologies that are consistent and transparent are essential to ensure fairness and rationality, consistency in decision making and ease of understanding by all the interested parties. [Applications for] The presentation of the results generated through surveillance (e.g. recognition of infection-free status or measures of disease frequency) should document the uncertainties, the assumptions made, and the potential effect of these on the final estimate.

3. Surveillance ~~General requirements~~ for demonstration of freedom from disease ~~[infection]~~

This section describes surveillance to demonstrate freedom from disease.

3.1. Objectives ~~[Population]~~

~~[The target population to which the demonstration of freedom from infection applies is all individuals of all species susceptible to the infection in a country, zone or aquaculture establishment.]~~

Appendix XXXII (contd)

Appendix V (contd)

The *study population* may be the same as the *target population* or a subset of it. The *study population* should be (in order of preference):

- The appropriate *study population* as defined in the relevant disease chapter of the *Aquatic Code* (if such a definition exists);
- A subset of the *target population* that defines a group of animals which, if infection were present, would be most likely to have a higher prevalence of infection than the *target population*. This subset should be defined in terms of:
 - species;
 - time (e.g. season or month of year);
 - stage of life cycle or growth period;
 - production system and/or management characteristics;
 - location;
 - readily identifiable physical or behavioural characteristics.
- The same as the *target population*;
- A subset of the *target population* with the same or lower probability of infection. The nature and impact of any biases on the results of the analysis must be considered, documented and taken into account in the analysis.]

The objective of this kind of surveillance system is to contribute on an on-going basis evidence to demonstrate freedom from disease in a particular country, zone or compartment with a known confidence and reference to a predetermined design prevalence and diagnostic test characteristics. The level of confidence and the design prevalence will depend on the testing situation, disease and host population characteristics and on the resources available.

A single such survey can contribute evidence adding to an on-going collection of health data (see also Section 5. Specific requirements for complex non-survey data sources). However, single surveys in isolation rarely, if ever, provide sufficient evidence that an aquatic animal disease is absent and must be augmented with on-going targeted evidence collection (e.g. ongoing disease sampling or passive detection capabilities) to substantiate claims of freedom from disease.

3.2. Population

The *population* of *epidemiological units* must be clearly defined. The *target population* consists of all individuals of all species susceptible to the disease or infection in a country, zone or compartment to which the surveillance results apply. Sometimes components of the target population are at higher risk of being the point of introduction for an exotic disease. In these cases, it is advisable to focus surveillance efforts on this part of the population, such as farms on a geographical border.

The design of the survey will depend on the size and structure of the *population* being studied. If the *population* is relatively small and can be considered to be homogenous with regards to risk of infection, a single-stage survey can be used.

In larger *populations* where a sampling frame is not available, or when there is a likelihood of clustering of disease, multi-stage sampling is required. In two-stage sampling, at the first stage of sampling, groups of animals (e.g. ponds, farms or villages) are selected. At the second stage, animals are selected for testing from each of the selected groups.

In the case of a complex (e.g. multi-level) population structure, multi-level sampling (ref) may be used and the data analysed accordingly in survey design.

3.3. Sources of evidence

Surveillance data may originate [~~Evidence of freedom from infection may be based on a~~] from a number of different sources, including:

- structured, population-based surveys using one or more *tests to detect* [~~for the presence of~~] the agent;
- other [~~surveillance, including~~] structured non-random [~~surveillance~~]sources, such as:
 - sentinel sites;
 - disease notifications and laboratory investigation records;
 - academic and other scientific studies;
- a knowledge of the biology of the agent, including environmental, host *population* distribution, known geographical distribution, vector distribution and climatic information;
- history of imports of potentially infected material;
- biosecurity measures in place;
- [• ~~evaluation of the official services; or~~]
- any other sources of information that provide contributory evidence regarding disease or [~~that~~] infection [~~is not present~~] in the country, zone or compartment [~~aquaculture establishment~~].

The sources of evidence [~~used to demonstrate freedom from infection~~] must be fully described. In the case of a structured survey, this must include a description of the sampling strategy used for the selection of *units* for testing. For complex *surveillance systems*, a full description of the system is required including consideration of any biases that may be inherent in the system. Evidence to support claims of freedom of disease can use structured non-random sources of information provided any potential error is to detect rather than miss positive cases (i.e. it should be biased towards detection).

3.4. Statistical methodology

Analysis of *test* results from a survey shall be in accordance with the provisions of this chapter and consider the following factors:

- The survey design;
- The sensitivity and specificity of the *test*, or *test system*;
- The design prevalence (or prevalences where a multi-stage design is used);
- The results of the survey.

Analysis of data for evidence of freedom from infection involves estimating the probability (a) that the evidence observed (the results of surveillance) could have been produced under the null hypothesis that infection is present in the *population* at a specified prevalence(s) (the design prevalence[s]). The *confidence* in (or, equivalently, the sensitivity of) the *surveillance system* that produced the evidence is equal to 1-a. If the *confidence* level exceeds a pre-set threshold, the evidence is deemed adequate to demonstrate freedom from infection.

The required level of *confidence* in the *surveillance system* (probability that the system would detect infection if infection were present at the specified level) must be greater than or equal to 95%.

The power (probability that the system would report that no infection is present if infection is truly not present) may be set to any value. By convention, this is often set to 80%, but may be adjusted according to the country's or zone's requirements.

Different statistical methodologies for the calculation of the probability *a*, including both quantitative and qualitative approaches, are acceptable as long as they are based on accepted scientific principles.

The methodology used to calculate the *confidence* in the *surveillance system* must be scientifically based and clearly documented, including references to published work describing the methodology.

[3.4. Clustering of infection

~~Infection in a country, zone or aquaculture establishment usually clusters rather than being uniformly distributed through a *population*. Clustering may occur at a number of different levels (e.g. a cluster of moribund fish in a pond, a cluster of ponds in a farm, or a cluster of farms in a zone). Except when dealing with demonstrably homogenous *populations*, approaches to demonstrating freedom must take this clustering into account in the design and the statistical analysis of the data, at least at what is judged to be the most significant level of clustering for the particular animal *population* and infection.~~

3.5. Design prevalence]

Statistical analysis of surveillance data often requires assumptions about population parameters or test characteristics. These are usually based on expert opinion, previous studies on the same or different populations, expected biology of the agent, and so on. The uncertainty around these assumptions must be quantified and considered in the analysis (e.g. in the form of prior probability distributions in a Bayesian setting).

For surveillance systems used to demonstrate freedom from specific diseases, calculation of the *confidence* of a *surveillance system* is based on the null hypothesis that infection is present in the *population*. The level of infection is specified by the design prevalence. In the simplest case, this is the prevalence of infection in a homogenous *population*. More commonly, in the presence of a complex (e.g. multi-level) population structure ~~disease clustering, two more than one~~ design prevalence value is required, for instance, the animal-level prevalence (proportion of ~~fish~~ infected animals in an infected farm) and the group-level prevalence (proportion of infected farms in the country, zone or compartment ~~[aquaculture establishment]~~). Further levels of clustering may be considered, requiring further design prevalence values.

The values for design prevalence used in calculations must be those specified in the relevant disease chapter (if present) of this *Aquatic Manual*. If not specified for the particular disease, justification for the selection of design prevalence values must be provided, and should be based on the following guidelines:

- At the individual animal level, the design prevalence is based on the biology of the infection in the *population*. It is equal to the minimum expected prevalence of infection in the *study population*, if the infection had become established in that *population*. It is dependent on the dynamics of infection in the *population* and the definition of the *study population* (which may be defined to maximise the expected prevalence in the presence of infection).

- A suitable design prevalence value at the animal level (e.g. prevalence of infected animals in a cage) may be:
 - between 1% and 5% for infections that are present in a small part of the population e.g. are transmitted slowly or are at the early stages of an outbreak, etc.; ~~[and]~~
 - over 5% for highly transmissible infections ~~[more contagious infections]~~.

If reliable information on the expected prevalence in an infected population is not available, a value of 2% should be used for the design prevalence.

- At higher levels (e.g. cage, pond, farm, village, etc.) the design prevalence usually reflects the prevalence of infection that is practically and reasonably able to be detected by a *surveillance system*. Detection of infection at the lowest limit (a single infected *unit* in the *population*) is rarely feasible in large *populations*. The expected behaviour of the infection may also play a role. Infections that have the ability to spread rapidly between farms may have a higher farm-level design prevalence than slow-moving infections.

A suitable design prevalence value for the first level of clustering, (e.g. proportion of infected farms in a zone) may be up to 2%.

When surveillance data are used to estimate incidence and prevalence measures for the purpose of describing disease occurrence in terms of animal unit, time and place. These measures can be calculated for an entire population and specific time period, or for subsets defined by host characteristics (e.g. age-specific incidence). Incidence estimation requires on-going surveillance to detect new cases while prevalence is the estimated proportion of infected individuals in a population at a given time point. The estimation process must consider test sensitivity and specificity.

3.5. Clustering of infection

Infection in a country, zone or compartment ~~[aquaculture establishment]~~ usually clusters rather than being uniformly distributed through a *population*. Clustering may occur at a number of different levels (e.g. a cluster of moribund fish in a pond, a cluster of ponds in a farm, or a cluster of farms in a zone). Except when dealing with demonstrably homogenous *populations*, surveillance must take this clustering into account in the design and the statistical analysis of the data, at least at what is judged to be the most significant level of clustering for the particular animal *population* and infection.

~~[3.5. Expected prevalence]~~

3.6. Test characteristics

All surveillance involves performing one or more *tests* for evidence of the presence of current or past infection, ranging from detailed laboratory examinations to farmer observations. The performance level of a *test* at the *population* level is described in terms of its *sensitivity* and *specificity*. Imperfect sensitivity and/or specificity impact on the interpretation of surveillance results and must be taken into account in the analysis of surveillance data. For example, in the case of a test with imperfect specificity, if the population is free of disease or has a very low prevalence of infection, all or a large proportion of positive tests will be false. Subsequently, samples that test positive can be confirmed or refuted using a highly specific test. Where more than one test is used in a *surveillance system* (sometimes called using tests in series or parallel), the *sensitivity* and *specificity* of the test combination must be calculated ~~[using a scientifically valid method]~~.

All calculations must take the performance level (sensitivity and specificity) of any *tests* used into account. The values of sensitivity and specificity used for calculations must be specified, and the method used to determine or estimate these values must be documented. ~~[Where]~~ Test sensitivity and specificity can be different when applied to different populations and testing scenarios. For example, test sensitivity may be lower when testing carrier animals with low level infections compared to moribund animals with clinical disease. Alternatively, specificity depends on the presence of cross-reacting agents, the distribution of which may be different under different conditions or regions. Ideally, test performance should be assessed under the conditions of use otherwise increased uncertainty exists regarding their performance. In the absence of local assessment of tests, values for sensitivity and/or specificity for a particular test that are specified in this Aquatic Manual may be used but the increased uncertainty associated with these estimates should be incorporated into the analysis of results ~~[these values may be used without justification].~~

Pooled testing involves the pooling of specimens from multiple individuals and performing a single *test* on the pool. Pooled testing is an acceptable approach in many situations. Where pooled testing is used, the results of testing must be interpreted using sensitivity and specificity values that have been determined or estimated for that particular pooled testing procedure and for the applicable pool sizes being used. Analysis of the results of pooled testing must, where possible, be performed using accepted, statistically based methodologies, which must be fully documented, including published references.

3.7. Multiple sources of information ~~[evidence]~~

Where multiple different data sources providing evidence of freedom from infection exist ~~[or are generated]~~, each of these data sources may be analysed accordingly ~~[to the provisions of Sections B.3, B.4 (for structured surveys) and B.5 (for complex data sources)]~~. The resulting estimates of the *confidence* in each data source may be combined to provide an overall level of *confidence* for the combined data sources.

The methodology used to combine the estimates from multiple data sources:

- must be scientifically valid, and fully documented, including references to published material; and
- should, where possible, take into account any lack of statistical independence between different data sources.

Surveillance information gathered from the same country, zone or compartment at different times (e.g. repeated annual surveys) may provide cumulative evidence of animal health status. Such evidence gathered over time may be combined to provide an overall level of confidence. However, a single larger survey, or the combination of data collected during the same time period from multiple random or non-random sources, may be able to achieve the same level of confidence in a shorter period of time.

~~[Surveillance information gathered from the same country, zone or aquaculture establishment at different times may provide cumulative evidence of freedom from infection. Such evidence gathered over time may be combined into an overall level of confidence. For instance, repeated annual surveys may be analysed to provide a cumulative level of confidence. However, a single (larger) survey may be able to achieve the same level of confidence in just 1 year.]~~

Analysis of surveillance information gathered intermittently or continuously over time should, where possible, incorporate the time of collection of the information to take into account the decreased value of older information. The sensitivity, specificity and completeness of data from each source should also be taken into account for the final overall confidence level estimation.

~~[3.8. Survey design~~

~~The most important unit of diagnosis is the epidemiological unit.]~~

3.8. Sampling

The objective of sampling from a *population* is to select a subset of *units* from the *population* that is representative of the *population* with respect to the characteristic of interest (in this case, the presence or absence of infection). The survey design may involve sampling at several levels. For sampling at the level of the *epidemiological units* or higher *units*, a formal *probability sampling* (e.g. simple random sampling) method must be used. Sampling should be carried out in such a way as to provide the best likelihood that the sample will be representative of the *population*, within the practical constraints imposed by different environments and production systems.

[3.9. Sampling methods]

When sampling below the level of the *epidemiological unit* (e.g. individual animal), the sampling method used should provide the best practical chance of generating a sample that is representative of the *population* of the chosen *epidemiological unit*. Collecting a truly representative sample of individual animals (whether from a pond, cage or fishery) is often very difficult. To maximise the chance of finding infection, the aim should be to bias the sampling towards infected animals, e.g. selecting moribund animals, life stages with a greater chance of active infection, etc.

Biased or targeted sampling in this context involves sampling from a defined *study population* that has a different probability of infection than the *target population* of which it is a subpopulation. Once the *study population* has been identified, the objective is still to select a representative sample from this subpopulation.

The sampling method used at all levels must be fully documented and justified.

3.9. Sample size

The number of *units* to be sampled from a *population* should be calculated using a statistically valid technique that takes at least the following factors into account:

- The sensitivity and specificity of the *diagnostic test*, or *test system*;
- The design prevalence (or prevalences where a multi-stage design is used);
- The level of *confidence* that is desired of the survey results.

Additionally, other factors may be considered in sample size calculations, including (but not limited to):

- The size of the *population* (but it is acceptable to assume that the *population* is infinitely large);
- The desired power of the survey;
- Uncertainty about [or variability in estimates of] sensitivity and specificity.

The specific sampling requirements will need to be tailor-made for each individual disease, taking into account its characteristics and the specificity and sensitivity of the accepted testing methods for detecting the disease agent in host populations.

Appendix XXXII (contd)

Appendix V (contd)

FreeCalc¹⁸ is a suitable software for the calculation of sample sizes at varying parameter values. The table below provides examples of sample sizes generated by the software for a type I and type II error of 5% (i.e. 95% confidence and 95% statistical power). However, this does not mean that a type 1 and type 2 error of 0.05 should always be used. For example, using a test with sensitivity and specificity of 99%, 528 units should be sampled. If 9 or less of those units test positive, the population can still be considered free of the disease at a design prevalence of 2% provided that all effort is made to ensure that all presumed false positives are indeed false. This means that there is a 95% confidence that the prevalence is 2% or lower.

In the case in which the values of Se and Sp are not known (e.g. no information is available in the specific disease chapter in the *Aquatic Manual*), they should not automatically be assumed to be 100%. All positive results should be included and discussed in any report regarding that particular survey and all efforts should be made to ensure that all presumed false positives are indeed false.

¹⁸ FreeCalc – Cameron, AR. Software for the calculation of sample size and analysis of surveys to demonstrate freedom from disease. Available for free download from <http://www.ausvet.com.au>.

Appendix XXXII (contd)

Appendix V (contd)

Design prevalence	Sensitivity (%)	Specificity (%)	Sample size	Maximum number of false +ve if the population is free
2	100	100	149	0
2	100	99	524	9
2	100	95	1671	98
2	99	100	150	0
2	99	99	528	9
2	99	95	1707	100
2	95	100	157	0
2	95	99	542	9
2	95	95	1854	108
2	90	100	165	0
2	90	99	607	10
2	90	95	2059	119
2	80	100	186	0
2	80	99	750	12
2	80	95	2599	148
5	100	100	59	0
5	100	99	128	3
5	100	95	330	23
5	99	100	59	0
5	99	99	129	3
5	99	95	331	23
5	95	100	62	0
5	95	99	134	3
5	95	95	351	24
5	90	100	66	0
5	90	99	166	4
5	90	95	398	27
5	80	100	74	0
5	80	99	183	4
5	80	95	486	32
10	100	100	29	0
10	100	99	56	2
10	100	95	105	9
10	99	100	29	0
10	99	99	57	2
10	99	95	106	9
10	95	100	30	0
10	95	99	59	2
10	95	95	109	9
10	90	100	32	0
10	90	99	62	2
10	90	95	123	10
10	80	100	36	0
10	80	99	69	2
10	80	95	152	12

[Detailed guidelines are to be provided in the next (fifth) edition of the *Aquatic Manual*. In the meantime, the sampling procedures given in Chapters 1.1, 1.2 and 1.3 may be applied.]

3.10. Quality assurance

Surveys should include a documented quality assurance system, to ensure that field and other procedures conform to the specified survey design. Acceptable systems may be quite simple, as long as they provide verifiable documentation of procedures and basic checks to detect significant deviations of procedures from those documented in the survey design.

4. Specific requirements for complex non-survey data sources for freedom from disease

Data sources that provide evidence of freedom from infection, but are not based on structured population-based surveys may also be used to demonstrate freedom, either alone or in combination with other data sources. Different methodologies may be used for the analysis of such data sources, but the methodology must comply with the provisions of Section B.3. The approach used should, where possible, also take into account any lack of statistical independence between observations.

Analytical methodologies based on the use of step-wise probability estimates to describe the *surveillance system* may determine the probability of each step either by:

- the analysis of available data, using a scientifically valid methodology; or where no data are available,
- the use of estimates based on expert opinion, gathered and combined using a formal, documented and scientifically valid methodology.

Where there is significant uncertainty and/or variability in estimates used in the analysis, stochastic modelling or other equivalent techniques should be used to assess the impact of this uncertainty and/or variability on the final estimate of confidence.

5. Specific requirements for structured survey design and analysis to assess disease occurrence

This section describes surveillance to estimate parameters of disease occurrence.

5.1. Objectives

The objective of this kind of surveillance system is to contribute on an on-going basis evidence to assess the occurrence and distribution of disease or infection in a particular country, zone or compartment. This will provide information for domestic disease control programmes and relevant disease occurrence information to be used by trading partners for qualitative and quantitative risk assessment.

A single such survey can contribute evidence adding to an on-going collection of health data (see also Section 5. Specific requirements for complex non-survey data sources).

5.2. Population

The population of epidemiological units must be clearly defined. The target population consists of all individuals of all species susceptible to the disease or infection in a country, zone or compartment to which the surveillance results apply. Some local areas within a region may be known to be free of the disease of concern, allowing resources to be concentrated on known positive areas for greater precision of prevalence estimates and only verification of expected 0 prevalence areas.

The design of the survey will depend on the size and structure of the *population* being studied. If the *population* is relatively small and can be considered to be homogenous with regards to risk of infection, a single-stage survey can be used.

In larger *populations* where a sampling frame is not available, or when there is a likelihood of clustering of disease, multi-stage sampling is required. In two-stage sampling, at the first stage of sampling, groups of animals (e.g. ponds, farms or villages) are selected. At the second stage, animals are selected for testing from each of the selected groups.

In the case of a complex (e.g. multi-level) population structure, multi-level sampling (ref) may be used and the data analysed accordingly.

5.3. Sources of evidence

Surveillance data may originate from a number of different sources, including:

- structured, population-based surveys using one or more *tests* to detect the agent;
- other structured non-random sources, such as:
 - sentinel sites;
 - disease notifications and laboratory investigation records;
 - academic and other scientific studies;
- a knowledge of the biology of the agent, including environmental, host *population* distribution, known geographical distribution, vector distribution and climatic information;
- history of imports of potentially infected material;
- biosecurity measures in place;
- any other sources of information that provide contributory evidence regarding disease or infection in the country, zone or compartment.

The sources of evidence must be fully described. In the case of a structured survey, this must include a description of the sampling strategy used for the selection of *units* for testing. For complex *surveillance systems*, a full description of the system is required including consideration of any biases that may be inherent in the system. Evidence to support changes in prevalence/incidence of endemic disease must be based on valid, reliable methods to generate precise estimates with known error.

5.4. Statistical methodology

Analysis of survey data should be in accordance with the provisions of this chapter and should consider the following factors:

- The survey design;
- The sensitivity and specificity of the *test*, or *test system*;
- The results of the survey.

For surveillance systems used to describe disease patterns, the purpose is to estimate prevalence or incidence with confidence intervals or probability intervals. The magnitude of these intervals expresses the precision of the estimates and is related to sample size. Narrow intervals are desirable but will require larger sample sizes and more dedication of resources. The precision of the estimates and the power to detect differences in prevalence between populations or between time points depends not only on sample size, but also on the actual value of the prevalence in the population or the actual difference. For this reason, when designing the surveillance system, a prior estimate/assumption of expected prevalence or expected difference in prevalence must be made.

For the purpose of describing disease occurrence, measures of animal unit, time and place can be calculated for an entire population and specific time period, or for subsets defined by host characteristics (e.g. age-specific incidence). Incidence estimation requires on-going surveillance to detect new cases in a specified time period while prevalence is the estimated proportion of infected individuals in a population at a given time point. The estimation process must consider test sensitivity and specificity.

Statistical analysis of surveillance data often requires assumptions about population parameters or test characteristics. These are usually based on expert opinion, previous studies on the same or different populations, expected biology of the agent, information contained in the specific disease chapter of the *Aquatic Manual* and so on. The uncertainty around these assumptions must be quantified and considered in the analysis (e.g. in the form of prior probability distributions in a Bayesian setting).

When surveillance objectives are to estimate prevalence/incidence or changes in disease patterns, statistical analysis must account for sampling error. Analytic methods should be thoroughly considered and consultation with biostatistician/quantitative epidemiologist consulted beginning in the planning stages and continued throughout the programme.

5.5. Clustering of infection

Infection in a country, zone or compartment usually clusters rather than being uniformly distributed through a *population*. Clustering may occur at a number of different levels (e.g. a cluster of moribund fish in a pond, a cluster of ponds in a farm, or a cluster of farms in a zone). Except when dealing with demonstrably homogenous *populations*, surveillance must take this clustering into account in the design and the statistical analysis of the data, at least at what is judged to be the most significant level of clustering for the particular animal *population* and infection. For endemic diseases, it is important to identify characteristics of the population which contribute to clustering and thus provide efficiency in disease investigation and control.

5.6. Test characteristics

All surveillance involves performing one or more *tests* for evidence of the presence of current or past infection, ranging from detailed laboratory examinations to farmer observations. The performance level of a *test* at the *population* level is described in terms of its *sensitivity* and *specificity*. Imperfect sensitivity and/or specificity impact on the interpretation of surveillance results and must be taken into account in the analysis of surveillance data. For example, in populations with low prevalence of infection, a large proportion of positive tests may be false unless the tests used have perfect specificity. To ensure detection in such instances, a highly sensitive test is frequently used for initial screening and then confirmed with highly specific tests.

All calculations must take the performance level (sensitivity and specificity) of any tests used into account. The values of sensitivity and specificity used for calculations must be specified, and the method used to determine or estimate these values must be documented. Test sensitivity and specificity can be different when applied to different populations and testing scenarios. For example, test sensitivity may be lower when testing carrier animals with low level infections compared to moribund animals with clinical disease. Alternatively, specificity depends on the presence of cross-reacting agents, the distribution of which may be different under different conditions or regions. Ideally, test performance should be assessed under the conditions of use otherwise increased uncertainty exists regarding their performance. In the absence of local assessment of tests, values for sensitivity and/or specificity for a particular test that are specified in this Aquatic Manual may be used but the increased uncertainty associated with these estimates should be incorporated into the analysis of results.

Pooled testing involves the pooling of specimens from multiple individuals and performing a single test on the pool. Pooled testing is an acceptable approach in many situations. Where pooled testing is used, the results of testing must be interpreted using sensitivity and specificity values that have been determined or estimated for that particular pooled testing procedure and for the applicable pool sizes being used. Analysis of the results of pooled testing must, where possible, be performed using accepted, statistically based methodologies, which must be fully documented, including published references.

Test results from surveillance for endemic disease will provide estimates of apparent prevalence (AP). Using diagnostic sensitivity (DSe) and diagnostic specificity (DSp) as described in chapter 1.1.2 of this Aquatic Manual, true prevalence (TP) should be calculated with the following formula:

$$TP = (AP + DSp - 1) / (DSe + DSp - 1)$$

In addition, it should be remembered that different laboratories may obtain conflicting results for various test, host, or procedure-related reasons. Therefore, sensitivity and specificity parameters should be validated for the particular laboratory and process.

5.7. Multiple sources of information

Where multiple different data sources providing information on infection or disease are generated, each of these data sources may be analysed and presented separately.

Surveillance information gathered from the same country, zone or compartment at different times and similar methodology (e.g. repeated annual surveys) may provide cumulative evidence of animal health status and changes. Such evidence gathered over time may be combined (e.g. using Bayesian methodology) to provide more precise estimates and details of disease distribution within a population.

Apparent changes in disease occurrence of endemic diseases may be real or due to other factors influencing detection proficiency.

5.8. Sampling

The objective of sampling from a population is to select a subset of units from the population that is representative of the population with respect to the characteristic of interest (in this case, the presence or absence of infection). The survey design may involve sampling at several levels. For sampling at the level of the epidemiological units or higher units, a formal probability sampling (e.g. simple random sampling) method must be used. Sampling should be carried out in such a way as to provide the best likelihood that the sample will be representative of the population, within the practical constraints imposed by different environments and production systems.

When sampling below the level of the *epidemiological unit* (e.g. individual animal), the method used should be probability-based sampling. Collecting a true probability-based sample is often very difficult and care should therefore be taken in the analysis and interpretation of results obtained using any other method, the danger being that inferences could not be made about the sampled population.

The sampling method used at all levels must be fully documented and justified.

5.9. Sample size

The number of *units* to be sampled from a *population* should be calculated using a statistically valid technique that takes at least the following factors into account:

- The sensitivity and specificity of the *diagnostic test* (single or in combination):
- Expected prevalence or incidence in the *population* (or prevalences/incidences where a multi-stage design is used):
- The level of *confidence* that is desired of the survey results.
- The *precision* desired (i.e. the width of the *confidence* or *probability intervals*).

Additionally, other factors may be considered in sample size calculations, including (but not limited to):

- The size of the *population* (but it is acceptable to assume that the *population* is infinitely large):
- Uncertainty about sensitivity and specificity.

The specific sampling requirements will need to be tailor-made for each individual disease, taking into account its characteristics and the specificity and sensitivity of the accepted testing methods for detecting the disease agent in host populations.

A number of software packages, e.g. Survey Tool Box, WinPEPI (add links and refs) can be used for the calculation of sample sizes.

In the case in which the values of *Se* and *Sp* are not known (e.g. no information is available in the specific disease chapter in the *Aquatic Manual*), they should not automatically be assumed to be 100%. Assumed values should be produced in consultation with subject-matter experts.

5.10. Quality assurance

Surveys should include a documented quality assurance system, to ensure that field and other procedures conform to the specified survey design. Acceptable systems may be quite simple, as long as they provide verifiable documentation of procedures and basic checks to detect significant deviations of procedures from those documented in the survey design.

PART 2

6. EXAMPLE SURVEILLANCE SYSTEMS FOR FREEDOM FROM DISEASE

The following examples describe surveillance systems and approaches to the analysis of evidence for demonstrating freedom from disease ~~[that are able to meet the requirements of this chapter]~~. The purpose of these examples is:

- to illustrate the range of approaches that may be acceptable;
- to provide practical guidance and models that may be used for the design of specific surveillance systems; and
- to provide references to available resources that are useful in the development and analysis of surveillance systems.

While these examples demonstrate ways in which freedom from infection may be successfully demonstrated, they are not intended to be prescriptive. Countries are free to use different approaches, as long as they meet the requirements of this chapter.

The examples deal with the use of structured surveys and are designed to illustrate different survey designs, sampling schemes, the calculation of sample size, and analysis of results. It is important to note that alternative approaches to demonstrating freedom using complex non-survey-based data sources are also currently being developed and may soon be published¹⁹.

Example 1 – one-stage structured survey (farm certification ~~[accreditation]~~)

Context

A freshwater aquaculture industry raising fish in tanks has established a farm certification ~~[accreditation]~~ scheme. This involves demonstrating farm-level freedom from a particular (hypothetical) disease (Disease X). The disease does not spread very quickly, and is most common during the winter months, with adult fish at the end of the production cycle being most severely affected. Farms consist of a number of grow-out tanks, ranging from 2 to 20, and each tank holds between 1000 and 5000 fish.

Objective

The objective is to implement surveillance that is capable of providing evidence that an individual farm is free from Disease X. (The issue of national or zone freedom, as opposed to farm freedom, is considered in the next example.)

¹⁹ International EpiLab, Denmark, Research Theme 1: Freedom from disease.
http://www.vetinst.dk/high_uk.asp?page_id=196

Approach

The accreditation scheme establishes a set of standard operating procedures and requirements for recognition of freedom, based on the guidelines given in this chapter. These require farms to undertake a structured survey capable of producing 95% confidence that the disease would be detected if it were present. Once farms have been surveyed without detecting disease, they are recognised as free, as long as they maintain a set of minimum biosecurity standards. These standards are designed to prevent the introduction of Disease X into the farm (through the implementation of controls specific to the method of spread of that disease) and to ensure that the disease would be detected rapidly if it were to enter the farm (based on evidence of adequate health record keeping and the prompt investigation of unusual disease events). The effective implementation of these biosecurity measures is evaluated with annual on-farm audits conducted by independent auditors.

Survey standards

Based on the guidelines given in this chapter, a set of standards are established for the conduct of surveys to demonstrate freedom from infection with causative agent of Disease X. These standards include:

- The level of confidence required of the survey is 95% (i.e. Type I error = 5%).
- The power of the survey is arbitrarily set at 95% (i.e. Type II error = 5%, which means that there is a 5% chance of concluding that a non-diseased farm is infected).
- The target population is all the fish on the farm. Due to the patterns of disease in this production system, in which only fish in the final stages of grow-out, and only in winter are affected, the study population is defined as grow-out fish during the winter months.
- The issue of clustering is considered. As fish are grouped into tanks, this is the logical level at which to consider clustering. However, when a farm is infected, the disease often occurs in multiple tanks, so there is little evidence of strong clustering. Also, the small number of tanks on a single farm means that it is difficult to define a design prevalence at the tank level (i.e. the proportion of infected tanks that the survey should be able to detect on the farm). For these reasons, it is decided to treat the entire grow-out population of each farm as a single homogenous population.
- Stratification is also considered. In order to ensure full representation, it is decided to stratify the sample size by tank, proportional to the population of each tank.
- The design prevalence at the animal level is determined based on the epidemiology of the disease. The disease does not spread quickly, however, in the defined target population, it has been reported to affect at least 10% of fish, if the population is infected. In order to take the most conservative approach, an arbitrarily low design prevalence of 2% is used. A prevalence of 10% may have been used (and would result in a much smaller sample size), but the authorities were not convinced by the thought that the population could still be infected at a level of say 5%, and disease still not be detected.
- The test used involves destructive sampling of the fish, and is based on an antigen-detection enzyme-linked immunosorbent assay (ELISA). Disease X is present in some parts of the country (hence the need for a farm-level accreditation programme). This has provided the opportunity for the sensitivity and the specificity of the ELISA to be evaluated in similar populations to those on farms. A recent study (using a combination of histology and culture as a gold standard) estimated the sensitivity of the ELISA to be 98% (95% confidence interval 96.7–99.2%), and the specificity to be 99.4% (99.2–99.6%). Due to the relatively narrow confidence intervals, it was decided to use the point estimates of the sensitivity and specificity rather than complicate calculations by taking the uncertainty in those estimates into account.

Sample size

The sample size required to meet the objectives of the survey is calculated to take the population size, the test performance, the confidence required and the design prevalence into account. As the population of each farm is relatively large, differences in the total population of each farm have little effect on the calculated sample size. The other parameters for sample size calculation are fixed across all farms. Therefore, a standard sample size (based on the use of this particular ELISA, in this population) is calculated. The sample size calculations are performed using the *FreeCalc* software²⁰. Based on the parameters listed above, the sample size required is calculated to be 410 fish per farm. In addition, the program calculates that, given the imperfect specificity, it is still possible for the test to produce up to five false-positive reactors from an uninfected population using this sample size. The authorities are not comfortable with dealing with false-positive reactors, so it is decided to change the test system to include a confirmatory test for any positive reactors. Culture is selected as the most appropriate test, as it has a specificity that is considered to be 100%. However, its sensitivity is only 90% due to the difficulty of growing the organism.

As two tests are now being used, the performance of the test system must be calculated, and the sample size recalculated based on the test system performance.

Using this combination of tests (in which a sample is considered positive only if it tests positive to both tests), the specificity of the combined two tests can be calculated by the formula:

$$Sp_{Combined} = Sp_1 + Sp_2 - (Sp_1 \times Sp_2)$$

which produces a combined specificity of $1 + 0.994 - (1 \times 0.994) = 100\%$

The sensitivity may be calculated by the formula:

$$Se_{Combined} = Se_1 \times Se_2$$

which produces a combined sensitivity of $0.9 \times 0.98 = 88.2\%$

These new values are used to calculate the survey sample size yielding a result of 169 fish. It is worth noting that attempts to improve the performance of a test (in this case increase specificity) generally result in a decrease in the performance of the other aspect of the test performance (sensitivity in this example). However, in this case, the loss of sensitivity is more than compensated for by the decreased sample size due to the improved specificity.

It is also worth noting that, when using a test system with 100% specificity, the effective power of the survey will always be 100%, regardless of the figure used in the design. This is because it is not possible to make a Type II error, and conclude that the farm is infected when it is not.

A check of the impact of population size on the calculated sample size is worthwhile. The calculated sample size is based on an infinitely large population. If the population size is smaller, the impact on sample size is shown in the following table:

²⁰ FreeCalc – Cameron, AR. Software for the calculation of sample size and analysis of surveys to demonstrate freedom from disease. Available for free download from <http://www.ausvet.com.au>.

Population size	Sample size
1000	157
2000	163
5000	166
10,000	169

Based on these calculations, it is clear that, for the population sizes under consideration, there is little effect on the sample size. For the sake of simplicity, a standard sample size of 169 is used, regardless of the number of grow-out fish on the farm.

Sampling

The selection of individual fish to include in the sample should be done in such a manner as to give the best chance of the sample being representative of the study population. A fuller description of how this may be achieved under different circumstances is provided in *Survey Toolbox*²¹. An example of a single farm will be used to illustrate some of the issues.

One farm has a total of eight tanks, four of which are used for grow-out. At the time of the survey (during winter), the four grow-out tanks have 1850, 4250, 4270 and 4880 fish, respectively, giving a total population of 15,250 grow-out fish.

Simple random sampling from this entire population is likely to produce sample sizes from each tank roughly in proportion to the number of fish in each tank. However, proportional stratified sampling will guarantee that each tank is represented in proportion. This simply involves dividing the sample size between tanks in proportion to their population. The first tank has 1850 fish out of a total of 15,250, representing 12.13%. Therefore 12.13% of the sample (21 fish) should be taken from the first tank. Using a similar approach the sample size for the other three tanks is 47, 47 and 54 fish, respectively.

Once the sample for each tank is determined, the problem remains as to how to select 21 fish from a tank of 1850 so that they are representative of the population. Several options exist.

- If the fish can be handled individually, random systematic sampling may be used. This is likely to be the case if, for example:
 - fish are harvested during winter and samples can be collected at harvest; or
 - routine management activities involving handling the fish (such as grading or vaccination) are conducted during the winter.

If fish are handled, systematic sampling simply involves selecting a fish at regular intervals. For instance, to select 21 from 1850, the sampling interval should be $1850/21 = 88$. This means that every 88th fish from the tank should be sampled. To ensure randomness, it is good practice to use a random number between 1 and 88 (in this case) to select the *first* fish (e.g. using a random number table), and then select every 88th fish after that.

²¹ Survey Toolbox for Aquatic Animal Diseases – A Practical Manual and Software Package. Cameron A.R. (2002). Australian Centre for International Agricultural Research (ACIAR), Monograph No. 94, 375 pp. ISBN 1 86320 350 8. Printed version available from ACIAR <http://www.aciar.gov.au> Electronic version available for free download from <http://www.ausvet.com.au>.

- If fish cannot be handled individually (by far the most common, and more difficult, circumstance) then the fish to be sampled must be captured from the tanks. Fish should be captured in the most efficient and practical way possible, however every effort should be made to try to ensure that the sample is representative. In this example, a dip net is the normal method used for capturing fish. Using a dip net, convenience sampling would involve capturing 21 fish by repeatedly dipping at one spot and capturing the easiest fish (perhaps the smaller ones). This approach is strongly discouraged. One method of increasing the representativeness is to sample at different locations in the tank – some at one end, some at either side, some at the other end, some in the middle, some close to the edge. Additionally, if there are differences among the fish, an attempt should be made to capture fish in such a way as to give different groups of fish a chance of being caught (i.e. do not just try to catch the small ones, but include big ones as well).

This method of collecting a sample is far from the ideal of random sampling, but due to the practical difficulties of implementing random sampling of individual fish, this approach is acceptable, as long as the efforts made to increase the representativeness of the sample are both genuine and fully documented.

Testing

Specimens are collected, processed and tested according to standardised procedures developed under the certification ~~[accreditation]~~ programme and designed to meet the requirements of this *Aquatic Manual*. The testing protocol dictates that any specimens that test positive to ELISA be submitted for culture, and that any positive culture results indicate a true positive specimen (i.e. that the farm is not free from disease). It is important that this protocol be adhered to exactly. If a positive culture is found, then it is not acceptable to retest it, unless further testing is specified in the original testing protocol, and the impact of such testing accounted for in the test system sensitivity and specificity estimates (and therefore the sample size).

Analysis

If the calculated sample size of 169 is used, and no positive reactors are found, then the survey will have a confidence of 95%. This can be confirmed by analysing the results using the *FreeCalc* software mentioned above (which reports a confidence level of 95.06%).

It may happen in some cases that the survey is not conducted exactly as planned, and the actual sample size is less than the target sample size. However, the size of the farm may also be smaller. In these cases, it is advisable to analyse the farm data on a farm-by-farm basis. For example, if only 165 specimens were collected from a farm with only 2520 fish, the resulting confidence would still be 95%. If only 160 fish were collected, the confidence is only 94.5%. If a rigid target of 95% confidence is used, then this survey would fail to meet that target and more evidence would be required.

Example 2 – two-stage structured survey (national freedom)

Context

A country aims to declare freedom from Disease Y of crustaceans. The industry in this country is based largely on small-holder ponds, grouped closely together in and around villages. The disease is reasonably highly contagious, and causes mass mortality mid to late in the production cycle, with affected animals becoming moribund and dying in a matter of days. Affected animals show few characteristic signs, but an infected pond will almost invariably break down with mass mortality unless harvested beforehand. It is more common in late summer, but can occur at any time of year. It also occurs occasionally early in the production cycle. In this country, there are some limitations to the availability of laboratory facilities and the transport infrastructure. However, there is a relatively large government structure, and a comprehensive network of fisheries officers.

Objective

The objective is to establish national freedom from Disease Y. The surveillance system must meet the requirements of ~~Part 1 of~~ this chapter, but must also be able to be practically implemented in this small-holder production system.

Approach

The aquaculture authorities decide to use a survey to gather evidence of freedom, using a two-stage survey design (sampling villages at the first level, and ponds at the second). Laboratory testing of specimens from a large number of farms is not considered feasible, so a combined test system is developed to minimise the need for expensive laboratory tests.

The unit of observation and analysis is, in this case, the pond, rather than the individual animal. This means that the diagnosis is being made at the pond level (an infected pond or a non-infected pond) rather than at the animal level.

The survey is therefore a survey to demonstrate that no villages are infected (using a random sample of villages and making a village-level diagnosis). The test used to make a village-level diagnosis is, in fact, another survey, this time to demonstrate that no ponds in the village are affected. A test is then performed at the pond level (farmer observation followed, if necessary, by further laboratory testing).

Survey standards

- The confidence to be achieved by the survey is 95%. The power is set at 95% (but is likely to be virtually 100% if the test system used achieves nearly 100% specificity, as demonstrated in the previous example).
- The target population is all ponds stocked with shrimp in the country during the study period. The study population is the same, except that those remote areas to which access is not possible are excluded. As outbreaks can occur at any time of year, and at any stage of the production cycle, it is decided not to further refine the definition of the population to target a particular time or age.
- Three tests are used. The first is farmer observation, to determine if mass mortality is occurring in a particular pond. If a pond is positive to the first test (i.e. mass mortality is detected), a second test is applied. The second test used is polymerase chain reaction (PCR). Cases positive to PCR are further tested using transmission experiments.
 - Farmer observation can be treated as a test just like any other. In this case, the observation of mass mortality is being used as a test for the presence of Disease Y. As there are a variety of other diseases that are capable of causing mass mortality, the test is not very specific. On the other hand, it is quite unusual for Disease Y to be present, and not result in mass mortality, so the test is quite sensitive. A standard case definition is established for 'mass mortality' (for instance, greater than 20% of the pond's population of shrimp observed dead in the space of less than 1 week). Based on this definition, farmers are able to 'diagnose' each pond as having mass mortality. Some farmers may be over-sensitive and decide that mass mortality is occurring when only a small proportion of shrimp are found dead (false positives, leading to a decrease in specificity) while a small number of others fail to recognise the mortalities, decreasing sensitivity.

In order to quantify the sensitivity and specificity of farmer observation of mass mortalities, as a test for Disease Y, a separate study is carried out. This involves both a retrospective study of the number of mass mortality events in a population that is thought to be free from disease, as well as a study of farmers presented with a series of mortality scenarios, to assess their ability to accurately identify a pond with mass mortality. By combining these results, it is estimated that the sensitivity of farmer-reported mass mortalities as a test for Disease Y is 87% while the specificity is 68%.

Appendix XXXII (contd)

Appendix V (contd)

- When a farmer detects a pond with mass mortality, specimens are collected from moribund shrimp following a prescribed protocol. Tissue samples from 20 shrimp are collected, and pooled for PCR testing. In the laboratory, the ability of pooled PCR to identify a single infected animal in a pool of 20 has been studied, and the sensitivity of the procedure is 98.6%. A similar study of negative specimens has shown that positive results have occasionally occurred, probably due to laboratory contamination, but maybe also because of the presence of non-viable genetic material from another source (shrimp-based feed stuffs are suspected). The specificity is therefore estimated at 99%.
- Published studies in other countries have shown that the sensitivity of transmission tests, the third type of test to be used, is 95%, partly due to variability in the load of the agent in inoculated material. The specificity is agreed to be 100%.
- Based on these figures, the combined test system sensitivity and specificity are calculated using the formulae presented in Example 1, first with the first two tests, and then with the combined effect of the first two tests and the third test. The result is a sensitivity of 81.5% and a specificity of 100%.
- The design prevalence must be calculated at two levels. First, the pond-level design prevalence (the proportion of ponds in a village that would be infected if disease were present) is determined. In neighbouring infected countries, experience has shown that ponds in close contact with each other are quickly infected. It is unusual to observe an infected village with fewer than 20% of ponds infected. Conservatively, a design prevalence of 5% is used. The second value for design prevalence applies at the village level, or the proportion of infected villages that could be identified by the survey. As it is conceivable that the infection may persist in a local area without rapid spread to other parts of the country, a value of 1% is used. This is considered to be the lowest design prevalence value for which a survey can be practically designed.
- The population of villages in the country is 65,302, according to official government records. Those with shrimp ponds number 12,890, based on records maintained by the aquaculture authorities. These are generated through a five-yearly agricultural census, and updated annually based on reports of fisheries officers. There are no records available of the number of ponds in each of these villages.

Sample size

Sample size is calculated for the two levels of sampling, first the number of villages to be sampled and then the number of ponds to be sampled. The number of villages to be sampled depends on the sensitivity and the specificity of the test used to classify villages as infected or not infected. As the 'test' used in each village is really just another survey, the sensitivity is equal to the confidence and the specificity is equal to the power of the village-level survey. It is possible to adjust both confidence and power by changing the sample size in the village survey (number of ponds examined), which means that we can determine, within certain limits, what sensitivity and specificity we achieve.

This allows a flexible approach to sample size calculation. If a smaller first-stage sample size is desired (a small number of villages), a high sensitivity and specificity are needed, which means that the number of ponds in each village that need to be examined is larger. A smaller number of ponds will result in lower sensitivity and specificity, requiring a larger number of villages. The approach to determining the optimal (least cost) combination of first- and second-stage sample sizes is described in *Survey Toolbox*.

A further complication is presented by the fact that each village has a different number of ponds. In order to achieve the same (or similar) confidence and power (sensitivity and specificity) for each village, a different sample size may be required. The authorities choose to produce a table of sample sizes for the number of ponds to sample in each village, based on the total ponds in each village.

Appendix XXXII (contd)

Appendix V (contd)

An example of one possible approach to determining the sample size follows:

The target sensitivity (confidence) achieved by each village-level survey is 95%. The target specificity is 100%. Using the *FreeCalc* software, with a design prevalence of 1% (the survey is able to detect disease if 1% or more villages are infected), the first-stage sample size is calculated as 314 villages. Within each village, the test used is the combined test system described above with a sensitivity of 81.5% and a specificity of 100%. Based on these figures the following table is developed, listing the number of ponds that need to be sampled in order to achieve 95% sensitivity.

Population	Sample size
30	29
40	39
60	47
80	52
100	55
120	57
140	59
160	61
180	62
200	63
220	64
240	64
260	65
280	65
300	66
320	66
340	67
360	67
380	67
400	67
420	68
440	68
460	68
480	68
500	68
1000	70

Sampling

First-stage sampling (selection of villages) is done using random numbers and a sampling frame based on the fisheries authorities list of villages with shrimp ponds. The villages are listed on a spreadsheet with each village numbered from 1 to 12,890. A random number table (such as that included in *Survey Toolbox*) or software designed for the generation of random numbers (such as EpiCalc²²) is used.

The second stage of sampling involves random selection of ponds within each village. This requires a sampling frame, or list of each pond in the village. The fisheries authorities use trained local fisheries officers to coordinate the survey. For each selected village, the officer visits the village and convenes a meeting of all shrimp farmers. At the meeting, they are asked how many ponds they have and a list of farmers' names and the number of ponds is compiled. A simple random sample of the appropriate number of ponds (between 29 and 70, from the table above, depending on the number of ponds in the village) is selected from this list. This is done either using software (such as Survey Toolbox's *RandomAnimal* program), or manually with a random number table or decimal dice for random number selection. Details of this process are described in *Survey Toolbox*. This selection process identifies a particular pond in terms of the name of the owner, and the sequence number amongst the ponds owned (e.g. Mr Smith's 3^d pond). Identification of the actual pond is based on the owners own numbering system for the ponds.

Testing

Once ponds have been identified, the actual survey consists of 'testing those ponds'. In practice, this involves the farmers observing the ponds during one complete production cycle. The local fisheries officer makes weekly visits to each farmer to check if any of the selected ponds have suffered mass mortality. If any are observed (i.e. the first test is positive), 20 moribund shrimp are collected for laboratory examination (first PCR, and then, if positive, transmission experiments).

Analysis

Analysis is performed in two stages. First, the results from each village are analysed to ensure that they meet the required level of confidence. If the target sample size is achieved (and only negative results obtained), the confidence should be 95% or greater in each village. At the second stage, the results from each village are analysed to provide a country level of confidence. Again, if the target sample size (number of villages) is achieved, this should exceed 95%.

Example 3 – spatial sampling and the use of tests with imperfect specificity

Context

A country has an oyster culture industry, based primarily on rack culture of oysters in 23 estuaries distributed along the coastline. In similar regions in other countries, Disease Z causes mortalities in late summer/early autumn. During an outbreak a high proportion of oysters are affected, however, it is suspected that the agent may be present at relatively low prevalence in the absence of disease outbreaks.

Objective

The national authorities wish to demonstrate national freedom from Disease Z. If the disease should be detected, a secondary objective of the survey is to collect adequate evidence to support zoning at the estuary level.

²² <http://www.myatt.demon.co.uk/epicalc.htm>

Approach

The authorities conclude that clinical surveillance for disease outbreaks is inadequate because of the possibility of low level subclinical infections. It is therefore decided to base surveillance on a structured two-stage survey, in which sampled oysters are subjected to laboratory testing. The first stage of the survey is the selection of estuaries. However, due to the objective of providing evidence for zoning (should disease be found in any of the estuaries), it is decided to use a census approach and sample every estuary. In essence this means that there will be 23 separate surveys, one for each estuary. A range of options for sampling oysters are considered, including sampling at harvest or marketing, or using farms (oyster leases) as a level of sampling or stratification. However the peak time of activity of the agent does not correspond to the harvest period, and the use of farms would exclude the significant numbers of wild oysters present in the estuaries. It is therefore decided to attempt to simulate simple random sampling from the entire oyster population in the estuary, using a spatial sampling approach.

Survey standards

- The target population is all of the oysters in each of the estuaries. The study population is the oysters present during the peak disease-risk period in late summer early autumn. Wild and cultured oysters are both susceptible to disease, and may have associated with them different (but unknown) risks of infection. They are therefore both included in the study population. As will be described below, sampling is based on mapping. Therefore the study population can more accurately be described as that population falling within those mapped areas identified as oyster habitats.
- A design prevalence value is only required at the oyster level (as a census is being used at the estuary level). While the disease is often recognised with very high prevalence during outbreaks, a low value is used to account for the possibility of persistence of the agent in the absence of clinical signs. A value of 2% is selected.
- The test used is histopathology with immuno-staining techniques. This test is known to produce occasional false-positive results due to nonspecific staining, but is very sensitive. Published studies indicate values of 99.1% for sensitivity and 98.2% for specificity. No other practical tests are available. This means that it is not possible to definitively differentiate false positives from true positives, and that in a survey of any size, a few false positives are expected (i.e. 1.8%).
- The confidence is set at 95% and the power at 80%. In the previous examples, due to the assumed 100% specificity achieved by use of multiple tests, the effective power was 100%. In this case, with imperfect specificity, there will be a risk of falsely concluding that a healthy estuary is infected, so the power is not 100%. The choice of a relatively low figure (80%) means that there is a 1 in 5 chance of falsely calling an estuary infected when it is not infected, but it also dramatically decreases the survey costs, through a lower sample size.

Sample size

Based on the assumption that the sampling procedure will mimic simple random sampling, the sample size (number of oysters to sample per estuary) can be calculated with *FreeCalc*. The population size (number of oysters per estuary) is assumed to be very large. The calculated sample size, using the sensitivity, specificity and design prevalence figures given above, is 450. *FreeCalc* also reports that, based on this sample size and the specificity of the test, it is possible to get 10 or fewer false-positive test results, and still conclude that the population is free from disease. This is because, if the population were infected at 2% or greater, the anticipated number of positive reactors from a sample of 450 would be greater than 10. In fact, we would expect 9 true positives ($450 \times 2\% \times 99.1\%$) and 8 false positives ($450 \times 98\% \times 1.8\%$) or a total of 17 positives if the population were infected at a prevalence of 2%.

This illustrates how probability theory and adequate sample size can help differentiate between true- and false-positive results when there is no alternative but to use a test with imperfect specificity.

Sampling

The aim is to collect a sample of 450 oysters that represent an entire estuary. Simple random sampling depends on creating a sampling frame listing every oyster (not possible) and systematic sampling depends on being able to (at least conceptually) line up all the oysters (again, not possible). The authorities decide to use spatial sampling to approximate simple random sampling. Spatial sampling involves selecting random points (defined by coordinates), and then selecting oysters near the selected points. In order to avoid selecting many points with no oysters nearby, the estuary is first mapped (the fisheries authorities already have digital maps defining oyster leases available). To these maps areas with significant concentrations of wild oysters are also added, based on local expertise. Pairs of random numbers are generated such that the defined point falls within the defined oyster areas. Other schemes are considered (including using a rope marked at regular intervals, laid out on a lease to define a transect, and collecting an oyster adjacent to each mark on the rope) but the random coordinate approach is adopted.

Survey teams then visit each point by boat (using a GPS [Global Positioning System] unit to pinpoint the location). A range of approaches is available for selecting which oyster to select from a densely populated area, but it should involve some effort at randomness. Survey staff opt for a simple approach: when the GPS receiver indicates that the site has been reached, a pebble is tossed in the air and the oyster closest to the point where it lands is selected. Where oysters are arranged vertically (e.g. wild oysters growing up a post), a systematic approach is used to determine the depth of the oyster to select. First, an oyster at the surface, next, an oyster halfway down, and thirdly, an oyster as deep as can be reached from the boat.

This approach runs the risk of bias towards lightly populated areas, so an estimate of the relative density of oysters at each sampling point is used to weight the results (see *Survey Toolbox* for more details).

Testing

Specimens are collected, processed, and analysed following a standardised procedure. The results are classified as definitively positive (showing strong staining in a highly characteristic pattern, possibly with associated signs of tissue damage), probably positive (on the balance of probabilities, but less characteristic staining), and negative.

Analysis

The interpretation of the results when using a test with imperfect specificity is based on the assumption that, in order to conclude that the population is free from infection, any positive result identified is really a false positive. With a sample size of 450, up to 10 false positives may be expected while still concluding that the population is free from disease. However, if there is reasonable evidence that there is even a single *true* positive, then the population cannot be considered free. This is the reason for the classification of positive results into definitive and probable positives. If there are any definitive positives at all, the population in that estuary must be considered infected. The probable positives are consistent with false positives, and therefore up to 10 may be accepted. Using *FreeCalc* the actual confidence achieved based on the number of (presumed) false positives detected can be calculated. For instance, if 8 'probably positive' results were detected from an estuary, the confidence level for the survey would be 98.76%. On the other hand, if 15 'probably positive' results were detected, the confidence is only 61.9%, indicating that the estuary is likely to be infected.

Appendix XXXII (contd)

Appendix V (contd)

Discussion

Normally, it may be safely assumed that a surveillance system aimed at demonstrating freedom from disease is 100% specific. This is because any suspected occurrence of disease is investigated until a definitive decision can be made. If the conclusion is that the case is truly a case of disease, then there is no issue of declaring freedom – the disease is known to be present. This example presents a different situation where, due to lack of suitable tests, it is not possible for the surveillance system to be 100% specific. This may represent an unusual situation in practice, but illustrates that methods exist for dealing with this sort of problem. In practice, a conclusion that a country (or estuary) is free from infection, in the face of a small (but statistically acceptable) number of positive results, will usually be backed up by further evidence (such as the absence of clinical disease).

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Aquatic Manual disease chapter template January 2007

CHAPTER XXX.

DISEASE NAME

1. Case definition

(Please start this chapter with a simple definition of the disease)

“For the purpose of this chapter, DISEASE NAME is considered to be INFECTION WITH PATHOGEN NAME.”

Case definition for infection, for diseased animal and for disease holding unit

Case definition in endemic situations and in zero prevalence situations

2. Information for the design of surveillance programmes

(Information on some of the following items is required for the design of surveillance programmes as described in Chapter 1.1.4 of the *Aquatic Manual*. Information on other items is needed to provide details on possible risk management and disease control measures as described in the *Aquatic Code*.)

There is no need to split the sections a) to d) into subsections to address each item. Also, it is acknowledged that it will not be possible to provide – and reference – scientific data on each of these items for each disease; some aspects will be well covered, while there will be a dearth of information on others. Authors may wish to draw attention to areas where there is a significant lack of information.)

For the following factors, what are the high-risk groups? Age, season, water temperature

How do these factors affect sensitivity and specificity? Age, season, water temperature

What is the preferred test for each of those categories?

Sensitivity and specificity estimates from previous studies – how were the evaluations made and what situations do they concern?

Are there any tests that have proven to be useful in environments rather than hosts? Equipment, water or soil samples and intermediate hosts/carrier

Can samples be preserved and how?

Incubation time

Opportunities for evidence collection and/or sampling at different points in the production cycle (alternative sources of evidence)

Appendix XXXII (contd)Appendix VI (contd)**a) Agent factors**

- Aetiological agent, agent strains
- Survival outside the host (i.e. in the natural environment)
- Stability of the agent (describe effective inactivation methods)
- Life cycle

b) Host factors

- Susceptible host species (common and Latin names)
- Susceptible stages of the host
- Species or sub-population predilection (probability of detection)
- Target organs and infected tissue
- Persistent infection with lifelong carriers
- Vectors
- Known or suspected wild carriers

c) Disease pattern

- Transmission mechanisms, coefficient of transmission, animal to animal, pond to pond, farm to farm, etc., under different conditions and through different pathways. When introduced into a naïve farm or area, does it have an obvious disease pattern (temporal/spatial distribution)?
- If detailed information on transmission is not available, provide qualitative information on the likelihood of transmission to occur under different situations (e.g. if one infected fish is introduced into a pond, what proportion of the population would be expected to be infected at different points in time)
- Within fish spread of the pathogen depending on different points of entry and under different conditions
- If available, can a causal web or quantified risk factors be provided? Risk factors means factors that either increase or decrease the risk
- What management practices increase or decrease the risk?
- Sources of stock – are they bought from outside, are they tested
- Prevalence (describe commonly observed prevalence in wild and farmed populations for the detection method used, under different conditions)
- Geographical distribution
- Mortality and morbidity
- Economic and/or production impact of the disease

d) Control and prevention

- Vaccination
- Chemotherapy
- Immunostimulation
- Resistance breeding
- Restocking with resistant species
- Blocking agents
- General husbandry practices

3. Sampling for surveillance purposesSurveillance to demonstrate the absence of *disease or infection*

- = Sampling frequency and duration (to establish freedom and to maintain status)
- = Risk factors that promote introduction of agents (e.g. isolation and avoiding exposure might lead lower requirement for continuous sampling)
- = Optimum water temperature
- = Optimum age range or development stage of fish
- = Selection of individual specimens
- = Preservation of samples for submission
- = Number of fish to be sampled
- = Best organs or tissues

Surveillance to determine the occurrence or distribution of *disease or infection*

- Comment on fish / tissues that are not appropriate (i.e. when it is never possible to detect)
- Priority areas for testing (fish: tissues, etc to be sampled for prevalence comparisons)
- Optimal diagnostic test combinations to determine prevalence (include ranking of costs)

4. Diagnostic methods

(Please provide a description of diagnostic methods.)

The diagnostic methods should include the entire gambit of a disease investigation in a population or an individual animal, i.e. provide descriptions of the clinical, histological etc picture, and not simply the agent detection methods. In previous editions of the *Aquatic Manual*, this information was often captured in the introduction.

Appendix XXXII (contd)Appendix VI (contd)

It is acknowledged that not all methods listed will be applicable to all diseases. Only the ones that are appropriate should be listed and described)

a) Field diagnostic methods

(This includes observation of the animal and its environment, and gross clinical examinations)

- Clinical signs
- Behavioural changes: specify which animals are likely to be affected or not affected by the disease or infection within a tank? If different for acute or chronic conditions, provide details.

b) Clinical methods

(This includes methods that focus on the effects of the pathological agent on the host, rather than on agent detection)

- Gross pathology
- Clinical chemistry
- Microscopic pathology
 - Wet mounts
 - Smears
 - Fixed sections
- Electron microscopy/cytopathology

c) Agent detection and identification methods

(This includes methods that detect, possibly isolate and amplify, and identify the agent. For each method, information on the items in the text box on the right hand side should be provided. This information is required to allow the reader to follow the technique, but also to provide the necessary data – e.g. specificity and sensitivity – that are required for the development of a sampling and surveillance programme.)

- **Direct detection methods**

- i) Microscopic methods**

- Wet mounts
 - Smears
 - Fixed sections

- ii) Agent isolation and identification**

- Cell culture/artificial media
 - Antibody-based antigen detection methods (IFAT, ELISA,)

- Molecular techniques (PCR, ISH, sequencing...)
- Agent purification
- **Indirect detection methods**
 - Serological methods

⇒ Samples to be taken
⇒ Technical procedure <ul style="list-style-type: none"> ▪ How to use positive/negative controls
⇒ Levels of validation <ul style="list-style-type: none"> ▪ Specificity and sensitivity ▪ 'Gold' standard
⇒ Interpretation of results
⇒ Availability of test (from Reference Laboratories, commercial sources or easily synthesised)

4. Rating of tests against purpose of use

(This information is needed to determine which test is appropriate for various purposes. For example, a particular method may be highly suitable to diagnose clinical cases of disease in individual animals of a certain age group, but the same method may be rather unsuitable for assessing the infection status of large numbers of clinically healthy animals. It is an assessment of the test's 'fitness for purpose'.)

The methods currently available for surveillance, detection, and diagnosis of WSSV are listed in Table 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 (at least stages 1 and 2 of Figure 1 of chapter 1.1.2), their routine nature and the fact that they have been used widely without dubious results, makes them acceptable.

Appendix XXXII (contd)

Appendix VI (contd)

Table 1. WSV surveillance, detection and diagnostic methods

Method	Surveillance				Presumptive	Confirmatory
	Larvae	PLs	Juveniles	Adults		
Gross signs	D	D	C	C	C	D
Bioassay	D	D	D	D	C	B
Direct LM	D	D	C	C	C	C
Histopathology	D	C	C	C	A	A
Transmission EM	D	D	D	D	D	A
Antibody-based assays	D	D	C	C	A	B
DNA Probes - <i>in situ</i>	D	D	C	C	A	A
PCR	D	B	A	A	A	A
Sequence	D	D	D	D	D	A

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

5. Corroborative diagnostic criteria

(Please define, based on the information provided in 1.-4., what constitutes a suspect case of disease, and a confirmed case of disease. This information is required, for example for the purpose of disease investigations, especially in cases where 'free' status is threatened. It would also be required when surveillance of healthy populations yields controversial results, for example, positive PCR signals in the absence of any other evidence of infection.

The definitions of 'suspect' and 'confirmed' will most likely be a combination of positive results from a range of different methods as described under 3.

For example, a certain level of mortality at the right time of the year (see under 2b), in susceptible animals (see under 2a.), together with matching clinical signs (see under 3a), liver lesions and histopathology (see under 3b) could be sufficient for suspicion of DISEASE X. Several combinations may be possible. A confirmed case could be defined where in addition to the above, the agent has been detected. However, detection of viable agents without any disease signs could also constitute a confirmed case. The definitions may differ between different species and may depend on whether case is outside the known geographical/host range.

In accordance with Article VVVV of the *Aquatic Code*, all cases in other species should be referred immediately to the appropriate OIE Reference Laboratory for confirmation, whether or not clinical signs are associated with the case.)

a) Definition of suspect case

b) Definition of confirmed case

6. Prescribed diagnostic/detection methods to declare freedom

(Please prescribe the methods, based on the information provided in 1.-3., and assessed in 4., for *targeted surveillance* to declare freedom from infection as outlined in the *Aquatic Code*)

Appendix XXXII (contd)

Appendix VI (contd)

REFERENCES

(Please provide a list key references that confirm the information in the chapter and references that provide useful additional information. References should be to documents that are readily accessible.)

*
* *

Steps in a surveillance system

1. Clearly define the objective and measurable outcomes
Prioritise the diseases to be included in the surveillance system (e.g. using a risk based approach)
2. Define the populations (reference, target, study, etc.)
3. Develop a case definition for each outcome
4. Define the sources of data
 - Active
 - sampling strategy (frequency, population(s), what kinds of observations, collection of samples and diagnostic tests),
 - training of people who will be gathering the data,
 - strictly defined procedures for data collection (standardisation of data collection, instruments of measurement, exposure definition),
 - decide whether you want to collect data on risk factors.
 - Passive
 - Who are the eyes of the system (vets, farmers, laboratories)? What should they do if they see something?
 - Education.
5. Allocation of responsibilities
6. Allocation of resources
7. Flow of information
8. Compilation of data at each level and presentation at the next level
9. Data quality (cross check with other sources)
10. Actions at each level
11. Overall evaluation of results
12. Feedback
13. Link outcome of surveillance system to contingency plans



**First International Conference of OIE Reference Laboratories and Collaborating Centres
Florianopolis (Brazil), 3-5 December 2006**

**Special Workshop on pathogen strain differentiation for OIE listing
and notification of diseases by strain/genotype**

3 December 2006

Introduction and background

Barry Hill

Vice-President, Aquatic Animal Health Standards Commission

OIE Aquatic Animal Commission's Position Paper on Pathogen Strain Differentiation

Franck Berthe, Barry Hill and Don Lightner

OIE Aquatic Animal Health Standards Commission

This, in my view, is a very slippery slope

Jim Winton (presented *in absentia* by Barry Hill)

OIE Reference Laboratory for Infectious Haematopoietic Necrosis

Western Fisheries Research Center, Seattle, Washington, USA

Distinguishing between different virus species and different strains of a viral species

Frederick Kibenge

OIE Reference Laboratory for Infectious Salmon Anaemia,

Atlantic Veterinary College, University of Prince Edward Island, Canada

Phylogenetic analysis and epidemiological tracing of viral haemorrhagic septicaemia virus (VHSV)

Niels Jørgen Olesen, Sanne Madsen, Katja Einer-Jensen, Helle Frank Skall and

Niels Lorenzen

OIE Reference Laboratory for VHS,

Danish Institute for Food and Veterinary Research, Århus, Denmark

Pathogenicity and genotypes of VHSV: what do we know?

Iveta Matejusova and Michael Snow

FRS Marine Laboratory, Aberdeen, United Kingdom

The Classical Swine Fever (CSF) database of the European Community Reference Laboratory

Irene Greiser-Wilke et al.

Institute of Virology – Hannover, Germany (OIE Ref and EU ref lab for CSF)

**Infectious hypodermal & hematopoietic necrosis disease and runt deformity syndrome (RDS) -
significance of strain differences?**

Don Lightner, Department of Veterinary Science and Microbiology, University of Arizona, USA

Open discussion

Moderator – Barry Hill

Appendix XXXIII (contd)**Conclusions:**

1. The issue of differentiating genotypes of an agent of a listed disease is of increasing relevance, especially for listing and reporting of certain diseases of aquatic animals.
2. This will be an ongoing issue for the foreseeable future and will require further scientific debate of the advantages and disadvantages of differentiating between genotypes for listing and reporting purposes.
3. While there are established methods for genotyping certain disease agents of aquatic animals, there is need for greater standardization of the methods by Reference Laboratories to achieve harmonization of reporting on the occurrence of a disease where genotype is a concern.
4. The issue of listing disease by genotype may be of increasing importance, but it must be recognized that in doing so the list of aquatic animal diseases will significantly increase and add to the complexity of reporting.
5. If genotyping becomes a requirement for reporting purposes, it must be recognized that not all labs in Member Countries can perform such assays, and this will lead to increased demand for the services of the Reference Laboratories.
6. Proposal to distinguish a strain or genotype of a pathogen as the cause of a disease of concern must be accompanied by a robust and validated diagnostic, or typing, technique.

Recommendations:

1. The issue of whether or not distinct genotypes of OIE listed diseases should be listed as such and have individual reporting requirements should be discussed in a wider forum at the next meeting of OIE Reference Laboratories.
2. Because different laboratories are using different methods for genotyping, OIE Reference Labs should be requested by OIE to take the lead in developing and harmonizing methods for differentiation of genotypes.
3. The implications of differentiating between genotypes for OIE notification and reporting criteria should be considered by the Aquatic Animals Health Standards Commission.

ABSTRACTS

OIE Aquatic Animal Health Standards Commission Position Paper on Pathogen Strain Differentiation

The number and power of techniques that are available for the description of pathogens, typing of isolates and development of diagnostic tests has significantly increased over the past three decades along with development of biotechnologies (1). Molecular biology plays a particularly prominent role in this respect as related techniques such as polymerase chain reaction (PCR), and sister techniques are often perceived as the ultimate perfect tests. With permanent quest for improving specificity and sensitivity of tests, targeting genes of phylogenetic interest – such as rDNA sub-units – has rapidly become a common approach. This has inconspicuously but readily drawn taxonomy in the front line. The paradox here is that while taxonomists have become more and more rare (2), flurries of sequence datasets have literally revolutionised taxonomy.

Taxonomy is the study of organisms for the purpose of their systematic. Classically, it proceeds through three major steps that are: 1) description of organisms, 2) delineation of taxons, a step that is also called classification, and 3) use of specific characteristics for identification purpose. In this context, information provided by the characterisation of an organism may eventually be used in a diagnostic procedure. With time, enthusiasm for and success of DNA sequencing have both led to the practice of taxonomy by non-taxonomists. This has sometimes resulted in confusion between the three tiers of taxonomy: description, classification and identification.

Indeed the delineation of biological organisms into taxonomic groups inherently contains an arbitrary facet that may be difficult to accept if not clearly understood²³. Furthermore, the commonly accepted assumption that DNA sequences *per se* overcome that subjectivity is one of hazardous misperceptions. Here we want to stress that taxonomic judgement must be based on a polyphasic approach. The polyphasic approach uses a spectrum of independent characteristics, e.g. morphological, biochemical, molecular, serological, epidemiological, etc. (3, 4).

Pathogen differentiation will influence listing and reporting, which in turn can have serious implications for international trade in live animals and their products. For the purpose of this paper, we consider pathogen strain differentiation in the particular context of the OIE standards for aquatic animal health.

When listing a disease, it is of critical importance to know whether its causative agent has been clearly established (5). It is important to know whether only certain strains of the pathogen cause the disease of concern. There are examples where only some virulent strains, but not all strains, of the same species cause the disease of concern. In such cases, robust differential diagnostic means are essential to avoid inaccurate reporting and implementation of inappropriate control measures.

Based on the above considerations, the Aquatic Animals Commission proposes a set of guiding principles for appropriate pathogen differentiation.

Guiding principles for appropriate pathogen differentiation

1. Taxonomy consists of the description of organisms, their classification, and identification by specific characteristics.
2. Taxon characteristic(s) may eventually be used in diagnostic procedures.

²³ If taking the image of organising some filing cabinet, one senses the pressing need to determine where a file should be placed, and the need for users to be able to retrieve those files. Obviously, the design of a filing method is of a central importance. Ideally, the filing method should avoid, on one hand, multiplying folders which ultimately would contain single files, and, on the other hand, reducing the number of folders with a low level of identity. Both extreme situations render filing useless and file retrieving cumbersome. Where this image is trivial, it is not without having connections with taxonomy.

Appendix XXXIII (contd)

3. However, taxonomy is part of a cognitive science and distinct from development of diagnostic assays.
4. When and where applicable, guidelines established by international committees for taxonomy must be followed.
5. Taxonomic decisions should be based on a polyphasic approach and proposals to recognise certain strains of a pathogen must similarly be, where applicable, based on considerations of, for example, virulence, pathology, epidemiology, molecular information, and ultrastructure characteristics.
6. Proposals to distinguish a strain or type of a pathogen as the cause of the disease of concern must be accompanied by a robust and validated diagnostic, or typing, technique.
7. For the purpose of OIE aquatic animal standards, proposals for recognition of distinct pathogen strains of OIE listed diseases of aquatic animal should be submitted to the OIE Aquatic Animals Commission, which may propose their inclusion as new standards in the *Aquatic Code* and *Manual* for adoption by Member Countries. This must be subject to regular review.
8. Pathogen strains may eventually be proposed for listing in place of entire pathogen species.

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This, in my view, is a very slippery slope

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Any recommendations along these lines need to be very careful to avoid appearing salmon-centric (or showing another species bias). For example, de-listing marine VHS genotypes (whatever these are) may represent a low risk to trout growers in Europe, but the marine strains we have here on the west coast are highly pathogenic for marine fish species and I doubt that those developing marine aquaculture programs (e.g. cod and halibut) would be excited about any proposal that put them at greater risk than their freshwater counterparts. Also, strains recently introduced into the Great Lakes (presumptively from marine fish on the Atlantic coast of North America) have proven to be exceptionally pathogenic for freshwater species. The second problem is that as more strains or isolates of viruses and bacterial are sequenced, it will be increasingly difficult to draw such boundaries in a biologically or geographically relevant manner. Furthermore, we are years away for identifying virulence determinants for many of the fish pathogens or from any real understanding about the factors that drive the evolution of these strains (and how stable they are to new selection pressure). Finally, this increases the ability for OIE member countries to define themselves as free of a given pathogen/disease based upon minor sequence differences of undetermined importance.

Appendix XXXIII (contd)**Distinguishing between different virus species and different strains of a viral species**

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The advent of nucleotide sequence determination has revolutionized biology and largely rationalized taxonomy, including that of viruses. The large increase in nucleotide sequences belonging to different viruses is driving the need for better descriptions of virus isolates. The World Animal Health Organization (OIE) Aquatic Animal Health Standards Commission is considering differentiating between genotypes of certain OIE listed pathogens for regulation and reporting purposes. The International Committee on Taxonomy of Viruses (ICTV) is concerned with taxonomy and nomenclature of viruses from the highest hierarchical level of virus order down to the lowest taxon of virus species [1], but not with any of the several sub-species designations in common use (strain, variant, isolate, type, sub-type, serotype, genotype, etc), although these sub-species demarcations can recognize significant biological differences. Whereas the creation or elimination, (re)naming, and (re)assignment of a virus species, genus, (sub)family, or order are all taxonomic acts that require public scrutiny and debate, leading to formal approval by the full membership of the ICTV, the naming of a virus isolate (or any of the virus sub-species), and its assignment to a pre-existing species are not considered taxonomic acts and are typically accomplished by publication of a paper describing the virus isolate (or any of the virus sub-species) in the peer-reviewed virology literature. Thus, for the subdivision of genera into species, the ICTV in 1991 endorsed the following polythetic definition: "*a virus species is a polythetic (polythetic in this case means variable) class of viruses that constitute a replicating lineage and occupy a particular ecological niche*". Although genome sequence relatedness is becoming an increasingly dominant species demarcation criterion for most genera, the polythetic definition of virus species, requires that it should not be the only criterion. To differentiate between individual species, it is necessary to rely on a consensus group of properties that are not present in all members of a genus or family, for example: genome sequence relatedness, natural host range, cell and tissue tropism, pathogenicity and cytopathology, mode of transmission, physicochemical properties, and antigenic properties. However, this rule does not apply to demarcations below the species level, which could appropriately be based on a single criterion, if desired. There is no international agreement below the species level; the differentiation between strains, groups, serotypes, variants, etc., is variable for each virus family and group of virologists. Thus, the criteria for sub-species designations clearly will depend on the everyday needs of practicing virologists. With the rapid increase in sequencing, it has become increasingly urgent to organize nomenclature below the species level. The pairwise sequence comparison (PASC) system [2] could be a good tool to further address the question and come up with family-specific descriptions of different virus sub-species. Moreover, because virus sub-species are real physical entities, management of their designations or identities requires establishment of virus databases (in OIE Reference labs?), similar to the ones set up by the World Health Organization (WHO) for influenza viruses.

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Phylogenetic analysis and epidemiological tracing of viral haemorrhagic septicaemia virus (VHSV)

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Viral haemorrhagic septicaemia (VHS) caused by the rhabdovirus VHSV is one of the most important viral diseases in European rainbow trout farming. Until 1989, the virus was mainly isolated from freshwater salmonids, especially rainbow trout. However, in the last 17 years, it has also been isolated from an increasing number of free-living marine fish species. The marine and fresh water isolates are serologically indistinguishable, but differ with respect to pathogenicity on the host species: Marine VHSV isolates do in general not induce disease in rainbow trout. Recent studies have shown that the marine virus group possesses the majority of diversity. So far, attempts on identifying a marker that may link genetic and pathogenic characteristics has been unsuccessful. A large amount of VHSV sequence data have been generated since 1998, and parallel phylogenetic analysis using various genomic regions from a fixed virus panel reveal that the overall genotyping of VHSV is identical regardless of which target region is selected (Einer-Jensen et al. 2005). However, the choice and especially the length of the target region did on the other hand influence the ability to characterise the isolates into genetic sub-lineages, and this observation should be kept in mind during epidemiological analyses. VHSV isolates can be divided into 4 genogroups, I, II, III, and IV. Genogroup IV and I are further divided into subgroups. The rainbow trout pathogenic isolates primarily cluster in Genotype Ia, Id and Ie, whereas most marine isolates from the Baltic Sea cluster in Ib. The advantages and disadvantages of discriminating between genotypes of VHSV at notification to the OIE will be discussed. At several occasions, the existing sequence data have proved to be informative when used for epidemiological tracing of new VHSV outbreaks. Case stories from 2006 as well as plans on generation and management of additional sequence information will be given.

Keywords (relevant for discussion): Notification of VHSV, management of sequence data, phylogenetic analysis and epidemiological tracing.

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Appendix XXXIII (contd)**Pathogenicity and genotypes of VHSV: what do we know?**Iveta Matejusova and Michael Snow

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VHSV is present in a wide range of naturally occurring host species in the marine environment. Four robust genetic groups of the virus are universally recognized. These genetic groupings have been shown to be robust, consistent and independent of the region of the genome selected for the analysis. Isolates can be readily assigned to genotype using a universally available simple RFLP-based methodology, or by reference laboratories using a PCR and sequencing approach. Experimental and field observations, suggest that genotypic classification can be a useful indicator of risk to a particular sector of aquaculture. Only GI isolates appear to present a significant risk to rainbow trout, with isolates from each subgroup (a-e) having caused disease in farms. Current fish health legislation, implemented to protect the rainbow trout industry against a serious disease threat, fails to differentiate subgroups of VHSV, despite only GI presenting an apparent high risk to this species. The consequence of detection of non-GI VHSV isolates in marine farms has hindered investment in and diversification of new species mariculture. The new Directive will introduce flexibility in the listing of diseases, which provides the opportunity to argue for legislative differentiation and separate management of different genotypes of VHSV based on risk. This could allow sustainable development of mariculture, whilst maintaining protection of the rainbow trout sector that VHS legislation was originally designed to protect. A modeling approach is now being used to formally investigate the risks posed by different genotypes of VHSV and evaluate the likely consequence of any change in legislation.

The presence of an integrated IHHNV-related sequence in some stocks of *Penaeus monodon* originating from Africa, Australia, and Andaman Sea

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We found an IHHNV-related sequence within the shrimp genome in populations of *Penaeus monodon* from Africa, Australia, and Andaman Sea. IHHNV is a single-stranded DNA virus that has caused severe mortality and stunted growth in penaeid shrimp. Recently, IHHNV-related sequences were found in samples of *P. monodon* from Madagascar and Tanzania. These sequences vary considerably from that of IHHNV found in association with viral epidemics. Laboratory bioassays were carried out to determine if either of these IHHNV-related sequences is infectious. Results indicated that the Africa type IHHNV-related sequences are not infectious. With the shrimp containing the IHHNV-related sequences, we performed genome walking at the 3' end of an IHHNV-related sequence and found that this virus-related sequence is part of the *P. monodon* genome.

These virus-related sequences have high similarity (86 and 92% identities in nucleotide sequence) to the viral genome, which has often generated false positive reactions during PCR screening of these stocks. A PCR assay (IHHNV309F/R primers) was subsequently developed that can only detect IHHNV but that does not react with IHHNV-related sequences. The sequence of primers matches (100%) the target sequence in IHHNV (Hawaii and Thailand origins) but have several mismatched nucleotides with the integrated IHHNV-related sequence. To determine if all *P. monodon* contain the integrated IHHNV-related sequence, a pair of primers MG831F/R, which bridges the insertion site of IHHNV-related sequence, was used to test *P. monodon* collected from various regions. Representative samples from SE Asia did not contain the integrated IHHNV-related sequence. The integrated IHHNV-related sequence was found in some stocks originating from Africa, Australia and Andaman Sea. These PCR assay will be useful for the development of SPF *P. monodon* stocks.

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COMMISSION WORK PLAN FOR 2007/2008	
<i>Aquatic Animal Health Code</i>	
<ul style="list-style-type: none"> • Ongoing review of the list of diseases <ul style="list-style-type: none"> • Review emerging diseases 	
<ul style="list-style-type: none"> • Finalise disease chapter for <i>Gyrodactylus salaris</i> after Member Countries' comments • Update the disease chapters for Part 2 	
<ul style="list-style-type: none"> • Prepare text for disease chapters for gaining and regaining freedom for compartments 	
<ul style="list-style-type: none"> • Harmonise horizontal chapters with those in the <i>Terrestrial Code</i> <ul style="list-style-type: none"> • Zoning and compartmentalisation • Aquatic animal health surveillance • Model certificates • Handling and disposal of carcasses and wastes of aquatic animals 	
<ul style="list-style-type: none"> • Finalise guidelines on animal health issues related to aquatic animal feed after Member Countries' comments received 	
<ul style="list-style-type: none"> • Aquatic animal welfare guidelines 	
<ul style="list-style-type: none"> • Antimicrobial resistance in the field of aquatic animals 	
<i>Manual of Diagnostic Tests for Aquatic Animals</i>	
<ul style="list-style-type: none"> • Finalise general surveillance chapter and develop guidelines for surveillance for individual diseases with the assistance of <i>ad hoc</i> groups and other experts after Member Countries' comments received 	
<ul style="list-style-type: none"> • Revise Chapter on methods for disinfection of aquaculture establishments 	
Meetings	
<ul style="list-style-type: none"> • Make presentations on the activities of the Aquatic Animals Commission at the Conferences of the OIE Regional Commissions 	
<ul style="list-style-type: none"> • Assist in the implementation of recommendations adopted by the OIE Regional Commission for Asia, the Far East and Oceania in 2003, and endorsed by the OIE International Committee of the OIE in 2004 	
Other issues	
<ul style="list-style-type: none"> • Depending on an affirmative decision by the OIE International Committee, develop a list of diseases and draft chapters for the <i>Aquatic Code</i> and <i>Aquatic Manual</i> with the assistance of the <i>ad hoc</i> Group on Amphibian Diseases 	
<ul style="list-style-type: none"> • Keep updating the Commission's web pages 	
<ul style="list-style-type: none"> • Consider new candidates for OIE Reference Laboratories for listed diseases 	
<ul style="list-style-type: none"> • Develop a version of the PVS that addresses aquatic animal health with the assistance of an <i>ad hoc</i> Group. 	
<ul style="list-style-type: none"> • Coordination of a publication on "Changing trends in managing aquatic animal disease emergencies" under the <i>Rev. Sci. Tech.</i> series 	

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