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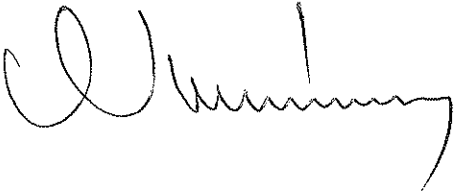

Dear Director General,

Please find attached, for your informal information, the intended positions of the European Union (EU) on the reports of the Terrestrial and Aquatic Animal Health Commissions to be proposed for adoption at the General Session in May 2011 in Paris.

Furthermore, please find attached the EU comments to the proposed new version of the OIE Organic and General Rules and related texts, as well as EU comments on the 2011-2013 OIE programme of work for the implementation of the 5th Strategic Plan.

I trust you will find this useful and I thank you for your continued cooperation.

Yours sincerely,

<p>Dr. Endre Kardeván Chief Veterinary Officer Hungary</p>	<p>Bernard Van Goethem Director, Veterinary and International Affairs DG Health and Consumers- European Commission</p>
	

Annexes: 3

Copy: All Directors / Chief Veterinary Officers of the EU 27 and Croatia, Iceland, Liechtenstein, Norway, Switzerland and Turkey.

Dr. B. Vallat
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ANNEX 2



Organisation
Mondiale
de la Santé
Animale

World
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for Animal
Health

Organización
Mundial
de Sanidad
Animal

Original: English
February 2011

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

Paris, 14–18 February 2011

The OIE Aquatic Animal Health Standards Commission (the Aquatic Animals Commission) met at the OIE Headquarters from 14 to 18 February 2011.

Details of participants and the adopted agenda are given at [Annexes I and II](#).

On behalf of Dr Bernard Vallat, Director General, Dr Gillian Mylrea, Deputy Head International Trade Department, welcomed members and thanked them for their ongoing work in support of the OIE.

The Aquatic Animals Commission strongly encouraged Members to participate in the development of the OIE's international standards by sending comments on this report. The Aquatic Animals Commission reiterated that it would be very helpful if comments were submitted as specific proposed text changes, supported by a scientific rationale. Members are requested not to use the automatic 'track-change' function provided by word processing software in preparation of their comments. The Commission also reminded Members that they should follow the established convention in recommending modification of text in the *Aquatic Animal Health Code* (hereinafter referred to as the *Aquatic Code*), i.e. propose new text (shown as double underline) and propose text deletions (shown as ~~strike through~~) and provide a scientific justification for all changes proposed.

The Aquatic Animals Commission reviewed various *Aquatic Code* draft texts from its October 2010 report in the light of Member comments. The outcome of the Commission's work is presented at [Annexes 3 to 25](#) in this report. Amendments made to the *Aquatic Code* chapters during the October 2010 meeting are shown as double underlined text, with deleted text in ~~strike through~~, while amendments made at this meeting (February 2011) are shown in a similar manner but with coloured background to distinguish the two groups of amendments.

The table below summarises the texts as presented in the Annexes. [Annexes 3 to 16](#) are proposed text for adoption at the 79th General Session in May 2011; [Annex 17 and 18](#) are presented for Member comments; [Annexes 19 to 25](#) for Members information.

Members are invited to submit their comments to the OIE on [Annexes 17 and 18](#) of this report. Comments must reach OIE Headquarters prior to **2 September 2011** in order to be considered at the next meeting of the Aquatic

Animals Commission, which will be held on 3-7 October 2011. Comments should be sent to the International Trade Department at: trade.dept@oie.int.

Texts proposed for adoption	Annex number
Glossary	Annex 3
Criteria for listing aquatic animal diseases (Chapter 1.2.)	Annex 4
Diseases listed by the OIE (Chapter 1.3.)	Annex 5
Principles for responsible and prudent use of antimicrobial agents in veterinary medicine (new Chapter 6.3)	Annex 6
Disinfection of salmonid eggs (Article 10.4.13., Article 10.5.13. and Article 10.9.13.)	Annex 7
Quality of aquatic animal health services (Chapter 3.1.)	Annex 8
Criteria to assess safety of aquatic animal commodities (Chapter 5.3.)	Annex 9
Control of hazards in aquatic animal feeds (Chapter 6.1.)	Annex 10
Introduction to the recommendations for controlling antimicrobial resistance (Chapter 6.2.)	Annex 11
Welfare of farmed fish during transport (Chapter 7.2.)	Annex 12
Welfare aspects of stunning and killing of farmed fish for human consumption (Chapter 7.3.)	Annex 13
Taura syndrome (Article 9.5.3.) Epizootic haematopoietic necrosis (Chapter 10.1.3.)	Annex 14
Listed aquatic commodities in Articles X.X.3. and X.X.11. (amphibians and fish) / X.X.12. (crustaceans and molluscs) all disease chapters (except epizootic haematopoietic necrosis, Taura syndrome, <i>B. ostreae</i>)	Annex 15
<i>Aquatic Manual</i> – chapters on amphibian diseases	Annex 16
Texts for Members' comment	Annex number
Aquatic Code – Killing of farmed fish for disease control purposes (new Chapter 7.4.)	Annex 17
<i>Aquatic Manual</i> – Criteria for listing species as susceptible to infection with a specific pathogen	Annex 18
Annexes for Members' information	Annex number
Amended and new product assessments	Annex 19
Aquatic Animals Commission Work Plan 2010/2011	Annex 20
Report of the <i>ad hoc</i> Group on the OIE List of Aquatic Animals Diseases (Finfish Team)	Annex 21
Report of the <i>ad hoc</i> Group on Pathogen Differentiation for Aquatic Animal Diseases	Annex 22
Report of the <i>ad hoc</i> Group on Safety of Products Derived from Aquatic Animals	Annex 23
Report of the <i>ad hoc</i> Group on Responsible Use of Antimicrobials in Aquatic Animals	Annex 24
Canada's assessment of pancreas disease	Annex 25

1. Activities and progress of *ad hoc* Groups

1.1. Report of the *ad hoc* Group on Safety of Products Derived from Aquatic Animals

Dr Berthe, Chair of the *ad hoc* Group on Safety of Products Derived from Aquatic Animals, summarised progress made at the *ad hoc* Group meeting held from 25-26 January 2011.

The *ad hoc* Group report is provided for information at [Annex 23](#)

[Agenda Items 2.6., 2.11., 2.12., and 2.13. provide details of specific *ad hoc* Group items addressed by the Aquatic Animals Commission.]

1.2. Report of the *ad hoc* Group on Pathogen Differentiation for Aquatic Animal Diseases

Dr Berthe, member of the *ad hoc* Group on Pathogen Differentiation for Aquatic Animal Diseases, gave a summary of progress made at the *ad hoc* Group meeting held from 27 to 28 January, 2011.

The Aquatic Animals Commission reviewed the *ad hoc* Group report and agreed with the Group's recommendations.

The Commission recommended that the *ad hoc* Group continue with the development of the criteria. While the Commission agreed that the suggested pathogens (ISAV, VHSV, *Marteilia refringens*, IHNV and YHV) were relevant for conducting a literature review, the Commission recommended that the *ad hoc* Group first address ISAV to produce a worked example for the Commission to consider. The Commission requested that the *ad hoc* Group report on this work to the Commission's October 2011 meeting.

The *ad hoc* Group report is provided for information at [Annex 22](#)

1.3. Report of the *ad hoc* Group on the OIE List of Aquatic Animals Diseases (Finfish Team)

Dr Hill, member of the *ad hoc* Group on the OIE List of Aquatic Animals Diseases (Finfish Team), gave a summary of progress made at the *ad hoc* Group's electronic consultations, which were held in December 2010 and January 2011.

The Aquatic Animals Commission considered the report of the *ad hoc* Group. The *ad hoc* Group members had reviewed the assessment provided by Chile that pancreas disease meets the listing criteria. The *ad hoc* Group also undertook its own assessment against the criteria in Chapter 1.3. The Commission noted the conclusion of the *ad hoc* Group that neither the data provided by Chile nor its own assessment provided sufficient evidence to satisfy Criteria 6 and 7. The Commission therefore invited Chile to submit additional supporting evidence in relation to Criteria 6 and 7, taking into consideration the comments made in the *ad hoc* Group's assessment.

Subsequent to the meeting of the *ad hoc* Group, OIE received an assessment of pancreas disease conducted by Canada against the OIE criteria for listing aquatic animal diseases. The Commission wished to thank Canada for this document, which reached similar conclusions to those of the *ad hoc* Group and is presented for Member information at [Annex 25](#).

The *ad hoc* Group report is provided for information at [Annex 21](#)

1.4. Report of the *ad hoc* Group on Responsible Use of Antimicrobials in Aquatic Animals

The Aquatic Animals Commission reviewed the report of the *ad hoc* Group on Responsible Use of Antimicrobials in Aquatic Animals electronic meeting held during February 2011. The Commission addressed the following items:

Chapter 6.3. Principles for responsible and prudent use of antimicrobial agents

Refer to agenda Item 2.4. for details on this draft chapter.

Discussion paper 'Susceptibility testing in aquaculture: current status and the way forward'

The Aquatic Animals Commission reviewed the *ad hoc* Group's draft discussion paper on susceptibility testing in aquaculture. The main problem described in this paper is the lack of standardized methods for susceptibility testing. The *ad hoc* Group suggested developing a list of bacteria in relation to which there is a most urgent need for standardised methods of susceptibility testing. The *ad hoc* Group also asked the Commission to make additional suggestions to stimulate and promote progress in this area. The Commission accepted the *ad hoc* Group recommendations and supported the development of a priority list of bacteria for which test methods should be established.

As noted in the draft discussion paper, the Clinical and Laboratory Standards Institute (CLSI), based in Pennsylvania, USA has developed guidelines for susceptibility testing in aquatic animals. However,

detailed test protocols and interpretive criteria are only available for a limited number of bacteria. The Commission asked the International Trade Department to make arrangements for direct contact between the CLSI and the *ad hoc* Group to discuss in more detail what steps might be taken to address current problems.

Advisory document on the Responsible and Prudent Use of Antimicrobial Agents in Aquatic Animals

The Commission reviewed the state of play with the advisory document, developed by the *ad hoc* Group, on the Responsible and Prudent Use of Antimicrobial Agents in Aquatic Animals (see Annex XXXIV in the February 2010 Commission report). The objective in producing this advisory document had been to provide guidance on the responsible and prudent use of antimicrobial agents in aquatic animals, via publication of the document on the OIE Internet site.

However, in light of the progress achieved with the draft new Chapter 6.3. 'Principles for responsible and prudent use of antimicrobial agents', including the potential for adoption in the *Aquatic Code* in May 2011, the Commission considered that to publish a similar, but more detailed, set of recommendations on the OIE Internet site could cause confusion.

The Commission asked the *ad hoc* Group to compare the text of Chapter 6.3., once it has been adopted, with the text of the advisory paper and highlight key points that may need to be addressed by the Commission in the future.

Discussion paper on risk analysis and antimicrobial resistance in aquatic animals

The Commission was informed that the *ad hoc* Group is continuing to develop a discussion paper on risk analysis and that it expected to complete this paper in time for the Commission's October 2011 meeting.

The *ad hoc* Group report is provided for information at [Annex 24](#).

2. OIE Aquatic Animal Health Code – Member comments

2.1. General Comments

The Aquatic Animals Commission welcomed the contribution of Australia, Canada, Chile, China (Peoples Republic of), Chinese Taipei, European Union (EU), Mexico, New Zealand, Norway, Switzerland, Thailand and the United States of America and the International Council for Animal Welfare (ICFAW).

2.2. Glossary

EU position

The EU cannot support the proposed amendments of the definition of "feed".

Rationale:

The proposed definition is not consistent with the definitions of "feed" neither in the Terrestrial Code nor in Codex Alimentarius standards. The EU would prefer to keep the current definition until the issue of the use of live and frozen fish as feed has been further elaborated, a work already foreseen by the Aquatic Animal Code Commission itself.

Whilst reviewing Member comments on Chapter 6.1., the Commission modified the definition of 'feed' to clarify that it includes living organisms (see agenda Item 2.8.):

'Feed means any material (single or multiple), ~~whether including living organisms and~~ processed, semi-processed or raw material that is intended to be fed directly to *aquatic animals*.'

The Commission considered that this modification, which leads to an element of overlap between the definitions for 'feed' and 'live feed', was necessary to address confusion on the part of some Members about the scope of the Chapter 6.1. It undertook to review this situation at its next meeting and, if necessary, further revise the Glossary.

The revised Glossary, proposed for adoption at the 79th General Session in May 2011, is presented at [Annex 3](#)

2.3. Listed Diseases (Chapter 1.3.)

EU position

The EU can support the adoption of the amended chapter.

The EU would propose that this chapter is revised in due course taking into account the ongoing work in this area undertaken by the OIE Terrestrial Code Commission.

A Member proposed that the disease name ‘Gyrodactylosis (*Gyrodactylus salaris*)’ be changed to ‘Infection with *Gyrodactylus salaris*’ and provided a rationale for this. The Aquatic Animals Commission agreed with this proposal as this is in line with the approach to the more recently adopted chapters on amphibian and mollusc diseases. The Commission intends to take this approach with all listed diseases. If the new name, ‘Infection with *Gyrodactylus salaris*’, is adopted, the relevant disease chapter in the *Aquatic Code* (Chapter 10.3.) and the *Aquatic Manual* (Chapter 2.3.3.) will be amended accordingly.

The Commission met with Dr Karim Ben Jebara, Head of the OIE Information Department, and reviewed the proposed amendments to Chapter 1.2. Criteria for listing diseases in the *Terrestrial Code*. The Commission compared these proposed changes with the text in Chapter 1.2. Criteria for listing diseases in the *Aquatic Code* and made a number of amendments for harmonization of the two Codes.

The revised Chapter 1.2. and Chapter 1.3., proposed for adoption at the 79th General Session in May 2011, are presented at [Annex 4](#) and [Annex 5](#), respectively.

2.4. Principles for Responsible and Prudent Use of Antimicrobial Agents in Veterinary Medicine (new Chapter 6.3.)

EU position

The EU thanks the OIE Aquatic Code Commission for taking on board the majority of EU comments. However, the EU can only support the adoption of this chapter if the following amendments are made:

- 1. In second paragraph of Article 6.3.7 the word "recommend" is deleted and in second paragraph of Article 6.3.8 the word "recommendation" is deleted.**

Rationale:

Veterinary antimicrobial agents should only be used upon prescription or administration by veterinarians or other aquatic animal health professionals authorised to prescribe veterinary medicines. This is an essential part of a strategy to ensure responsible and prudent use of antimicrobial agents in aquatic animals, and would create consistency with the equivalent Articles in the Terrestrial Code.

- 2. In the title and in paragraphs 2, 3, 4, 6 and 7 of Article 6.3.7, and in second paragraph of Article 6.3.8, the words "authorised to prescribe veterinary medicines" are added after the words "aquatic animal health professionals"**

Rationale:

The EU acknowledges that in the veterinary field, not only veterinarians, but also other aquatic animal health professionals are carrying out important duties and agree that this should be recognised in this Article. However, it is essential that professionals prescribing veterinary medicines have appropriate knowledge of aquatic animal clinical pathology and pharmacology. To ensure this the right to prescribe veterinary medicines should be limited to aquatic animal health professionals who are specifically authorised to do so under relevant legislation.

- 3. The second sentence of Paragraph 7 of Article 6.3.7 is deleted.**

Rationale:

As regards responsible and prudent use of antimicrobials, including the extra-label/off-label use, the conditions should, taking into the potential public health concerns, be

equivalent for products intended for the national market as products intended for export. The EU is therefore of the opinion that it is not appropriate to include the second sentence of Paragraph 7 of Article 6.3.7. Furthermore, this sentence does not fit well into the systematics of the chapter. The chapter lays down principles for responsible and prudent use in each Member country. The sentence is the only one which is referring to import/export. Moreover, similar provisions are not included in the equivalent Terrestrial Code chapter. Finally, as the OIE standards governing trade between OIE Members are regulated in other parts of the Code and including provision on import/export in this chapter may lead to confusion and trade problems.

In addition to the above comments, the EU would also suggest to replace the word "effectiveness" with "efficacy" in the first sentence of Paragraph 1 of Article 6.3.5 and in the last sentence of Paragraph 8 of Article 6.3.7.

Rationale:

When referring to antimicrobial agent, the common term used is "efficacy". "Efficacy" is also used several places in this Chapter. This amendment would therefore contribute to consistency in the terminology of the Code.

The Aquatic Animals Commission reviewed the recommendations of the *ad hoc* Group on Responsible Use of Antimicrobial Agents in Aquatic Animals in response to Member comments on the proposed new Chapter 6.3. 'Responsible and Prudent Use of Antimicrobial Agents in Veterinary Medicine' and agreed with the proposed amendments.

The *ad hoc* Group on the responsible use of antimicrobials in aquatic animals held a telephone meeting to address OIE Members' comments on the draft Chapter 6.3. 'Principles for responsible and prudent use of antimicrobials in aquatic animals'.

Contrary to the recommendation of a Member, the Commission agreed with the *ad hoc* group on the need to make provision for an aquatic animal health professional who is authorised to prescribe or recommend the use of antimicrobial agents, given that veterinarians are not centrally involved with aquatic animal production in many countries. Including text on the responsibilities of this individual reflects the reality of aquatic animal production, especially in developing countries.

The Commission also agreed with the *ad hoc* Group that the availability of registered antimicrobial agents for use in aquatic animal production is very limited compared with terrestrial animal production. In aquatic animal production there is an extreme shortage of authorised antimicrobial agents; in addition, there are many different species used in aquatic animal production. The Commission agreed that the chapter should make provision to address these problems including providing for off-label use, under appropriate conditions. With the passage of time this situation may improve.

Following a Member's comment, the Commission asked the *ad hoc* Group to give further consideration to aquatic animal feeds containing antimicrobial agents, and the responsibilities of aquatic animal feed producers and aquatic animal producers in regards to feed, at the next meeting and develop some additional details for inclusion in the chapter.

The revised Chapter 6.3. Principles for Responsible and Prudent Use of Antimicrobial Agents in Aquatic Animals, proposed for adoption at the 79th General Session in May 2011, is presented at [Annex 6](#).

2.5. Disinfection of salmonid eggs (Article 10.4.13., Article 10.5.13. and Article 10.9.13.)

EU position

The EU thanks the Aquatic Commission for this addition and supports the adoption of the amended chapter, but has one comment:

In point c of Paragraph 2 of Article 10.5.13, the words "OIE Members" should, for clarity and consistency, be replaced by the words "Competent Authority of the importing country".

The Aquatic Animals Commission considered Member comments but made no amendments to these articles.

The revised articles (Article 10.4.13., Article 10.5.13. and Article 10.9.13.), proposed for adoption at the 79th General Session in May 2011, are presented at [Annex 7](#).

2.6. Quality of Aquatic Animal Health Services (Chapter 3.1.)

EU position

The EU can support the adoption of the amended chapter.

The Aquatic Animals Commission considered Member comments but made no amendments.

The Commission considered that the proposed text changes were to clarify intent and it did not amend the text as it considered that it was clear as written and that it was also important to keep this chapter aligned with the *Terrestrial Code* chapter, where appropriate.

The revised Chapter 3.1. Quality of Aquatic Animal Health Services, proposed for adoption at the 79th General Session in May 2011, is presented at [Annex 8](#).

2.7. Criteria to assess safety of aquatic animal commodities (Chapter 5.3.)

EU position

The EU can support the adoption of the amended chapter.

The Aquatic Animals Commission reviewed the recommendations of the *ad hoc* Group on Safety of Commodities Derived from Aquatic Animals in response to Member comments on amendments to Article 5.3.2. The Aquatic Animals Commission agreed with the proposed amendments.

The Commission noted some problems with the internet version of Chapter 5.3. (English, Spanish and French versions), which has some numbering and formatting errors, and Article 5.3.2. in the printed version, which also had some numbering and formatting errors. The Commission requested that OIE Headquarters amend the web version and the 2011 printed edition of the *Aquatic Code*.

The revised Chapter 5.3. Criteria to assess the safety of aquatic animal commodities, proposed for adoption at the 79th General Session in May 2011, is presented at [Annex 9](#).

2.8. Control of hazards in aquatic animal feeds (Chapter 6.1.)

EU position

The EU can only support the proposed amendments if the following comment is taken account of:

In the second sentence of the second paragraph of Article 6.1.1 the words "are also important references" should be replaced by "may be relevant sources of guidance".

Rationale:

The term "reference" is ambiguous and could be wrongfully interpreted as a legal reference.

In addition, the EU would like to highlight the importance of being clear when in the OIE standards citing sources that are not international standards relevant under SPS, whether these sources are to be considered "references" (i.e. part of the standard) or only source of information. As it may create legal uncertainty, the EU would prefer that a cautionary approach was taken on this matter and limit the number of such external sources of information. On the other hand, the OIE should make cross-references to other standards and to significant collaboration with other organisations, such as "in synergy with Codex", where appropriate.

The Aquatic Animals Commission considered Member comments and made relevant amendments.

A Member commented that the introduction and scope appeared to be in conflict with respect to public health concerns. The Commission amended the introduction to clarify that this chapter covers both aquatic animal health and public health.

The Commission received several comments from a Member about the risks associated with phytoplankton production for use as aquatic animal feed. The Commission considered that this topic had been addressed in a general way in the chapter but that more detailed recommendations could be provided, and asked the OIE International Trade Department to obtain advice from an expert for consideration by the Commission at its next meeting.

Two Members commented on the risks of using whole live or frozen fish as feed. The Commission noted that this is a widespread and growing practice and that some aquaculture sectors, particularly new sectors depend on this source of feed. The Commission decided to review the issue in more detail and asked the OIE International Trade Department to obtain advice from FAO and other experts as appropriate for future consideration by the Commission.

The Commission modified the definition of feed in the Glossary to clarify that it includes living organisms. As stated in point 2.2 above, the Commission considered that this modification, which leads to an element of overlap between the definitions for 'feed' and 'live feed', was necessary to address confusion on the part of some Members about the scope of the Chapter 6.1. It undertook to review this situation at its next meeting and, if necessary, further revise the Glossary.

The Commission agreed with a Member's proposal to delete Article 6.1.5. as certification is covered elsewhere in the *Aquatic Code*. Relevant provisions were included as a new point in Article 6.1.4.

The revised Chapter 4.5. Control of Hazards in Aquatic Animal Feeds, proposed for adoption at the 79th General Session in May 2011, is presented at [Annex 10](#).

2.9. Introduction to the recommendations for controlling antimicrobial resistance (Chapter 6.2.)

EU position

The EU can support the adoption of the amended chapter.

The Aquatic Animals Commission considered Member comments but made no amendments.

Two Members again proposed the addition of text referring to OIE collaboration with Codex Alimentarius Commission (CAC). The Aquatic Animals Commission again noted that all the OIE work on animal production food safety is conducted in active collaboration with the CAC and therefore considered there was no need to make a specific statement to this effect in individual articles in the *Aquatic Code*. Furthermore, the proposed text aligns with the equivalent text in the *Terrestrial Code*.

A Member asked if the proposed *Aquatic Code* chapters will in future cover antivirals, anthelmintics and treatments for ectoparasites. The Commission clarified that the chapters will cover the substances that meet the definition proposed for 'antimicrobial agent', which means that antivirals may be addressed in future but not anthelmintics and treatments for ectoparasites, unless OIE Members call for the definition to be modified.

In order to maintain harmonization with the corresponding chapter in the *Terrestrial Code*, the Commission did not accept text amendments proposed by two Members.

The revised Chapter 6.1. Introduction to the Recommendations for Controlling Antimicrobial Resistance, proposed for adoption at the 79th General Session in May 2011, is presented at [Annex 11](#).

2.10. Welfare of farmed fish during transport (Chapter 7.2.)

EU position

The EU can support the adoption of the proposed changes in the chapter of welfare of farmed fish during transport at OIE's 79th General Session in May 2011.

The EU would request the OIE to consider several proposals for amendments of the chapter. These proposals are included in Annex 12.

The Aquatic Animals Commission considered Member comments and made relevant amendments.

The revised Chapter 7.2. Welfare of Farmed Fish During Transport, proposed for adoption at the 78th General Session in May 2010, is presented at [Annex 12](#).

2.11. Welfare aspects of stunning and killing of farmed fish for human consumption (Chapter 7.3.)

EU position

The EU welcomes the revision of the Chapter on Welfare aspects of stunning and killing of farmed fish for human consumption and thanks the OIE for having taken into account most of the EU comments submitted earlier this year.

The EU can support adoption of the proposed changes in the chapter at OIE's 79th General Session in May 2011.

The EU would request the OIE to consider several proposals for amendments of the chapter. These proposals are included in Annex 13.

The Aquatic Animals Commission considered comments from Members and the International Council for Animal Welfare (ICFAW), and made relevant amendments.

The Commission did not accept some ICFAW recommendations due to the fact that the OIE policy to date, in both the *Terrestrial* and *Aquatic Code*, is generally not to adopt quantitative measures. Some other suggestions from ICFAW were not accepted as the Commission considered these were already covered in the existing text.

A Member recommended that a description and assessment of pharmacological methods for stunning be added to the chapter. The Commission noted that the inclusion of pharmacological methods was considered and reported in the February 2009 Commission report. The Commission decided not to include pharmacological methods for stunning because more information on the food safety aspects of pharmacological methods is needed before proposing text for adoption. The Commission invited Members to provide technical information on pharmacological methods that are currently authorised in their country for the stunning and killing of fish intended for human consumption.

In reviewing the chapter, the Commission decided to replace 'chemicals' with 'pharmacological substances' as this was more appropriate.

The revised Chapter 7.3. Welfare aspects of stunning and killing of farmed fish for human consumption, proposed for adoption at the 79th General Session in May 2011, is presented at [Annex 13](#).

2.12. Taura syndrome (Article 9.5.3.) and epizootic haematopoietic necrosis (Article 10.1.3.)

EU position

The EU can support the adoption of the amended chapter.

The Aquatic Animals Commission reviewed the recommendations of the *ad hoc* Group on Safety of Commodities Derived from Aquatic Animals in response to Member comments on amendments to Article 9.5.3. Taura syndrome and Article 10.1.3. Epizootic haematopoietic necrosis.

The Aquatic Animals Commission agreed with the proposed amendments.

The revised Article 9.5.3. and Article 10.1.3., proposed for adoption at the 79th General Session in May 2011, are presented at [Annex 14](#).

2.13. Listed aquatic products in Articles X.X.3. and X.X.11. (amphibians and fish) / X.X.12. (crustaceans and molluscs) (all disease chapters except epizootic haematopoietic necrosis, Taura syndrome, *B. ostreae*)

EU position

The EU thanks the Aquatic Commission for the inclusion of points i) and j) in Article 10.3.3 on *Gyrodactylus salaris* and supports the adoption of all the amended chapters, but has a comment on Article 3 points g and h of Article 10.3.3:

The EU would propose that the original wording of points g) and h) of Article 10.3.3 are retained, but with "7.5" replaced by "25".

Rationale:

The crucial risk mitigation measure for these two products are the the salinity of the water in which the animals are kept before harvesting. According to scientific studies (Arnulf Soleng and Tor A. Bakke, Salinity tolerance of *Gyrodactylus salaris* (Platyheminthes, Monogenea: laboratory studies, Can. J. Fish. Aquat. Sci. 54 1837-1854 (1997)) *Gyrodactylus salaris* will only survive for considerably less than an hour if kept in full strength seawater. Consequently, the EU finds it inadequate to add the criterion that the animals must have been reared in such water for at least 2 months. This is also a considerably longer time period than what is required for live animals in Article 10.3.8 which refers to 14 days in water of at least 25 ppt. The requirements should not be more restrictive for products than those for live animals. Furthermore, the term "full strength seawater" is a rather ambiguous one and in the interest of maintaining coherence between the different Articles of the Chapter, the EU would prefer to refer to ppt. 25 ppt.

The Aquatic Animals Commission reviewed the recommendations of the *ad hoc* Group on Safety of Commodities Derived from Aquatic Animals in response to Member comments on amendments to the aquatic product listings in Articles X.X.3. and X.X.11. (amphibians and fish) / X.X.12. (crustaceans and molluscs) for all disease chapters, and agreed with the Group's recommendations.

The Commission reminded Members that the revised product listings are based on product assessments conducted by the *ad hoc* Group using the criteria listed in Articles 5.3.1. and 5.3.2.

OIE Headquarters reminded Members that due to the large size of the product assessment document, it was only provided in English in the Report of the October 2010 Meeting of the Aquatic Animals Commission (Annex XVIII). However, translation to French and Spanish has now been completed and the relevant Annexes have been updated in the French and Spanish versions of the October 2010 Meeting of the Aquatic Animals Commission and are now available on the OIE website.

The Commission noted that the products 'fish roe' and 'chilled fish products from which the skin, fins and gills have been removed' proposed for inclusion in Article 10.3.3. (Gyrodactylosis) are newly listed products supported by new assessments kindly provided by the EU and Norway. The inclusion of 'chilled fillets and steaks' proposed in Article 10.2.12. (EUS) are based on a revised product assessment. The product description for 'chilled fillets or steaks and chilled eviscerated fish that have been reared for at least 2 months in full strength seawater' in Article 10.3.3. (Gyrodactylosis) were amended.

The revised product assessments for 'chilled, eviscerated fish' and 'chilled fillets and steaks' for inclusion in Article 10.3.3. (Gyrodactylosis) and for 'chilled fillets and steaks' for inclusion in Article 10.2.12. (EUS); and new product assessments for 'fish roe' and 'chilled fish products from which the skin, fins and gills have been removed' for inclusion in Article 10.3.3. (Gyrodactylosis) are provided at [Annex 19](#) for Member information.

The revised Articles X.X.3. and X.X.11. (amphibians and fish)/ X.X.12. (crustaceans and molluscs) for all disease chapters (except epizootic haematopoietic necrosis, Taura syndrome and Infection with *B. ostreae*), proposed for adoption at the 79th General Session in May 2011, are presented at [Annex 15](#).

2.14. Killing of farmed fish for disease control purposes (new Chapter 7.4.)

The Aquatic Animals Commission appreciated the large number of Member comments on this text and amended the text where considered appropriate.

The revised Chapter 7.4. Killing of farmed fish for disease control purposes is presented at [Annex 17](#) for Member comments.

EU comments

The EU notes that previous EU comments concerning the prior stunning of fish were not taken into account; however, the report of the meeting of the OIE Aquatic Animal Health Standards Commission does not explain the basis for the proposed changes and for refusing the EU comments. Specific comments are included in Annex 17.

3. Other relevant activities

3.1. Harmonisation of OIE Codes

The Aquatic Animals Commission continues to work to ensure ongoing harmonisation of the two OIE Codes. The Commission reviewed the proposed amendments to Chapter 1.2. Criteria for Listing Diseases in the *Terrestrial Code* and proposed some amendments to Chapter 1.2. Criteria for Listing Diseases in the *Aquatic Code* to align of these two chapters (see details in Item 2.3.).

3.2. PVS Tool – Application to Aquatic Animal Health Services – update

Dr Kahn advised the Commission of the state of play with the PVS evaluation of Aquatic Animal Health Services. The OIE has developed a revised PVS Tool for use in evaluations of AAHS based on a pilot evaluation of a Member. The revised Tool will be used in an assessment of another OIE Member shortly, and some additional work undertaken, principally in the development of indicators.

The Commission commended this work and encouraged Members to request that the OIE carry out evaluations with a view to obtaining needed investments on the parts of governments and donors to strengthen aquatic animal health services.

3.3. 5th Strategic Plan (2011–2015)

The Aquatic Animals Commission reviewed the OIE 5th Strategic Plan (2011-2015) and noted the broader remit proposed for the Commission.

3.4. Communication

Dr Kahn informed the Aquatic Animals Commission that the Terrestrial Code Commission is proposing a new chapter on communication for inclusion in the *Terrestrial Code*. The Commission welcomed this addition and proposed to review the chapter once adopted and consider inclusion of an equivalent chapter in the *Aquatic Code*.

3.5. Ad hoc Group on Veterinary Education

Dr Kahn updated the Commission about the OIE work on veterinary education. The ad hoc Group has met twice and has drafted recommendations on the ‘minimum competencies of day 1 veterinary graduates to assure the delivery of quality Veterinary Services, as described by the OIE’. In view of the strong support of Members for the work of the OIE in strengthening the quality of Veterinary Services by establishing the PVS Pathway, and the direct relevance of veterinary education to the performance of Veterinary Services, at its meeting on 1-11 February 2011, the Terrestrial Code Commission proposed to make direct reference to these recommendations by including a short reference to the minimum competencies in the *Terrestrial Code*.

Dr Kahn noted that in 2011 the veterinary profession is celebrating its 250 years anniversary. The OIE is involved in several important initiatives relevant to the Vet2011 celebrations.

The second global Conference on Veterinary Education will take place in Lyon on 13-14 May 2011. This conference will feature presentations on the OIE’s work on veterinary education. It is hoped that in May 2011 the World Assembly will adopt a resolution supporting the OIE recommendations on veterinary

education, specifically the day 1 competencies of graduates to enable Veterinary Services to fulfil the OIE mandate.

The Aquatic Animals Commission undertook to follow this work with interest.

4. Cooperation with FAO

Dr Subasinghe, representing the FAO, provided a summary of current and ongoing FAO activities relevant to aquatic animal health globally. He stressed the need for a concerted effort to assist Southern African countries in the Zambezi basin to address the impacts of fast spreading EUS in the region. He also stressed the urgent need for assisting Southeast Asian countries, in particular Indonesia, to reduce the risks of Infectious Myonecrosis Virus (IMNV) in white shrimp, *Penaeus /Litopennaeus vannamei*. He also explained FAO's ongoing and planned capacity building activities for biosecurity in the Pacific and Western Balkan regions, Asia and Africa. Dr Subasinghe emphasised the urgent need to improve aquatic biosecurity globally and requested the OIE to expand collaboration with FAO in assisting the Members.

Dr Subasinghe informed the Commission that the 29th Session of the FAO Committee on Fisheries (COFI) held in February 2011 approved the Technical Guidelines on Aquaculture Certification. He mentioned that COFI recognised the existing standards and guidelines set by international organisations such as the OIE for aquatic animal health and welfare, Codex Alimentarius Commission for food safety and the International Labour Organisation for socio-economic aspects. COFI recommended that FAO develop an evaluation framework to assess the conformity of public and private certification schemes with the FAO aquaculture certification guidelines. Dr Subasinghe noted that for animal health and food safety the OIE and Codex standards are the relevant standards to follow. Dr Subasinghe noted that the OIE will formally be invited to all statutory meetings of the FAO Fisheries and Aquaculture Department and will be informed of all follow-up activities on aquaculture certification.

The Commission agreed with the importance of further strengthening collaboration between FAO and OIE on improving aquatic biosecurity globally.

5. OIE Conferences and relevant meetings

Members of the Aquatic Animals Commission or other OIE representatives attended the following OIE conferences and meetings and delivered a presentation on the work of the Aquatic Animals Commission:

- 24th Conference of the OIE Regional Commission for Europe (Kazakhstan, 20–24 September, 2010);
- 20th Conference of the OIE Regional Commission for the Americas (Uruguay, 16–19 November 2010);
- 9th Meeting of the NACA Regional Advisory Group on Aquatic Animal Health (Bangkok, 8–10 November 2010);
- Tahiti Aquaculture 2010: 'Conference for Sustainable Aquaculture on Tropical Islands' (Tahiti, 6–10 December);
- 29th Session of the Committee on Fisheries (COFI) (31 January–4 February, Rome, Italy).

6. Upcoming OIE Conferences and relevant meetings

Members of the Aquatic Animals Commission or other OIE representatives will attend the following OIE conferences and meetings and deliver a presentation on the work of the Aquatic Animals Commission:

- 19th Conference of the OIE Regional Commission for Africa (Kigali, Rwanda, 14–18 February 2011);
- OIE Global Conference on Wildlife: animal health and biodiversity – Preparing the future (23–25 February, Paris, France);

- 15th International Conference of the European Association of Fish Pathologists (Split, Croatia, 12–16 September 2011).

7. OIE Regional aquatic animal focal points training workshops

Members of the Aquatic Animals Commission have attended/ will attend and deliver presentations at the following OIE regional aquatic animal focal points training workshops:

For Europe: Dubrovnik, Croatia, 16–18 November, 2010;

For the Americas: Roatan, Honduras, 23–25 November, 2010;

For Far East, Asia Pacific: Ho Chi Minh City, Vietnam, 19–21 April, 2011.

8. OIE Global Conference on Aquatic Animal Health: ‘Aquatic Animal Health Programmes: their benefit to global food security’ 28-30 June 2011, Panama

The Aquatic Animals Commission noted the contents of the draft programme and aspects of their role as the Scientific Committee and are looking forward to the event.

9. Manual of Diagnostic Tests for Aquatic Animals, seventh edition 2012

Ms Sara Linnane, Scientific Editor, from the Scientific and Technical Department, joined the meeting for this agenda item.

Comments had been received from: Canada, Chile, China [People’s Rep. of], European Union, Japan, New Zealand, Norway, Switzerland, Thailand, and the United States of America.

9.1. Comments from OIE experts on a Member comment on the sixth edition of the *Aquatic Manual*

As recorded in the report of the meeting of the Aquatic Animals Commission held in October 2010, the Commission had decided to consult the OIE Reference Laboratory experts for technical advice on a comment that had been received from an OIE Member on the sixth edition of the *Aquatic Manual*. The comment concerned including the loop-mediated isothermal amplification (LAMP) diagnostic procedure in each disease chapter of the *Aquatic Manual*. Based on the advice received from the experts, the majority view was that it was not suitable to include this procedure in every disease chapter but only in those chapters for which there are publications in peer-reviewed journals and for which the test has been validated for diagnostic purposes. The authors of the chapters will be asked to consider if LAMP diagnostic techniques are sufficiently developed to merit inclusion in the chapter for which they are responsible.

9.2. Comments from OIE Members on draft *Aquatic Manual* chapters on amphibian diseases

EU position

The EU can support the adoption of the amended chapter.

The Aquatic Animals Commission reviewed Member comments on the draft chapters for diseases of amphibians: Infection with *Batrachochytrium dendrobatidis* and Infection with ranavirus. As the comments were of a technical nature, the Commission sent them to the authors for immediate review, then considered the revised draft chapters, and accepted the proposed changes.

The two draft chapters, Infection with *Batrachochytrium dendrobatidis* and Infection with ranavirus, are proposed for adoption and presented at [Annex 16](#).

If adopted, they will be included in the web version of the *Aquatic Manual*.

9.3. Comments from OIE Members on draft text on disinfection of eggs

Comments, some of a highly technical nature, had been received on the draft text on disinfection of eggs. It is clear that Members would like the chapter to include scientific references to the methods mentioned in the chapter. The Aquatic Animals Commission could not deal with this issue during its meeting as external experts would need to be consulted. The Commission identified a number of experts who could work electronically on the chapter, providing the missing references. The Commission requested that this work be completed prior to its meeting in October 2011.

9.4. Criteria for listing species as susceptible to infection with a specific pathogen

The Aquatic Animals Commission discussed the issue of listing susceptible species in the *Aquatic Code* and the *Aquatic Manual* and proposed a broader approach to this issue. The Commission concluded that these criteria (previously provided in the 'Guidance document for listing species as susceptible to infection with a specific pathogen') be used to assess species for susceptibility for the disease specific chapters in the *Aquatic Code* and the *Aquatic Manual*, rather than only as a guidance document for authors of *Aquatic Manual* disease specific chapters.

The Aquatic Animals Commission considered Member comments and OIE Reference Laboratory expert comments received following circulation of the 'Guidance document for listing species as susceptible to infection with a specific pathogen' in the report of the Aquatic Animals Commission meeting held in September 2009. The Commission amended these criteria, as relevant, and proposed that these be included as a new chapter in the *Aquatic Manual*.

The electronic meeting of the *ad hoc* Group proposed by the Aquatic Animals Commission at its October 2010 meeting did not take place. In light of the revised approach to this issue, the Aquatic Animals Commission recommended that a new *ad hoc* Group on Listing Species as Susceptible to Infection with a Specific Pathogen be convened to finalise these criteria and to develop a worked example using these criteria for KHVD. The Commission requested that the *ad hoc* Group meet prior to its meeting in October 2011.

Two Members submitted their assessments for WSD using the criteria circulated in the report of the meeting of the Aquatic Animals Commission held in September 2009. The Commission acknowledged the work that these assessments involved and considered these assessments to be useful for future work on this issue.

The revised criteria for listing species as susceptible to infection with a specific pathogen are presented at [Annex 18](#) for Member comments.

10. OIE Reference Laboratories and Collaborating Centres

10.1. New applications for Reference Laboratory status

At its meeting in October 2010, the Commission had postponed reaching a final decision on three applications for OIE Reference Laboratory status, until assurances were received that the laboratories have the capacity for speedy receipt and shipment of samples and reference reagents and materials. The Delegates of the two countries concerned had provided such assurances. The Commission therefore, recommends acceptance of the following applications for OIE Reference Laboratory status:

OIE Reference Laboratories for White spot disease and Infectious hypodermal and hematopoietic necrosis
Maricultural Organism Disease Control and Molecular Pathology Laboratory, Yellow Sea Fisheries
Research Institute (YSFRI), Chinese Academy of Fishery Sciences #106 Nanjing Road, Qingdao,
Shandong Province 266071, CHINA (PEOPLE'S REP. OF)

Tel: (+86-532) 85.82.30.62 ext. 802; Fax: (+86-532) 85.81.15.14; E-mail: huangjie@ysfri.ac.cn;
aqudis@public.qd.sd.cn

Web site: www.ysfri.ac.cn

Designated Reference Expert: Dr Jie Huang

OIE Reference Laboratory for Spring viraemia of carp

The Laboratory of Aquatic Animal Diseases, Shenzhen Exit & Entry Inspection and Quarantine Bureau,

AQSIQ, 2049 Heping Road, Shenzhen, 518001, CHINA (PEOPLE'S REP. OF)
 Tel: (+86-755) 25.58.84.10; Fax: (+86-755) 25.58.86.30; E-mail: liuhong@szciq.gov.cn
 Designated Reference Expert: Dr Hong Liu

OIE Reference Laboratory for Infection with Abalone herpes-like virus
 Australian Animal Health Laboratory (AAHL), CSIRO Livestock Industries, 5 Portarlington Road, East
 Geelong, Victoria 3220, AUSTRALIA
 Tel: (+61-3) 52.27.51.18; Fax: (+61-3) 52.27.55.55; E-mail: mark.crane@csiro.au
 Designated Reference Expert: Dr Mark Crane

10.2. Review nominations for replacement experts

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

Infection with *Mikrocytos mackini*

Dr Gary Meyer to replace Dr Susan Bower at the Pacific Biological Station, Fisheries and Oceans Canada, Nanaimo, British Columbia, CANADA

Infection with *Perkinsus marinus* and *P. olseni*, Infection with *Haplosporidium costale*, and *H. nelsoni*

Dr Ryan Carnegie to replace Dr Eugene Bureson at the Virginia Institute of Marine Science, College of William and Mary Gloucester Point, United States of America.

10.3. Review of annual reports of OIE Reference Laboratory and Collaborating Centre activities in 2010

Reports had been received from all but three of the OIE Reference Laboratories for Aquatic Animals and from the two Collaborating Centres. The Aquatic Animals Commission carefully reviewed the reports received. It was impressed, in general, with the quality of the work carried out by the laboratories and expressed its gratitude to the experts for their efforts. The Commission noted that some experts did not appear to have followed the instructions sent with the report template such that it was not clear if the information provided was for the laboratory's activities specifically as an OIE Reference Laboratory or its activities as a national laboratory for the disease in question. Some laboratories reported low or no activities in a number of categories in the report template. These laboratories would be requested to clarify whether this is because of lack of requests or an inability to fulfil the mandate.

The full set of reports for 2010 would be supplied to Members and to all the Reference Laboratories and Collaborating Centres on a CD-ROM.

11. Laboratory Twinning Projects

Dr Hamilton from the OIE Scientific and Technical Department joined the meeting for this agenda item. He provided the following update on twinning projects.

Twinning for EUS between Thailand and Zambia: The twinning proposal has been updated to account for comments arising from the previous Commission meeting. The proposal is awaiting administrative clearance in Thailand. The Commission noted that there was an urgent need to build capacity for EUS in Southern Africa, and efforts should be made to facilitate approval of this project.

Twinning for Crustacean diseases between Cuba and Italy/USA: The proposal has run into administrative delays and it is uncertain whether these can be resolved. The Commission suggested that Cuba may wish to consider twinning with the OIE Reference Laboratory for white spot disease in Chinese Taipei.

The Aquatic Animals Commission will be represented at the OIE twinning feedback session to be held in Paris, 30-31 March 2011. Following this meeting it is likely that the twinning guide will be updated and will be presented to the Commission for comment.

Lina Awada from the Scientific and Technical Department updated the Commission on an OIE project that involves targeting geographic areas that could benefit from improved laboratory capacity through the OIE twinning program. The list of priority areas for laboratory capacity development will be undertaken by studying different criteria (such as animal density, exports, the capacity of existing laboratories, the importance of livestock production in GDP, etc.), for diseases of livestock, aquatic animals and bees. This assessment will also involve expert opinion. The second phase of the project will focus on East Africa, which has been identified as an area that requires development of laboratory capacity.

The Commission welcomed this initiative, offered to provide relevant information where useful and looked forward to seeing the outcome of the project.

12. Review of the Aquatic Animals Commission's work plan for 2011/12

The Aquatic Animals Commission reviewed and updated its work plan, which is provided at [Annex 20](#) for Members' information.

13. Date of the next meeting

3–7 October 2011

.../Annexes

**MEETING OF THE OIE
AQUATIC ANIMAL HEALTH STANDARDS COMMISSION
Paris, 14–18 February 2011**

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

Paris, 14–18 February 2011

Adopted agenda

- 1. Activities and progress of *ad hoc* Groups**
 - 1.1. Report of the *ad hoc* Group on Safety of Products Derived from Aquatic Animals**
 - 1.2. Report of the *ad hoc* Group on Pathogen Differentiation for Aquatic Animal Diseases**
 - 1.3. Report of the *ad hoc* Group on the OIE List of Aquatic Animals Diseases (Finfish Team)**
 - 1.4. Report of the *ad hoc* Group on Responsible Use of Antimicrobials in Aquatic Animals**
- 2. OIE *Aquatic Animal Health Code* – Member comments**
 - 2.1. General Comments**
 - 2.2. Glossary**
 - 2.3. Listed Diseases (Chapter 1.3.)**
 - 2.4. Principles for Responsible and Prudent Use of Antimicrobial Agents in Veterinary Medicine (new Chapter 6.3.)**
 - 2.5. Disinfection of salmonid eggs (Article 10.4.13., Article 10.5.13. and Article 10.9.13.)**
 - 2.6. Quality of Aquatic Animal Health Services (Chapter 3.1.)**
 - 2.7. Criteria to assess safety of aquatic animal commodities (Chapter 5.3.)**
 - 2.8. Control of hazards in aquatic animal feeds (Chapter 6.1.)**
 - 2.9. Introduction to the recommendations for controlling antimicrobial resistance (Chapter 6.2.)**
 - 2.10. Welfare of farmed fish during transport (Chapter 7.2.)**
 - 2.11. Welfare aspects of stunning and killing of farmed fish for human consumption (Chapter 7.3.)**
 - 2.12. Taura syndrome (Article 9.5.3.) and epizootic haematopoietic necrosis (Article 10.1.3.)**
 - 2.13. Listed aquatic products in Articles X.X.3. and X.X.11. (amphibians and fish) / X.X.12. (crustaceans and molluscs) (all disease chapters except epizootic haematopoietic necrosis, Taura syndrome, *B. ostreae*)**

- 2.14. Killing of farmed fish for disease control purposes (new Chapter 7.4.)
 3. Other relevant activities
 - 3.1. Harmonisation of OIE Codes
 - 3.2. PVS Tool – Application to Aquatic Animal Health Services – update
 - 3.3. 5th Strategic Plan (2011-2015)
 - 3.4. Communication
 - 3.5. Ad hoc Group on Veterinary Education
 4. Cooperation with FAO
 5. OIE Conferences and relevant meetings
 6. Upcoming OIE Conferences and relevant meetings
 7. OIE Regional aquatic animal focal points training workshops
 8. OIE Global Conference on Aquatic Animal Health: ‘Aquatic Animal Health Programmes: their benefit to global food security’ 28-30 June 2011, Panama
 9. Manual of Diagnostic Tests for Aquatic Animals, seventh edition 2012
 - 9.1. Comments from OIE experts on a Member comment on the sixth edition of the *Aquatic Manual*
 - 9.2. Comments from OIE Members on draft *Aquatic Manual* chapters on amphibian diseases
 - 9.3. Comments from OIE Members on draft text on disinfection of eggs
 - 9.4. Criteria for listing species as susceptible to infection with a specific pathogen
 10. OIE Reference Laboratories and Collaborating Centres
 - 10.1. New applications for Reference Laboratory status
 - 10.3. Review nominations for replacement experts
 - 10.3. Review of annual reports of OIE Reference Laboratory and Collaborating Centre activities in 2010
 11. Laboratory Twinning Projects
 12. Review of the Aquatic Animals Commission’s work plan for 2011/12
 13. Date of the next meeting
-

GLOSSARY**EU position**

The EU cannot support the proposed amendments of the definition of "feed".

Rationale:

The proposed definition is not consistent with the definitions of "feed" neither in the Terrestrial Code nor in Codex Alimentarius standards. The EU would prefer to keep the current definition until the issue of the use of live and frozen fish as feed has been further elaborated, a work already foreseen by the Aquatic Animal Code Commission itself.

Feed

means any material (single or multiple), ~~whether~~ including living organisms and processed, semi-processed or raw material that is intended to be fed directly to *aquatic animals*.

— text deleted

CHAPTER 1.2.

CRITERIA FOR LISTING AQUATIC ANIMAL DISEASES

EU position

The EU can support the adoption of the amended chapter.

The EU would propose that this chapter is revised in due course taking into account the ongoing work in this area undertaken by the OIE Terrestrial Code Commission.

Article 1.2.1.

Criteria for listing an aquatic animal disease

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

No.	Criteria (A-C)	Parameters that support a listing	Explanatory notes
A. Consequences			
1.		The disease has been shown to cause significant production losses at a national or multinational (zonal or regional) level.	There is a general pattern that the disease will lead to losses in susceptible ¹ species, and that morbidity or mortality are related primarily to the agent and not management or environmental factors. (Morbidity includes, for example, loss of production due to spawning failure.) The direct economic impact of the disease is linked to its morbidity, mortality and effect on product quality.
2.	Or	The disease has been shown to or scientific evidence indicates that it is likely to negatively affect wild aquatic animal populations that are an asset worth protecting for economic or ecological reasons.	Wild aquatic animal populations can be populations that are commercially harvested (wild fisheries) and hence are an economic asset. However, the asset could be ecological or environmental in nature, for example, if the population consists of an endangered species of aquatic animal or an aquatic animal potentially endangered by the disease.
3.	Or	The agent is of public health concern.	
And			

B. Spread			
4.		Infectious aetiology of the disease is proven.	
No.	Criteria (A-C)	Parameters that support a listing	Explanatory notes
5.	Or	An infectious agent is strongly associated with the disease, but the aetiology is not yet known.	Infectious diseases of unknown aetiology can have equally high-risk implications as those diseases where the infectious aetiology is proven. Whilst disease occurrence data are gathered, research should be conducted to elucidate the aetiology of the disease and the results be made available within a reasonable period of time.
No.	Criteria (A-C)	Parameters that support a listing	Explanatory notes
6.	And	Potential for Likelihood of international spread, including via live animals, their products or fomites.	International trade in aquatic animal species susceptible to the disease exists or is likely to develop and, under international trading practices, the entry and establishment of the disease is a likely risk.
7.	And	Several countries or countries with zones may be declared free of the disease based on the general surveillance principles outlined in Chapter 1.4. of the <i>Aquatic Code</i> .	Free countries/zones could still be protected. Listing of diseases that are ubiquitous or extremely widespread would render notification unfeasible. However, individual countries that run a control programme on such a disease can propose its listing provided they have undertaken a scientific evaluation to support their request. Examples may be the protection of broodstock from widespread diseases, or the protection of the last remaining free zones from a widespread disease.
And			
C. Diagnosis			
8.		A repeatable and robust means of detection/diagnosis exists.	A diagnostic test should be widely available and preferably has undergone a formal standardisation and validation process using routine field samples (See <i>Aquatic Manual</i> .) or a robust case definition is available to clearly identify cases and allow them to be distinguished from other pathologies.

Article 1.2.2.

Criteria for listing an emerging aquatic animal disease

A newly recognised *disease* or a known *disease* behaving differently may be proposed for listing if it meets the criteria 1 or 2, and 3 or 4. Such proposals should be accompanied by a *case definition* for the *disease* under consideration.

No.	Parameters that support a listing	Explanatory notes
1.	Infectious aetiology of the disease is proven.	
Or		
No.	Parameters that support a listing	Explanatory notes
2.	An infectious agent is strongly associated with the disease, but the aetiology is not yet known.	Infectious diseases of unknown aetiology can have equally high-risk implications as those diseases where the infectious aetiology is proven. Whilst disease occurrence data are gathered, research should be conducted to elucidate the aetiology of the disease and the results be made available within a reasonable period of time.
And		
3.	The agent is of public health concern.	
Or		
4.	Significant spread in naive populations of wild or cultured aquatic animals.	The disease has exhibited significant morbidity, mortality or production losses at a zone, compartment or country level. 'Naive' means animals previously unexposed either to a new disease or a new form of a known disease.

— text deleted

'Susceptible' is not restricted to 'susceptible to clinical disease' but includes 'susceptible to covert infections'.

CHAPTER 1.3.

DISEASES LISTED BY THE OIE

EU position

The EU can support the adoption of the amended chapter.

Preamble: The following *diseases* are listed by the OIE according to the criteria for listing an *aquatic animal disease* (see Article 1.2.1.) or criteria for listing an *emerging aquatic animal disease* (see Article 1.2.2.).

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

Article 1.3.1.

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

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

Article 1.3.2.

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

- Infection with abalone herpes-like virus
- Infection with *Bonamia ostreae*
- Infection with *Bonamia exitiosa*
- Infection with *Marteilia refringens*
- Infection with *Perkinsus marinus*
- Infection with *Perkinsus olseni*
- Infection with *Xenobalotus californiensis*.

Annex 5 (contd)

Article 1.3.3.

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

- Crayfish plague (*Aphanomyces astaci*)
- Infectious hypodermal and haematopoietic necrosis
- Infectious myonecrosis
- Necrotising hepatopancreatitis
- Taura syndrome
- White spot disease
- White tail disease
- Yellow head disease.

Article 1.3.4.

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

- Infection with *Batrachochytrium dendrobatidis*
- Infection with ranavirus.

-
-
- text deleted

CHAPTER 6.3.

**PRINCIPLES FOR RESPONSIBLE AND
PRUDENT USE OF ANTIMICROBIAL AGENTS
IN
~~VETERINARY MEDICINE~~ AQUATIC ANIMALS**

EU position

The EU thanks the OIE Aquatic Code Commission for taking on board the majority of EU comments. However, the EU can only support the adoption of this chapter if the following amendments are made:

1. In second paragraph of Article 6.3.7 the word "recommend" is deleted and in second paragraph of Article 6.3.8 the word "recommendation" is deleted.

Rationale: Veterinary antimicrobial agents should only be used upon prescription or administration by veterinarians or other aquatic animal health professionals authorised to prescribe veterinary medicines. This is an essential part of a strategy to ensure responsible and prudent use of antimicrobial agents in aquatic animals, and would create consistency with the equivalent Articles in the Terrestrial Code.

2. In the title and in paragraphs 2, 3, 4, 6 and 7 of Article 6.3.7, and in second paragraph of Article 6.3.8, the words "authorised to prescribe veterinary medicines" are added after the words "aquatic animal health professionals"

Rationale: The EU acknowledges that in the veterinary field, not only veterinarians, but also other aquatic animal health professionals are carrying out important duties and agree that this should be recognised in this Article. However, it is essential that professionals prescribing veterinary medicines have appropriate knowledge of aquatic animal clinical pathology and pharmacology. To ensure this the right to prescribe veterinary medicines should be limited to aquatic animal health professionals who are specifically authorised to do so under relevant legislation.

3. The second sentence of Paragraph 7 of Article 6.3.7 is deleted.

Rationale: As regards responsible and prudent use of antimicrobials, including the extra-label/off-label use, the conditions should, taking into the potential public health concerns, be equivalent for products intended for the national market as products intended for export. The EU is therefore of the opinion that it is not appropriate to include the second sentence of Paragraph 7 of Article 6.3.7. Furthermore, this sentence does not fit well into the systematics of the chapter. The chapter lays down principles for responsible and prudent use in each Member country. The sentence is the only one which is referring to import/export. Moreover, similar provisions are not included in the equivalent Terrestrial Code chapter. Finally, as the OIE standards governing trade between OIE Members are regulated in other parts of the Code and including provision on import/export in this chapter may lead to confusion and trade problems.

In addition to the above comments, the EU would also suggest to replace the word "effectiveness" with "efficacy" in the first sentence of Paragraph 1 of Article 6.3.5 and in the last sentence of Paragraph 8 of Article 6.3.7.

Rationale:

When referring to antimicrobial agent, the common term used is "efficacy". "Efficacy" is also used several places in this Chapter. This amendment would therefore contribute to consistency in the terminology of the Code.

Article 6.3.1.

Purpose

These principles recommendations provide guidance for the responsible and prudent use of antimicrobial agents in *aquatic animals*, with the aim of protecting both animal and human health. The *Competent Authorities* responsible for the registration and marketing authorisation of products registration and the control of all groups organisations involved in the production, distribution and use of veterinary antimicrobials agents have specific obligations.

Article 6.3.2.

Objectives of responsible and prudent use

Responsible and prudent use includes a set of practical measures and recommendations intended to reduce the risk associated with the selection and dissemination of antimicrobial resistant micro-organisms and antimicrobial resistance determinants in *aquatic animal* production to:

1. maintain the efficacy of *antimicrobial agents* both for veterinary and human medicine and to ensure the rational use of antimicrobials in *aquatic animals* with the purpose of optimising both their efficacy and safety;
2. comply with the ethical obligation and economic need to keep *aquatic animals* in good health;
3. prevent or reduce the transfer of both resistant micro-organisms and ~~or~~ resistance determinants from *aquatic animals* to humans and terrestrial animals;
4. ~~maintain the efficacy of *antimicrobial agents* used in human medicine and prolong the usefulness of the antimicrobials;~~
5. ~~prevent the contamination of animal derived food with antimicrobial residues that exceed the established maximum residue limit (MRL) occurring in the food;~~
6. ~~protect consumer health by ensuring the safety of food of *aquatic animal* origin.~~

Article 6.3.3.

Definitions

Antimicrobial agent: means a naturally occurring, semi-synthetic or synthetic substance that at in vivo concentrations exhibits antimicrobial activity (kill or inhibit the growth of micro-organisms). Anthelmintics and substances classed as disinfectants or antiseptics are excluded from this definition.

Pharmacovigilance of antimicrobial agent: means the detection and investigation of the effects of the use of these products, mainly aimed at safety and efficacy in animals and safety in people exposed to the products.

Article 6.3.4.

Responsibilities of the regulatory *Competent Authorities*

The national *Regulatory* *Competent Authorities*, which are responsible for granting marketing authorization for antimicrobials agents, have a significant role in specifying the terms of the authorization and in providing the appropriate information to the *veterinarian* or other *aquatic animal* health professional through product labeling and/or by other means, in support of prudent use of veterinary antimicrobial agents drugs in *aquatic animals*.

It is the responsibility of regulatory Competent Authorities to develop up-to-date guidelines on data requirements for evaluation of veterinary antimicrobial drug agent applications.

~~National governments~~ Competent Authorities in cooperation with animal and public health professionals should adopt a proactive approach to promote prudent use of *antimicrobial agents* in *aquatic animals* as an element of a comprehensive national strategy for the containment of antimicrobial resistance.

Other Elements of the national a comprehensive strategy should include good animal husbandry practices, vaccination policies and development of animal health care at the farm level, and consultation with a *veterinarian* or other *aquatic animal* health professional, all of which should contribute to reduction of the prevalence of animal disease requiring antimicrobial treatment.

Regulatory The Competent Authorities should expeditiously grant marketing authorizations when criteria of quality, efficacy, and safety are met.

The examination of ~~dossiers/drug-marketing authorization~~ applications should include an assessment of the risks to ~~both animals, and humans~~ and the environment resulting from the use of antimicrobial agents in *aquatic animals*. The evaluation should focus on each individual ~~veterinary antimicrobial agent drug~~ veterinary medicinal product ~~but and~~ take into consideration the class of antimicrobials to which the particular active principle substance belongs. The safety evaluation should include consideration of the potential impact of the proposed use in *aquatic animals* on human health, including the human health impact of antimicrobial resistance developing in food-borne micro-organisms found in *aquatic animals*. An assessment of the impact of the proposed use on the environment should be conducted.

The regulatory authority Competent Authorities should aim to ensure that advertising of antimicrobial agents complies with national relevant legislation and marketing authorizations granted and discourage direct advertising ~~to aquatic animal producers~~ other than to those legally entitled to prescribe the antimicrobial agent.

Information collected through pharmacovigilance programmes, including on lack of efficacy, should form part of the *Competent Authority's* comprehensive strategy to minimize antimicrobial resistance.

~~Regulatory~~ Competent Authorities should disseminate, to *veterinarians* or other *aquatic animal* health professionals, information on trends in antimicrobial resistance collected during surveillance programmes and should monitor the performance of susceptibility testing laboratories.

~~The~~ Competent Authorities and stakeholders should work together to provide for develop effective procedures for the safe collection and destruction of unused or out-of-date *antimicrobial agents*.

Article 6.3.5.

Responsibilities of the veterinary pharmaceutical industry

The veterinary pharmaceutical industry has responsibilities for providing information requested by the Regulatory Competent Authorities on the quality, effectiveness and safety of *antimicrobial agents*. The responsibilities of the veterinary pharmaceutical industry covers pre- and post- marketing phases, including manufacturing, sale, importation, labeling, ~~and~~ advertising issues and pharmacovigilance.

The veterinary pharmaceutical industry has the responsibility to provide the ~~regulatory~~ Competent Authority with the information necessary to evaluate the amount of *antimicrobial agents* marketed. The veterinary pharmaceutical industry should ensure that the advertising of *antimicrobial agents* directly to the *aquatic animal* producer is discouraged.

Article 6.3.6.

Responsibilities of wholesale and retail distributors

Distributors should ensure that their activities are in compliance with the relevant national or regional legislation.

Distributors should ensure that information for the appropriate use and disposal of the *antimicrobial agent* preparation should accompany all distributed products and should also be responsible for maintaining and disposing of the product under according to the manufacturer recommendations.

~~Distributors should have responsibilities in collection and destruction of antimicrobial agents that have passed their expiry date.~~

Article 6.3.7.

Responsibilities of veterinarians and other aquatic animal health professionals

Responsibilities of *veterinarians* or other *aquatic animal* health professionals include identifying, preventing and treating *aquatic animal diseases*, as well as the promotion of sound animal husbandry methods, hygiene procedures, vaccination and other alternative strategies to minimise the need for *antimicrobial* use in *aquatic animals*.

Veterinarians or other *aquatic animal* health professionals should only prescribe, dispense, administer or recommend antimicrobial a specific course of treatment with an antimicrobial agent for *aquatic animals* under their care.

The responsibilities of *veterinarians* or other *aquatic animal* health professionals are to carry out a proper thorough clinical examination assessment of the *aquatic animal(s)*, including as appropriate: and make a diagnosis, based on the clinical examination, post-mortem examination, bacteriology with culture and sensitivity, and other laboratory tests to arrive at the most definitive diagnosis possible before initiating a specific course of treatment with an antimicrobial agent. ~~the results of laboratory tests and.~~ Evaluation of environmental factors and husbandry at the production site (e.g. water quality) should be considered as potential primary factors leading to infection and should be addressed prior to recommending a course of antimicrobial agent treatment.

If therapy with an *antimicrobial agent* is deemed appropriate necessary it should be initiated as soon as possible. The selection of the agent should be based on the knowledge and experience of the *veterinarian* or other *aquatic animal* health professional.

As soon as possible, susceptibility testing of the target micro-organism should be used to confirm the choice of treatment. Results of all susceptibility tests should be ~~communicated~~ retained and should be available to the national Competent Authority.

The *veterinarian* or other *aquatic animal* health professional should indicate precisely to the *aquatic animal* producer the treatment regime, including the dose, the treatment intervals, the duration of the treatment, the withdrawal period and the amount of antimicrobial agents drug to be delivered, depending on the dosage and the number of *aquatic animals* to be treated.

~~The *veterinarian* or other *aquatic animal* health professional may prescribe or recommend in appropriate circumstances~~ The use of *antimicrobial agents* extra-label/off-label, may be permitted in appropriate circumstances in conformity with the relevant national legislation. For products destined for export, the and any requirements of importing countries should be considered.

Records on the use of *antimicrobial agents* should be kept in conformity with the relevant national legislation. *Veterinarians* or *aquatic animal* health professionals should also periodically review farm records on the use of the *antimicrobial agents* to ensure compliance with their directions and use these records to evaluate the effectiveness of treatment regimens. Suspected adverse reactions, as well as including a lack of

effectiveness, should be reported to the *Competent Authority*. ~~The~~ Associated susceptibility data should accompany the report of lack of effectiveness.

~~Veterinarians or other *aquatic animal* health professionals should periodically review farm records on the use of *antimicrobial agents* to ensure compliance with their directions and use these records to evaluate the efficacy of treatment regimens.~~

Article 6.3.8.

Responsibilities of aquatic animal producers

Aquatic animal producers should implement health programmes on their farms in order to promote *aquatic animal* health and food safety. This can be done through adequate planning of culture strategies to maintain *aquatic animal* health through biosecurity programmes, husbandry, nutrition, vaccination strategies, maintenance of good water quality, etc.

Aquatic animal producers should use antimicrobial agents only on the prescription or recommendation of a *veterinarian* or other *aquatic animal* health professional, and follow directions on the dosage, method of application, and withdrawal period.

Aquatic animal producers should ensure that *antimicrobial agents* are properly stored, handled, and disposed.

Aquatic animal producers should keep adequate records of *antimicrobial agents* used, bacteriological and susceptibility tests, and ~~to~~ make such records available to the *veterinarian* or other *aquatic animal* health professional.

Aquatic animal producers should inform the *veterinarian* or other *aquatic animal* health professional of recurrent disease problems and lack of efficacy of *antimicrobial agent* treatment regimes.

Article 6.3.9.

Training of ~~antimicrobial~~ users of antimicrobial agents

The training of users of *antimicrobial agents* should involve all the relevant organisations, such as Competent relevant regulatory ~~regulatory~~ authorities, pharmaceutical industry, veterinary schools, research institutes, and veterinary professional organisations and other approved users such as *aquatic animal* owners.

Article 6.3.10.

Research

To address the significant lack of information for numerous species of *aquatic animals*, ~~relevant~~ Competent the relevant regulatory authorities and other stakeholders should encourage public-funded and industry-funded research.

— text deleted

CHAPTER 10.4.

INFECTIOUS HAEMATOPOIETIC NECROSIS

EU position

The EU thanks the Aquatic Commission for this addition and supports the adoption of the amended chapter, but has one comment:

In point c of Paragraph 2 of Article 10.4.13, the words "OIE Members" should, for clarity and consistency, be replaced by the words "Competent Authority of the importing country".

[...]

Article 10.4.13.

Importation of disinfected eggs for aquaculture from a country, zone or compartment not declared free from infectious haematopoietic necrosis

1. When importing disinfected eggs of the species referred to in Article 10.4.2. for *aquaculture*, from a country, *zone* or *compartment* not declared free from IHN, the *Competent Authority* of the *importing country* should assess the *risk* associated with at least:
 - a) the IHN virus status of the water to be used during the *disinfection* of the eggs;
 - b) the level of infection with IHN virus in broodstock (ovarian fluid and milt); and
 - c) the temperature and pH of the water to be used for *disinfection*.
2. If the *Competent Authority* of the *importing country* concludes that the importation is acceptable, it should apply the following risk mitigation measures including:
 - a) the eggs should be disinfected prior to importing, according to the methods described in Chapter 1.1.3. of the *Aquatic Manual* (under study) or those specified by the *Competent Authority* of the *importing country*; and
 - b) between *disinfection* and the import, eggs should not come into contact with anything which may affect their health status;
 - c) the OIE Members may wish to consider internal measures, such as renewed *disinfection* of the eggs upon arrival in the *importing country*.
3. When importing disinfected eggs of the species referred to in Article 10.4.2. for *aquaculture*, from a country, *zone* or *compartment* not declared free from IHN, 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 the procedures described in point 2 of Article 10.4.13. have been fulfilled.

[...]

— text deleted

CHAPTER 10.5.

INFECTIOUS SALMON ANAEMIA

EU position

The EU thanks the Aquatic Commission for this addition and supports the adoption of the amended chapter, but has one comment:

In point c of Paragraph 2 of Article 10.5.13, the words "OIE Members" should, for clarity and consistency, be replaced by the words "Competent Authority of the importing country".

[...]

Article 10.5.13.

Importation of disinfected eggs for aquaculture from a country, zone or compartment not declared free from infectious salmon anaemia

1. When importing disinfected eggs of the species referred to in Article 10.5.2. for *aquaculture*, from a country, *zone* or *compartment* not declared free from ISA, the *Competent Authority* of the *importing country* should assess the *risk* associated with at least:
 - a) the ISA virus status of the water to be used during the *disinfection* of the eggs;
 - b) the level of infection with ISA virus in broodstock (ovarian fluid and milt); and
 - c) the temperature and pH of the water to be used for *disinfection*.
2. If the *Competent Authority* of the *importing country* concludes that the importation is acceptable, it should apply the following *risk* mitigation measures including:
 - a) the eggs should be disinfected prior to importing, according to the methods described in Chapter 1.1.3. of the *Aquatic Manual* (under study) or those specified by the *Competent Authority* of the *importing country*; and
 - b) between *disinfection* and the import, eggs should not come into contact with anything which may affect their health status;
 - c) the OIE Members may wish to consider internal measures, such as renewed *disinfection* of the eggs upon arrival in the *importing country*.
3. When importing disinfected eggs of the species referred to in Article 10.5.2. for *aquaculture*, from a country, *zone* or *compartment* not declared free from ISA, 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 the procedures described in point 2 of Article 10.5.13. have been fulfilled.

[...]

 — text deleted

CHAPTER 10.9.

VIRAL HAEMORRHAGIC SEPTICAEMIA

EU position

The EU thanks the Aquatic Commission for this addition and supports the adoption of the amended chapter, but has one comment:

In point c of Paragraph 2 of Article 10.9.13, the words "OIE Members" should, for clarity and consistency, be replaced by the words "Competent Authority of the importing country".

[...]

Article 10.9.13.

Importation of disinfected eggs for aquaculture from a country, zone or compartment not declared free from viral haemorrhagic septicaemia

1. When importing disinfected eggs of the species referred to in Article 10.9.2. for *aquaculture*, from a country, *zone* or *compartment* not declared free from **ISA-VHS**, the *Competent Authority* of the *importing country* should assess the *risk* associated with at least:
 - a) the VHS virus status of the water to be used during the *disinfection* of the eggs;
 - b) the level of infection with VHS virus in broodstock (ovarian fluid and milt); and
 - c) the temperature and pH of the water to be used for *disinfection*.
2. If the *Competent Authority* of the *importing country* concludes that the importation is acceptable, it should apply the following *risk* mitigation measures including:
 - a) the eggs should be disinfected prior to importing, according to the methods described in Chapter 1.1.3. of the *Aquatic Manual* (under study) or those specified by the *Competent Authority* of the *importing country*; and
 - b) between *disinfection* and the import, eggs should not come into contact with anything which may affect their health status;
 - c) the OIE Members may wish to consider internal measures, such as renewed *disinfection* of the eggs upon arrival in the *importing country*.
3. When importing disinfected eggs of the species referred to in Article 10.9.2. for *aquaculture*, from a country, *zone* or *compartment* not declared free from VHS, 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 the procedures described in point 2 of Article 10.9.13. have been fulfilled.

[...]

— text deleted

CHAPTER 3.1.

QUALITY OF AQUATIC ANIMAL HEALTH SERVICES

EU position

The EU can support the adoption of the amended chapter.

Article 3.1.1.

The quality of the *Aquatic Animal Health Services* depends on a set of factors, which include of OIE Members need to embody the fundamental principles of an ethical, organisational, legislative, regulatory and technical nature. The *Aquatic Animal Health Services* shall conform to these fundamental principles, regardless of the political, economic or social situation of their country.

Compliance with these fundamental principles by a Member's *Aquatic Animal Health Service* is important in the establishment and maintenance of confidence in its aquatic *animal health status* and *international aquatic animal health certificates* provided by the *Aquatic Animal Health Service* of other Members.

These fundamental principles are presented in Article 3.1.2. Other factors to consider when evaluating *Aquatic Animal Health Services* are described in the *Aquatic Code* (*notification*, principles of certification, etc.).

The ability of *Aquatic Animal Health Services* to deliver appropriate services, monitor and control *aquatic animal diseases* based on Members' *aquatic animal health* legislation and regulations, can be measured through an evaluation or audit whose general principles are described in Articles 3.1.3. and 3.1.4.

A procedure for evaluating *Aquatic Animal Health Services* by OIE experts, on a voluntary basis, is described in Article 3.1.5.

Article 3.1.2.

Fundamental principles of quality

Aquatic Animal Health Services should comply with the following principles to ensure the quality of their activities:

1. Professional judgement

Aquatic Animal Health Services should ensure that personnel have the relevant qualifications, scientific expertise and experience to give them the competence to make sound professional judgements.

2. Independence

Care should be taken to ensure that the *Aquatic Animal Health Service* personnel are free from any commercial, financial, hierarchical, political or other pressures which may inappropriately influence their judgement or decisions.

3. Impartiality

Aquatic Animal Health Services should be impartial. In particular, all the parties affected by their activities have a right to expect their services to be delivered under reasonable and non-discriminatory conditions.

4. Integrity

Aquatic Animal Health Services are responsible for ensuring that the work of each of their personnel is of a consistently high level of integrity. Any fraud, corruption or falsification should be identified, documented and corrected.

5. Objectivity

Aquatic Animal Health Services should conduct themselves, in an objective, transparent and non-discriminatory manner.

6. Aquatic animal health legislation and regulations

Aquatic animal health legislation and regulations are a fundamental element that supports good governance and provides the legal framework for all key activities of the *Aquatic Animal Health Service*.

Legislation and regulations should be suitably flexible to allow for judgements of equivalence and efficient responses to changing situations. In particular, they should define and document the responsibilities and structure of the organisations in charge of traceability and control of *aquatic animal* movements, *aquatic animal disease* control and reporting systems, epidemiological *surveillance* and communication of epidemiological information.

7. General organisation

Aquatic Animal Health Services should be able to demonstrate by means of an appropriate legislation and regulations, sufficient financial resources and effective organisation that they are in a position to have control of the establishment and application of aquatic animal health measures, and of international *aquatic animal* health certification activities.

Aquatic Animal Health Services should have at their disposal effective systems for *aquatic animal disease surveillance, diagnosis and notification of disease* problems that may occur in the national territory, in accordance with the provisions of the *Aquatic Code*. They should at all times endeavour to improve their performance in terms of *aquatic animal* health information systems and *aquatic animal disease* control.

Aquatic Animal Health Services should define and document the responsibilities and structure of the organisation (in particular the chain of command) in charge of issuing *international aquatic animal health certificates*.

Each position within the *Aquatic Animal Health Services* that has an impact on their quality should be described.

These job descriptions should include the requirements for education, training, technical knowledge and experience.

8. Quality policy

Aquatic Animal Health Services should define and document their policy and objectives for, and commitment to, quality, and should ensure that this policy is understood, implemented and maintained at all levels in the organisation. Where conditions allow, they may implement a quality system corresponding to their areas of activity and appropriate for the type, range and volume of

work that they have to perform. The recommendations provided in this chapter describe a suitable reference system, which should be used if a Member chooses to adopt a quality system.

9. Procedures and standards

Aquatic Animal Health Services should develop and document appropriate procedures and standards for all providers of relevant activities and associated facilities. These procedures and standards may for example relate to:

- a) programming and management of activities, including international *aquatic animal* health certification activities;
- b) prevention, control and *notification of disease outbreaks*;
- c) *risk analysis*, epidemiological *surveillance* and zoning;
- d) inspection and sampling techniques;
- e) diagnostic tests for *aquatic animal diseases*;
- f) preparation, production, registration and control of *biological products* for use in the *diagnosis* or prevention of *diseases*;
- g) border controls and import regulations;
- h) *disinfection*;
- i) treatments intended to inactivate pathogens in *aquatic animal* products.

Where there are standards in the *Aquatic Code* or in the *Aquatic Manual*, *Aquatic Animal Health Services* should comply with these standards when applying *aquatic animal* health measures and when issuing *international aquatic animal health certificates*.

10. Information, complaints and appeals

Aquatic Animal Health Services should undertake to reply to requests from *Aquatic Animal Health Services* of other Members or any other authority, in particular ensuring that any requests for information, complaints or appeals that are presented are dealt with in a timely manner.

A record should be maintained of all complaints and appeals and of the relevant action taken by *Aquatic Animal Health Services*.

11. Documentation

Aquatic Animal Health Services should have at their disposal a reliable and up-to-date documentation system suited to their activities.

12. Self-evaluation

Aquatic Animal Health Services should undertake periodical self-evaluation especially by documenting achievements against goals, and demonstrating the effectiveness of their organisational components and resource adequacy.

A procedure for evaluating *Aquatic Animal Health Services* by OIE experts, on a voluntary basis, is described in Article 3.1.5.

13. Communication

Aquatic Animal Health Services should have effective internal and external systems of communication covering administrative and technical staff and parties affected by their activities.

14. Human and financial resources

Responsible authorities should ensure that adequate resources are made available to implement effectively the above activities.

Article 3.1.3.

For the purposes of the *Aquatic Code*, every Member should recognise the right of another Member to undertake, or request it to undertake, an evaluation of its *Aquatic Animal Health Services* where the initiating Member is an actual or a prospective importer of *aquatic animal commodities* and/or where the evaluation is to be a component of a *risk analysis* process that is to be used to determine or review *sanitary measures* which apply to such trade.

A Member has the right to expect that the evaluation of its *Aquatic Animal Health Services* will be conducted in an objective and transparent manner. A Member undertaking an evaluation should be able to justify any measure taken as a consequence of its evaluation.

Article 3.1.4.

A Member which intends to conduct an evaluation of another Member's *Aquatic Animal Health Services* should provide notice in writing, and allow sufficient time for the other Member to comply with the request. This notice should define the purpose of the evaluation and details of the information required.

On receipt of a formal request for information to enable an evaluation of its *Aquatic Animal Health Services* by another Member, and following bilateral agreement of the evaluation process and criteria, a Member should expeditiously provide the Member requesting the evaluation with meaningful and accurate information of the type requested.

The evaluation process should take into account the fundamental principles and other factors of quality laid down in Article 3.1.1. and in Article 3.1.2. It should also take into consideration the specific circumstances regarding quality, as described in Article 3.1.1., prevailing in the countries concerned.

The outcome of an evaluation conducted by a Member should be provided in writing as soon as possible, and in any case within 4 months of receipt of the relevant information, to the Member which has undergone the evaluation. The evaluation report should detail any findings that affect trade prospects. The Member which conducts the evaluation should clarify in detail any points of the evaluation on request.

In the event of a dispute between two Members over the conduct or the conclusions of the evaluation of *Aquatic Animal Health Services*, the matter should be dealt with having regard to the procedures set out in Article 3.1.3.

Article 3.1.5.

Evaluation facilitated by OIE experts under the auspices of the OIE

The OIE has established procedures for the evaluation of *Aquatic Animal Health Services* of Members. Members can make a request to the OIE for an evaluation of their *Aquatic Animal Health Services*.

The World Assembly of OIE Delegates may endorse a list of approved experts to facilitate the evaluation process.

Under these procedures, the Director General of the OIE recommends an expert(s) from that list.

The expert(s) facilitate(s) the evaluation of the *Aquatic Animal Health Services* of the Member using the OIE PVS Tool: Application to *Aquatic Animal Health Services* applied as appropriate to the context of the evaluation.

The expert(s) produce(s) a report in consultation with the *Aquatic Animal Health Services* of the Member.

The report is submitted to the Director General of the OIE and, with the consent of the Member, published by the OIE.

— text deleted

CHAPTER 5.3.

**CRITERIA TO ASSESS THE SAFETY OF
AQUATIC ANIMAL COMMODITIES**

EU position

The EU can support the adoption of the amended chapter.

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

Article 5.3.1.

Criteria to assess the safety of aquatic animals and aquatic animal products for any purpose from a country, zone or compartment not declared free from disease X

In all *disease* chapters, point 1 of Article X.X.3. lists *aquatic animals* and *aquatic animal products* that can be traded for any purpose from a country, *zone* or *compartment* not declared free from *disease X*. The criteria for inclusion of *aquatic animals* and *aquatic animal products* in point 1 of Article X.X.3. are based on the absence of the *pathogenic agent* in the traded *aquatic animals* and *aquatic animal products* or inactivation of the *pathogenic agent* by treatment or processing.

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

It is assumed that treatment or processing (i) uses standardised protocols, which include the steps considered critical in the inactivation of the *pathogenic agent* of concern; (ii) is conducted according to Good Manufacturing Practices; and (iii) that any other steps in the treatment, processing and subsequent handling of the *aquatic animal product* do not jeopardise the safety of the traded *aquatic animal product*.

Criteria

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

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

AND

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

OR

2. Even if the *pathogenic agent* is present in, or contaminates the tissues from which the *aquatic animal* or *aquatic animal product* is derived, the treatment or processing to produce the *aquatic animal* or *aquatic animal product* to be traded inactivates the *pathogenic agent*:
 - a) physical (e.g. temperature, drying, smoking);

AND/OR

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

AND/OR

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

Article 5.3.2.

Criteria to assess the safety of aquatic animals or aquatic animal products for retail trade for human consumption from a country, zone or compartment not declared free of a disease

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

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

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

Criteria

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

1. the *aquatic animal* or *aquatic animal product* is prepared and packaged for retail trade for human consumption; AND

EITHER

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

OR

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

— text deleted

CHAPTER 6.1.

CONTROL OF HAZARDS IN AQUATIC ANIMAL FEEDS

EU position

The EU can only support the proposed amendments if the following comment is taken account of:

In the second sentence of the second paragraph of Article 6.1.1 the words "are also important references" should be replaced by "may be relevant sources of guidance".

Rationale:

The term "reference" is ambiguous and could be wrongfully interpreted as a legal reference.

In addition, the EU would like to highlight the importance of being clear when in the OIE standards citing sources that are not international standards relevant under SPS, whether these sources are to be considered "references" (i.e. part of the standard) or only source of information. As it may create legal uncertainty, the EU would prefer that a cautionary approach was taken on this matter and limit the number of such external sources of information. On the other hand, the OIE should make cross-references to other standards and to significant collaboration with other organisations, such as "in synergy with Codex", where appropriate.

Article 6.1.1.

Introduction

One of the key objectives of the *Aquatic Code* is to help OIE Members trade safely in *aquatic animals* and *aquatic animal products* by developing relevant *aquatic animal* health measures. These recommendations address *aquatic animal* health hazards and food safety hazards in *aquatic animal feed*. A key objective is to prevent the spread, via *aquatic animal feed*, of diseases from an infected country, zone or compartment to a free country, a free zone or a free compartment.

These recommendations complement the Codex Alimentarius Commission (CAC) Code of Practice on Good Animal Feeding (CAC/RCP 54-2004). The FAO Technical Guidelines for Responsible Fisheries: Aquaculture Development: 1. Good aquaculture feed manufacturing practice (2001) and the FAO/ IFIF Good Practices for the Feed Industry (2010) are also important references. OIE Members are encouraged to consult these publications. These recommendations should be read in conjunction with relevant recommendations of the OIE *Terrestrial Animal Health Code*. The Food and Agriculture Organization of the United Nations (FAO) has published recommendations relevant to terrestrial and *aquatic animal feed* (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]) and there is a Codex Alimentarius Commission (CAC) standard (Code of Practice on Good Animal Feeding [CAC/RCP 54-2004]). OIE Members are encouraged to consult these publications.

Key considerations relevant to *aquatic animal feed* are as follows:

1. Concentration of *aquaculture establishments* heightens the risk of disease transmission, whether the pathogen enters the culture system via *feed* or other means.

2. For many *aquatic animal* species, predation (including cannibalism) is their natural way of feeding in their natural habitat.
3. Historically, animal proteins used in *feed* were mainly sourced from the marine environment, due to the nutritional needs of *aquatic animals* and for reasons of economy. This practice increases the *risk* of *disease* transmission, especially when *aquatic animals* are fed live or whole *aquatic animals* 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.
4. The usage of *feed* in moist form (moisture content equal to or greater than 70%), semi-moist form (moisture content between 15 and 70%), and dry form (a moisture content equal to or less than 15%) implies different levels of *risk* due to the processing applied to the *feed*.
5. With the increasing number of species being farmed (especially marine finfish), the use of *live feed* and moist feed has increased. It is likely that these industries will in future use formulated *feed* as appropriate technologies are developed.
6. 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 contaminated 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.
7. 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 *feed* (and *feed ingredients*) that are appropriate to the species and its culture system. As more and more *aquatic animal* species are being cultured, it is difficult to make recommendations for all *pathogenic agent*/host species combinations.

Article 6.1.2.

Scope

These recommendations document *risk* mitigation measures, including traceability and certification, to deal with *aquatic animal* health *risks* associated with trade in *aquatic animal feed* and *feed ingredients*. They recommend the control of hazards through adherence to recommended practices during the production (harvest, handling, storage, processing and distribution) and use of both commercial and on-farm produced *feed* (and *feed ingredients*) for *aquatic animals*. Hazards include pathogens that cause OIE listed diseases and other agents that cause an adverse effect on animal and/or public health. While *aquatic animals* grown for food are the main focus, the same principles apply to *feed* for *aquatic animals* used for other purposes.

Article 6.1.3.

General principles

1. Roles and responsibilities

The *Competent Authority* has the legal power to set and enforce regulatory requirements related to animal *feed*, 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. Refer to Chapter 3.1. of the *Aquatic Code*.

It is a particular responsibility of the *Competent Authority* to set and enforce the regulatory requirements pertaining to the use of veterinary *drugs products*, *aquatic animal disease* control and the food safety aspects that relate to the management of live *aquatic 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 requirements. All personnel involved in the harvest, 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. Appropriate *contingency plans* should be developed in case of a *feed-borne outbreak of disease*. Equipment for producing, storing and transporting *feed* should be kept clean and maintained in good working order.

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).

2. Regulatory standards for feed safety

All *feed* and *feed ingredients* should meet regulatory standards for *feed* safety. Scientific evidence, including the sensitivity of analytical methods, and on the characterisation of *risks*, should be taken into account in defining limits and tolerances for hazards.

3. Risk analysis

Internationally accepted principles and practices for *risk analysis* (see Section 2. 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 hazards.

4. 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 or adopt suitable international standards or recommendations.

Where appropriate, Hazard Analysis and Critical Control Point (HACCP; as defined in the Annex to the Recommended International Code of Practice on General Principles of Food Hygiene [CAC/RCP 1-1969]) principles should be followed to control hazards that may occur in *feed*.

5. Relationship between prions and aquatic animal species

Scientific knowledge is lacking on the relationship between prions and *aquatic animal* species. There is no evidence to suggest that the use of terrestrial animal by-products as ingredients in *aquatic animal feed* as currently practiced in *aquaculture* 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.

6. Bioaccumulation

Chemical hazards, such as heavy metals, dioxins and polychlorinated biphenyls (PCB) persist in certain tissues and therefore tend to accumulate through the food chain.

7. Geographic and environmental considerations

Aquatic and terrestrial harvest areas for *feed* 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* and the location of *aquaculture establishments*.

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 the use of fertiliser in the production of microalgae, industrial operations and waste treatment plants that generate pollutants and other hazardous products. The potential accumulation of pollutants in the food chain through *feed* needs to be considered.

8. Zoning and compartmentalisation

Feed is an important components of biosecurity and needs to be considered when defining a compartment or zone in accordance with Chapter 4.1. of the Aquatic Code.

9. Sampling and analysis

Sampling and analytical protocols for *feed* should be based on scientific principles and procedures, and OIE standards where applicable.

10. 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 the Codex Code of Practice on Good Animal Feeding (CAC/RCP 54-2004).~~

Labelling should be informative, unambiguous, legible and conspicuously easily visible placed on the package if sold in package form and on the waybill and other sales accompanying documents if sold in bulk, un-packaged form, and should comply with regulatory requirements and Section 4.2. Labelling of Codex Code of Practice on Good Animal Feeding (CAC/RCP 54-2004), including listing of ingredients and instructions on the handling, storing and use. All claims made on a label should be able to be substantiated.

11. 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 harvest, handling, storage, processing, distribution and use of *feed* and *feed ingredients*. Operators have full responsibility for implementing systems for quality control. Where such systems are applied, the *Competent Authority* should verify that they meet all regulatory requirements.

12. Assurance and certification

Feed business operators manufacturers are responsible for demonstrating assuring the safety of their feed products establishments under their control. Competent Authorities are responsible for providing assurances domestically and to trading partners that regulatory requirements have been met. For international trade in aquatic animal product based feeds, Competent Authorities are required responsible to provide international aquatic animal health certificates.

13. Hazards associated with aquatic animal feed

- a) Biological hazards

Biological hazards that may occur in *feed* and *feed ingredients* include agents such as bacteria, viruses, fungi and parasites. The scope of these recommendations covers *OIE listed diseases* and other agents that cause an adverse effect on animal and/or public health.

b) 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 products, and** pesticides and radionuclides.

c) Physical hazards

Physical hazards that may occur in *feed* and *feed ingredients* include foreign objects (such as pieces of glass, metal, plastic or wood).

14. Contamination

Procedures to minimise the *risk* of contamination during the production, processing, storage, distribution (including transport) and the use of feed or feed ingredients should be included in current regulations and standards. Scientific evidence, including the sensitivity of analytical methods and on the characterisation of *risk*, 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*.

15. Antimicrobial resistance

Concerning the use of antimicrobials in animal *feed* refer to Section 6.X. of the *Aquatic Code* (under development study).

16. Management of information

The *Competent Authority* should establish requirements for the provision of information by the private sector in accordance with the regulatory framework.

The private sector should maintain records, in a readily accessible form, on the production, distribution, importation 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 *aquatic animal* health and/or public health concerns. The private sector should provide information to the *Competent Authority* in accordance with the regulatory framework.

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 Chapters 4.1. and 4.2. of the *OIE Terrestrial Animal Health Code*; Section 4.3 of CAC/RCP 54-2004).

Article 6.1.4.

Recommended approaches to **aquatic animal health risk mitigation**

1. Commodities

a) Safe commodities

Some *commodities* undergo extensive processing such as heat treatment, acidification, extrusion and extraction. There may be a negligible *risk* that pathogens will survive in such products if they have been produced in accordance with Good Manufacturing Practice. Such *aquatic animal products* are listed in *disease-specific* chapters in the *Aquatic Code* in Article X.X.3.

b) ~~Other e~~ Commodities not listed as safe commodities

Competent Authorities should consider the following *risk* mitigation measures:

- i) sourcing *feed* and *feed ingredients* from a *disease free country, free zone or free compartment*; or
- ii) confirmation (e.g. by testing) that pathogens are not present in the *commodity*; or
- iii) treatment (e.g. by heat or acidification) of the *commodity* using a method approved by the *Competent Authority* to inactivate pathogens; or
- iv) use of *feed* only in populations that are not susceptible to the pathogen(s) in question and where *aquatic animals* that are susceptible to the pathogen(s) in question will not come into contact with the *feed* or its waste products.

In addition, *risks* associated with the disposal of effluents and waste material from *feed* processing plants and *aquaculture establishments* should be considered.

e) ~~Whole fish (fresh or frozen)~~

The practice of trading fresh or frozen whole marine fish for use as *aquatic animal feed* presents a significant *risk* of introducing *diseases* into populations and should be avoided where possible. *Risk* mitigation measures include sourcing fish only from stocks where there is no evidence of *infection* with any of the *OIE listed diseases* or treatments that inactivate aquatic animal pathogens.

2. Feed production

To prevent contamination by pathogens 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 batches as appropriate;
- b) buildings and equipment for processing and transporting *feed* and *feed ingredients* should be constructed in a manner that facilitates hygienic operation, maintenance and cleaning and prevents contamination;
- c) in particular, *feed* manufacturing plants should be designed and operated to avoid cross-contamination between batches;
- d) processed *feed* and *feed ingredients* should be stored separately from unprocessed *feed ingredients*, under appropriate storage 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 and 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 place and date of production.

3. Importing countries

Competent Authorities should consider the following measures:

- a) imported *feed* and *feed ingredients* should be delivered to *feed* manufacturing plants or *aquaculture* facilities for processing and 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 pathogens should only be used in a *zone* or *compartment* that does not contain *species susceptible* to the *disease* in question;
- d) the importation of raw unprocessed feed derived from *aquatic animals* to feed aquatic animal species should be avoided where possible;
- e) introduction of internal measures to address the risks associated with raw commodities for human consumption being diverted to use as feed.

4. Certification procedures

When importing *feed* and *feed ingredients* of *aquatic animal* origin other than those mentioned in point 1a) of Article 6.1.4., 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*).

Specific provisions for *OIE listed diseases* may be found in relevant *disease* chapters of the *Aquatic Code*.

The certificate should be in accordance with the Model Certificate in Chapter 5.10.

~~Article 6.1.5.~~

Certification procedures for feeds and feed ingredients of aquatic animal origin

When importing *feed* and *feed ingredients* of *aquatic animal* origin other than those mentioned in point 1a) of Article 6.1.4., 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:

1. that *feed* and *feed ingredients* of *aquatic animal* origin were obtained from a country, *zone* or *compartment* that is free from relevant *aquatic animal diseases*; or
2. that *feed* and *feed ingredients* of *aquatic animal* origin were tested for relevant *aquatic animal diseases* and shown to be free of these *diseases*; or
3. that *feed* and *feed ingredients* of *aquatic animal* origin have been processed to ensure that they are free of relevant *aquatic animal diseases*.

Specific provisions for *OIE listed diseases* may be found in relevant *disease* chapters of the *Aquatic Code*.

The certificate should be in accordance with the Model Certificate in Chapter 5.10.

Article 6.1.65.

Risk pathways for pathogen transmission and contamination through harvest, manufacture and use of aquatic animal feed

1. Pathogens can be introduced into feed in the following ways:

- a) via the harvest of infected *aquatic animals*;
- b) during storage, processing and transport, due to poor hygienic practices, the presence of pests, or residues of previous batches of feed remaining in processing lines, *containers* or transport *vehicles*.

2. *Aquatic animals* can be exposed to *pathogenic agents* in feed in the following ways:

- a) Direct exposure

The use of unprocessed feed derived from *aquatic animals* to feed *aquatic animals* presents a potential direct route of exposure. For example feeding salmonid offal to salmonids presents a heightened *risk* of *disease* transmission because tissue from a *susceptible species* is being fed to a *susceptible species*.

- b) Indirect exposure

Pathogens in feed may be transmitted to *aquatic animals* in *aquaculture* and wild *aquatic animals* via contamination of the environment or *infection* of non-target species.

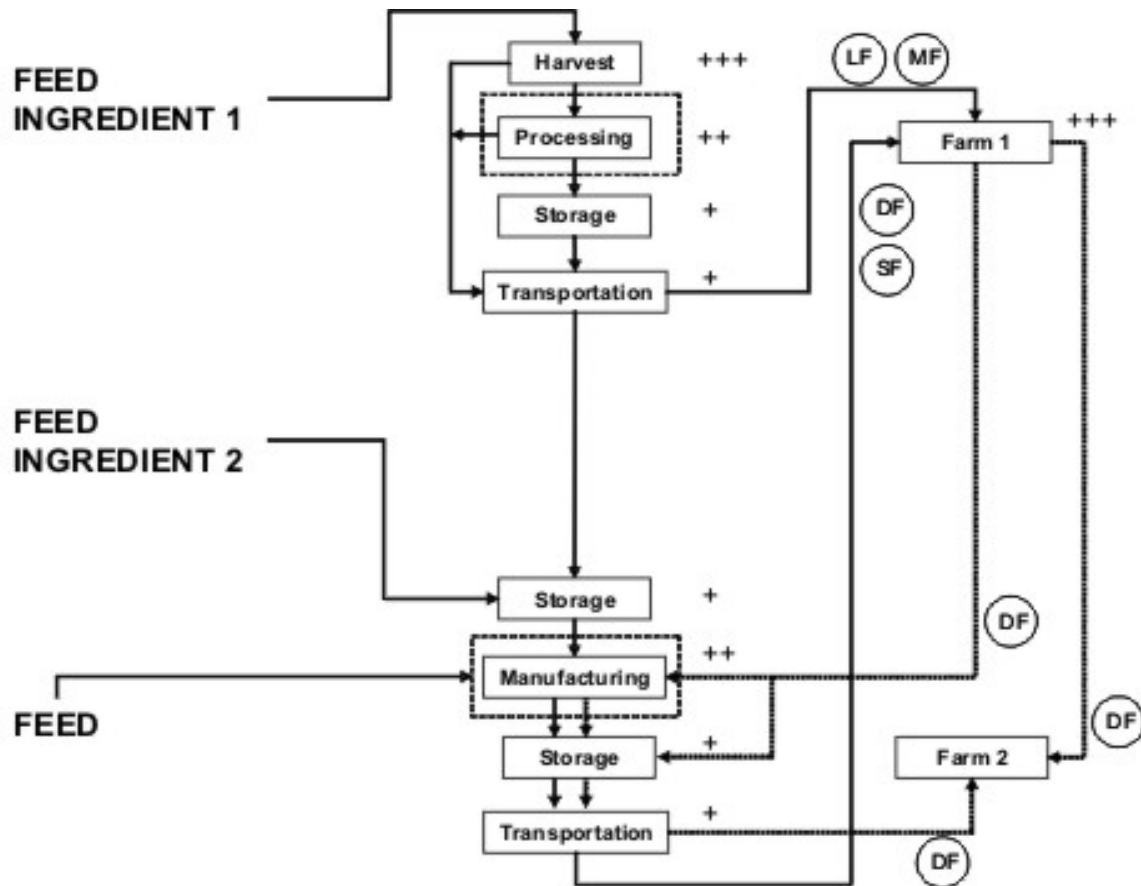
Figure 1 illustrates the possible pathways for transmission of pathogens within the *feed* production and utilisation process.

Feed ingredients of aquatic origin used in *aquaculture* can be a source of pathogens (viruses, bacteria and parasites) to cultured *aquatic animal* species. In *aquaculture establishments* pathogens in *feed* can infect the animals directly (via consumption of *feed*) or indirectly via environmental sources. *Live feed* and moist *feed* are more likely to contain pathogens because their ingredients are either in a raw state or subject to minimal treatment.

Feed and *feed ingredients* harvested from infected countries, *zones* or *compartments* may have a high pathogen load. *Feed* and *feed ingredients* from these sources should be processed (e.g. using heat or chemical treatments) to reduce, or eliminate, the pathogen load. After processing care should be taken to avoid post processing contamination during storage and transportation of these *commodities*. For example, when two or more batches of *ingredients* of different sanitary status are handled, stored and/or transported together without appropriate biosecurity measures, there is a *risk* of cross-contamination of the *feed*.

An *aquaculture* facility can also be a source of pathogens in *aquatic animal feed*. For example, *feed* can be contaminated with pathogens through poor hygiene practices at an infected *aquaculture establishment*. If the *feed* is redistributed from the *aquaculture* facility to the manufacturing facility for recycling, or distributed to another farm, pathogens can be transferred to other *aquaculture establishments*.

Figure 1: Risk chart of pathogen transmission and contamination through harvest, manufacture and use of aquatic animal feed



LF	Live feed	
MF	Moist feed	
SF	Semi-moist feed	
DF	Dry feed	
+++	High risk of pathogen presence	
++	Moderate risk of pathogen presence	
+	Low risk of pathogen presence	
		→ Possibility for risk reduction
	 Redistribution or recycling of finished feed

 — text deleted

CHAPTER 6.2.

**INTRODUCTION TO THE RECOMMENDATIONS FOR
CONTROLLING ANTIMICROBIAL RESISTANCE**

EU position

The EU can support the adoption of the amended chapter.

Article 6.2.1.

Objectives

The purpose of this section is to provide guidance for Members to appropriately address the selection and dissemination of resistant micro-organisms and antimicrobial resistance determinants from the use of antimicrobial agents in *aquatic animals*.

Antimicrobial agents are essential drugs for human and animal health and welfare. The OIE recognises the need for access to antimicrobial agents in veterinary medicine: antimicrobial agents are essential for treating and controlling ~~and preventing~~ infectious *diseases* in *aquatic animals*. The OIE therefore considers that ensuring continued access to effective antimicrobial agents is important.

The OIE recognises that antimicrobial resistance is a global public and animal health concern that is influenced by the usage of antimicrobial agents in humans, animals and elsewhere. Those working in the human, animal and plant sectors have a shared responsibility to address the risk factors for the selection and dissemination of antimicrobial resistance. Arising from its mandate for the protection of animal health and food safety, the OIE developed these chapters to provide guidance to Members in regard to risks in the animal sector.

The application of *risk assessment* and *risk management* measures should be based on relevant international standards on *risk analysis* and supported by sound data and information when available. The guidance provided in these chapters should be consulted as part of the standard approach to reduce the risk associated with the selection and dissemination of antimicrobial resistant micro-organisms and antimicrobial resistance determinants.

— text deleted

CHAPTER 7.2.

WELFARE OF FARMED FISH DURING TRANSPORT

EU position

The EU can support the adoption of the proposed changes in the chapter of welfare of farmed fish during transport at OIE's 79th General Session in May 2011.

For future revisions of this chapter the EU asks the below amendments to be considered as indicated in the text below.

Article 7.2.1.Scope

Preamble: ~~Transport is stressful to fish.~~ This chapter provides information recommendations to minimise the effect of transport on the welfare of farmed fish (hereafter referred to as fish). It applies to their transport by air, by sea or on land within a country and between countries, and only considers the issues related to their welfare.

Recommendations for measures to control the *aquatic animal* health *risks* related to the transport of fish are included in Chapter 5.4. ~~on~~ Control of aquatic animal health risks associated with transport of aquatic animals.

Article 7.2.~~1~~2.**Responsibilities**

All personnel handling fish throughout the transportation process are responsible for ensuring that consideration is given to the potential impact on the welfare of the fish.

The roles of each of the various personnel are defined below:

1. The responsibilities of the *Competent Authority* for the exporting and importing jurisdiction include:
 - a) establishing minimum standards for fish welfare during transport, including examination before, during and after their transport, appropriate certification, ~~and~~ record keeping, awareness and training of personnel involved in transport;
 - ~~b) ensuring awareness and training of personnel involved in transport;~~
 - eb) ensuring implementation of the standards, including possible accreditation of transport companies.
2. Owners and managers of fish at the start and at the end of the journey are responsible for:
 - a) the general health of the fish and their fitness for transport at the start of the journey and to ensure the overall welfare of the fish during the transport regardless of whether these duties are subcontracted to other parties;
 - b) ensuring trained and competent personnel supervise operations at their facilities for fish to be loaded and unloaded in a manner that causes minimum stress and injury;

- c) having a *contingency plan* available to enable humane killing of the fish at the start and at the end of the journey, as well as during the journey, if required;
 - d) ensuring fish have a suitable environment to enter at their destination that ensures their welfare is maintained.
3. Transport companies, in cooperation with the farm owner/manager, are responsible for planning the transport to ensure that the transport can be carried out according to fish health and welfare standards including:
 - a) using a well maintained *vehicle* that is appropriate to the species to be transported;
 - b) ensuring trained and competent staff are available for loading and unloading; and to ensure swift, humane killing of the fish, if required;
 - c) having *contingency plans* to address emergencies and minimise stress during transport;
 - d) selecting suitable equipment for loading and unloading of the *vehicle*.
 4. The person in charge of supervising the transport is responsible for all documentation relevant to the transport, and practical implementation of recommendations for welfare of fish during transport.

Article 7.2.23.

Competence

All parties supervising transport activities, including loading and unloading, should have an appropriate knowledge and understanding to ensure that the welfare of the fish is maintained throughout the process. Competence may be gained through formal training and/or practical experience.

1. All persons handling live fish, or who are otherwise responsible for live fish during transport, should be competent according to their responsibilities listed in Article 7.2.1.
2. *Competent Authority*, farm owners/managers, and transport companies have a responsibility in providing training to their respective staff and other personnel.
3. Any necessary training should address species-specific knowledge and may include practical experience on:
 - a) fish behaviour, physiology, general signs of *disease* and poor welfare;
 - b) operation and maintenance of equipment relevant to fish health and welfare;
 - c) water quality and suitable procedures for water exchange;
 - d) methods of live fish handling during transport, loading and unloading (species-specific aspects when relevant);
 - e) methods for inspection of the fish, management of situations frequently encountered during transport such as changes in water quality parameters, adverse weather conditions, and emergencies;
 - f) methods for the humane killing of fish in accordance with Chapter 7.4. on the Humane killing of fish for disease control purposes (in preparation);
 - g) logbooks and record keeping.

Article 7.2.34.

Planning the transport1. General considerations

Adequate planning is a key factor affecting the welfare of fish during transportation. The pre-transport preparation, the duration and route of a transport should be determined by the purpose of the transport e.g. biosecurity issues, transport of fish for stocking farms or resource enhancement, for slaughter/killing for disease control purposes. Before the transport starts, plans should be made in relation to:

- a) type of *vehicle* and transport equipment required;
- b) route – such as distance, expected weather and/or sea conditions;
- c) nature and duration of the transport;
- d) need for care of the fish during the transport;
- e) emergency response procedures related to fish welfare;
- f) assessment of the necessary biosecurity level (e.g. washing and *disinfection* practices, safe places for changing water, treatment of transport water) (refer to Chapter 5.4.).

2. Vehicle design and maintenance

- a) *Vehicles* and *containers* used for transport of fish should be appropriate to the species, size, weight and number of fish to be transported.
- b) *Vehicles* and *containers* should be maintained in good mechanical and structural condition to prevent predictable and avoidable damage of the *vehicle* that may directly or indirectly affect the welfare of transported fish.

EU Comments

For future revision of this chapter, the EU would suggest OIE to add to point 2 b) the following text: "Vehicles and containers also may need to be insulated to prevent temperature fluctuation. Containers should be fixed to the vehicle to avoid containers moving around inside the vehicle."

Rationale:

Exposure to high temperature fluctuations is stressful to fish. Furthermore, the temperature can increase so much that the temperature is not any more within the species tolerance level. Uncontrolled movement of the containers could result in fish being injured.

- c) *Vehicles* (if relevant) and *containers* should have adequate circulation of water and equipment for oxygenation as required to meet variations in the conditions during the journey and the needs of the animals being transported, including the closing of valves in well boats for biosecurity reasons.

EU Comments

Equipment for the removal of waste such as carbondioxide is also necessary and the EU would propose the following rephrasing of point 2 c):

"Vehicles and containers should have adequate circulation of water and equipment for oxygenation and removal of waste (e.g. CO₂) as required to meet variations in the conditions during the journey and the needs of the animals being transported, including the closing of valves in well boats for biosecurity reasons."

Rationale:

Removal of waste from the water is equally important to maintain a good water quality that satisfies the species needs. In addition for air breathers a layer of carbondioxide on the water should be avoided.

- d) The fish should be accessible to inspection en route, if necessary, to ensure that fish welfare can be assessed.
- e) Documentation that focuses on fish welfare and thus carried with the *vehicle* should include a transport logbook of stocks received, contact information, mortalities and disposal/storage logs.

3. Water

- a) Water quality (e.g. oxygen, CO₂ and NH₃ level, pH, temperature, salinity) should be appropriate for the species being transported and method of transportation.
- b) Equipment to monitor and maintain water quality may be required depending on the length of the transport. Annex 12 (contd)

4. Preparation of fish for the transport

- a) Prior to transport, feed should be withheld from the fish, taking into consideration the fish species and life stage to be transported.
- b) The ability of the fish to cope with the stress of transport should be assessed based on health status, previous handling and recent transport history of the fish. Generally, only fish that are fit for transport should be loaded. Transport for disease control purposes should be in accordance with Chapter X.X. on the humane killing of fish for disease control purposes (in preparation).
- c) Reasons for considering of unfitness of fish for transport includes:
 - i) displaying clinical signs of *disease*;
 - ii) significant physical injuries or abnormal behaviour, such as rapid ventilation or abnormal swimming;
 - iii) recent exposure to stressors that adversely affect behaviour or physiological state (for example extreme temperatures, chemical agents);
 - iv) insufficient or excessive length of fasting.

5. Species-specific recommendations

Transport procedures should take account of variations in the behaviour and specific needs of the transported fish species. Handling procedures that are successful with one species may be ineffective or dangerous for another species.

Some species or life stages may need to be physiologically prepared prior to entering a new environment, such as by feed deprivation or osmotic acclimatisation.

6. Contingency plans

There should be a *contingency plan* that identifies the important adverse fish welfare events that may be encountered during the transport, the procedures for managing each event and the action to be taken

in such an event. For each event, the plan should document the actions to be undertaken and the responsibilities of all parties involved, including communications and record keeping.

Article 7.2.45.

Documentation

1. Fish should not be loaded until the required documentation is complete.
2. The documentation accompanying the consignment (the transport log) should include:
 - a) description of the consignment (e.g. date, time, and place of loading, species, biomass load);
 - b) description of the transport plan (e.g. including route, water exchanges, expected time, date and place of arrival and unloading and receiver contact information).
3. The transport log should be made available to the dispatcher and the receiver of the consignment as well as to the *Aquatic Animal Health Service* upon request. Transport logs from previous journeys should be kept after completion of the transport for a period of time as specified by the *Aquatic Animal Health Service*.

Article 7.2.56.

Loading the fish

1. The issues which should be addressed to avoid unnecessary stress and injury to the fish include:

EU Comments

Stress and injury should not be automatically linked, as in this case in 7.2.6, point 1. The EU suggests the OIE to consider the following rephrasing: "... to avoid injury and unnecessary stress and injury to the fish include:"

This is a general comment as the same or similar phrasing is to be found in this chapter (e.g. article 7.2.2. point 2 b) and in chapter 7.3.

Rationale:

Stress and injury should not be presented to be inseparable. In most cases it is not possible to handle fish (e.g. crowd, load, unload) given the equipment that currently is in use, without causing fish a certain amount of stress. Handling stress can be reduced but never completely eliminated. However it should be possible to avoid injury by correct operation of the equipment or with good technique.

- a) crowding procedure in farm pond, tank, net or cage prior to loading;
- b) equipment (such as nets, pumps, pipes and fittings) that are both improperly constructed, (for example with e.g. sharp bends or protrusions) or improperly operated (by e.g. overloading the system with fish of incorrect size or number of fish per time unit according to the equipment's capacity);
- c) water quality - some species of fish should be acclimatised if there is a likelihood of the fish being transported in water of a significantly different temperature or other water parameters.

EU Comments

The EU would suggest OIE to consider rephrasing 1(c) as follows: "...fish should not be transported be acclimatised if there is the likelihood of the fish being transported in water of a significantly different temperature or other parameters, unless they have been acclimatised."

This should also apply to article 7.2.8. Unloading the fish, point 2, second sentence.

Rationale: This creates a clearer and stronger point.

2. The density of fish in a *vehicle* and/or *container* should be in accordance with scientific data where available and not exceed what is generally accepted for a given species and a given situation.
3. Loading should be carried out, or supervised, by operators with knowledge and experience of the behaviour and other characteristics of the fish species being loaded to ensure that the welfare of the fish is maintained.

Article 7.2.67.

Transporting the fish

1. General considerations

- a) Periodic inspections should take place during the transport to verify that acceptable welfare is being maintained.
- b) Ensure that water quality is monitored and the necessary adjustments made to avoid extreme conditions.
- c) Travel in a manner that minimises uncontrolled movements of the fish that may lead to stress and injury.

2. Sick or injured fish

- a) In the event of a fish health emergency during transport, the *vehicle* operator should initiate the *contingency plan* (see point 6 of Article 7.2.3.).
- b) If the killing of fish is necessary during the transport, it should be carried out humanely in accordance with Chapter 7.4. Killing of farmed fish for disease control purposes (in preparation), and in compliance with relevant legislation.

Annex 12 (contd)

Article 7.2.78.

Unloading the fish

1. The principles of good fish handling during loading apply equally during unloading.
2. Fish should be unloaded as soon as possible after arrival at the destination, allowing sufficient time to ensure that the unloading procedure does not cause harm to the fish. Some species of fish should be acclimatised if there is a likelihood of the fish being unloaded into water of a significantly different quality (such as temperature, salinity, pH).
3. Moribund or seriously injured fish should be removed and humanely killed in accordance with Chapter 7.4. Killing of farmed fish for disease control purposes (in preparation).

Article 7.2.89.

Post-transport activities

1. The person in charge of receiving the fish should closely observe them during the post-transport period, and keep appropriate records.

2. Fish showing abnormal clinical signs should be humanely killed in accordance with Chapter 7.4. Killing of farmed fish for disease control purposes (in preparation) or isolated and examined by a *veterinarian* or other qualified personnel, who may recommend treatment.

3. Significant problems associated with transport should be evaluated to prevent recurrence of such problems.

CHAPTER 7.3.

WELFARE ASPECTS OF STUNNING AND KILLING
OF FARMED FISH FOR HUMAN CONSUMPTION**EU position**

The EU welcomes the revision of the Chapter on Welfare aspects of stunning and killing of farmed fish for human consumption and thanks the OIE for having taken into account most of the EU comments submitted earlier this year.

The EU can support adoption of the proposed changes in the chapter at OIE's 79th General Session in May 2011.

The EU asks OIE to consider several amendments to be included in future revisions of this chapter as indicated in the text below.

Article 7.3.1.

Scope

These recommendations apply to the stunning and killing of farmed fish species for human consumption.

These recommendations address the need to ensure the welfare of farmed fish, intended for human consumption, during stunning and killing including transport and holding immediately prior to stunning.

This chapter describes general principles that should be applied to ensure the welfare of fish for stunning and killing and also applies to fish killed for disease control purposes and intended for human consumption. Specific measures applicable to emergency killing for disease control purposes not intended for human consumption are addressed in Chapter 7.4. Humane Killing of Farmed Fish for Disease Control Purposes (under development).

As a general principle, fish should be stunned before killing, and the stunning method should ensure immediate and irreversible loss of consciousness. If the stunning is not irreversible, fish should be killed before consciousness is recovered.

EU Comments

The EU proposes that the following text is considered inserted in the first sentence of the final paragraph of the scope: "... or for methods that do not produce immediate loss of consciousness the induction phase should be without avoidable stress."

Rationale:

Methods of slaughter should minimize stress, and this is especially true if there is any delay in the loss of consciousness of fish.

Article 7.3.2.

Personnel

Persons engaged in the handling, stunning and killing of fish play an important role in their welfare. Personnel handling fish for stunning and killing should be experienced and competent in the handling of fish, and understand their behaviour patterns as well as the underlying principles necessary to carry out their tasks. Some stunning and killing methods may pose a risk to the personnel; therefore training should cover occupational health and safety implications of any methods used.

Article 7.3.3.

Transport

If fish are to be transported prior to stunning and killing, this should be done in accordance with OIE recommendations on the welfare of farmed fish during transport (see Chapter 7.2).

Article 7.3.4.

Design of holding facilities

1. The holding facilities should be designed and specifically constructed to hold a certain fish species or group of fish species.
2. The holding facilities should be of a size that allows holding a certain number of fish for processing in a given timeframe without compromising the welfare of the fish.
3. Operations should be conducted with minimal injury and stress to the fish.
4. The following recommendations may help to achieve this:
 - a) nets and tanks should be designed to minimise physical injuries;

EU Comments

The EU proposes to add the words "and maintained" under point 4(a) as to read as follows: "nets and tanks should be designed and maintained to minimise physical injuries".

Rationale:

Just like other constructs or equipment mentioned in this text, nets should be maintained properly so that they do not compromise the welfare of the fish.

- b) water quality should be suitable for the fish species and stocking density;
- c) equipment for transferring fish, including pumps and pipes, should be designed and maintained to minimise injury.

Article 7.3.5.

Unloading, transferring and loading

1. Fish should be unloaded, transferred and loaded under conditions that minimise injury and stress to the fish.
2. The following points should be considered:
 - a) Water quality (e.g. temperature, oxygen and CO₂ levels, pH and salinity) should be assessed on arrival of fish prior to their unloading, and corrective action taken if required.
 - b) Where possible any injured or moribund fish should be separated and killed humanely.

- c) The crowding periods of fish should be as short and infrequent as possible to avoid stressful conditions arising.
- d) The handling of fish during transfers should be minimised and preferably fish should not be handled out of water. If fish need to be removed from water, this period should be kept as short as possible.
- e) Where feasible, and when applicable, fish should be allowed to swim directly into a stunning device without handling to avoid handling stress.
- f) Equipment used to handle fish, for example nets and dip nets, pumping devices and brailing devices, should be designed, constructed and operated to minimise physical injuries (e.g. pumping height, pressure and speed are important factors to consider).
- g) Fish should not be fasted (deprived of food) before killing for longer than is necessary (e.g. to clear the gut or to reduce undesirable organoleptic properties).

EU Comments

The EU would suggest deleting the second parenthesis of point 2 g) in future revisions.

Rationale:

The withdrawal of feed should be as short as possible, but how long this period is may vary due to water temperature, species etc. Clearing the gut does not usually take long, but it might be required to deprive the fish of feed for longer to achieve the required effect as regards organoleptic properties. These examples can thus be slightly misleading.

- eh) There should be a *contingency plan* to address emergencies and minimise stress during unloading, transferring and loading fish.

Article 7.3.6.

Stunning and killing methods

1. General considerations

- a) The *Competent Authority* should approve the stunning and killing methods for fish. The choice of method should take account of species-specific information where available.
- b) All handling, stunning and killing equipment should be maintained and operated appropriately; it should be tested on a regular basis to ensure that performance is adequate.
- c) Effective stunning should be verified by the absence of consciousness.
- d) A backup stunning system is necessary. If Any fish mis-stunned, or regaining consciousness before death, the fish should be re-stunned as soon as possible.
- e) Stunning should not take place if killing is likely to be delayed such that the fish will recover or partially recover consciousness.
- f) While absence of consciousness may be difficult to recognise, signs of correct stunning include
 - i) loss of body and respiratory movement (loss in opercular activity); ii) loss of visual evoked response (VER); iii) loss of vestibulo-ocular reflex (VOR, eye rolling).

EU Comments

In article 7.3.6, section 1, subsection f, the EU would suggest OIE to consider deleting "loss of visual evoked response (VER)" while inserting "and no response to handling" at the end of the sentence.

Rationale:

This method is relevant to use in scientific research but is impractical to use in an abattoir. On the other hand in most cases a reaction to handling indicates some state of consciousness.

2. Mechanical stunning and killing methods

- a) Percussive stunning is achieved by a blow of sufficient strength to the head applied above or immediately adjacent to the brain in order to damage the brain. Mechanical stunning may be achieved either manually or using specially developed equipment.
- b) Spiking or coring are irreversible stunning and killing methods of fish based on physical damage to the brain by inserting a spike or core into the brain.
- c) Shooting using a free bullet may be used for killing large fish (such as tuna). The fish may either be crowded in a net and shot in the head from the surface, or individual fish may be killed by shooting in the head from under the water (commonly called lupara).
- d) Mechanical stunning is generally irreversible if correctly applied.

3. Electrical stunning and killing methods

- a) Electrical stunning involves the application of an electrical current of sufficient strength, frequency and duration, and suitable frequency to cause immediate loss of consciousness and insensibility of the fish. The conductivity of fresh and brackish water varies, so it is essential to establish the parameters of the electrical current to ensure proper stunning.

EU Comments

In article 7.3.6, 3(a), the EU suggests OIE to consider adding "sea" in the introductory part of the last sentence, which would then read: "The conductivity of fresh, brackish, and "sea" water varies, so it is essential to"

Rationale:

Sea water is also used as a medium, and standards must be developed based on this.

- b) The electrical stunning device should be constructed and used for the specific fish species and their environment.
- c) Electrical stunning may be reversible. In such cases fish should be killed before consciousness is recovered.
- d) Fish should be confined beneath the surface of the water, and there should be a uniform distribution of electrical current in the stunning tank or chamber.
- e) In semi-dry electrical stunning systems, fish should enter the device head first to ensure rapid and efficient stunning.

4. Other killing methods

The following methods are known to be used for killing fish: chilling with ice in holding water, carbon dioxide (CO₂) in holding water; chilling with ice and CO₂ in holding water; salt or ammonia baths; asphyxiation by removal from water; exsanguination without stunning. However, they have been shown to result in poor fish welfare. Therefore, these it is preferable to use the methods should not be used if it is feasible to use the methods described in points 2 and 3 of this Article, as appropriate to the fish species.

EU Comments

The EU would propose to rename point 4 to: "other stunning/killing methods".

Furthermore for the sake of clarity when it is not feasible to use any of the more humane methods described in point 2 or 3, we would suggest to add a final sentence concerning the choice of one of the methods described in point 4. For this reason the text needs to be altered and should read:

"The following methods are known to be used for stunning/killing fish: chilling with ice in holding water, carbon dioxide (CO₂) in holding water; chilling with ice and CO₂ in holding water; salt or ammonia baths; asphyxiation by removal from water; exsanguination without stunning. However, in different degrees, they have been shown to result in poor fish welfare. Therefore these methods should not be used if it is feasible to use the methods described in points 2 and 3 (stunning/killing) and 5 (killing) of this Article, as appropriate to the fish species. Where it is necessary to use one of these methods, the method selected should be the one that causes the least adverse effect, taking into account the available scientific knowledge for the species concerned."

Rationale:

The methods mentioned here (point 4) all have in different degrees a negative impact on fish welfare, and are in many cases not used solely as a killing method but either as a stunning method or as a combined stunning and killing method.

The EU also strongly recommends that a new point is added to article 7.3.6 as follow:

"Point 5 on exsanguination.

This killing method is the preferred method to be used after stunning in order to ensure that fish remain unconscious till death supervenes. "

Rationale:

Point 5: The only killing method commonly in use that does not have an adverse impact on fish welfare when used in conjunction with prior stunning, exsanguination, is however not listed.

It is clearly stated in the scope that fish need to be killed following the use of a reversible stunning method. The alteration would ensure a greater consistency in the chapter.

~~Article 7.3.7.~~

~~Examples of stunning/killing methods for fish groups~~

The following methods enable humane killing for the following fish groups:

1. ~~Percussive stunning: carp, catfish, salmonids, halibut;~~
2. ~~Spiking or coring: salmonids, tuna;~~
3. ~~Free bullet: tuna;~~
4. ~~Electrical stunning: carp, catfish, eel, salmonids, tilapia.~~

Article 7.3.87.

Summary table of some stunning/killing methods for fish and their respective welfare issues

A combination of methods described in the table below may be used.

Stunning/ killing method	Specific method	Key fish welfare concerns/requirements	Advantages	Disadvantages

Stunning/ killing method	Specific method	Key fish welfare concerns/requirements	Advantages	Disadvantages	
Mechanical	Percussive stunning	The blow should be of sufficient force and delivered above or adjacent to the brain in order to render immediate unconsciousness. Fish should be quickly removed from the water, restrained and given a quick blow to the head, delivered either manually by a club or by automated percussive stunning. The effectiveness of stunning should be checked, and fish be re-stunned if necessary. It can be a stun / kill method.	Immediate loss of consciousness. Suitable for medium to large sized fish.	Hand operated equipment may be hampered by uncontrolled movement of the fish. Mis-stunning may result from a too weak blow. Injuries may occur. Manual percussive stunning is only practicable for the killing of a limited number of fish <u>of a similar size</u> .	
Stunning/ killing method	Specific method	Key fish welfare concerns/requirements	Advantages	Disadvantages	
Mechanical	Spiking or coring		The spike should be aimed on the skull in a position to penetrate the brain of the fish and the impact of the spike should produce immediate unconsciousness. Fish should be quickly removed from the water, restrained and the spike immediately inserted into the brain. It is a stun / kill method.	Immediate loss of consciousness. Suitable for medium to large sized fish. For small tuna, spiking under the water avoids exposure of fish to air. The pineal window of tuna facilitates spiking for this species.	Inaccurate application may cause injuries. Difficult to apply if fish agitated. It is only practicable for the killing of a limited number of fish.
	Free bullet		The shot should be carefully aimed at the brain. The fish should be positioned correctly and the shooting range should be as short as practicable. It is a stun / kill method.	Immediate loss of consciousness. Suitable for large sized fish (e.g. large tuna).	Shooting distance; calibre need to be adapted. Excessive crowding and noise of guns may cause stress reaction. Contamination of the working area due to release of body fluids may present a biosecurity risk. May be hazardous to operators.
Electrical	Electrical stunning	Involves the application of an electrical current of sufficient strength, frequency and duration to cause immediately unconsciousness. It can be a stun / kill method. Equipment should be designed and maintained correctly.	Immediate loss of consciousness. Suitable for small to medium sized fish. Suitable for large numbers of fish, and the fish do not have to be removed from the water.	Difficult to standardise for all species. Optimal control parameters are unknown for some species. May be hazardous to operators.	
	Semi-dry electrical stunning	The head of the fish should enter the system first so electricity is applied to the brain first. Involves the application of an electrical current of sufficient strength, frequency and duration to cause immediately unconsciousness. Equipment should be designed and maintained correctly.	Good visual control of stunning and the ability for re-stunning of individual fish.	Misplacement of the fish may result in improper stunning. Optimal control parameters are unknown for some species. Not suitable for mixed sizes of fish	Semi-dry electrical stunning

Note : the terms small, medium and large fish should be interpreted relative to the species in question.

Examples of stunning/killing methods for fish groups

The following methods enable humane killing for the following fish groups:

1. percussive stunning: carp, salmonids;

2. spiking or coring: salmonids, tuna;

EU Comment

The EU proposes that "salmonids" is considered deleted so that the phrasing is "spiking or coring: salmonids, tuna."

Rationale:

Even though spiking is a method that is proved efficacious in stunning salmonids in the laboratory, the method requires a high level of precision to ensure that the spike penetrates the brain. Given the throughput of fish at the abattoir, it will be difficult to achieve such precision. The method is thus considered impractical for commercial slaughter of salmonids.

3. free bullet: tuna;

4. electrical stunning: carp, eel, salmonids.

CHAPTER 9.5.

TAURA SYNDROME

EU position

The EU can support the adoption of the amended chapter.

[...]

Article 9.5.3.

Importation or transit of aquatic animals and aquatic animal products for any purpose from a country, zone or compartment not declared free from Taura syndrome

1. *Competent Authorities* should not require any TS related conditions, regardless of the TS status of the *exporting country, zone or compartment* when authorising the importation or transit of the following *aquatic animals and aquatic animal products* from the species referred to in Article 9.4.2. intended for any purpose and complying with Article 5.3.1.:
 - a) heat sterilised hermetically sealed crustacean products (i.e. a heat treatment at 121°C for at least 3.6 minutes or any time/temperature equivalent);
 - b) cooked crustacean products that have been subjected to heat treatment at 70°C for at least 30 minutes ~~(or to any time/temperature equivalent treatment~~ which has been demonstrated to inactivate TSV);
 - c) pasteurised crustacean products that have been subjected to heat treatment at 90°C for 10 minutes ~~(or to any time/temperature pasteurisation~~ equivalent which has been demonstrated to inactivate TSV);
 - d) crustacean oil;
 - e) crustacean *meal*; and
 - f) chemically extracted chitin.

[...]

 — text deleted

CHAPTER 10.1.

EPIZOOTIC HAEMATOPOIETIC NECROSIS

EU position

The EU can support the adoption of the amended chapter.

[...]

Article 10.1.3.

Importation or transit of aquatic animals and aquatic animal products for any purpose from an exporting country, zone or compartment not declared free from epizootic haematopoietic necrosis

1. *Competent Authorities* should not require any EHN related conditions, regardless of the EHN status of the *exporting country, zone or compartment* when authorising the importation or transit of the following *aquatic animals* and *aquatic animal products* from the species referred to in Article 10.1.2. intended for any purpose and complying with Article 5.3.1.:
 - a) heat sterilised hermetically sealed fish products (i.e. a heat treatment at 121°C for at least 3.6 minutes or any time/temperature equivalent);
 - b) pasteurised fish products that have been subjected to heat treatment at 90°C for 10 minutes (or ~~to~~ any time/temperature ~~pasteurisation~~ equivalent which has been demonstrated to inactivate EHNV);
 - c) mechanically dried eviscerated fish (i.e. a heat treatment at 100°C for at least 30 minutes or any time/temperature equivalent which has been demonstrated to inactivate EHNV);
 - d) fish skin leather;
 - e) fish oil; and
 - f) fish meal.

[...]

ARTICLES X.X.3 AND X.X.11./12.

CHAPTER 8.1.

**INFECTION WITH
BATRACHOCHYTRIUM DENDROBATIDIS**

EU position

The EU can support the adoption of the amended chapter.

[...]

Article 8.1.3.

Importation or transit of aquatic animals and aquatic animal products for any purpose from a country, zone or compartment not declared free from *B. dendrobatidis*

1. *Competent Authorities* should not require any *B. dendrobatidis* related conditions, regardless of the *B. dendrobatidis* status of the *exporting country, zone or compartment* when authorising the importation or transit of the following *aquatic animals* and *aquatic animal products* from the species referred to in Article 8.1.2. intended for any purpose and complying with Article 5.3.1.:
 - a) ~~[commodities treated in a manner that inactivates the disease agent e.g. canned products; leather made from amphibian skin; dried amphibian products (including air dried, flame dried and sun dried)] (under study).~~
 - a) heat sterilised hermetically sealed amphibian products (i.e. a heat treatment at 121°C for at least 3.6 minutes or any time/temperature equivalent);
 - b) cooked amphibian products that have been subjected to heat treatment at 100°C for at least one minute (or any time/temperature equivalent which has been demonstrated to inactivate *B. dendrobatidis* [e.g. 60°C for at least 5 minutes]);
 - c) pasteurised amphibian products that have been subjected to heat treatment at 90°C for at least 10 minutes (or any time/temperature equivalent which has been demonstrated to inactivate *B. dendrobatidis* [e.g. 60°C for at least 5 minutes]);
 - d) mechanically dried amphibian products (i.e. a heat treatment of 100°C for at least 30 minutes or any time/temperature equivalent which has been demonstrated to inactivate *B. dendrobatidis* [e.g. 60°C for at least 5 minutes]; and
 - e) amphibian skin leather.
2. When authorising the importation or transit of *aquatic animals* and *aquatic animal products* of a species referred to in Article 8.1.2., other than those referred to in point 1 of Article 8.1.3., *Competent Authorities* should require the conditions prescribed in Articles 8.1.7. to 8.1.12. relevant to the *B. dendrobatidis* status of the exporting country, zone or compartment.
3. When considering the importation or transit of aquatic animals and aquatic animal products from an exporting country, zone or compartment not declared free of *B. dendrobatidis* of a species not

covered in Article 8.1.2. but which could reasonably be expected to pose a risk of transmission for *B. dendrobatidis*, *Competent Authorities* should conduct a *risk analysis* in accordance with the recommendations in the *Aquatic Code*. The *exporting country* should be informed of the outcome of this assessment.

[...]

Article 8.1.12.

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

1. *Competent Authorities* should not require any *B. dendrobatidis* related conditions, regardless of the *B. dendrobatidis* status of the *exporting country*, *zone* or *compartment* when authorising the importation or transit of the following *commodities* which have been prepared and packaged for retail trade and complying with Article 5.3.2.:

~~i) skinned frog legs with feet removed;~~

~~ii) skinned amphibian meat or carcasses, with heads, hands and feet removed] (under study).~~

a) amphibian meat (skin off, fresh or frozen).

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

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

 — text deleted

CHAPTER 8.2.

INFECTION WITH RANAVIRUS

EU position

The EU can support the adoption of the amended chapter.

[...]

Article 8.2.3.

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

1. *Competent Authorities* should not require any ranavirus related conditions, regardless of the ranavirus status of the *exporting country, zone or compartment* when authorising the importation or transit of the following *aquatic animals* and *aquatic animal products* from the species referred to in Article 8.2.2. intended for any purpose and complying with Article 5.3.1.:
 - a) ~~[commodities treated in a manner that inactivates the disease agent e.g. canned products; leather made from amphibian skin; dried amphibian products (including air dried, flame dried and sun dried)] (under study);~~
 - a) heat sterilised hermetically sealed amphibian products (i.e. a heat treatment at 121°C for at least 3.6 minutes or any time/temperature equivalent);
 - b) cooked amphibian products that have been subjected to heat treatment at 65°C for at least 30 minutes (or any time/temperature equivalent which has been demonstrated to inactivate all virus species of the genus Ranavirus in the family Iridoviridae [with the exception of epizootic haematopoietic necrosis virus and European catfish virus]);
 - c) pasteurised amphibian products that have been subjected to heat treatment at 90°C for at least 10 minutes (or any time/temperature equivalent which has been demonstrated to inactivate all virus species of the genus Ranavirus in the family Iridoviridae [with the exception of epizootic haematopoietic necrosis virus and European catfish virus]);
 - d) mechanically dried amphibian products (i.e. a heat treatment at 100°C for at least 30 minutes or any time/temperature equivalent which has been demonstrated to inactivate all virus species of the genus Ranavirus in the family Iridoviridae [with the exception of epizootic haematopoietic necrosis virus and European catfish virus]).
2. When authorising the importation or transit of *aquatic animals* and *aquatic animal products* of a species referred to in Article 8.2.2., other than those referred to in point 1 of Article 8.2.3., *Competent Authorities* should require the conditions prescribed in Articles 8.2.7. to 8.2.12. relevant to the ranavirus status of the *exporting country, zone or compartment*.
3. When considering the importation or transit of *aquatic animals* and *aquatic animal products* from an *exporting country, zone or compartment* not declared free of ranavirus of a species not covered in Article 8.2.2. but which could reasonably be expected to pose a *risk* of transmission for ranavirus,

Competent Authorities should conduct a *risk analysis* in accordance with the recommendations in the *Aquatic Code*. The *exporting country* should be informed of the outcome of this assessment.

[...]

Article 8.2.12.

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

1. *Competent Authorities* should not require any ranavirus related conditions, regardless of the ranavirus status of the *exporting country*, *zone* or *compartment* when authorising the importation or transit of the following *commodities* which have been prepared and packaged for retail trade and complying with Article 5.3.2.:
 - ~~i) skinned frog legs with feet removed;~~
 - ~~ii) skinned amphibian meat or carcasses, with heads, hands and feet removed] (under study).~~
 - a) no commodities listed.

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

2. When importing *aquatic animals* or *aquatic animal products*, other than those referred to in point 1 above, of the species referred to in Article 8.2.2. from a country, *zone* or *compartment* not declared free from ranavirus, the *Competent Authority* of the *importing country* should assess the *risk* and apply appropriate *risk* mitigation measures.

— text deleted

CHAPTER 9.1.

**CRAYFISH PLAGUE
(APHANOMYCES ASTACI)**

EU position

The EU can support the adoption of the amended chapter.

[...]

Article 9.1.3.

Importation or transit of aquatic animals and aquatic animal products for any purpose from a country, zone or compartment not declared free from crayfish plague

1. *Competent Authorities* should not require any crayfish plague related conditions, regardless of the crayfish plague status of the *exporting country, zone or compartment* when authorising the importation or transit of the following *aquatic animals* and *aquatic animal products* from the species referred to in Article 9.1.2.intended for any purpose and complying with Article 5.3.1.:

- ~~a) [*commodities treated in a manner that inactivates the pathogenic agent e.g. boiled, canned or pasteurised products and some ready to eat meals; and crayfish oil and crayfish meal intended for use in feed;*~~
- ~~b) chemically extracted chitin;~~
- ~~c) crayfish products made non-infectious through processing as dry feed (e.g. pelleted or extruded feed);~~
- ~~d) frozen crayfish products that have been subjected to -20°C or lower temperatures for at least 72 hours] under study.~~
- a) heat sterilised hermetically sealed crayfish products (i.e. a heat treatment at 121°C for at least 3.6 minutes or any time /temperature equivalent);
- b) cooked crayfish products that have been subjected to heat treatment at 100°C for at least one minute (or any time/temperature equivalent which has been demonstrated to inactivate *A. astaci*);
- c) pasteurised crayfish products that have been subjected to heat treatment at 90°C for at least 10 minutes (or any time/temperature equivalent which has been demonstrated to inactivate *A. astaci*);
- d) frozen crayfish products that have been subjected to -20°C or lower temperatures for at least 72 hours;
- e) crayfish oil;
- f) crayfish meal; and

g) chemically extracted chitin.

2. When authorising the importation or transit of *aquatic animals* and *aquatic animal products* of a species referred to in Article 9.1.2., other than those referred to in point 1 of Article 9.1.3., *Competent Authorities* should require the conditions prescribed in Articles 9.1.7. to 9.1.11. relevant to the crayfish plague status of the *exporting country, zone or compartment*.
3. When considering the importation or transit of *aquatic animals* and *aquatic animal products* from an *exporting country, zone or compartment* not declared free of crayfish plague of a species not covered in Article 9.1.2. but which could reasonably be expected to pose a *risk* of transmission for crayfish plague, *Competent Authorities* should conduct a *risk analysis* in accordance with the recommendations in the *Aquatic Code*. The *exporting country* should be informed of the outcome of this assessment.

[...]

Article 9.1.11.

Importation of aquatic animals and aquatic animal products for retail trade for human consumption from a country, zone or compartment not declared free from crayfish plague

1. *Competent Authorities* should not require any crayfish plague related conditions, regardless of the crayfish plague status of the *exporting country, zone or compartment* when authorising the importation or transit of the following *commodities* which have been prepared and packaged for retail trade and complying with Article 5.3.2.:

a) ~~[commodity(ies)] under study~~a) no commodities listed.

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

2. When importing *aquatic animals* or *aquatic animal products*, other than those referred to in point 1 above, of the species referred to in Article 9.1.2. 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.

— text deleted

CHAPTER 9.2.

**INFECTIOUS HYPODERMAL
AND HAEMATOPOIETIC NECROSIS**

EU position

The EU can support the adoption of the amended chapter.

[...]

Article 9.2.3.

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

1. *Competent Authorities* should not require any IHHN related conditions, regardless of the IHHN status of the *exporting country, zone or compartment* when authorising the importation or transit of the following *aquatic animals and aquatic animal products* from the species referred to in Article 9.2.2. intended for any purpose and complying with Article 5.3.1.:
 - a) ~~[commodities treated in a manner that inactivates the pathogenic agent e.g. boiled, canned or pasteurised products and some ready-to-eat meals; and crustacean oil and crustacean meal intended for use in feed;~~
 - b) ~~chemically extracted chitin;~~
 - e) ~~crustacean products made non-infectious through processing as dry feed (e.g. pelleted or extruded feed)] under study.~~
 - a) heat sterilised hermetically sealed crustacean products (i.e. a heat treatment at 121°C for at least 3.6 minutes or any time/temperature equivalent);
 - b) cooked crustacean products that have been subjected to heat treatment at 90°C for at least 20 minutes (or any time/temperature equivalent which has been demonstrated to inactivate IHHNV);
 - c) crustacean oil; and
 - d) crustacean meal.
2. When authorising the importation or transit of *aquatic animals and aquatic animal products* of a species referred to in Article 9.2.2., other than those referred to in point 1 of Article 9.2.3., *Competent Authorities* should require the conditions prescribed in Articles 9.2.7. to 9.2.11. relevant to the IHHN status of the *exporting country, zone or compartment*.
3. When considering the importation or transit of *aquatic animals and aquatic animal products* from an *exporting country, zone or compartment* not declared free of IHHN of a species not covered in Article 9.2.2. but which could reasonably be expected to pose a *risk* of transmission for IHHN,

Competent Authorities should conduct a *risk analysis* in accordance with the recommendations in the *Aquatic Code*. The *exporting country* should be informed of the outcome of this assessment.

[...]

Article 9.2.11.

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

1. *Competent Authorities* should not require any IHHN related conditions, regardless of the IHHN status of the *exporting country*, *zone* or *compartment* when authorising the importation or transit of the following *commodities* which have been prepared and packaged for retail trade and complying with Article 5.3.2.:

a) ~~[commodity(ies)] under study.~~

a) frozen peeled shrimp (shell off, head off).

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

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

 — text deleted

CHAPTER 9.3.

INFECTIOUS MYONECROSIS

EU position

The EU can support the adoption of the amended chapter.

[...]

Article 9.3.3.

Importation or transit of aquatic animals and aquatic animal products for any purpose from a country, zone or compartment not declared free from infectious myonecrosis

1. *Competent Authorities* should not require any IMN related conditions, regardless of the IMN status of the *exporting country, zone or compartment* when authorising the importation or transit of the following *aquatic animals* and *aquatic animal products* from the species referred to in Article 9.3.2. intended for any purpose and complying with Article 5.3.1.:
 - a) ~~[commodities treated in a manner that inactivates the disease agent e.g. boiled, canned or pasteurised products and some ready-to-eat meals; and crustacean oil and crustacean meal intended for use in feed;~~
 - b) ~~chemically extracted chitin;~~
 - e) ~~crustacean products made non infectious through processing as dry feed (e.g. pelleted or extruded feed)] (under study).~~
 - a) heat sterilised hermetically sealed crustacean products (i.e. a heat treatment at 121°C for at least 3.6 minutes or any time/temperature equivalent);
 - b) cooked crustacean products that have been subjected to heat treatment at 100°C for at least 3 minutes (or any time/temperature equivalent which has been demonstrated to inactivate IMNV);
 - c) crustacean oil;
 - d) crustacean meal; and
 - e) chemically extracted chitin.
2. When authorising the importation or transit of *aquatic animals* and *aquatic animal products* of a species referred to in Article 9.3.2., other than those referred to in point 1 of Article 9.3.3., *Competent Authorities* should require the conditions prescribed in Articles 9.3.7. to 9.3.11. relevant to the IMN status of the *exporting country, zone or compartment*.
3. When considering the importation or transit of *aquatic animals* and *aquatic animal products* from an *exporting country, zone or compartment* not declared free of IMN of a species not covered in Article 9.3.2. but which could reasonably be expected to pose a *risk* of transmission for IMN, *Competent Authorities*

should conduct a *risk analysis* in accordance with the recommendations in the *Aquatic Code*. The *exporting country* should be informed of the outcome of this assessment.

[...]

Article 9.3.11.

Importation of aquatic animals and aquatic animal products for retail trade for human consumption from a country, zone or compartment not declared free from infectious myonecrosis

1. *Competent Authorities* should not require any IMN related conditions, regardless of the IMN status of the *exporting country*, *zone* or *compartment* when authorising the importation or transit of the following *commodities* which have been prepared and packaged for retail trade and complying with Article 5.3.2.:

a) ~~[commodity(ties)] under study.~~

a) frozen peeled shrimp (shell off, head off).

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

2. When importing *aquatic animals* or *aquatic animal products*, other than those referred to in point 1 above, of the species referred to in Article 9.3.2. 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.

— text deleted

CHAPTER 9.4.

NECROTISING HEPATOPANCREATITIS

EU position

The EU can support the adoption of the amended chapter.

[...]

Article 9.4.3.

Importation or transit of aquatic animals and aquatic animal products for any purpose from a country, zone or compartment not declared free from necrotising hepatopancreatitis

1. *Competent Authorities* should not require any NHP related conditions, regardless of the NHP status of the *exporting country, zone or compartment* when authorising the importation or transit of the following *aquatic animals* and *aquatic animal products* from the species referred to in Article 9.4.2. intended for any purpose and complying with Article 5.3.1.:
 - a) ~~[commodity(ies)] under study.~~
 - a) heat sterilised hermetically sealed crustacean products (i.e. a heat treatment at 121°C for at least 3.6 minutes or any time/temperature equivalent);
 - b) cooked crustacean products that have been subjected to heat treatment at 100°C for at least 3 minutes (or any time/temperature equivalent which has been demonstrated to inactivate the NHP bacterium);
 - c) pasteurised crustacean products that have been subjected to heat treatment at 63°C for at least 30 minutes (or any time/temperature equivalent which has been demonstrated to inactivate the NHP bacterium);
 - d) crustacean oil;
 - e) crustacean meal; and
 - f) chemically extracted chitin.
2. When authorising the importation or transit of *aquatic animals* and *aquatic animal products* of a species referred to in Article 9.4.2., other than those referred to in point 1 of Article 9.4.3., *Competent Authorities* should require the conditions prescribed in Articles 9.4.7. to 9.4.11. relevant to the NHP status of the *exporting country, zone or compartment*.
3. When considering the importation or transit of *aquatic animals* and *aquatic animal products* from an *exporting country, zone or compartment* not declared free of NHP of a species not covered in Article 9.4.2. but which could reasonably be expected to pose a *risk* of transmission for NHP, *Competent Authorities*

should conduct a *risk analysis* in accordance with the recommendations in the *Aquatic Code*. The *exporting country* should be informed of the outcome of this assessment.

[...]

Article 9.4.11.

Importation of aquatic animals and aquatic animal products for retail trade for human consumption from a country, zone or compartment not declared free from necrotising hepatopancreatitis

1. *Competent Authorities* should not require any NHP related conditions, regardless of the NHP status of the *exporting country*, *zone* or *compartment* when authorising the importation or transit of the following *commodities* which have been prepared and packaged for retail trade and complying with Article 5.3.2.:

a) ~~[commodity(ies)] under study.~~

a) frozen peeled shrimp (shell off, head off).

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

2. When importing *aquatic animals* or *aquatic animal products*, other than those referred to in point 1 above, of the species referred to in Article 9.4.2. 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.

— text deleted

CHAPTER 9.6.

WHITE SPOT DISEASE

EU position

The EU can support the adoption of the amended chapter.

[...]

Article 9.6.3.

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

1. *Competent Authorities* should not require any WSD related conditions, regardless of the WSD status of the *exporting country, zone or compartment* when authorising the importation or transit of the following *aquatic animals and aquatic animal products* from the species referred to in Article 9.6.2. intended for any purpose and complying with Article 5.3.1.:
 - ~~a) commodities treated in a manner that inactivates the pathogenic agent e.g. boiled, canned or pasteurised products and some ready-to-eat meals; and crustacean oil and crustacean meal intended for use in feed;~~
 - ~~b) chemically extracted chitin;~~
 - ~~e) crustacean products made non-infectious through processing as dry feed (e.g. pelleted or extruded feed)] (under study).~~
 - a) heat sterilised hermetically sealed crustacean products (i.e. a heat treatment at 121°C for at least 3.6 minutes or any time/ temperature equivalent);
 - b) cooked crustacean products that have been subjected to heat treatment at 60°C for at least one minute (or any time/ temperature equivalent which has been demonstrated to inactivate WSSV);
 - c) pasteurised crustacean products that have been subjected to heat treatment at 90°C for at least 10 minutes (or any time / temperature equivalent which has been demonstrated to inactivate WSSV);
 - d) crustacean oil;
 - e) crustacean meal; and
 - f) chemically extracted chitin.
2. When authorising the importation or transit of *aquatic animals and aquatic animal products* of a species referred to in Article 9.6.2., other than those referred to in point 1 of Article 9.6.3., *Competent Authorities* should require the conditions prescribed in Articles 9.6.7. to 9.6.11. relevant to the WSD status of the *exporting country, zone or compartment*.

3. When considering the importation or transit of *aquatic animals* and *aquatic animal products* from an *exporting country, zone* or *compartment* not declared free of WSD of a species not covered in Article 9.6.2. but which could reasonably be expected to pose a *risk* of transmission for WSD, *Competent Authorities* should conduct a *risk analysis* in accordance with the recommendations in the *Aquatic Code*. The *exporting country* should be informed of the outcome of this assessment.

[...]

Article 9.6.11.

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

1. *Competent Authorities* should not require any WSD related conditions, regardless of the WSD status of the *exporting country, zone* or *compartment* when authorising the importation or transit of the following *commodities* which have been prepared and packaged for retail trade and complying with Article 9.6.2.:

a) ~~[commodity(ies)] (under study);~~

a) frozen peeled shrimp or decapod crustacea (shell off, head off).

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

2. When importing *aquatic animals* or *aquatic animal products*, other than those referred to in point 1 above, of the species referred to in Article 9.6.2. 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.

 — text deleted

CHAPTER 9.7.

WHITE TAIL DISEASE

EU position

The EU can support the adoption of the amended chapter.

[...]

Article 9.7.3.

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

1. *Competent Authorities* should not require any WTD related conditions, regardless of the WTD status of the *exporting country, zone or compartment* when authorising the importation or transit of the following *aquatic animals and aquatic animal products* from the species referred to in Article 9.7.2. intended for any purpose and complying with Article 5.3.1.:
 - a) ~~[commodities treated in a manner that inactivates the pathogenic agent e.g. boiled, canned or pasteurised products and some ready-to-eat meals; and crustacean oil and crustacean meal intended for use in feed;~~
 - b) ~~chemically extracted chitin;~~
 - e) ~~crustacean products made non-infectious through processing as dry feed (e.g. pelleted or extruded feed)] (under study).~~
 - a) heat sterilised hermetically sealed crustacean products (i.e. a heat treatment at 121°C for at least 3.6 minutes or any time/temperature equivalent);
 - b) cooked crustacean products that have been subjected to heat treatment at 60°C for at least 60 minutes (or any time/temperature equivalent which has been demonstrated to inactivate MrNV);
 - c) pasteurised crustacean products that have been subjected to heat treatment at 90°C for at least 10 minutes (or any time/temperature equivalent that has been shown to inactivate MrNV);
 - d) crustacean oil;
 - e) crustacean meal; and
 - f) chemically extracted chitin.
2. When authorising the importation or transit of *aquatic animals and aquatic animal products* of a species referred to in Article 9.7.2., other than those referred to in point 1 of Article 9.7.3., *Competent Authorities* should require the conditions prescribed in Articles 9.7.7. to 9.7.11. relevant to the WTD status of the *exporting country, zone or compartment*.

3. When considering the importation or transit of *aquatic animals* and *aquatic animal products* from an *exporting country, zone* or *compartment* not declared free of WTD of a species not covered in Article 9.7.2. but which could reasonably be expected to pose a *risk* of transmission for WTD, *Competent Authorities* should conduct a *risk analysis* in accordance with the recommendations in the *Aquatic Code*. The *exporting country* should be informed of the outcome of this assessment.

[...]

Article 9.7.11.

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

1. *Competent Authorities* should not require any WTD related conditions, regardless of the WTD status of the *exporting country, zone* or *compartment* when authorising the importation or transit of the following *commodities* which have been prepared and packaged for retail trade and complying with Article 9.7.2.:

a) ~~[commodity(ies)] (under study).~~

a) frozen peeled shrimp (shell off, head off).

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

2. When importing *aquatic animals* or *aquatic animal products*, other than those referred to in point 1 above, of the species referred to in Article 9.7.2. 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.

 — text deleted

CHAPTER 9.8.

YELLOW HEAD DISEASE

EU position

The EU can support the adoption of the amended chapter.

[...]

Article 9.8.3.

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

1. *Competent Authorities* should not require any YHD related conditions, regardless of the YHD status of the *exporting country, zone or compartment* when authorising the importation or transit of the following *aquatic animals and aquatic animal products* from the species referred to in Article 9.8.2. intended for any purpose and complying with Article 5.3.1.:
 - ~~a) commodities treated in a manner that inactivates the pathogenic agent e.g. boiled, canned or pasteurised products and some ready-to-eat meals; and crustacean oil and crustacean meal intended for use in feed;~~
 - ~~b) chemically extracted chitin;~~
 - ~~e) crustacean products made non infectious through processing as dry feed (e.g. pelleted or extruded feed)] under study.~~
 - a) heat sterilised hermetically sealed crustacean products (i.e. a heat treatment at 121°C for at least 3.6 minutes or equivalent);
 - b) cooked crustacean products that have been subjected to heat treatment at 60°C for at least 15 minutes (or any time/temperature equivalent which has been demonstrated to inactivate YHV);
 - c) pasteurised crustacean products that have been subjected to heat treatment at 90°C for at least 10 minutes (or any time/temperature equivalent which has been demonstrated to inactivate YHV);
 - d) crustacean oil;
 - e) crustacean meal; and
 - f) chemically extracted chitin.
2. When authorising the importation or transit of *aquatic animals and aquatic animal products* of a species referred to in Article 9.8.2., other than those referred to in point 1 of Article 9.8.3., *Competent Authorities* should require the conditions prescribed in Articles 9.8.7. to 9.8.11. relevant to the YHD status of the *exporting country, zone or compartment*.

3. When considering the importation or transit of *aquatic animals* and *aquatic animal products* from an *exporting country, zone* or *compartment* not declared free of YHD of a species not covered in Article 9.8.2. but which could reasonably be expected to pose a *risk* of transmission for YHD, *Competent Authorities* should conduct a *risk analysis* in accordance with the recommendations in the *Aquatic Code*. The *exporting country* should be informed of the outcome of this assessment.

[...]

Article 9.8.11.

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

1. *Competent Authorities* should not require any YHD related conditions, regardless of the YHD status of the *exporting country, zone* or *compartment* when authorising the importation or transit of the following *commodities* which have been prepared and packaged for retail trade and complying with Article 5.3.2.:

a) ~~[commodity(ies)] under study.~~

a) frozen peeled shrimp or decapod crustacea (shell off, head off).

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

2. When importing *aquatic animals* or *aquatic animal products*, other than those referred to in point 1 above, of the species referred to in Article 9.8.2. 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.

 — text deleted

CHAPTER 10.2.

EPIZOOTIC ULCERATIVE SYNDROME

EU position

The EU can support the adoption of the amended chapter.

[...]

Article 10.2.3.

Importation or transit of aquatic animals and aquatic animal products for any purpose from a country, zone or compartment not declared free from epizootic ulcerative syndrome

1. *Competent Authorities* should not require any EUS related conditions, regardless of the EUS status of the *exporting country, zone or compartment* when authorising the importation or transit of the following *aquatic animals and aquatic animal products* from the species referred to in Article 10.2.2. intended for any purpose and complying with Article 5.3.1.:
 - a) ~~[commodities treated in a manner that inactivates the pathogenic agent e.g. leather made from fish skin, pasteurised products and some ready-to-eat meals; and fish oil and fish meal intended for use in feed] (under study);~~
 - a) heat sterilised hermetically sealed fish products (i.e. a heat treatment at 121°C for at least 3.6 minutes or any time/temperature equivalent);
 - b) pasteurised fish products that have been subjected to heat treatment at 90°C for at least 10 minutes (or any time/temperature equivalent which has been demonstrated to inactivate *Aphanomyces invadans*);
 - c) mechanically dried eviscerated fish (i.e. a heat treatment at 100°C for at least 30 minutes or any time/temperature equivalent which has been demonstrated to inactivate *Aphanomyces invadans*);
 - d) fish oil;
 - e) fish meal;
 - f) frozen eviscerated fish; and
 - g) frozen fillets or steaks.
2. When authorising the importation or transit of *aquatic animals and aquatic animal products* of a species referred to in Article 10.2.2., other than those referred to in point 1 of Article 10.2.3., *Competent Authorities* should require the conditions prescribed in Articles 10.2.7. to 10.2.12. relevant to the EUS status of the *exporting country, zone or compartment*.
3. When considering the importation or transit of *aquatic animals and aquatic animal products* from an *exporting country, zone or compartment* not declared free of EUS of a species not covered in Article 10.2.2. but which could reasonably be expected to pose a *risk* of transmission for EUS,

Competent Authorities should conduct a *risk analysis* in accordance with the recommendations in the *Aquatic Code*. The *exporting country* should be informed of the outcome of this assessment.

[...]

Article 10.2.12.

Importation of aquatic animals and aquatic animal products for retail trade for human consumption from a country, zone or compartment not declared free from epizootic ulcerative syndrome

1. *Competent Authorities* should not require any EUS related conditions, regardless of the EUS status of the *exporting country*, *zone* or *compartment* when authorising the importation or transit of the following *commodities* which have been prepared and packaged for retail trade and complying with Article 5.3.2.:
 - a) ~~[eviscerated fish (chilled or frozen);~~
 - b) ~~fillets or cutlets (chilled or frozen);~~
 - e) ~~dried eviscerated fish (including air dried, flame dried and sun dried)] (under study).~~
 - a) no commodities listed fillets or steaks (chilled).

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

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

 — text deleted

CHAPTER 10.3.

GYRODACTYLOSIS

EU position

The EU thanks the Aquatic Commission for the inclusion of points i) and j) in Article 10.3.3 and supports the adoption of the amended chapter but has a comment on Article 3 points g and h:

The EU would propose that the original wording of points g) and h) of Article 10.3.3 are retained, but with "7.5" replaced by "25".

Rationale:

The crucial risk mitigation measure for these two products are the the salinity of the water in which the animals are kept before harvesting. According to scientific studies (Arnulf Soleng and Tor A. Bakke, Salinity tolerance of *Gyrodactylus salaris* (Platyheminthes, Monogenea: laboratory studies, Can. J. Fish. Aquat. Sci. 54 1837-1854 (1997)) *Gyrodactylus salaris* will only survive for considerably less than an hour if kept in full strength seawater. Consequently, the EU finds it inadequate to add the criterion that the animals must have been reared in such water for at least 2 months. This is also a considerably longer time period than what is required for live animals in Article 10.3.8 which refers to 14 days in water of at least 25 ppt. The requirements should not be more restrictive for products than those for live animals. Furthermore, the term "full strength seawater" is a rather ambiguous one and in the interest of maintaining coherence between the different Articles of the Chapter, the EU would prefer to refer to ppt. 25 ppt.

[...]

Article 10.3.3.

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

1. *Competent Authorities* should not require any gyrodactylosis related conditions, regardless of the gyrodactylosis status of the *exporting country, zone or compartment* when authorising the importation or transit of the following *aquatic animals* and *aquatic animal products* from the species referred to in Article 10.3.2. intended for any purpose and complying with Article 5.3.1.:
 - ~~a) [commodities treated in a manner that inactivates the pathogenic agent e.g. leather made from fish skin, pasteurised products and some ready-to-eat meals; and fish oil and fish meal intended for use in feed;~~
 - ~~b) chilled products of fish, where the head, fins and skin have been removed] (under study).~~
 - a) heat sterilised, hermetically sealed fish products (i.e. a heat treatment at 121°C for at least 3.6 minutes or any time/temperature equivalent);
 - b) pasteurised fish products that have been subjected to a heat treatment at 63°C for at least 30 minutes (or any time/temperature equivalent which has been demonstrated to inactivate *G. salaris*);

- c) mechanically dried, eviscerated fish (i.e. a heat treatment at 100°C for at least 30 minutes or any time/temperature equivalent which has been demonstrated to inactivate *G. salaris*);
 - d) naturally dried, eviscerated fish (i.e. sun-dried or wind-dried);
 - e) frozen eviscerated fish that have been subjected to -18°C or lower temperatures;
 - f) frozen fish fillets or steaks that have been subjected to -18°C or lower temperatures;
 - g) chilled eviscerated fish that have been reared for at least 2 months in full strength seawater harvested from seawater of at least 7.5 ppt or higher;
 - h) chilled fish fillets or steaks derived from fish reared for at least 2 months in harvested from full strength seawater of at least 7.5 ppt or higher;
 - i) chilled fish products from which the skin, fins and gills have been removed;
 - j) fish roe;
 - k) fish oil;
 - l) fish meal; and
 - m) fish skin leather.
2. When authorising the importation or transit of *aquatic animals* and *aquatic animal products* of a species referred to in Article 10.3.2., other than those referred to in point 1 of Article 10.3.3., *Competent Authorities* should require the conditions prescribed in Articles 10.3.7. to 10.3.12. relevant to the gyrodactylosis status of the *exporting country, zone or compartment*.
 3. When considering the importation or transit of *aquatic animals* and *aquatic animal products* from an *exporting country, zone or compartment* not declared free of gyrodactylosis of a species not covered in Article 10.3.2. but which could reasonably be expected to pose a *risk* of transmission for gyrodactylosis, *Competent Authorities* should conduct a *risk analysis* in accordance with the recommendations in the *Aquatic Code*. The *exporting country* should be informed of the outcome of this assessment.

[...]

Article 10.3.12.

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

1. *Competent Authorities* should not require any gyrodactylosis related conditions, regardless of the gyrodactylosis status of the *exporting country, zone or compartment* when authorising the importation or transit of the following *commodities* which have been prepared and packaged for retail trade and complying with Article 5.3.2.:
 - a) ~~[fish (chilled or frozen);~~
 - b) ~~fillets or cutlets (chilled or frozen);~~
 - e) ~~dried fish (including air dried, flame dried and sun dried);~~
 - d) ~~smoked salmonids] (under study).~~

a) no commodities listed.

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

2. When importing *aquatic animals* or *aquatic animal products*, other than those referred to in point 1 above, of the species referred to in Article 10.3.2. from a country, ~~zone~~ or *compartment* not declared free from gyrodactylosis, the *Competent Authority* of the *importing country* should assess the *risk* and apply appropriate *risk* mitigation measures.

— text deleted

CHAPTER 10.4.

INFECTIOUS HAEMATOPOIETIC NECROSIS

EU position

The EU can support the adoption of the amended chapter.

[...]

Article 10.4.3.

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

1. *Competent Authorities* should not require any IHN related conditions, regardless of the IHN status of the *exporting country, zone or compartment* when authorising the importation or transit of the following *aquatic animals and aquatic animal products* from the species referred to in Article 10.4.2. intended for any purpose and complying with Article 5.3.1.:
 - a) ~~[commodities treated in a manner that inactivates the disease agent e.g. leather made from fish skin, pasteurised products and some ready-to-eat meals; and fish oil and fish meal intended for use in feed] (under study).~~
 - a) heat sterilised, hermetically sealed fish products (i.e. a heat treatment at 121°C for at least 3.6 minutes or any time/temperature equivalent);
 - b) pasteurised fish products that have been subjected to a heat treatment at 90°C for at least 10 minutes (or any time/temperature equivalent which has been demonstrated to inactivate IHNV);
 - c) mechanically dried, eviscerated fish (i.e. a heat treatment at 100°C for at least 30 minutes or any time/temperature equivalent which has been demonstrated to inactivate IHNV);
 - d) fish oil;
 - e) fish meal; and
 - f) fish skin leather.
2. When authorising the importation or transit of *aquatic animals and aquatic animal products* of a species referred to in Article 10.4.2., other than those referred to in point 1 of Article 10.4.3., *Competent Authorities* should require the conditions prescribed in Articles 10.4.7. to 10.4.12. relevant to the IHN status of the *exporting country, zone or compartment*.
3. When considering the importation or transit of *aquatic animals and aquatic animal products* from an *exporting country, zone or compartment* not declared free of IHN of a species not covered in Article 10.4.2. but which could reasonably be expected to pose a *risk* of transmission for IHN, *Competent Authorities* should conduct a *risk analysis* in accordance with the recommendations in the *Aquatic Code*. The *exporting country* should be informed of the outcome of this assessment.

[...]

Article 10.4.12.

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

1. *Competent Authorities* should not require any IHN related conditions, regardless of the IHN status of the *exporting country*, *zone* or *compartment* when authorising the importation or transit of the following *commodities* which have been prepared and packaged for retail trade and complying with Article 5.3.2.:

~~a) [eviscerated fish (chilled or frozen);~~

~~b) fillets or cutlets (chilled or frozen);~~

~~e) dried eviscerated fish (including air dried, flame dried and sun dried)] (under study).~~

a) fish fillets or steaks (frozen or chilled).

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

2. When importing *aquatic animals* or *aquatic animal products*, other than those referred to in point 1 above, of the species referred to in Article 10.4.2. from a country, *zone* or *compartment* not declared free from IHN, the *Competent Authority* of the *importing country* should assess the *risk* and apply appropriate *risk* mitigation measures.

 — text deleted

CHAPTER 10.5.

INFECTIOUS SALMON ANAEMIA

EU position

The EU can support the adoption of the amended chapter.

[...]

Article 10.5.3.

Importation or transit of aquatic animals and aquatic animal products for any purpose from a country, zone or compartment not declared free from infectious salmon anaemia

1. *Competent Authorities* should not require any ISA related conditions, regardless of the ISA status of the *exporting country, zone or compartment* when authorising the importation or transit of the following *aquatic animals* and *aquatic animal products* from the species referred to in Article 10.5.2. intended for any purpose and complying with Article 5.3.1.:
 - a) ~~[commodities treated in a manner that inactivates the disease agent e.g. leather made from fish skin, pasteurised products and some ready-to-eat meals; and fish oil and fish meal intended for use in feed] (under study).~~
 - a) heat sterilised, hermetically sealed fish products (i.e. a heat treatment at 121°C for at least 3.6 minutes or any time/temperature equivalent);
 - b) pasteurised fish products that have been subjected to a heat treatment at 90°C for at least 10 minutes (or to any time/temperature equivalent which has been demonstrated to inactivate ISAV);
 - c) mechanically dried, eviscerated fish (i.e. a heat treatment at 100°C for 30 minutes or any time/temperature equivalent which has been demonstrated to inactivate ISAV);
 - d) fish oil;
 - e) fish meal; and
 - f) fish skin leather.
2. When authorising the importation or transit of *aquatic animals* and *aquatic animal products* of a species referred to in Article 10.5.2., other than those referred to in point 1 of Article 10.5.3., *Competent Authorities* should require the conditions prescribed in Articles 10.5.7. to 10.5.12. relevant to the ISA status of the *exporting country, zone or compartment*.
3. When considering the importation or transit of *aquatic animals* and *aquatic animal products* from an *exporting country, zone or compartment* not declared free of ISA of a species not covered in Article 10.5.2. but which could reasonably be expected to pose a *risk* of transmission for ISA, *Competent Authorities* should conduct a *risk analysis* in accordance with the recommendations in the *Aquatic Code*. The *exporting country* should be informed of the outcome of this assessment.

[...]

Article 10.5.12.

Importation of aquatic animals and aquatic animal products for retail trade for human consumption from a country, zone or compartment not declared free from infectious salmon anaemia

1. *Competent Authorities* should not require any ISA related conditions, regardless of the ISA status of the *exporting country, zone or compartment* when authorising the importation or transit of the following *commodities* which have been prepared and packaged for retail trade and complying with Article 5.3.2.:

a) ~~[eviscerated fish (chilled or frozen);~~

b) ~~fillets or cutlets (chilled or frozen);~~

e) ~~dried eviscerated fish (including air dried, flame dried and sun dried)] (under study).~~

a) fish fillets or steaks (frozen or chilled).

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

2. When importing *aquatic animals* or *aquatic animal products*, other than those referred to in point 1 above, of the species referred to in Article 10.5.2. from a country, *zone* or *compartment* not declared free from ISA, the *Competent Authority* of the *importing country* should assess the *risk* and apply appropriate *risk* mitigation measures.

— text deleted

CHAPTER 10.6.

KOI HERPESVIRUS DISEASE

EU position

The EU can support the adoption of the amended chapter.

[...]

Article 10.6.3.

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

1. *Competent Authorities* should not require any KHVD related conditions, regardless of the KHVD status of the *exporting country, zone or compartment* when authorising the importation or transit of the following *aquatic animals* and *aquatic animal products* from the species referred to in Article 10.6.2. intended for any purpose and complying with Article 5.3.1.:
 - a) ~~[commodities treated in a manner that inactivates the disease agent e.g. leather made from fish skin, pasteurised products and some ready-to-eat meals; and fish oil and fish meal intended for use in feed] (under study).~~
 - a) heat sterilised hermetically sealed fish products (i.e. a heat treatment at 121°C for at least 3.6 minutes or any time/temperature equivalent);
 - b) pasteurised fish products that have been subjected to heat treatment at 90°C for at least 10 minutes (or to any time/temperature equivalent which has been demonstrated to inactivate KHV);
 - c) mechanically dried eviscerated fish (i.e. a heat treatment at 100°C for at least 30 minutes or any time/temperature equivalent which has been demonstrated to inactivate KHV);
 - d) fish oil; and
 - e) fish meal.
2. When authorising the importation or transit of *aquatic animals* and *aquatic animal products* of a species referred to in Article 10.6.2., other than those referred to in point 1 of Article 10.6.3., *Competent Authorities* should require the conditions prescribed in Articles 10.6.7. to 10.6.12. relevant to the KHVD status of the *exporting country, zone or compartment*.
3. When considering the importation or transit of *aquatic animals* and *aquatic animal products* from an *exporting country, zone or compartment* not declared free of KHVD of a species not covered in Article 10.6.2. but which could reasonably be expected to pose a *risk* of transmission for KHVD, *Competent Authorities* should conduct a *risk analysis* in accordance with the recommendations in the *Aquatic Code*. The *exporting country* should be informed of the outcome of this assessment.

[...]

Article 10.6.12.

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

1. *Competent Authorities* should not require any KHVD related conditions, regardless of the KHVD status of the *exporting country, zone or compartment* when authorising the importation or transit of the following *commodities* which have been prepared and packaged for retail trade and complying with Article 5.3.2.:

a) ~~[eviscerated fish (chilled or frozen);~~

b) ~~fillets or cutlets (chilled or frozen);~~

e) ~~dried eviscerated fish (including air dried, flame dried and sun dried)] (under study).~~

a) fish fillets or steaks (frozen or chilled).

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

2. When importing *aquatic animals* or *aquatic animal products*, other than those referred to in point 1 above, of the species referred to in Article 10.6.2. 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.

 — text deleted

CHAPTER 10.7.

RED SEA BREAM IRIDOVIRAL DISEASE

EU position

The EU can support the adoption of the amended chapter.

[...]

Article 10.7.3.

Importation or transit of aquatic animals and aquatic animal products for any purpose from a country, zone or compartment not declared free from red sea bream iridovirus

1. *Competent Authorities* should not require any RSIVD related conditions, regardless of the RSIVD status of the *exporting country, zone or compartment* when authorising the importation or transit of the following *aquatic animals* and *aquatic animal products* from the species referred to in Article 10.7.2. intended for any purpose and complying with Article 5.3.1.:
 - ~~a) [commodities treated in a manner that inactivates the disease agent e.g. leather made from fish skin, pasteurised products and some ready-to-eat meals; and fish oil and fish meal intended for use in feed](under study).~~
 - a) heat sterilised hermetically sealed fish products (i.e. a heat treatment at 121°C for at least 3.6 minutes or any time/temperature equivalent);
 - b) pasteurised fish products that have been subjected to heat treatment at 90°C for at least 10 minutes (or any time/temperature equivalent which has been demonstrated to inactivate RSIV);
 - c) mechanically dried eviscerated fish (i.e. a heat treatment at 100°C for at least 30 minutes or any time/temperature equivalent which has been demonstrated to inactivate RSIV);
 - d) fish skin leather;
 - e) fish oil; and
 - f) fish meal.
2. When authorising the importation or transit of *aquatic animals* and *aquatic animal products* of a species referred to in Article 10.7.2., other than those referred to in point 1 of Article 10.7.3., *Competent Authorities* should require the conditions prescribed in Articles 10.7.7. to 10.7.12. relevant to the RSIVD status of the *exporting country, zone or compartment*.
3. When considering the importation or transit of *aquatic animals* and *aquatic animal products* from an *exporting country, zone or compartment* not declared free of RSIVD of a species not covered in Article 10.7.2. but which could reasonably be expected to pose a *risk* of transmission for RSIVD, *Competent Authorities* should conduct a *risk analysis* in accordance with the recommendations in the *Aquatic Code*. The *exporting country* should be informed of the outcome of this assessment.

[...]

Article 10.7.12.

Importation of aquatic animals and aquatic animal products for retail trade for human consumption from a country, zone or compartment not declared free from red sea bream iridovirus

1. *Competent Authorities* should not require any RSIVD related conditions, regardless of the RSIVD status of the *exporting country*, *zone* or *compartment* when authorising the importation or transit of the following *commodities* which have been prepared and packaged for retail trade and complying with Article 5.3.2.:

a) ~~[eviscerated fish (chilled or frozen)];~~

b) ~~fillets or cutlets (chilled or frozen);~~

e) ~~dried eviscerated fish (including air dried, flame dried and sun dried)] (under study).~~

a) fillets or steaks (chilled or frozen).

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

2. When importing *aquatic animals* or *aquatic animal products*, other than those referred to in point 1 above, of the species referred to in Article 10.7.2. from a country, *zone* or *compartment* not declared free from RSIVD, the *Competent Authority* of the *importing country* should assess the *risk* and apply appropriate *risk* mitigation measures.

— text deleted

CHAPTER 10.8.

SPRING VIRAEemia OF CARP

EU position

The EU can support the adoption of the amended chapter.

[...]

Article 10.8.3.

Importation or transit of aquatic animals and aquatic animal products for any purpose from a country, zone or compartment not declared free from spring viraemia of carp

1. *Competent Authorities* should not require any SVC related conditions, regardless of the SVC status of the *exporting country, zone or compartment* when authorising the importation or transit of the following *aquatic animals* and *aquatic animal products* from the species referred to in Article 10.8.2. intended for any purpose and complying with Article 5.3.1.:
 - a) ~~[commodities treated in a manner that inactivates the disease agent e.g. leather made from fish skin, pasteurised products and some ready-to-eat meals; and fish oil and fish meal intended for use in feed] (under study).~~
 - a) heat sterilised hermetically sealed fish products (i.e. a heat treatment at 121°C for at least 3.6 minutes or equivalent);
 - b) pasteurised fish products that have been subjected to heat treatment at 90°C for at least 10 minutes (or any time/temperature equivalent which has been demonstrated to inactivate SVCV);
 - c) mechanically dried eviscerated fish (i.e. a heat treatment at 100°C for at least 30 minutes or any time/temperature equivalent which has been demonstrated to inactivate SVCV);
 - d) fish oil; and
 - e) fish meal.
2. When authorising the importation or transit of *aquatic animals* and *aquatic animal products* of a species referred to in Article 10.8.2., other than those referred to in point 1 of Article 10.8.3., *Competent Authorities* should require the conditions prescribed in Articles 10.8.7. to 10.8.12. relevant to the SVC status of the *exporting country, zone or compartment*.
3. When considering the importation or transit of *aquatic animals* and *aquatic animal products* from an *exporting country, zone or compartment* not declared free of SVC of a species not covered in Article 10.8.2. but which could reasonably be expected to pose a *risk* of transmission for SVC, *Competent Authorities* should conduct a *risk analysis* in accordance with the recommendations in the *Aquatic Code*. The *exporting country* should be informed of the outcome of this assessment.

[...]

Article 10.8.12.

Importation of aquatic animals and aquatic animal products for retail trade for human consumption from a country, zone or compartment not declared free from spring viraemia of carp

1. *Competent Authorities* should not require any SVC related conditions, regardless of the SVC status of the *exporting country, zone or compartment* when authorising the importation or transit of the following *commodities* which have been prepared and packaged for retail trade and complying with Article 5.3.2.:

~~a) [eviscerated fish (chilled or frozen);~~

~~b) fillets or cutlets (chilled or frozen);~~

~~e) dried eviscerated fish (including air dried, flame dried and sun dried)] (under study).~~

a) fillets or steaks (chilled or frozen).

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

2. When importing *aquatic animals* or *aquatic animal products*, other than those referred to in point 1 above, of the species referred to in Article 10.8.2. from a country, *zone or compartment* not declared free from SVC, the *Competent Authority* of the *importing country* should assess the *risk* and apply appropriate *risk* mitigation measures.

— text deleted

CHAPTER 10.9.

VIRAL HAEMORRHAGIC SEPTICAEMIA

EU position

The EU can support the adoption of the amended chapter.

[...]

Article 10.9.3.

Importation or transit of aquatic animals and aquatic animal products for any purpose from a country, zone or compartment not declared free from viral haemorrhagic septicaemia

1. *Competent Authorities* should not require any ~~RSIVD~~ **VHSV** related conditions, regardless of the ~~RSIVD~~ **VHSV** status of the *exporting country, zone or compartment* when authorising the importation or transit of the following *aquatic animals* and *aquatic animal products* from the species referred to in Article 10.9.2. intended for any purpose and complying with Article 5.3.1.:
 - ~~a) [commodities treated in a manner that inactivates the disease agent e.g. leather made from fish skin, pasteurised products and some ready-to-eat meals; and fish oil and fish meal intended for use in feed] (under study).~~
 - a) heat sterilised, hermetically sealed fish products (i.e. a heat treatment at 121°C for at least 3.6 minutes or any time/temperature equivalent);
 - b) pasteurised fish products that have been subjected to a heat treatment at 90°C for at least 10 minutes (or to any time/temperature equivalent which has been demonstrated to inactivate VHSV);
 - c) mechanically dried, eviscerated fish (i.e. a heat treatment at 100°C for at least 30 minutes or any time/temperature equivalent which has been demonstrated to inactivate VHSV);
 - d) naturally dried, eviscerated fish (i.e. sun-dried or wind-dried);
 - e) fish oil;
 - f) fish meal; and
 - g) fish skin leather.
2. When authorising the importation or transit of *aquatic animals* and *aquatic animal products* of a species referred to in Article 10.9.2., other than those referred to in point 1 of Article 10.9.3., *Competent Authorities* should require the conditions prescribed in Articles 10.9.7. to 10.9.12. relevant to the RSIVD status of the *exporting country, zone or compartment*.
3. When considering the importation or transit of *aquatic animals* and *aquatic animal products* from an *exporting country, zone or compartment* not declared free of RSIVD of a species not covered in Article 10.9.2. but which could reasonably be expected to pose a *risk* of transmission for RSIVD,

Competent Authorities should conduct a *risk analysis* in accordance with the recommendations in the *Aquatic Code*. The *exporting country* should be informed of the outcome of this assessment.

[...]

Article 10.9.12.

Importation of aquatic animals and aquatic animal products for retail trade for human consumption from a country, zone or compartment not declared free from viral haemorrhagic septicaemia

1. *Competent Authorities* should not require any VHS related conditions, regardless of the VHS status of the *exporting country*, *zone* or *compartment* when authorising the importation or transit of the following *commodities* which have been prepared and packaged for retail trade and complying with Article 5.3.2.:

~~a) eviscerated fish (chilled or frozen);~~

~~b) fillets or cutlets (chilled or frozen);~~

~~e) dried eviscerated fish (including air dried, flame dried and sun dried) (under study).~~

a) fillets or steaks (chilled or frozen).

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

2. When importing *aquatic animals* or *aquatic animal products*, other than those referred to in point 1 above, of the species referred to in Article 10.9.2. from a country, *zone* or *compartment* not declared free from VHS, the *Competent Authority* of the *importing country* should assess the *risk* and apply appropriate *risk* mitigation measures.

 — text deleted

CHAPTER 11.1.

INFECTION WITH ABALONE HERPES-LIKE VIRUS

EU position

The EU can support the adoption of the amended chapter.

[...]

Article 11.1.3.

Importation or transit of aquatic animals and aquatic animal products for any purpose from a country, zone or compartment not declared free from abalone herpes-like virus

1. *Competent Authorities* should not require any abalone herpes-like virus related conditions, regardless of the abalone herpes-like virus status of the *exporting country, zone or compartment* when authorising the importation or transit of the following *aquatic animals* and *aquatic animal products* from the species referred to in Article 11.1.2. intended for any purpose and complying with Article 5.3.1.:
 - a) ~~[commodity(ies)] under study.~~
 - a) heat sterilised hermetically sealed abalone products (i.e. a heat treatment at 121°C for at least 3.6 minutes or any time/temperature equivalent);
 - b) mechanically dried abalone products (i.e. a heat treatment at 100°C for at least 30 minutes or any time/temperature equivalent which has been demonstrated to inactivate AbHV).
2. When authorising the importation or transit of *aquatic animals* and *aquatic animal products* of a species referred to in Article 11.1.2., other than those referred to in point 1 of Article 11.1.3., *Competent Authorities* should require the conditions prescribed in Articles 11.1.7. to 11.1.11. relevant to the abalone herpes-like virus status of the *exporting country, zone or compartment*.
3. When considering the importation or transit of *aquatic animals* and *aquatic animal products* from an *exporting country, zone or compartment* not declared free of infection with abalone herpes-like virus of a species not covered in Article 11.1.2. but which could reasonably be expected to pose a *risk* of transmission for abalone herpes-like virus, *Competent Authorities* should conduct a *risk analysis* in accordance with the recommendations in the *Aquatic Code*. The *exporting country* should be informed of the outcome of this assessment.

[...]

Article 11.1.11.

Importation of aquatic animals and aquatic animal products for retail trade for human consumption from a country, zone or compartment not declared free from abalone herpes-like virus

1. *Competent Authorities* should not require any abalone herpes-like virus related conditions, regardless of the abalone herpes-like virus status of the *exporting country, zone or compartment* when authorising the

importation or transit of the following *commodities* which have been prepared and packaged for retail trade and complying with Article 5.3.2.:

a) ~~[commodity(ies)] under study.~~

a) off the shell, eviscerated abalone meat (chilled or frozen).

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

2. When importing *aquatic animals* or *aquatic animal products*, other than those referred to in point 1 above, of the species referred to in Article 11.1.2. from a country, *zone* or *compartment* not declared free from abalone herpes-like virus, the *Competent Authority* of the *importing country* should assess the *risk* and apply appropriate *risk* mitigation measures.

— text deleted

CHAPTER 11.2.

INFECTION WITH *BONAMIA EXITIOSA***EU position**

The EU can support the adoption of the amended chapter.

[...]

Article 11.2.3.

Importation or transit of aquatic animals and aquatic animal products for any purpose from a country, zone or compartment not declared free from *B. exitiosa*

1. *Competent Authorities* should not require any *B. exitiosa* related conditions, regardless of the *B. exitiosa* status of the *exporting country, zone or compartment* when authorising the importation or transit of the following *aquatic animals* and *aquatic animal products* from the species referred to in Article 11.2.2. intended for any purpose and complying with Article 5.3.1.:
 - ~~a) [commodities treated in a manner that inactivates the pathogenic agent e.g. canned or pasteurised products] (under study).~~
 - a) frozen oyster meat; and
 - b) frozen half-shell oysters.
2. When authorising the importation or transit of *aquatic animals* and *aquatic animal products* of a species referred to in Article 11.2.2., other than those referred to in point 1 of Article 11.2.3., *Competent Authorities* should require the conditions prescribed in Articles 11.2.7. to 11.2.11. relevant to the *B. exitiosa* status of the *exporting country, zone or compartment*.
3. When considering the importation or transit of *aquatic animals* and *aquatic animal products* from an *exporting country, zone or compartment* not declared free of *B. exitiosa* of a species not covered in Article 11.2.2. but which could reasonably be expected to pose a *risk* of transmission for *B. exitiosa*, *Competent Authorities* should conduct a *risk analysis* in accordance with the recommendations in the *Aquatic Code*. The *exporting country* should be informed of the outcome of this assessment.

[...]

Article 11.2.11.

Importation of aquatic animals and aquatic animal products for retail trade for human consumption from a country, zone or compartment not declared free from *B. exitiosa*

1. *Competent Authorities* should not require any *B. exitiosa* related conditions, regardless of the *B. exitiosa* status of the *exporting country, zone or compartment* when authorising the importation or transit of the following *commodities* which have been prepared and packaged for retail trade and complying with Article 5.3.2.:

a) ~~[off the shell (chilled or frozen);~~

b) ~~half-shell (chilled)] (under study).~~

a) chilled oyster meat; and

b) chilled half-shell oysters.

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

2. When importing *aquatic animals* or *aquatic animal products*, other than those referred to in point 1 above, of the species referred to in Article 11.2.2. from a country, *zone* or *compartment* not declared free from *B. exitiosa*, the *Competent Authority* of the *importing country* should assess the *risk* and apply appropriate *risk* mitigation measures.

— text deleted

CHAPTER 11.4.

INFECTION WITH *MARTEILIA REFRINGENS***EU position**

The EU can support the adoption of the amended chapter.

[...]

Article 11.4.3.

Importation or transit of aquatic animals and aquatic animal products for any purpose from a country, zone or compartment not declared free from *M. refringens*

1. *Competent Authorities* should not require any *M. refringens* related conditions, regardless of the *M. refringens* status of the *exporting country, zone or compartment* when authorising the importation or transit of the following *aquatic animals* and *aquatic animal products* from the species referred to in Article 11.4.2. intended for any purpose and complying with Article 5.3.1.:
 - a) ~~[commodities treated in a manner that inactivates the pathogenic agent e.g. canned or pasteurised products] (under study).~~
 - a) heat sterilised hermetically sealed mollusc products (i.e. a heat treatment at 121°C for at least 3.6 minutes or any time /temperature equivalent).
2. When authorising the importation or transit of *aquatic animals* and *aquatic animal products* of a species referred to in Article 11.4.2., other than those referred to in point 1 of Article 11.4.3., *Competent Authorities* should require the conditions prescribed in Articles 11.4.7. to 11.4.11. relevant to the *M. refringens* status of the *exporting country, zone or compartment*.
3. When considering the importation or transit of *aquatic animals* and *aquatic animal products* from an *exporting country, zone or compartment* not declared free of *M. refringens* of a species not covered in Article 11.4.2. but which could reasonably be expected to pose a *risk* of transmission for *M. refringens*, *Competent Authorities* should conduct a *risk analysis* in accordance with the recommendations in the *Aquatic Code*. The *exporting country* should be informed of the outcome of this assessment.

[...]

Article 11.4.11.

Importation of aquatic animals and aquatic animal products for retail trade for human consumption from a country, zone or compartment not declared free from *M. refringens*

1. *Competent Authorities* should not require any *M. refringens* related conditions, regardless of the *M. refringens* status of the *exporting country, zone or compartment* when authorising the importation or transit of the following *commodities* which have been prepared and packaged for retail trade and complying with Article 5.3.2.:
 - a) ~~[off the shell (chilled or frozen);~~

~~b) half shell (chilled)] (under study).~~

a) mollusc meat (chilled or frozen); and

b) half-shell oyster (chilled or frozen).

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

2. When importing *aquatic animals* or *aquatic animal products*, other than those referred to in point 1 above, of the species referred to in Article 11.4.2. from a country, *zone* or *compartment* not declared free from *M. refringens*, the *Competent Authority* of the *importing country* should assess the *risk* and apply appropriate *risk* mitigation measures.

— text deleted

CHAPTER 11.5.

INFECTION WITH *PERKINSUS MARINUS***EU position****The EU can support the adoption of the amended chapter.**

[...]

Article 11.5.3.

Importation or transit of aquatic animals and aquatic animal products for any purpose from a country, zone or compartment not declared free from *P. marinus*

1. *Competent Authorities* should not require any *P. marinus* related conditions, regardless of the *P. marinus* status of the *exporting country, zone or compartment* when authorising the importation or transit of the following *aquatic animals* and *aquatic animal products* from the species referred to in Article 11.5.2. intended for any purpose and complying with Article 5.3.1.:
 - a) ~~[commercially sterile canned or other heat treated products] (under study).~~
 - a) heat sterilised hermetically sealed mollusc products (i.e. a heat treatment at 121°C for at least 3.6 minutes or any time /temperature equivalent).
2. When authorising the importation or transit of *aquatic animals* and *aquatic animal products* of a species referred to in Article 11.5.2., other than those referred to in point 1 of Article 11.5.3., *Competent Authorities* should require the conditions prescribed in Articles 11.5.7. to 11.5.11. relevant to the *P. marinus* status of the *exporting country, zone or compartment*.
3. When considering the importation or transit of *aquatic animals* and *aquatic animal products* from an *exporting country, zone or compartment* not declared free of *P. marinus* of a species not covered in Article 11.5.2. but which could reasonably be expected to pose a *risk* of transmission for *P. marinus*, *Competent Authorities* should conduct a *risk analysis* in accordance with the recommendations in the *Aquatic Code*. The *exporting country* should be informed of the outcome of this assessment.

[...]

Article 11.5.11.

Importation of aquatic animals and aquatic animal products for retail trade for human consumption from a country, zone or compartment not declared free from *P. marinus*

1. *Competent Authorities* should not require any *P. marinus* related conditions, regardless of the *P. marinus* status of the *exporting country, zone or compartment* when authorising the importation or transit of the following *commodities* which have been prepared and packaged for retail trade and complying with Article 5.3.2.:
 - a) ~~[chemically preserved products (e.g. smoked, salted, pickled, marinated);~~

~~b) non-commercially sterile products (e.g. ready prepared meals) that have been heat treated in a manner to ensure the inactivation of the parasite] (under study).~~

a) mollusc meat (chilled and frozen); and

b) half-shell oysters (chilled and frozen).

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

2. When importing *aquatic animals* or *aquatic animal products*, other than those referred to in point 1 above, of the species referred to in Article 11.5.2. from a country, *zone* or *compartment* not declared free from *P. marinus*, the *Competent Authority* of the *importing country* should assess the *risk* and apply appropriate *risk* mitigation measures.

— text deleted

CHAPTER 11.6.

INFECTION WITH *PERKINSUS OLSENI***EU position****The EU can support the adoption of the amended chapter.**

[...]

Article 11.6.3.

Importation or transit of aquatic animals and aquatic animal products for any purpose from a country, zone or compartment not declared free from *P. olseni*

1. *Competent Authorities* should not require any *P. olseni* related conditions, regardless of the *P. olseni* status of the *exporting country, zone or compartment* when authorising the importation or transit of the following *aquatic animals* and *aquatic animal products* from the species referred to in Article 11.6.2. intended for any purpose and complying with Article 5.3.1.:
 - a) ~~[commercially sterile canned or other heat treated products] (under study).~~
 - a) heat sterilised hermetically sealed mollusc products (i.e. a heat treatment at 121°C for at least 3.6 minutes or any time/temperature equivalent).
2. When authorising the importation or transit of *aquatic animals* and *aquatic animal products* of a species referred to in Article 11.6.2., other than those referred to in point 1 of Article 11.6.3., *Competent Authorities* should require the conditions prescribed in Articles 11.6.7. to 11.6.11. relevant to the *P. olseni* status of the *exporting country, zone or compartment*.
3. When considering the importation or transit of *aquatic animals* and *aquatic animal products* from an *exporting country, zone or compartment* not declared free of *P. olseni* of a species not covered in Article 11.6.2. but which could reasonably be expected to pose a *risk* of transmission for *P. olseni*, *Competent Authorities* should conduct a *risk analysis* in accordance with the recommendations in the *Aquatic Code*. The *exporting country* should be informed of the outcome of this assessment.

[...]

Article 11.6.11.

Importation of aquatic animals and aquatic animal products for retail trade for human consumption from a country, zone or compartment not declared free from *P. olseni*

1. *Competent Authorities* should not require any *P. olseni* related conditions, regardless of the *P. olseni* status of the *exporting country, zone or compartment* when authorising the importation or transit of the following *commodities* which have been prepared and packaged for retail trade and complying with Article 5.3.2.:
 - a) ~~[chemically preserved products (e.g. smoked, salted, pickled, marinated)];~~

~~b) non-commercially sterile products (e.g. ready prepared meals) that have been heat treated in a manner to ensure the inactivation of the parasite] (under study).~~

a) mollusc meat (chilled and frozen); and

b) half-shell molluscs (chilled and frozen).

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

2. When importing aquatic animals or aquatic animal products, other than those referred to in point 1 above, of the species referred to in Article 11.6.2. from a country, zone or compartment not declared free from *P. olseni*, the Competent Authority of the importing country should assess the risk and apply appropriate risk mitigation measures.

— text deleted

CHAPTER 11.7.

INFECTION WITH *XENOHALIOTIS CALIFORNIENSIS***EU position**

The EU can support the adoption of the amended chapter.

[...]

Article 11.7.3.

Importation or transit of aquatic animals and aquatic animal products for any purpose from a country, zone or compartment not declared free from *X. californiensis*

1. *Competent Authorities* should not require any *X. californiensis* related conditions, regardless of the *X. californiensis* status of the *exporting country, zone or compartment* when authorising the importation or transit of the following *aquatic animals* and *aquatic animal products* from the species referred to in Article 11.7.2. intended for any purpose and complying with Article 5.3.1.:
 - a) ~~[commodities treated in a manner that inactivates the disease agent e.g. canned or pasteurised products] (under study).~~
 - a) heat sterilised hermetically sealed abalone products (i.e. a heat treatment at 121°C for at least 3.6 minutes or any time/temperature equivalent).
2. When authorising the importation or transit of *aquatic animals* and *aquatic animal products* of a species referred to in Article 11.7.2., other than those referred to in point 1 of Article 11.7.3., *Competent Authorities* should require the conditions prescribed in Articles 11.7.7. to 11.7.11. relevant to the *X. californiensis* status of the *exporting country, zone or compartment*.
3. When considering the importation or transit of *aquatic animals* and *aquatic animal products* from an *exporting country, zone or compartment* not declared free of *X. californiensis* of a species not covered in Article 11.7.2. but which could reasonably be expected to pose a *risk* of transmission for *X. californiensis*, *Competent Authorities* should conduct a *risk analysis* in accordance with the recommendations in the *Aquatic Code*. The *exporting country* should be informed of the outcome of this assessment.

[...]

Article 11.7.11.

Importation of aquatic animals and aquatic animal products for retail trade for human consumption from a country, zone or compartment not declared free from *X. californiensis*

1. *Competent Authorities* should not require any *X. californiensis* related conditions, regardless of the *X. californiensis* status of the *exporting country, zone or compartment* when authorising the importation or

transit of the following *commodities* which have been prepared and packaged for retail trade and complying with Article 5.3.2.:

a) ~~[off the shell, eviscerated abalone (chilled or frozen)] (under study).~~

a) off the shell, eviscerated abalone (chilled or frozen).

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

2. When importing *aquatic animals* or *aquatic animal products*, other than those referred to in point 1 above, of the species referred to in Article 11.7.2. from a country, ~~zone~~ or *compartment* not declared free from *X. californiensis*, the *Competent Authority* of the *importing country* should assess the *risk* and apply appropriate *risk* mitigation measures.

— text deleted

CHAPTER 2.1.1.

INFECTION WITH *BATRACHOCHYTRIUM DENDROBATIDIS*

EU position

The EU can support the adoption of the amended chapter.

1. Scope

For the purposes of this chapter, chytridiomycosis as a disease resulting from infection with the zoosporic fungus *Batrachochytrium dendrobatidis* (Fungi, Chytridiomycota, Rhizophydiales). The recommendations in this chapter apply to all species of Anura (frogs and toads), Caudata (salamanders, newts and sirens) and Gymnophiona (caecilians).

All protocols and reagents described in this chapter are available from the OIE Reference Laboratory.

All sampling, histology, histochemistry and TaqMan techniques have been validated (Hyatt *et al.*, 2007).

2. Disease information**2.1. Agent factors****2.1.1. Aetiological agent, agent strains**

Batrachochytrium dendrobatidis (Bd) was first described in 1998 and 1999 (Berger *et al.*, 1998; Longcore *et al.*, 1999) and is now the accepted cause of the potentially fatal disease chytridiomycosis (refer to the Australian Threat Abatement Plan for chytridiomycosis, which can be found at: <http://www.environment.gov.au/biodiversity/threatened/publications/tap/pubs/chytrid-background.pdf>). The disease has led to mass mortalities, population declines, and extinction (up to eight species in Australia) of amphibian populations and species around the world (Fisher *et al.*, 2009). Bd is now recognised for its ability to spread rapidly through amphibian populations (Lips *et al.*, 2006; Skerratt *et al.*, 2007), cause high rates of mortality (Lips *et al.*, 2006; Scholegel *et al.*, 2006) and persist at low host densities (Scholegel *et al.*, 2006; Woodhams & Alford, 2005). To date, Bd has been identified in all continents (36 countries) where wild amphibian populations exist. Bd infects over 350 amphibian species and has been implicated in driving the decline of over 200 of these species (Skerratt *et al.*, 2007).

Pathogenesis of this skin disease has been difficult to determine as no consistent pathological changes in internal organs have been detected. Two, not mutually exclusive, hypotheses have been published to explain the cause of death. The first is that Bd releases proteolytic enzymes or other active compounds that are absorbed through the permeable skin of the frog. The second suggests that damage to skin function results in disturbance of water and electrolyte balance (osmoregulation) of water or electrolyte balance resulting in death (Berger *et al.*, 1998). A recent report (Voyles *et al.*, 2009) presents data that support the second hypothesis.

2.1.2. Survival outside the host

Bd has been speculated, but not confirmed, to exist outside its host. Bd DNA has been recovered from rocks (Lips *et al.*, 2006), and Bd can be grown in the laboratory conditions on bird feathers and moist soil (Johnson & Speare, 2003; 2005; Lips *et al.*, 2006). Bd was detected in laboratory-based mesocosms from which Bd-infected *Rana sphenoccephala* had been removed.

2.1.3. Stability of the agent (effective inactivation methods)

Bd is susceptible to a broad range of chemical and physical treatments (Phillott *et al.*, 2010). Effective solutions include the quaternary ammonium compound, didecyl dimethyl ammonium chloride (e.g. Path X, 1 in 500 dilution for 30 seconds) or benzalkonium chloride (e.g. F10, 1 in 1500 dilution for 1 minute). Sodium hypochlorite is effective at concentrations of 1% and above. Also effective is exposure to 70% ethanol and 1 mg ml⁻¹ Virkon for 20 seconds. These chemicals can be used for disinfection in the laboratory, in amphibian husbandry, and in field work. For example, alcohol wipes can be used to disinfect scissors, calipers and other instruments between animals. Cultures of Bd do not survive complete drying, but in practice persistence of water in droplets allows survival of the pathogen up to 3 hours after 'drying' (Johnson *et al.*, 2003). Heating to above 37°C for 4 hours results in death of sporangia. Ultraviolet light used routinely for killing bacteria, fungi and viruses is ineffective.

Bd can grow, but may not thrive, on many different nitrogen sources (Piotrowski *et al.*, 2004) and in sterile lake water (Johnson & Speare, 2003; 2005; Piotrowski *et al.*, 2004). Additional anecdotal findings suggest it can survive and grow as a saprobe in the absence of frogs. Zoospores can remain motile for over 24 hours with approximately 50% and 5% remaining motile after 18 hours and 24 hours, respectively (Piotrowski *et al.*, 2004).

2.1.4. Life cycle

The life cycle of Bd has two main stages: the motile, waterborne, short-lived zoospore for dispersal, and the stationary, monocentric thallus, which develops into a zoosporangium for asexual amplification. Bd is adapted to living in the stratified epidermis of the skin. Thalli live inside epidermal cells, initially parasitising cells a few layers deep, and have a rate of development that coincides with the maturing of the cell as it moves outwards and keratinises. Bd grows initially in living cells but thalli complete their development as zoosporangia in dead superficial keratinised cells that lack organelles. Discharge tubes have the ability to merge with and dissolve the epidermal cell membrane and open on to the surface of the cell, usually the surface distal from the body. The distribution of sporangia in adults and tadpoles shows that a stratified, keratinising epidermis is a requirement of Bd when occurring as a parasite (Berger *et al.*, 1998; Marantelli *et al.*, 2004). Immature sporangia can also grow within the deeper cells that contain prekeratin. Resistant resting spores have not been found.

2.2. Host factors

2.2.1. Susceptible host species

As stated above, Bd has been identified on six continents, from two amphibian orders, 14 families and in over 350 species. Collectively, it can be stated that most, if not all, anurans and urodeles are susceptible to Bd infection; morbidity and mortality varies between species. Mortality in tadpoles has not, in the main, been reported (there is one report stating otherwise [Blaustein *et al.*, 2005]) and, to date, viable Bd has not been detected on eggs.

2.2.2. Susceptible stages of the host

Susceptible stages of the host are all age classes, larvae, metamorphs and adults (not eggs).

2.2.3. Species or subpopulation predilection (probability of detection)

Species vary greatly in their innate susceptibility. The microhabitat and environment that a species inhabits are also key determinants for infection and disease, as virulence is reduced at warmer temperatures (>26°C).

With the exception of eggs, Bd can be detected in all age classes, larvae, metamorphs and adults via a variety of techniques – visual inspection of tadpoles, light microscopy, immunohistochemistry, electron microscopy, immuno-electron microscopy and quantitative polymerase chain reaction.

2.2.4. Target organs and infected tissue

The target organ for collecting samples is the skin (Hyatt *et al.*, 2007). One reported clinical sign of chytridiomycosis is excess sloughing of skin from the epidermal surface. The sloughed skin is frequently derived from ventral surfaces of the abdomen, limbs and feet and is usually identified (Berger *et al.*, 2005a; 2005b; Pessier *et al.*, 1999) by hyperkeratosis and the presence of zoosporangia. Collection of skin at these sites is an obvious way to maximise the chances of detecting Bd.

2.2.5. Persistent infection with lifelong carriers

Some amphibian species carry sustained infections in the wild with little or no evidence of either morbidity or mortality. Whether such species are lifelong carriers is not known.

2.2.6. Vectors

Amphibian species can act as reservoirs of Bd without displaying signs of infection. The ability of Bd to survive in the environment or in sympatric species enables it to drive some species to extinction. Bd is a significant risk factor for approximately 97% of critically endangered amphibians (Smith *et al.*, 2006) and as stated above has been credited for causing the extinction of some species of amphibians (Fisher *et al.*, 2009).

2.2.7. Known or suspected wild aquatic animal carriers

Only amphibians are identified as sources and carriers of Bd.

2.3. Disease pattern

2.3.1. Transmission mechanisms

Bd infection can spread by animal to animal contact or via contact with waterborne motile zoospores. Long distance transmission is understood to occur by means other than water, including translocation of animals during international trade (Rowley *et al.*, 2007) and potentially by movement of contaminated water or moist soil.

2.3.2. Prevalence

Prevalence varies greatly with season, location and species. In infected populations, prevalence is usually at least 5% but 90% is not uncommon. In tropical regions, higher prevalences occur in winter and at higher altitudes. Details on its prevalence, based on intensive and global surveillance (via qPCR), can be found at <http://www.spatalepidemiology.net/bd-maps/>.

2.3.3. Geographical distribution

Bd infections have been reported on all continents (refer above). Up-to-date information (countries, regions, species – number tested, positive/negative, method of identification, year of report, International Union for Conservation of Nature [IUCN] status and locations are listed on <http://www.spatalepidemiology.net/bd-maps/>). At the time of publication of this chapter Bd has been reported in 72 countries, please refer to above web site for details.

2.3.4. Mortality and morbidity

Some species of amphibians coexist with Bd whereas others succumb to disease (Davidson *et al.*, 2003; Hanselmann *et al.*, 2004; Retallick *et al.*, 2004). Even within species, some populations can coexist with Bd whereas others decline to extinction (Briggs *et al.*, 2005). Bd should be regarded as a highly infectious and potentially fatal pathogen that can drive a species to extinction.

2.3.5. Environmental factors

Outbreaks of chytridiomycosis are, in the main, associated with seasons (cooler months), altitude (most declines are generally restricted to high-altitude populations), and breeding habitat. In respect to the latter declines are pronounced in stream-dwelling species. Severity of the population impact of the disease is also correlated with small distributions of populations that are less fecund (Williams & Hero, 1998). Note: operating within these identified 'factors' are more complex interactions and apparently inherent differences in susceptibility.

2.4. Control and prevention

2.4.1. Vaccination

None available.

2.4.2. Chemotherapy

Bd is susceptible to a range of antifungal agents and low levels of heat (>30°C) when tested *in vitro*, but there are few proven methods for clearing amphibians of Bd (Berger *et al.*, 2010). Heating (32°C for 5 days and 37°C for two periods of 8 hours, 24 hours apart) has been demonstrated as effective against chytridiomycosis in two amphibian species (Phillott *et al.*, 2010; Woodhams *et al.*, 2003). Heat should be tested and optimised in a range of species, and many temperate amphibians would not tolerate 37°C. Although itraconazole (0.01% for 5 minutes a day for 11 days) and formalin/malachite green baths both appear effective treatments for post-metamorphic frogs (Forzan *et al.*, 2008;

Hohreiter & Rigg 2001; Nichols & Lamirande, 2009; Parker *et al.*, 2002), these trials were not rigorous and toxicity is an issue, particularly for formalin/malachite green. However, itraconazole baths have been widely used in amphibian rescue and conservation programmes and anecdotal evidence suggests that it is effective for adults and subadults. Successful treatment of infected tadpoles of one species has been reported in a controlled trial using low dose itraconazole (1.5 mg litre⁻¹), but may have been associated with depigmentation (Garner *et al.*, 2009). Note: the water-soluble formulation of itraconazole is not widely available. Safe and effective treatment against Bd infections (adults and tadpoles) has been reported for voriconazole. The treatment consists of spraying once daily for 7 days at 1.25 mg litre⁻¹ (Martel *et al.*, 2010).

2.4.3. Immunostimulation

Not tested.

2.4.4. Resistance breeding

No resistance breeding programmes have been initiated; however some countries are developing national plans to control chytridiomycosis. These plans include implementing hygiene protocols for individuals dealing with wild amphibians and recognising that pristine, isolated areas containing highly vulnerable species need to be protected from Bd. Another activity (Genwin 2008) involves establishing captive populations for highly susceptible species threatened by advancing epidemic waves or already infected populations suffering slow, steady declines (the Amphibian Ark project: <http://www.amphibianark.org/>).

2.4.5. Restocking with resistant species

The presence of resistant species is thought to increase transmission to endangered species. Captive breeding programs have been undertaken to restock wild populations of endangered frogs.

2.4.6. Blocking agents

Not tested.

2.4.7. Disinfection

A summary of disinfection protocols are summarised in a recent paper by (Phillot *et al.*, 2010) where protocols are detailed in respect to capture, handling and holding of wild amphibians; skin disinfection before and after invasive procedures; marking of frogs; disinfection of skin, sealing of wounds and treatment of accessory equipment Table 1.1). These protocols are designed to provide within-site hygiene measures to minimise risk of Bd transmission among individuals. The paper also details protocols for entry, exit and between-site hygiene measures to prevent increased risk of Bd spread above background levels.

Table 1.1: Disinfection strategies suitable for killing *Bd* (Retallick *et al.*, 2004)

Application	Disinfectant	Concentration	Time
Disinfecting surgical equipment and other instruments (e.g. scales, callipers)	Benzalkonium chloride	2 mg ml ⁻¹	1 minute
	Ethanol	70%	1 minute
Disinfecting collection equipment and containers	Sodium hypochlorite	1%	1 minute
	Path X or Quaternary ammonium compound 128	1 in 500 dilution	0.5 minutes
	Trigene	1 in 5000 dilution	1 minute
	F10	1 in 5000 dilution	1 minute
	Virkon	2 mg ml ⁻¹	1 minute
	Potassium permanganate	1%	10 minutes
	Complete drying		>3 hours
	Heat	60°C	30 minutes
	Heat	37°C	8 hours
Disinfecting footwear	Sodium hypochlorite	1%	1 minute
	Path X or Quaternary ammonium compound 128	1 in 500 dilution	0.5 minutes
	Trigene	1 in 5000 dilution	1 minute
	F10	1 in 5000 dilution	1 minute
	Complete drying		>3 hours
Disinfecting cloth	Hot wash	60°C or greater	30 minutes

2.4.8. General husbandry practices

Where captive facilities pose a threat to wild populations the following should be observed:

- i) Water, wastes and other materials must be treated to kill *Bd* or stored, disposed of or removed to a site where it does not pose a threat. For example infected waters may be released to closed sewers that expel directly to a marine environment.
- ii) Livestock including food animals must be enclosed in such a way as to prevent escape to the external environment.
- iii) Persons, equipment and materials leaving the facility must undergo cleaning adequate to prevent the passage of *Bd*.
- iv) Amphibians being released to the wild must be kept in strict isolation and tested for *Bd* prior to release. If any specimens test positive treatment and retesting of all animals should be undertaken until they are clear of infection.
- v) When planning facilities, consideration of location and design should take into account the local status of *Bd* as well as the availability of safe supply of water and consumables and the safe disposal of wastes. For example farming operations are best undertaken in tropical lowland areas where *Bd* presents less of a threat to local species and where access to safer disposal of wastes is more readily available.
- vi) Where *Bd* is present locally care must be taken not to increase the load or introduce new strains to the local environment. Farming operations should ensure the load in waste water and other

materials is reduced to ambient levels before disposal to the environment and that new arrivals that potentially carry new stains are treated and cleared prior to introduction to other amphibians.

Where external sources represent a threat to the captive collections the following should be observed:

- i) Water and other incoming persons and materials should be treated to remove Bd unless they are known to come from safe sources.
- ii) Incoming amphibians should be isolated and tested. If any specimens test positive treatment and retesting of the entire group should be undertaken until they are clear of infection.

3. Sampling

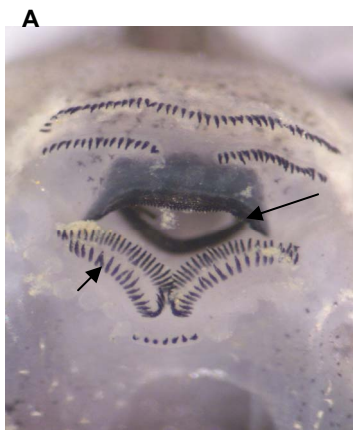
3.1. Selection of individual specimens

Bd replicates in the keratinised mouth parts of tadpoles and in the main on the ventral surfaces and toes of adult amphibians. The target organs are toe-clips (not recommended for ethical reasons), swabbing of skin (adults) and mouthparts (tadpoles), and bathing of whole animals (adults and tadpoles) (Hyatt *et al.*, 2007).

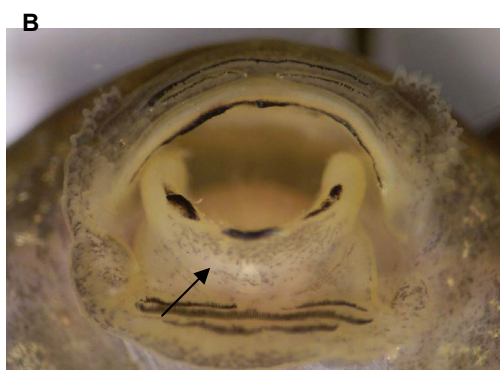
3.1.1. Toe clips (adults), oral discs (tadpoles/larvae, and swabs (adults and tadpoles).

Toe-clips for use in real-time TaqMan PCR can be excised in the field and stored dry or in 70% alcohol. Alternatively they can be harvested into 1.5 ml tubes and stored at -80°C prior to DNA extraction. Toe-clips to be processed for histology should be fixed in 10% neutral buffered formalin.

In the field, tadpole mouths (oral discs – refer to photographs below) can be swabbed using fine tip swabs¹. Oral discs can also be dissected and air dried onto filter paper. Infected animals can be identified via depigmentation (arrow, photograph B) of the jaw sheaths, shortened teeth or loss of teeth (Images supplied by Dr D. Obendorf; Department of Pathology, University of Tasmania).



Normal oral disc (pigmented tissues) of Common Froglet, *Crinia signifera*



Bd-affected Eastern Banjo Frog, *Limnodynastes dumerilii*

When swabbing frogs, the underside of the legs, feet and drink patch should be comprehensively swabbed (3–5 times) and the swab then broken off into a 1.5 ml tube. Mouthparts of tadpoles should be swabbed by inserting the swab into the mouth and twirling the swab several times.

3.1.2. Water bath and filters

¹ Medical Wire & Equipment Co. MW100-100.

As skin of infected frogs slough off into the surrounding environment, amphibians can be placed into containers containing Bd-free solutions (e.g. DS solution – details below). The ‘bath’ water can be analysed directly (refer below) following 15 minutes immersion. Alternatively, bath water can be filtered and filters (e.g. 0.45 µm filter²) stored dried (room temperature or 4°C) until analysis.

Reagents

Preparation of weak salt solution (DS)

KH_2PO_4 – 0.001 M

MgCl_2 – 0.0001 M

CaCl_2 – 0.00002 M

Stock #1 (phosphate stock):

KH_2PO_4 – 136.0 g

K_2HPO_4 – 174.18 g

$(\text{NH}_4)_2\text{HPO}_4$ – 132.07 g

Distilled water to 1000 ml

Stock #2 (calcium-magnesium stock):

$\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ – 36.76 g

$\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ – 50.83 mg

Distilled water to 500 ml

Make up to pH 7 with KOH (weak solution)

To make up the DS use 0.1ml of calcium-magnesium stock with 0.5 ml of phosphate stock in 1000 ml of distilled water

3.2. Preservation of samples for submission

Swabs and excised oral discs can be stored dry at ambient temperatures (up to 23°C). Note: exposure to prolonged high temperature (such as those in unattended automobiles) can reduce recovery of nucleic acid.

For light and electron microscopic examination, fix tissues in 10% neutral buffered formalin and 2.5% buffered glutaraldehyde respectively. Samples should be processed as described for Epizootic haematopoietic necrosis and Infection with ranavirus.

3.3. Pooling of samples

A maximum of five samples can be pooled (although low positives may be missed). Note: It is recommended that samples be pooled only for population studies where presence/absence data is sought (Hyatt *et al.*, 2007).

3.4. Best organs or tissues

Skin – ventral (adults), toes (adults), and mouthparts (tadpoles).

3.5. Samples/tissues that are not appropriate

Internal organs and eggs.

4. Diagnostic methods

4.1. Field diagnostic methods

4.1.1. Clinical signs

Clinical signs are absent in the majority of infected animals. The period of showing signs is typically short and limited to those amphibians that will die.

4.1.2. Behavioural changes

Central nervous system signs predominate. Behavioural change includes slow and uncoordinated movement, abnormal sitting posture, tetanic spasms, loss of righting reflex and paralysis.

4.2. Clinical methods

² Sartorius Minisart No. 16555.

4.2.1. Gross pathology

Gross changes to the skin may be noted in severe infections and include abnormal skin shedding (more frequently and in smaller pieces) and erythema. These clinical signs are not specific to chytridiomycosis

4.2.2. Clinical chemistry

In diseased green tree frogs (*Litoria caerulea*) plasma sodium and potassium concentrations are reduced by –20% and –50%, respectively (Voyles *et al.*, 2009).

4.2.3. Microscopic pathology

Microscopy includes examination of wet skin preparations (scrapings, smears or whole skin), histological sections of skin stained with haematoxylin and eosin, and immunohistochemistry of skin sections. These routine tests have a high positive predictive value. Details of these techniques and how to interpret results are described below.

4.2.4. Wet mounts

Samples of whole skin, from webbing or elsewhere, can be examined; the technique maintains the skin's anatomy and a large surface area can be examined. The advantage of this technique is that the sample can be oriented and the location of the suspected agent can aid in identification. For example, it can be ascertained whether suspected fungal profiles are within superficial cells and thus indicative of Bd or whether they are in deeper layers and thus profiles normal amphibian morphology. This technique is quick, inexpensive and, when used by skilled observers, is equivalent in sensitivity to staining with haematoxylin and eosin (Longcore *et al.*, 2007). It is useful in studying healthy frogs from which sheets of shedding skin cannot be obtained.

Infected tadpoles can often be identified in the field by the loss of colour on the jaw sheaths, which can be seen with the aid of a hand lens ($\times 10$). Tadpole mouth-parts can also be examined by cutting off pieces of the tooth-rows or jaw sheaths and squashing them under a cover-slip where clusters of sporangia can be observed.

In wet mounts and smears (refer below), round to oval intracellular sporangia usually occur in clumps. Old empty sporangia are the most prevalent stage in shedding skin although sporangia containing zoospores are commonly found. Discharge tubes (associated with zoosporangia), from which zoospores exit, usually point perpendicularly to the skin's surface and thus appear as small circles that can be difficult to discern. The observation of internal septa within sporangia increases confidence in the diagnosis. Epidermal cell nuclei are of similar size to sporangia but can be differentiated by their irregular, indistinct membranes and flat, granular, grey appearance.

4.2.5. Smears

Examination of skin scrapings, or smears, by light microscopy is a quick and simple method of Bd identification and can be performed on fresh, frozen or fixed samples (Berger *et al.*, 2009a). Shedding skin is lifted or scraped from the frog (using a scalpel or sterile plastic spoon) and is spread flat on a slide with a drop of water, covered by a cover-slip and the preparation examined with a compound light microscope. Ideally, an even monolayer of keratinised epidermal cells is obtained. Magnification of $\times 100$ is used initially to scan a section and then $\times 400$ is used to confirm the presence of sporangia. The round to oval intracellular sporangia (5–13 μm) occur in clumps within host cells. Old empty sporangia are the most prevalent stage in shedding skin although sporangia containing zoospores are commonly found.

Samples prepared as described above can be stained by the following protocols:

Lactophenol Cotton Blue:

- i) Place a drop of 70% alcohol on a microscope slide.
- ii) Immerse the specimen/material in the drop of alcohol.
- iii) Add one or two drops of the lactophenol/cotton blue before the alcohol dries out.
- iv) Holding the coverslip between forefinger and thumb, touch one edge of the drop of mountant with the coverslip edge, and lower gently, avoiding air bubbles. The preparation is now ready for examination.

Reagents (makes 1 litre):

Phenol:	200.0 g
Cotton Blue:	0.5 g
Glycerol:	400 ml
Lactic Acid:	200 ml
Deionised water:	200 ml

Congo-red: With a freshly prepared (and filtered) working solution of Congo red stain skin scrapings, or smears for 45–60 minutes. The chitinous walls of empty sporangia (walls) and exposed discharge tubes will stain brick-red. The walls of most immature, mature and empty sporangia also stain. Note: zoospores are not stained by this procedure. Epidermal cell nuclei stain pale orange with Congo-red if cells are damaged.

Reagents

Stock solution:	saturated
Congo Red stain:	1.0 g
Stock NaCl:	500 ml
(Stock NaCl 30.0 g + 200 ml distilled water):	200 ml

Working solution (Congo red)

Stock Congo red solution:	50.0 ml
1% sodium NaOH:	0.5 ml

Note: Filter through glass wool, solution should be clear, not cloudy, use immediately)

DipQuick³ can be used to stain for general diagnostic cytology. For the detection of Bd, use the stain to identify, zoospores and sporangia walls; the cytoplasm and host nuclei will also be stained. This is a proprietary stain and the manufacturers' protocol should be followed.

Note: These stains may improve accuracy and ease of diagnosis, but comparisons have not been carried out.

4.3. Agent detection and identification methods

4.3.1. Direct detection methods

4.3.1.1. Microscopic methods other than wet mounts and smears

4.3.1.1.1. Light microscopy: fixed sections

Prepare tissues as per conventional protocols. Section blocks at 5 µm and stained with haematoxylin and eosin (Drury & Wallington, 1980). Section such that a vertical section through the skin is achieved.

Digits are examined by sectioning a whole foot ventral-side down or by sectioning a single toe. For toes, the maximum length of *stratum corneum* is obtained from a longitudinal section rather than from a cross section. Digits are decalcified in EDTA for 48 hours at 37°C or in 10% formic acid for 3–5 days before processing. Alternatively with larger digits, for example from amphibians with a snout-vent length >60 mm, it is possible to remove skin from the underlying phalanx and section the skin without bone.

In the *stratum corneum* the chytrid is spherical or oval with discharge papillae projecting from the surface. Discharge papillae can be seen in histological sections, but are not common. Zoospores that develop in the zoosporangium escape through the open discharge tube. The wall of the zoosporangium is smooth, uniform in thickness and usually stains eosinophilic. The contents of the zoosporangia vary with the developmental stage of the chytrid; four stages can be identified: (1) The earliest stage contains a central basophilic, rather homogenous mass. (2) Zoosporangia become multinucleate and then the cytoplasm divides to form zoospores. Zoospores are basophilic and appear in cross-section as round or oval bodies, usually numbering about 4–10 depending on the plane of the section. (3) Once the zoospores are released via the discharge papilla, the empty zoosporangia remain. In some empty colonial stages, thin septa are visible dividing the sporangium into internal compartments. (4) The empty sporangium may collapse into an irregular shape. During

³ http://www.ihcworld.com/protocols/special_stains/diff_quick_ellis.htm

this terminal stage the empty shell sometimes becomes colonised by bacteria and these are seen in section as basophilic rods or cocci. Empty sporangia are the most common stage present in the sloughing surface layer. In histological sections the diameter of zoosporangia varies from 5 to 13 μm . They are a similar size to epidermal cell nuclei. Discharge tubes have a diameter of 2 μm and a variable length, usually between 2 and 4 μm , but sometimes as large as 10 μm . Zoospores are about 2 μm in diameter. Infection is usually associated with skin pathology and these changes can be used to detect, at low magnification, areas likely to be infected. Focal hyperkeratosis and erosions are common in the area adjacent to the organisms. Irregular thickening of the epidermis (hyperplasia) may be present. In some fatal cases extensive sloughing of the hyperkeratotic layer leaves the epidermis with few organisms. In these cases, however, Bd can be detected in low numbers in the slightly keratinised surface layer or may be seen in large numbers in the sloughed skin. Sporangia are not present in areas of extensive ulceration (Berger *et al.*, 2009b).

4.3.1.1.2. Electron microscopy

Preparation of samples (skin and toes) is described elsewhere (Berger *et al.*, 2005a). Studies on infected frog skin by electron microscopy shows a zone of condensed host cytoplasm, up to 2.5 μm thick, around some sporangia. This zone appears to be mainly fibrils with (no organelles). The more superficial epidermal cells contain larger sporangia and host nuclei and organelles such as mitochondria are located on one side of the cell. Near the skin surface the epidermal cytoplasm condenses into a thin layer around the fungal thalli and host organelles are lost as they are during normal epidermal cell maturation. Cell nuclei become dark and condensed but are not as flattened as in normal *stratum corneum*. Keratinisation appears to occur prematurely in infected cells below the skin's surface, compared with uninfected cells in the same epidermal layer. The cell junctions of infected cells usually appear normal. Some infected cells and uninfected cells near foci of infection are acutely swollen, although mitochondria and other organelles in these cells are intact. Nuclei of some infected cells in the *stratum granulosum* are shrunken and chromatolytic. Pathology in the deeper epidermal cells, as distant as the basal layer, includes focal shrinkage, increased intercellular spaces, vacuolation and dissolution of the cytoplasm. The hyperkeratosis appeared to be partly attributable to an increased turnover of epidermal cells. The swelling of epidermal cells near foci of infection suggests a hyperplastic response. Sporangia appear to initiate premature death and keratinisation of host cells. Thinning of the epidermis may occur when the germination of epidermal cells does not match the increased rate of sloughing caused by increased cell death. Other infected frogs may have a markedly thickened epidermis because of hyperplasia exceeding sloughing.

4.3.1.2. Agent isolation and identification

4.3.1.2.1. Isolation, culturing and archiving of Bd

4.3.1.2.1.1. Isolation

Collect animals (live, moribund or freshly dead) and keep cool and damp until examination. Identify Bd as described above.

- i) Examine skin with a dissecting microscope ($\times 10$ or $\times 20$).
- ii) Use a sharp and sterile needle to remove loose skin from between digits of foot and elsewhere on the ventral surface of the animal. If skin is not loose, use needle-nosed forceps and tear pieces from the leading edge of the skin between the hind digits or use a single edged razor blade to excise webbing from between toes. If larval animals have focal tooth loss or depigmentation of the jaw sheath, remove these areas with needle-nosed forceps.
- iii) Place the skin or jaw sheath on a microscope slide in a drop of sterile distilled water and cover with a coverslip. Observe with a compound microscope at $\times 100$ and $\times 400$.
- iv) Examine for the presence of sporangia (walled, spherical to oval bodies, 10–30 μm in diameter) inside epidermal cells. Note: some sporangia will be septate (i.e. contain divisions of the cell walls) that divide the fungal body, or thallus, into two or more sporangia. Some sporangia may contain zoospores and some will appear empty.
- v) Place skin on which Bd has been seen on a culture plate (9 cm) containing mTGh nutrient agar and antibiotics, which are added after autoclaving.
- vi) Use a sharpened and sterilised needle to draw and push a small ($< 1 \times 1$ mm) piece of infected skin ($\times 40$) through nutrient agar (9 cm culture plate). If pieces of skin are thick and do not tear, cut into small pieces with micro-scissors or cuticle scissors.
- vii) Every few mm take the needle away from the piece of skin and wipe through the agar (removes contaminating bacteria, yeast and fungal spores).

- viii) Reverse the direction of the skin and wipe it back and forth through the agar). Note: Bacterial and fungal contamination is less of a problem when working with tadpole tissue.
- ix) Following wiping the sample across the agar, place the cleaned skin on a fresh plate of mTGh agar. Ensure the opening of the new plate is minimal and wipe the sample into the agar with the needle. Repeat this process for additional pieces of skin; for each attempt it is recommended that at least six pieces of wiped skin be placed on each of two plates. Seal the nutrient agar plate with Parafilm® or other laboratory film stretched around the circumference of the plate.
- x) Incubate sealed plates at 17° to 23°C. During the next one to three weeks, check development by inverting the culture plate on the stage of a compound microscope (×100). Check plates for contaminants and remove fungal and bacterial colonies via sterilised scalpel. Examine for motile Bd zoospores (3–4 µm) near the skin (1–3 days post-inoculation) or for spherical outlines of growing thalli (usually within several days to 2 weeks). If hyphae are seen growing from the cleaned skin, aseptically remove the hyphal colony from the isolation plate with a sterile knife/scalpel.
- xi) When Bd colonies are observed on the skin, part of a colony may be aseptically removed and placed in a drop of water on a microscope slide. Observe with a compound microscope and identify sporangia and zoospores by comparison with published photographs (e.g. Berger *et al.*, 2002; Longcore *et al.*, 1999; and <http://www.umaine.edu/chytrids/Batrachochytrium/Photographs.html>)
- xii) Upon positive identification and sufficient growth (2 weeks – 1 month) aseptically transfer the colony (or part thereof) to a plate of 1% tryptone agar. The fungus grows best in groups, so be careful not to separate sporangia during transfer. Incubate for 1–2 weeks; if the culture is free of contaminating micro-organisms (e.g. bacteria and fungi), spread the colony on the plate and transfer a part of the colony to nutrient broth in a screw-capped 250 ml flask. For back-up, also transfer bits of the colony to fresh plates and seal with Parafilm®. Note: if possible work within a laminar-flow hood.
- xiii) For stocks, keep two sets of cultures in 1% liquid tryptone at 5°C. Refrigerated cultures in liquid medium remain viable for about four months.

Reagents

mTGh

8 g tryptone
2 g gelatin hydrolysate
10 g agar
1,000 ml distilled water

Add 200 mg litre⁻¹ penicillin-G and 200–500 mg litre⁻¹ streptomycin sulfate after autoclaving; if bacteria are still a problem add 1 mg litre⁻¹ ciprofloxacin.

1% Tryptone agar

10 g tryptone
10 g agar
1000 ml distilled water

1% Tryptone broth

10 g tryptone
1000 ml of distilled water

4.3.1.2.1.2. Cryo-archiving from broth culture

4.3.1.2.1.2.1. Preparation of cultures

- i) Take 2 ml of actively growing broth culture (1 week old).
- ii) Add to 13 ml of new broth in 25 cm² flask.
- iii) Incubate for 3–4 days ensuring the flasks are lying flat.

- iv) The cultures should have many very active zoospores, single small to medium sized sporangia attached to the plastic and some larger ones close to releasing zoospores. There should also be lots of small clumps of sporangia floating in the broth.
- v) Scrape the sides of the flask and spin the contents in a bench top centrifuge at 1700 **g** for 10 minutes.
- vi) Pour off the supernatant (note the supernatant contains large numbers of active zoospores).
- vii) Resuspend the pellet in 1ml 10% DMSO, 10% FCS in broth and freeze in cryo-container. 10% DMSO in broth or cryoprotectant (as below) also work but DMSO, FCS combination gives the best recovery.
- viii) Following thawing and plating onto TGhL plates add 1ml of DS.

4.3.1.2.1.2.2. Freezing cultures

- i) In a laminar flow cabinet, place the cryoprotectant (approximately 1 ml) into a 3 ml tube.
- ii) Scrape a portion of the cultured Bd and place in the cryoprotectant. If there are some areas of the culture with solid clumps then select a few of these, with the attached agar and add those to the 'portion'. Note: do not add more than approximately 0.5 cm³ of culture.
- iii) Freeze using an isopropanol-containing, plastic cryocontainer⁴.
- iv) Place the cryocontainers in a –80°C freezer overnight, then place the cryotubes in liquid nitrogen for permanent storage

4.3.1.2.1.2.3. Thawing of cultures

- i) Fill a container with water (43°C).
- ii) Place the cryotubes directly from liquid nitrogen into this water and, ensure they are kept beneath the water. Agitate for approximately 30 seconds.
- iii) Check the cryotubes by lifting them out of the water, check to determine if the cryoprotectant is thawed. If thawed then pour the contents into a sterile Petri dish; if not, return them to the warm water. It is crucial the sample is not over heated (Note: prolonged exposure of Bd at 43°C will kill the organism), Tip: as the contents starts to thaw hold the cryotube in your hand so the warmth of your hand facilitates thawing.
- iv) Place the sample onto the agar (TGhL) in the Petri dish.
- v) Pipette some of the liquid cryoprotectant, onto the sample (in the Petri dish).
- vi) Put on a few drops (approximately 1 ml) of DS onto the transferred sample and incubate (refer above). Examine the cultures over 7–10 days for movement of zoospores. If, after 4–7 days no movement is observed and the agar looks dry, place a few drops of DS on the culture. If no movement occurs after after 3 weeks, the cultures are probably dead.

Note: If there is contamination, transfer the culture to an uncontaminated area. If contamination is substantial use TGhL with antibiotics.

Reagents

DS

Refer to Section 3.1.2.

Cryo medium

Stock skimmed milk solution

Add 90 ml (30.5 g) of dry skimmed milk powder to 200 ml of room temperature distilled water in a 500 ml bottle. Mix well, then autoclave for no more than 15 minutes with a vent rate of 5–8 minutes. Note that the milk caramelises very easily, and you often have to throw away the result and repeat

⁴ Recommend Mr Frosty® containers (Nalgene)

this step. If the solution looks too brown when it comes out of the autoclave – throw it and re-do the stock. It should be a pale brown/cream colour.

Solution #1: Make up 100 ml of 20% glycerol in double-distilled water and autoclave on a normal cycle.

Solution #2: In a hood, make 17% sterile skimmed milk solution (refer above) by adding 17 ml of the skimmed milk solution to 83 ml of sterile water.

In a hood, make up 200 ml of cryo-protectant by adding equal volumes of solution #1 to solution #2. This should be kept sterile, by flaming the neck of the bottle on use.

4.3.1.3. Antibody-based antigen detection methods

It should be noted that antibodies used in all related methods cross-react with a range of fungi (Berger *et al.*, 2002). They should therefore be used with caution and in association with classical light and/or electron microscopy (refer to Section 4.3.1.).

4.3.1.3.1. Co-localisation of *Bd* and keratin by histochemistry and specialised staining

Sections from infected animals were processed via a modified version of the protocol (Berger *et al.*, 2002). Sections were incubated with immunoperoxidase (IPX) conjugated secondary antibody, resulting in red-brown staining of *Bd* set amongst the epithelial cells (counterstained with Lillie-Mayer's haematoxylin⁵).

Procedure # 1: Immunoperoxidase

- i) Dewax sections: 10 minutes in 60°C oven, xylene 1 minute (× 3), 100% Ethanol (tech grade) 1 minute × 2, 70% ethanol 1 minute, running tap water 1 minute, distilled water.
- ii) Rinse in PBS buffer to eliminate any air bubbles.
- iii) Apply primary antibody at desired dilution (determine empirically); use 0.1% skimmed milk powder/PBSA as diluent.
- iv) Incubate slides for 45 minutes at 37°C.
- v) Rinse slides in PBS for 5 minutes.
- vi) Block endogenous peroxide: Apply 3% H₂O₂ in distilled water, 200 µl slide⁻¹, for 20 minutes at RT.
- vii) Rinse slides PBS 5 minutes.
- viii) Add "Envision +"⁶ (anti-rabbit for polyclonal, anti-mouse when primary is MAb), 3–4 drops for 20 minutes, 37°C.
- ix) Rinse slides in PBS for 5 minutes.
- x) Remove slides from the sequenza cassettes and place in staining rack in jar of buffer. Lay slides out on horizontal staining rack wiping around sections with tissue and apply substrate solution 200 µl per slide.
- xi) Add 200 µl di-methyl formamide (DMF) to 200 mg AEC powder (3-amino-9-ethylcarbazole, Sigma), ensure powder is completely dissolved, add 10 ml 0.05 M acetate buffer pH 5.0, add 5 µl 30% H₂O₂
- xii) Check positive control section every 2 minutes until optimal staining is achieved, usually between 2–5 minutes.
- xiii) Stop reaction by washing slides in distilled water
- xiv) Counterstain in Lillie-Mayer Haematoxylin (Mod.) for 30–90 seconds, rinse in tap water, blue in Scott's Solution, rinse in running tap water.
- xv) Rinse slides in distilled water and mount in an aqueous mounting medium.

⁵ Sigma

⁶ "ENVISION +": Dako Peroxidase anti mouse Code 4000 (15 ml) or 4001 (110 ml)

Procedure #2. Alkaline phosphatase and Keratin stain

Histological and histochemical identification of Bd can be complicated by the sloughing of the superficial keratinised layer (*stratum corneum*) leading to misdiagnosis because sporangia are lost with the sloughed skin. Combining immunostaining for Bd with Hollande's Trichrome keratin stain helps determine whether a negative result could be due to loss of the keratin layer (Olsen *et al.*, 2004).

Although alkaline phosphatase (AP) is no more effective as a substrate than IPX, it is preferred because of a bleaching effect on the IPX substrate by subsequent keratin staining. AP has the advantage of enhanced contrast between the substrate and keratin stains.

- i) Dewax sections with xylene and hydrate through graded ethanols to running tap water.
- ii) Place in distilled water.
- iii) Incubated sections with Rabbit 667 anti-chytrid polyclonal antibody, (1/1000 in 1% [w/v] skim milk/Tris buffered saline [TBS]) for 45 minutes at 37°C.
- iv) Wash sections for 5 minutes with TBS.
- v) Incubate with ENVISION anti-rabbit/mouse Alkaline Phosphatase⁷ for 20 minutes at 37°C.
- vi) Wash again (5 minutes) with TBS.
- vii) Add BCIP/NBT substrate⁸ directly to the sections and incubate for 10 minutes.
- viii) Rinse slides with distilled water.

At this stage, if no further staining is required, slides should be mounted in a water based gel⁹ and sealed with a coverslip.

Keratin stain – Modified Hollande's Trichrome

- i) Take slides from distilled water (after immunolabelling) and incubate with 1% (w/v) phosphomolybdic acid¹⁰ (solution C) for 5 minutes at RT.
- ii) Rinse with distilled water and incubate with saturated (minimum of 11% [w/v]) Orange G¹¹ solution (solution D) for 5 minutes at RT.
- iii) The majority of solution D should be decanted and 0.2% (w/v) light green SF (Sigma) (solution E) added without rinsing for 2 minutes at RT.
- iv) Place slides (without rinsing) into 100% ethanol (×2), clear in xylene (×2) and mount in a xylene based mounting gel.
- v) Allow slides to dry until mounting medium had set, then view with a light microscope.

Interpretation of staining

The combined staining method results in a blue/purple colour for Bd, orange for keratin and pre-keratin and green for collagen and other sub-epidermal connective tissues.

Staining result		Interpretation
Keratin	Bd	
+	–	Frog was negative for Bd. Presence of keratin allows confidence in diagnosis
+	+	Frog was infected with Bd
–	–	Equivocal. A negative identification cannot be made as keratin is lacking and

⁷ DakoCytomation

⁸ DakoCytomation

⁹ For example Immunon™, Thermo Shandon

¹⁰ Ajax/Univar

¹¹ Gurr, Michrome #411

		Bd may be present in shed skin
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4.3.1.3.2. Detection of *Bd* using antigen-capture ELISA

Due to poor sensitivity and specificity an antigen-capture ELISA is not recommended for the detection of *Bd*.

4.3.1.3.3. Conventional and Immunoelectron microscopy

Principle of the test: skin can be examined by electron microscopy. Conventional electron microscopy (examination of ultra-thin sections) will generate data on *Bd* structure. The use of *Bd*-specific antibodies and gold-labelled anti-species antibodies permits both ultrastructure and antigenicity to be examined (Berger *et al.*, 2005).

Conventional transmission electron microscopy

Fix tissues as described in Berger *et al.* (1999). Briefly, 2.5% (v/v) buffered glutaraldehyde (cacodylate or phosphate) is used to fix cells for 40 minutes. Following primary fixation the cells are rinsed in the same buffer (3 × 20 minutes), post-fixed in 1% (w/v) buffered osmium tetroxide (1 hour), washed (3 × 5 minutes) in double-distilled/reverse osmosis (RO) water, dehydrated through graded alcohol (70–100%) and infiltrated and embedded in an epoxy resin (e.g. Spurr's or Epon).

Gold-labelling of sections

- i) For gold labelling of ultra-thin resin sections (Hyatt, 1991), attention must be given to fixation and embedding regimes. For example, cells should be fixed in 0.25% (v/v) glutaraldehyde with 2–4% paraformaldehyde. No secondary fixation is used and the cells are infiltrated and embedded in an acrylic resin¹².
- ii) Following fixation and embedding, cut and transfer ultrathin sections onto filmed nickel grids.
- iii) Cut sections from the appropriate blocks.
- iv) Block in 2% (w/v) skim milk powder in PBS-A (10 minutes).
- v) Block free aldehydes with 0.1 M glycine in PBS-A (20 minutes).
- vi) Wash in PBS-A (3 × 1 minute). This is an optional step used only if there is an excess of free aldehydes (a high background may be indicative of this).
- vii) If protein A-gold is not being used then block in normal species serum – this serum should be homologous to that complexed to gold. Recommended dilution is approximately 1/40 (10 minutes).
- viii) Incubate in primary antibody. If incubation details are unknown then perform initial reactions with 1/100 to 1/2700 dilutions (with three-fold dilutions). Dilute antibodies in 1% (v/v) cold water fish gelatin in PBS-A, (60 minutes, RT).
- ix) Rinse in 1% (v/v) coldwater fish gelatin in PBS-A, (6 × 3 minutes).
- x) Incubate in gold-labelled secondary antibody or protein A-gold or protein G-gold. Suggested dilution 1/40 in a PBS-A containing 1% (w/v) bovine serum albumin (BSA), 0.1% (v/v) Tween 20 and 0.1% (v/v) Triton X, 60 minutes, RT.
- xi) Rinse in PBS-A (6 × 3 minutes, RT).
- xii) Post-fix in 2.5% (v/v) glutaraldehyde in PBS-A (5 minutes, RT).
- xiii) Rinse in water (RO) (3 × 3 minutes, RT).
- xiv) Dry on filter paper (type not critical).
- xv) Stain in uranyl acetate and lead acetate.

Interpretation of results

Membranes (external) associated with zoospores and sporangia will be gold-labelled.

¹² such as LR White or HM20 Lowicryl

4.3.1.4. Molecular techniques, TaqMan PCR

Identification of Bd is possible using the described and validated (Boyle *et al.*, 2004; Hyatt *et al.*, 2007) real-time TaqMan assay. The assay can be completed in less than 24 hours at relatively low cost.

The Taqman RT-PCR uses a primer/probe set designed to target a highly conserved region 5.8, 18 and 28S DNA separated by internal transcribed spacers (ITS-1 and ITS-2) and an intergenic spacer to detect Bd from swabs, toe clips, filters and tadpole oral discs (fresh or desiccated). Sequences of 5.8, 18 and 28S rRNA are highly conserved, whereas the ITS region and intergenic spacer units evolve quickly. The assay has a sensitivity of 0.1 zoospore equivalents. It will also quantify the level of infection in animals.

As the assay is very sensitive, all possible precautions need to be taken to prevent contamination (the assay will quantitatively detect a single zoospore in the test sample). These include using disposable implements for each sample, wearing gloves, performing assays in a Class II Biological safety cabinet, aliquoting reagents for one-time use, using filtered tips, using dedicated pipettes, work in 'clean' area.

4.3.1.4.1 Preparation of swabs

Swabs recommended for uses: Medical Wire & Equipment Co (UK) MW 100-100 sourced from Biomirieux Aust.). Alternative swabs have not been validated. If using alternatives appropriate validation would be required.

- i) Swab underside of feet, legs and drink patch vigorously 2–3 times.
- ii) Break off swab into 1.5ml screw cap tube (with O-ring) containing 30–40 mg of 0.5 mm zirconium/silica beads¹³ and 50 µl PrepMan Ultra¹⁴.
- iii) Homogenise using a beadbeater (2 × 45 second). Centrifuge in a microfuge (30 seconds) between each homogenisation, to recover all material from tube, and again after second homogenisation.
- iv) Place screw-cap tubes in a suitable holder and heat samples (10 minutes at 100°C).
- v) Cool (2 minutes) at RT then microfuge (3 minutes).
- vi) Collect and store as much supernatant as possible – usually 20–40 µl.

When processing large numbers the supernatants can be stored in 96-well V-bottom plates in every second row – dilution (1/10 in water) can be made in the alternate rows of the plate. Harvested supernatants can be stored for a week at 4°C if the assay is being done in that time otherwise store frozen at –20°C. Seal the plates to prevent evaporation. A negative extraction control should be included each time to ensure there is no contamination (i.e. a clean swab in 50 µl PrepMan Ultra).

4.3.1.4.2 Preparation of toe clips and mouthparts

Extraction is same as for swabs. Use approximately (no more) than 1mg. Use new sterile scalpel blades on a clean Bd-free (i.e. has not been used before) surface, e.g. Petri dish. For large toes strip the skin off the toe using clean scalpels, petri dish and toothpick and add no more than 10% (w/v) to prevent the homogenisation system being overloaded; increase the volume of PrepMan Ultra if necessary. Place the sample directly into the tube with zirconium beads and PrepMan Ultra as described for swabs.

4.3.1.4.3. DNA extraction

DNA is extracted from toe-clips, swabs, filters or tadpole oral discs by extraction with PrepMan Ultra.

- i) 50 µl of PrepMan Ultra (200 µl for filters) is added to each sample along with 30–40 mg of Zirconium/silica beads 0.5 mm diameter¹⁵.
- ii) Homogenise sample for 45 sec in a bead-beater, e.g. a Mini Beadbeater 8¹⁶.

¹³ Biospec. Products – Cat. # 11079105z

¹⁴ Applied Biosystems. Cat. # 4318930

¹⁵ Biospec Products

- iii) Centrifuge (30 seconds, 13,000 **g** in a microfuge, ×2): this recovers all material from the tube.
- iv) Heat sample at 100°C (10 minutes), cool (2 minutes) and centrifuge (13,000 **g**, 3 minutes in a microfuge).
- v) Take 20% of supernatant and use immediately; sample can be stored at –20°C until used.

4.3.1.4.4. Preparation of standards

- i) Seed TGH plates are seeded with 2 ml of actively growing *B. dendrobatidis* culture and grown for 4 to 5 days.
- ii) Harvest zoospores are harvested by flooding plate twice with 2 ml DS solution.
- iii) Count zoospore in haemocytometer (× 4).
- iv) Pellet 10⁷ zoospores in a microfuge (13,000 **g**, 1 minute).
- v) Remove pellet and resuspend in 200 µl of PrepMan Ultra.
- vi) Boil the suspension for 10 minutes, cool 2 minutes, microfuge for 3 minutes and remove 150 µl of supernatant.
- vii) Dilute DNA in distilled water (2 × 10⁵ per ml genome equivalents) and aliquots stored at –20°C.

4.3.1.4.5. Internal controls

Inhibitors of the TaqMan assay, such as soil on the swabs, may be present after the extraction process resulting in false negatives being reported. The presence of inhibitors in samples can be detected by using an internal control. Applied Biosystems® produces a synthetic amplicon from a plasmid source whose sequence is not known to occur in nature. This is VIC-labelled and primer limited for use in multiplex assays¹⁷: 1 µl 10 × Exo IPC mix and 0.5 µl 50× Exo IPC DNA should be included in the master mix of 1 well of the three triplicates. The Ct values in the VIC layer should be comparable for controls and test samples. If the Ct value of the test sample in the VIC layer is two- to four-fold higher than the negative control then the sample should be diluted 1/100 or greater.

4.3.1.4.6. TaqMan assay

- i) Aliquot 20 µl of combined master mix/primers/probe per well.
- ii) Add 5 µl DNA at 1/10 dilution (in water) for samples prepared with PrepMan Ultra.
- iii) For each assay, standards of 100, 10, 1 & 0.1 zoospores must be used to construct a standard curve. Stocks of standards can be prepared and stored frozen and diluted as required.
- iv) An extraction control with no DNA (no template control) should also be included on each plate.
- v) All samples including standards should be done in triplicate.
- vi) Primers and Probe sequences:

<p>Primer 1 (Forward Primer): <i>ITS1-3 Bd</i>: 29 bases 5'-CCT-TGA-TAT-AAT-ACA-GTG-TGC-CAT-ATG-TC-3'</p>
<p>Primer 2 (Reverse Primer): <i>5.8S Bd</i>: 22 bases 5'-AGC-CAA-GAG-ATC-CGT-TGT-CAA-A-3'</p>
<p>Probe: Chytr MGB2 15 nucleotides – FAM Labelled. 5'-6FAM-CGA-GTC-GAA-CAA-AAT-MGBNFQ-3'</p>

¹⁶ Biospec Products

¹⁷ TaqMan Exogenous Internal Positive Control Reagents (VIC Probe) # 4308323

Within the OIE Reference Laboratory amplification is carried out in a ABI 7500 fast or 7900 Sequence Detection System thermal cycler using the following programme: 50°C for 2 minutes (uracil N-deglycosylase digest) 1 cycle; 95°C for 10 minutes (activation of the Taq Gold thermostable DNA polymerase present in the master mix), 1 cycle; 95°C for 15 seconds, 60°C for 1 minute; 45 cycles. Note: Primers and probe are resuspended as per manufacturer's instructions.

Cycling Conditions:

50°C	2 minutes
95°C	10 minutes
<i>45 Repeats of:</i>	
95°C	15 seconds
60°C	1 minute

4.3.1.4.7 *Interpretation of data*

After completion of the assay, results should be analysed using the following guide:

- i) Perform OBT (outliers, baseline and threshold), setting the baseline range at 3, 15 and the threshold bar to a Delta R_N of 0.1 (Bd and internal control)
- ii) If positive samples have a C_T value <18, reduce the upper baseline value from 15 to at least 3 lower than the sample C_T .

Determine if the assay is valid by visualising:

- i) Positive control wells: amplification curves on the FAM dye layer must have characteristic shape.
- ii) The exogenous positive control should not be more than twofold higher than its determined value in the negative control. If the C_T value is more than twofold higher, then the test has been inhibited and the sample should be diluted (1/100).
- iii) Non-template control and negative control extraction: determine the absence of contamination by observing no characteristic amplification curves on FAM layer. VIC dye layer must have C_T value greater than 39.
- iv) Standard curve: standards at concentrations of 1/100, 10, 1, 0.1 are within specified range of reference standard. R^2 is greater than 0.98.

If the test is deemed valid, the results for the test sample wells can be interpreted using the following criteria:

Positive results

Definition: Presence of specific amplicons, indicated by a characteristic amplification curve similar to the positive control with a C_T value less than or equal to 39 (in all three wells).

Negative results

Definition: Absence of specific amplicons, indicated by no characteristic amplification curve and having a C_T value greater than 41 (in all three wells).

Indeterminate results

Definition: presence of characteristic amplification curves similar to the positive control but with a C_T value between 39 and 41 or a low number of zoospore equivalents in only one or two wells). Such results necessitate a repeat of the assay.

5. Rating of tests against purpose of use

The methods currently available for surveillance, detection, and diagnosis of chytridiomycosis are listed in Table 5.1. The designations used in the Table indicate: a = the method is the recommended method for reasons of availability, utility, and diagnostic specificity and sensitivity; b = the method is a standard method with good diagnostic sensitivity and specificity; c = the method has application in some situations, but cost, accuracy, or other factors severely limits its application; d = the method is presently not recommended for this purpose; and NA = not applicable. These are somewhat subjective as suitability involves issues of reliability, sensitivity,

specificity and utility. Although not all of the tests listed as category a or b have undergone formal standardisation and validation (see Chapter 1.1.2 of this *Aquatic Manual*), their routine nature and the fact that they have been used widely without dubious results, makes them acceptable.

Table 5.1. Methods for targeted surveillance and diagnosis

Method	Targeted surveillance				Presumptive diagnosis	Confirmatory diagnosis
	Ova/mil t	Tadpole s	Metamorphs	Adults		
Gross signs	na	d	d	d	d	d
Histopathology	na	c	c	c	c	c
Immunoperoxidase stain	na	c	c	c	b	b
Transmission EM	na	d	d	d	c	c
Immuno-EM	na	d	d	d	c	c
Isolation	na	na	na	na	na	na
Antigen-capture ELISA	na	na	na	na	na	na
Antibody-capture ELISA	na	na	na	na	na	na
TaqMan PCR	na	a	a	a	a	a
PCR sequence analysis	na	b	b	b	b	a

Histopathology is highly specific; in diseased animals it is also highly sensitive. EM = electron microscopy; ELISA = enzyme-linked immunosorbent assay; PCR = polymerase chain reaction; na: not applicable.

6. Test(s) recommended for targeted surveillance to declare freedom from chytridiomycosis

The sampling and associated TaqMan assays described in this paper can be used for targeted surveillance of Bd. A peer-reviewed survey protocol, which can be used for the mapping of Bd within geographic regions, can be found in a recent paper (Skerratt *et al.*, 2010).

7. Corroborative diagnostic criteria

7.1. Definition of suspect case

Amphibian, apparently healthy or moribund which displays aberrant behaviour and has localised areas of sloughed skin. The skin must contain evidence of zoospore and sporangia structure which stain with antibodies obtained from the reference laboratory

7.2. Definition of confirmed case

Amphibian, apparently healthy, moribund or dead in which skin contains Bd by TaqMan assay. Note: Histology (hematoxylin and eosin sections) can be used with confidence by qualified pathologists as there are no other fungi present on amphibian with similar structure (sporangia with discharge tubes, zoospores, within cells of stratum corneum); however definitive definition is by TaqMan PCR.

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NB: There is an OIE Reference Laboratory for Infection with *Batrachochytrium dendrobatidis* (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).

INFECTION WITH RANAVIRUS

EU position

The EU supports the adoption of the amended chapter.

1. Scope

For the purpose of this chapter, ranavirus disease is considered to be systemic clinical or subclinical infection with a member of the genus *Ranavirus*. **It does not include epizootic haematopoietic necrosis virus, which is the aetiological agent for epizootic haematopoietic necrosis (EHN).**

2. Disease information

2.1. Agent factors

2.1.1. Aetiological agent, agent strains

Ranaviruses belong to the genus *Ranavirus* of the Family *Iridoviridae*. The type species is Frog virus 3 (FV3) (Chinchar *et al.*, 2005). Other species include Bohle virus (BIV), Epizootic haematopoietic necrosis virus (EHN), European catfish virus (ECV), European sheatfish virus (ESV) and Santee-Cooper ranavirus. There are many other tentative species in this genus. Since the recognition of disease caused by EHN in finfish in Australia in 1986, similar systemic necrotising iridovirus syndromes have been reported in amphibians. Ranaviruses have been isolated from healthy or diseased frogs, salamanders and reptiles in America, Europe, **Asia** and Australia (Chinchar, 2002; Drury *et al.*, 1995; Fijan *et al.*, 1991; Hyatt *et al.*, 2002; Speare & Smith, 1992; Wolf *et al.*, 1968; **Xia *et al.*, 2009**; Zupanovic *et al.*, 1998b). Ranaviruses have large (150–**170 480** nm), icosahedral virions, a double-stranded DNA genome 150–170 kb, and replicate in both the nucleus and cytoplasm with cytoplasmic assembly (Chinchar *et al.*, 2005). They possess common antigens that can be detected by several techniques.

Species	No. of isolates	Examples	Geographic source
<i>Ambystoma tigrinum virus</i>	2	<i>Ambystoma tigrinum virus</i> , Regina ranavirus	North America
<i>Bohle iridovirus</i>	1	<i>Bohle iridovirus</i>	Australia
<i>Frog virus 3</i>	12	<i>Frog virus 3</i>	Europe, North & South America
		<i>Box turtle virus 3</i>	Europe, North & South America
		<i>Bufo bufo United Kingdom virus</i>	Europe, North & South America

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		<i>Bufo marinus Venezuelan iridovirus 1</i>	Europe, America	North	&	South
		<i>Lucké triturus virus 1</i>	Europe, America	North	&	South
		<i>Rana temporaria United Kingdom virus</i>	Europe, America	North	&	South
		<i>Redwood Park virus</i>	Europe, America	North	&	South
		<i>Stickleback virus</i>	Europe, America	North	&	South
		<i>Tadpole edema virus</i>	Europe, America	North	&	South
		<i>Tadpole virus 2</i>	Europe, America	North	&	South
		<i>Tiger frog virus</i>	Europe, America	North	&	South
		<i>Tortoise virus 5</i>	Europe, America	North	&	South
<i>Tentative species</i>	3	<i>Rana esculenta iridovirus</i>	Europe, America	North	&	South
		<i>Testudo iridovirus</i>	Europe, America	North	&	South

2.1.2. Survival outside the host

All ranaviruses are probably extremely resistant to drying; EHNV can survive for months in water, in frozen fish tissues for more than 2 years (Langdon, 1989), and in frozen fish carcasses for at least a year (Whittington *et al.*, 1996). Santee-Cooper ranavirus remains viable in frozen fish tissues for at least 155 days (Plumb & Zilbert, 1999). Less is known about other ranaviruses, but given their similarity to EHNV they are presumed to have comparable stability. ATV was infectious for salamanders if present in moist but not dry pond sediment, but the duration of infectivity is unknown.

2.1.3. Stability of the agent (effective inactivation methods)

Ranaviruses (as exemplified via EHNV) are susceptible to 70% ethanol, 200 mg litre⁻¹ sodium hypochlorite or heating to 60°C for 15 minutes (19). If desiccated first, EHNV may survive heating to 60°C for 15 minutes (unpublished observations). 10⁷ plaque-forming units per ml of a ranavirus of amphibian origin was inactivated within 1 minute in a solution of 150 mg litre⁻¹ chlorhexidine (0.75% Nolvasan ®), 180 mg litre⁻¹ sodium hypochlorite (3% bleach) or 200 mg litre⁻¹ potassium peroxymonosulfate (1% Virkon ®) (Bryan *et al.*, 2009).

2.1.4. Life cycle

The route of infection is unknown but amphibians are susceptible experimentally following bath exposure injection and or exposure following laboratory induced abrasions. (Cunningham *et al.*, 2007; 2008).

2.2. Host factors**2.2.1. Susceptible host species**

Natural ranavirus infections are known from most of the major families of Anura and Caudata (Carey *et al.* 2003a; 2003b; Cullen & Owens, 2002; Daszak *et al.*, 2003).

2.2.2. Susceptible stages of the host

Susceptible stages of the host are all age classes, larvae, metamorphs and adults.

2.2.3. Species or sub-population predilection (probability of detection)

Not known.

2.2.4. Target organs and infected tissue

Amphibian target organs and tissues infected with ranaviruses may vary. Three examples are given: i) BIV: liver, kidney, spleen, lung and other parenchymal tissues (Cullen & Owens, 2002). ii) FV-3 infects proximal tubular epithelial cells in the kidney, the liver, and the gastrointestinal tract (Robert *et al.*, 2005). iii) United Kingdom ranavirus (RUK) infects epithelial cells, fibroblasts, lymphocytes, melanomacrophages and a small proportion of endothelial cells in many tissues, as well as hepatocytes and Kupffer cells in the liver, the epidermis and dermis (Cunningham *et al.*, 2008). *Ambystoma tigrinum virus* is found in skin, spleen, liver, renal tubular epithelial cells, and lymphoid and haematopoietic tissues of salamanders.

2.2.5. Persistent infection with lifelong carriers

Not known.

2.2.6. Vectors

Amphibians can become infected in the same way as fish and, as such, details associated with EHNV are included here. Possible vectors include nets, boats and other equipment, or in amphibians used for bait by recreational fishers. Birds are potential mechanical vectors, as ranaviruses can be carried in the gut, on feathers, feet and the bill. It should be noted that ranaviruses are likely to be inactivated at typical avian body temperatures (40–44°C). Nevertheless, it is possible that ranaviruses (as evidenced by EHNV) can be spread by regurgitation of ingested material within a few hours of feeding **is possible** (Whittington *et al.*, 1996). In addition amphibians have been shown to be infected by exposure to sediment from sites where ranavirus die-offs have occurred.

2.2.7. Known or suspected wild aquatic animal carriers

Not known.

2.3. Disease pattern

2.3.1. Transmission mechanisms

Ranavirus infections can occur from animal-to-animal contact, ingestion of infected, dying and dead individuals (e.g. Cullen & Owens, 2002; Picco & Collins, 2008). Viruses can also be spread between widely separated river systems and impoundments. Transmission is understood to occur by means other than water (refer above); mechanisms include translocation of live fish or bait by recreational fishers (e.g. Pico *et al.*, 2007).

2.3.2. Prevalence

Ranavirus infections have been reported on five continents including Asia (Gray *et al.*, 2009); its prevalence, based on intensive widespread serosurveillance, antigen detection, is not known.

2.3.3. Geographical distribution

Ranaviruses have been recovered from free-living or farmed, healthy or diseased frogs in America, continental Europe, the United Kingdom and Asia (Ariel *et al.*, 2009; Chinchar, 2002; Cunningham *et al.*, 1996; Drury *et al.*, 1995; Fijan *et al.*, 1991; Fox *et al.*, 2006; Green *et al.*, 2002; He *et al.*, 2002; Wolf *et al.*, 1968; Zhan *et al.*, 2001; Zupanovic *et al.*, 1998b) as well as diseased free-living spotted salamanders *Ambystoma maculatum* in North America (Docherty *et al.*, 2003; Jancovich *et al.*, 2003). Bohle iridovirus (BIV), which is distinct from FV-3, was isolated originally from diseased ornate burrowing frog *Limnodynastes ornatus* tadpoles in far north Queensland, Australia (Speare & Smith, 1992). It has not been isolated since, although there is serological evidence of ranavirus infection in cane toads *Bufo bufo* in that region (Whittington *et al.*, 1996). Another distinct species of ranavirus, *Ambystoma tigrinum* virus (ATV), is responsible for die-offs in the tiger salamander *A. tigrinum* (Jancovich *et al.*, 2005). Viruses closely related to FV-3 have also been recovered from reptiles. Wamena iridovirus (WIV) was isolated in Australia from diseased green pythons *Chondropython viridis* smuggled from West Papua (Irian Jaya) while THIV (TV-CH8) was recovered from diseased Hermann's tortoises *Testudo hermanni* in Europe. Both WIV and THIV had >97% nucleotide sequence homology with FV-3 in the regions of MCP that were examined (Hyatt *et al.*, 2002; Marshang *et al.*, 1999).

2.3.4. Mortality and morbidity

Mortality and morbidity vary from species to species. Laboratory infections and field data show that mortality can range from low (e.g. 0%) to >75–100% of infected animals of an experimental group depending on species, virus and age and health status of the host, following short infection times (Harp & Petranka, 2006; Hyatt *et al.*, 1998; Pearman *et al.*, 2004). However other experiments involving different host species and ranaviruses gave variable results (Brunner *et al.*, 2004; 2007; Cunningham *et al.*, 2007).

2.3.5. Environmental factors

Natural epizootics of amphibian ranaviruses appear to be similar for piscine ranaviruses (e.g. EHNV). Epizootics appear to be seasonal and can be related to poor husbandry (captive populations) and overcrowding (wild and captive). It has been assumed that for some amphibians such as salamanders (references) disease is related to the annual appearance of large numbers of non-immune young animals and their subsequent exposure to the virus in shallow waters (Brunner *et al.*, 2004; 2007; Green *et al.*, 2002; Greer & Collins, 2008; Greer *et al.*, 2008; Jancovich *et al.*, 1997; 2001; Rojas *et al.*, 2005).

2.4. Control and prevention

2.4.1. Vaccination

None available.

2.4.2. Chemotherapy

None available.

2.4.3. Immunostimulation

Not tested.

2.4.4. Resistance breeding

Not tested.

2.4.5. Restocking with resistant species

Not tested.

2.4.6. Blocking agents

Not tested.

2.4.7. Disinfection of eggs and larvae

Not tested.

2.4.8. General husbandry practices

Not tested.

3. Sampling

3.1. Selection of individual specimens

A simple method for preparation of tissues for cell culture and enzyme-linked immunosorbent assay (ELISA) has been validated in fish (Whittington & Steiner, 1993; Whittington *et al.*, 1999).

Bath large amphibians for 30 seconds in 70% ethanol; bath small amphibians for 5 seconds in 70% ethanol then rinse in sterile water. Dissect aseptically in a Class II biosafety cabinet.

Large amphibians: (>60 mm length) remove 0.1 g liver, kidney, spleen (\pm other organs in specific situations) and place in sterile 1.5 ml tubes. Tubes suitable for use with pestles for grinding tissues (see below) are available, but standard 1.5 ml tubes may be suitable. In some situations liver, kidney and spleen may be pooled in a single tube (see section 3.3).

Medium amphibian (30–60 mm length): scrape all viscera into the tube.

Small amphibian (<30 mm length): remove head and tail, place rest of animal into the tube.

3.2. Preservation of samples for submission

For cell culture and ELISA, freeze tubes containing tissues at temperatures from -20°C to -80°C until needed.

For light microscopic examination, fix tissues in 10% neutral buffered formalin.

3.3. Pooling of samples

The effect of pooling tissues from multiple animals on the sensitivity of diagnostic tests has not been evaluated. However, tissues for virus isolation are commonly pooled in lots of 5 or 10 individuals per test.

3.4. Best organs or tissues

Liver, kidney, spleen, lung, skin.

3.5. Samples/tissues that are not appropriate

Inappropriate tissues include gonads, gonadal fluids, milt and ova, since there is no evidence of reproductive tract infection and broodstock are not known to participate in an infection cycle.

4. Diagnostic methods

4.1. Field diagnostic methods

4.1.1. Clinical signs

There are two syndromes in frogs associated with ranavirus infection: a chronic ulcerative syndrome and an acute haemorrhagic syndrome (Cunningham *et al.*, 1996). Salamanders infected with *Ambystoma tigrinum* virus develop ulcerative dermatitis and enteritis. Affected larvae have small multifocal haemorrhages affecting subcutaneous tissue on the plantar surface of feet, the inguinal area, and the vent area, with ventral oedema and the skin may contain pale foci (Bollinger *et al.*, 1999; Docherty *et al.*, 2003).

4.1.2. Behavioural changes

Field and behaviour changes differ between species, life stage and severity of disease. Changes include lordosis, erratic swimming, lethargy and loss of equilibrium (Gray *et al.*, 2009).

4.2. Clinical methods

4.2.1. Gross pathology

There may be no gross lesions or nonspecific lesions. There are two syndromes in frogs associated with ranavirus infection: ulcerative syndrome and haemorrhagic syndrome (Cunningham *et al.*, 1996). In salamanders infected with *Ambystoma tigrinum* virus there may be ulcerative dermatitis, pale foci in the skin, small multifocal haemorrhages affecting subcutaneous tissue on the plantar surface of feet, the inguinal area, the vent, the subserosal surface of the intestine, and the liver may be pale and swollen; there may be ventral oedema (Bollinger *et al.*, 1999; Docherty *et al.*, 2003).

4.2.2. Clinical chemistry

Not applicable.

4.2.3. Microscopic pathology

BIV and FV3 cause multifocal multi-organ haemorrhage and necrosis (Cullen & Owens, 2002; Robert *et al.*, 2005). Salamanders infected with *Ambystoma tigrinum* virus develop necrosis in many tissues including spleen, liver, renal tubular epithelial cells, and lymphoid and haematopoietic tissues (Bollinger *et al.*, 1999). Amphophilic intracytoplasmic inclusion bodies may be present in cells in many organs together with single cell or variable sized areas of focal necrosis (Bollinger *et al.*, 1999; Docherty *et al.*, 2003). In skin there may be foci of spongiosis and ballooning degeneration, erosion and ulceration and hyperplasia of epidermal epithelial cells which may have intracytoplasmic inclusion bodies (Bollinger *et al.*, 1999).

4.2.4. Wet mounts

Not applicable.

4.2.5. Smears

Not tested.

4.2.6. Fixed sections

[Refer to Section 4.3. Text](#)

4.2.7. Electron microscopy/cytopathology

Affected tissues (e.g. kidney liver and spleen) contain cells exhibiting necrosis. Cells contain conspicuous cytoplasmic inclusions that are rarefied areas of the cytoplasm in which the viruses are assembled. Within the cytoplasm, aggregates (paracrystalline arrays) of large [\(ranaviruses can vary greatly in size ranging from approximately 150 nm to >170 nm\) \(175 nm ± 6 nm\)](#) non-enveloped icosahedral viruses are apparent; single viruses are also present. Complete viruses (containing electron-dense cores) bud/egress from the infected cells through the plasma membrane. The nuclei of infected cells are frequently located peripherally and are distorted in shape.

4.3. Agent detection and identification methods

4.3.1. Direct detection methods

4.3.1.1. Microscopic methods

Light microscopy: routine methods can be used for tissue fixation in 10% buffered neutral formalin, paraffin embedding, preparation of 10 µm sections and staining with H&E to demonstrate tissue necrosis and basophilic intracytoplasmic inclusion bodies. These inclusion bodies are indicative but not confirmatory for ranavirus. Formalin-fixed paraffin-embedded sections can also be stained using an immunoperoxidase method (see below) to identify ranavirus antigen associated with necrotic lesions.

Electron microscopy: Ultrathin routine sectioning methods can be used for preparation of tissues and cell cultures (Eaton *et al.*, 1991) to demonstrate tissue necrosis, presence of viruses and virus inclusion bodies. Tissues and cells fixed with an alternative fixation and embedding regime can be used for antigen detection (Hyatt, 1991).

Negative contrast electron microscopy: supernatants from dounce homogenised tissues (10% [w/v]) and cell cultures can be used to detect viruses. Ranaviruses have a definitive appearance. They vary in diameter (150–180 nm) and have a limiting cell-derived (plasma membrane) envelope that surrounds a capsid of skewed symmetry. Underlying the capsid is a *de novo* membrane that itself surrounds a core containing the double-stranded (ds) DNA and minor proteins. These preparations can also be used to confirm ranavirus antigenicity (Eaton *et al.*, 1991).

4.3.1.1.1. Wet mounts

Not applicable.

4.3.1.1.2. Smears

Not applicable.

4.3.1.1.3. Fixed sections

See Section 4.3.1.1 on microscopic methods.

4.3.1.2. Agent isolation and identification

4.3.1.2.1. Cell culture/artificial media

Preparation of amphibian tissues for virus isolation and ELISA

A simple method for preparation of tissues for cell culture and ELISA has been described (Whittington & Steiner, 1993; Whittington *et al.*, 1999) (see sampling Section 3.1).

- i) Freeze tubes containing tissues at -80°C until needed.
- ii) Add 0.5 ml of homogenising medium (minimal essential medium Eagle, with Earle's salts with glutamine [MEM] with 200 International Units [IU] ml^{-1} penicillin, 200 $\mu\text{g ml}^{-1}$ streptomycin and 4 $\mu\text{g ml}^{-1}$ amphotericin B) to each tube. Grind tissue to a fine mulch with a sterile fitted pestle.
- iii) Add another 0.5 ml of homogenising medium to each tube and mix with a pestle.
- iv) Add three sterile glass beads to each tube (3 mm diameter) and close the lid of the tube.
- v) Vortex the suspension vigorously for 20–30 seconds and place at 4°C for 2 hours.
- vi) Vortex the suspension again as above and centrifuge for 10 minutes at 2500 *g* in a benchtop microcentrifuge.
- vii) Transfer the supernatant, now called clarified tissue homogenate, to a fresh sterile tube. Homogenates may be frozen at -80°C until required for virus isolation and ELISA.

Cell culture/artificial media

Cell culture is the gold-standard test but is costly and time consuming. Ranaviruses grow well in many fish cell lines including BF-2 (bluegill fry ATCC CCL 91), FHM (fathead minnow; ATCC CCL 42), EPC (*epithelioma papulosum cyprini* [Fijan *et al.*, 1983]), and CHSE-214 (Chinook salmon embryo cell line; ATCC CRL 1681) at temperatures ranging from 15 to 22°C (Crane *et al.*, 2005), but BF-2 are preferred by the Reference Laboratory where an incubation temperature of 22°C both before and after inoculation with virus is used. The procedure for BF-2 cells is provided below. A procedure for CHSE-214 cells is provided under immunoperoxidase staining below (see Section 4.3.1.2.2). *Ambystoma tigrinum* virus produces CPE like that of EHNV in FHM, RTG and bullfrog tongue cells at 25°C (Docherty *et al.*, 2003). Others have used frog embryo fibroblasts at 27°C or FHM cells to isolate or propagate the United Kingdom isolates of FV-3 (Cunningham *et al.*, 1996, 2007).

The identity of viruses in cell culture is determined by immunostaining, ELISA, immuno-electron microscopy, polymerase chain reaction (PCR) or other methods.

Samples: tissue homogenates.

Cell culture technical procedure: cells are cultured (in flasks, tubes or multi-well plates) with growth medium (MEM + 10% fetal calf serum [FCS] with 100 IU ml^{-1} penicillin, 100 $\mu\text{g ml}^{-1}$ streptomycin and 2 $\mu\text{g ml}^{-1}$ amphotericin B). The cells are incubated until almost confluent at 22°C , which can take up to 4 days depending on the seeding rate. Medium is changed to a maintenance medium (MEM with 2% FCS and 100 IU ml^{-1} penicillin, 100 $\mu\text{g ml}^{-1}$ streptomycin and 2 $\mu\text{g ml}^{-1}$ amphotericin B) on the day of inoculation. A 1/10 dilution using homogenising medium is made of single or pooled homogenates. Each culture is inoculated with 100 μl of sample per ml of culture medium. This represents a 1/100 dilution of a 0.1 mg ml^{-1} tissue homogenate. One culture is inoculated with undiluted homogenate, and two with 1/10 homogenate. No adsorption step is used. As an alternative, two to three cultures can be inoculated directly with 10 μl undiluted homogenate per ml of culture medium. Note that a high rate of cell toxicity or contamination often accompanies the use of a large undiluted inoculum. The cultures are incubated at 22°C in an incubator for 6 days. Cultures are read at day 3 and day 6. Cultures are passed at least once to detect samples with low levels of virus. On day 6, the primary cultures (P1) are frozen overnight at -20°C , thawed, gently mixed and then the culture supernatant is inoculated onto fresh cells as before (P2), i.e. 100 μl P1 supernatant per ml culture

medium. Remaining P1 supernatants are transferred to sterile 5 ml tubes and placed at 4°C for testing by ELISA or PCR or another means to confirm the cause of cytopathic effect (CPE) as EHNV. P2 is incubated as above, and a third pass is conducted if necessary.

Interpretation of results

CPE is well developed and consists of focal lysis surrounded by rounded granular cells. This change extends rapidly to involve the entire monolayer, which detaches and disintegrates.

4.3.1.2.2. Antibody-based antigen detection methods

It should be noted that antibodies used in all related methods (immunoperoxidase, antigen-capture ELISA and immunoelectron microscopy) cross-react with all known ranaviruses (Hyatt *et al.*, 2000).

4.3.1.2.2.1. Detection of ranaviruses using immunoperoxidase staining of infected cell cultures

Principle of the test: ranaviruses replicate within cultured cells. The addition of a mild detergent permeabilises the cells allowing an affinity purified rabbit antibody to bind to intracellular viral proteins. Ranavirus is detected by a biotinylated anti-species antibody and a streptavidin–peroxidase conjugate. The addition of a substrate results in ‘brick-red’ staining in areas labelled with antibodies.

Samples: tissue homogenates.

Operating characteristics: when performed as described in this protocol, the staining is conspicuous and specific. However, the test has not been validated with respect to sensitivity or reproducibility.

Preparation of cells: the procedure described below is for CHSE-214 cells. Other recommended cell lines can also be used.

- i) CHSE-214, 24-well plates are seeded the day before use with 250,000 cells/well (or 4 million cells in 40 ml of growth medium per plate) in 1.5 ml of growth medium (Earle’s MEM with non-essential amino acids [EMEM], 10% FCS, 10 mM N-2-hydroxyethyl-piperazine-N-2-ethanesulfonic acid [HEPES], 2 mM glutamine, 100 IU penicillin and 100 µg streptomycin) and incubated in 5% CO₂ at 22°C overnight. (Note: cultures must be nearly confluent and have healthy dividing cells prior to use.)
- ii) Discard the medium, inoculate each well with 150 µl of a 10% suspension of ground tissue (e.g. liver, kidney or spleen), incubate for 1 hour (22°C) then add 1.5 ml of fresh maintenance medium (as for growth medium except 2% FCS) and return to the incubator (22°C).
- iii) Observe cultures for CPE. If no CPE occurs by day 10, pass the cultures on to fresh CHSE cells by collecting the cells and medium and adding 150 µl to the cells of the fresh plate; note that cells are not freeze–thawed. There is no need to discard the existing medium, just return the new plate to the incubator (22°C). Again, observe daily for CPE.
- iv) Fix cells (add 50 µl for 96-well plate cultures with 200 µl culture medium/well or 400 µl (for 24-well plate cultures with 1.6 ml culture medium/well) of a 20% formalin solution to each well), without discarding the culture medium when CPE is first observed. After incubation (22°C) for 1 hour at room temperature (RT), the medium/formalin mixture is discarded and the wells are rinsed twice with PBS-A (phosphate buffered saline, Ca⁺⁺ and Mg⁺⁺ free) to remove the formalin. More PBS-A is added if the plates are to be stored at 4°C.

Protocol

- i) Dilute primary anti-EHNV antibody and normal serum to working strength as described below (fixation protocol for immunocytochemistry) for the relevant agent in 1% skim milk (SM) solution (PBS-A (SM)) to the volume required for the test.
- ii) Remove PBS-A from wells (with fixed cell cultures) and wash wells twice with 0.05% (v/v) PBS/Tween 20 (PBST). Add 50 µl of primary antibody solutions to each well in a 96-well plate well or 200 µl in a 24-well plate well. Incubate on a plate shaker at 100–200 rpm at RT (22–24°C) for 15–30 minutes or without shaking at 37°C for 1 hour.

- iii) Dilute biotinylated anti-species serum (secondary antibody) in 0.1% SM solution as described in the fixation protocol (below) for the relevant agent to the volume required for the test.
- iv) Remove primary antibody solution and wash wells three times with PBST. Add secondary antibody to all wells. Incubate on a plate shaker at 100–200 rpm at RT for 15–30 minutes or without shaking at 37°C for 1 hour.
- v) Dilute streptavidin–peroxidase conjugate in 0.1% SM solution for the relevant agent to the volume required for the test.
- vi) Remove secondary antibody from wells and wash wells three times with PBST. Add conjugate to each well. Incubate on a plate shaker at 100–200 rpm at RT for 15–30 minutes or without shaking at 37°C for 1 hour.
- vii) Prepare stock substrate of 3-amino-9-ethylcarbazole (AEC) solution: dissolve one AEC tablet (20 mg) in 2.5 ml of dimethyl formamide.
- viii) Remove conjugate from wells. Wash (three times) with PBST.
- ix) Dilute dissolved AEC stock in 47.5 ml of acetate buffer (4.1 ml anhydrous sodium acetate in 1 litre of de-ionised water; the pH is adjusted to 5.0 with glacial acetic acid). Just before use, add 25 µl 30% hydrogen peroxide to AEC solution then add to each well. Incubate at RT for 20 minutes.
- x) Remove substrate solution and wash wells twice with deionised water to stop reaction.
- xi) To visualise all cells counterstain with Mayer's haematoxylin (50 µl/well or 200 µl/well) for 1 minute and rinse with deionised water.
- xii) Add 50 µl Scott's tap water and rinse with deionised water and air dry.

Interpretation of the results

Positive reaction: granular-like, focal, brick-red staining of cells indicates presence of virus identified by the diagnostic antibody.

Negative reaction: no red staining apparent – all cells should be stained pale blue due to counterstain.

Background staining: non-granular, non-focal, more generalised, pale, pinkish staining may occur throughout the culture. This background staining could be caused by any number of reasons, e.g. non-specific antibody reaction with non-viral components, inefficient washing, and expiration of other reagents.

Reagents for immunocytochemistry tests

20% Formaldehyde (PBS-A) saline

Formalin (36–38% formaldehyde)	54 ml
Distilled water	36 ml
10 × PBS-A	10 ml

10 × PBS-A

To make up 1 litre of 10 × PBS-A use:

NaCl	80.0 g
Na ₂ HPO ₄	11.5 g
KCl	2.0 g
KH ₂ PO ₄	2.0 g
Distilled water	1.0 litre

NOTE: some salts are supplied with extra water groups. If using these reagents adjust the masses to ensure the appropriate mass of salt is added, e.g. for Na₂HPO₄·2H₂O add 15 g instead of 11.5 g (156 mw/120 mw × 11.5 g = 14.95 g) to remove the effect of the water molecules.

4.3.1.2.2.2 Detection of ranavirus using antigen-capture ELISA

Antigen-capture ELISA has been validated to detect EHNV in cell cultures and directly in fish tissue homogenates. The same assay can be applied to amphibian tissues. The analytical sensitivity is 10³ to 10⁴ TCID₅₀ ml⁻¹. Specificity approaches 100% and sensitivity for direct detection in fish tissues is 60%

relative to the gold standard of virus isolation in BF-2 cells (Drury *et al.*, 1995; Marsh *et al.*, 2002; and unpublished data). ELISA is useful for both diagnosis and certification. Neutralisation tests cannot be used to identify EHNV because neutralising antibodies are not produced following immunisation of mammals or fish. Mouse monoclonal antibodies produced against EHNV are directed against major capsid protein (MCP) epitopes and are non-neutralising (unpublished data). Rabbit-anti-EHNV antibodies have been developed for use in antigen-capture ELISA, immunoperoxidase staining and immunoelectron microscopy (Hengstberger *et al.*, 1993; Hyatt *et al.*, 1991; Reddacliff & Whittington, 1996). Reagents and protocols are available from the reference laboratory.

Samples: tissue homogenate samples prepared using a validated protocol (see below), and cell cultures.

Principle of the test: EHNV particles are captured from the sample by an affinity purified rabbit antibody that is coated to the plate. EHNV is detected by a second antibody and a peroxidase-labelled conjugate using the chromogen ABTS (2,2'-azino-di-[3-ethyl-benzthiazoline]-6-sulphonic acid). The enzyme is inactivated after 20 minutes and the resulting optical density (OD) is compared with standards.

Test components and preparation of reagents

- i) Flat bottom microtitre plates are required.
- ii) Affinity purified rabbit anti-EHNV immunoglobulin and sheep anti-EHNV antiserum reagents are supplied in freeze-dried form. Reconstitute using 1 ml of purified water and allow the vial to stand at RT for 2 minutes. Mix the vial very gently. These reagents are stable when stored at -20°C for at least 4 years. For routine use in ELISA, it is recommended that working stocks of both antibodies be prepared as a 1/10 dilution in tris saline glycerol merthiolate TSGM (formula at end of this section). These are stable at -20°C for at least 5 years and do not solidify at this temperature.
- iii) The peroxidase labelled anti-sheep immunoglobulin conjugate (commercial reagent, KPL #14-23-06; 0.5 mg) is supplied as a freeze-dried powder. This reagent has displayed remarkable consistency in activity between different lots over a period of 15 years. The product should be reconstituted in sterile 50% glycerol water, dispensed in 150 μl aliquots and stored at -20°C as undiluted stock. A working stock is prepared by adding 900 μl of TSGM to 100 μl of undiluted stock. The working stock is also stored at -20°C and is stable for at least 1 year. New batches of this conjugate should be titrated against an older batch using standard protocols.
- iv) EHNV control antigen, heat-inactivated, is supplied as freeze-dried powder. Reconstitute in 1 ml sterile water and store in small aliquots at -20°C . Prepare dilutions using PBSTG (PBS + Tween + gelatin) on the same day the test is performed. Control EHNV antigen dilutions (A, B, D and F) cover the range of the signal response of the assay and enable a normalisation procedure to be undertaken.

Equipment

An automatic plate washer is recommended although plates can be washed by hand. The assay is sensitive to plate washing conditions. If the OD of the controls is unexpectedly low, and the conjugate and other reagents are within date, the plate washer should be adjusted so that washing pressure during filling of wells and aspiration of wells is minimised.

An automatic plate reader is recommended although plates can be read by eye.

Precision calibrated pipettes (e.g. Gilson) should be used to prepare dilutions of all reagents and to load reagents into microtitre plate wells.

Protocol

- i) Coat a 96-well ELISA plate (100 μl well⁻¹) with affinity purified rabbit-anti-EHNV diluted 1/12,800 in borate coating buffer. Incubate overnight at 4°C .
- ii) Wash plate five times with wash buffer (Milli-Q (MQ) purified water plus 0.05% Tween 20). Note that distilled and deionised water can also be used in this and all other steps.
- iii) Prepare a blocking solution: warm the solutions in a microwave oven or water bath to dissolve the gelatin, then cool to RT.

- iv) Block remaining binding sites using blocking solution (100 $\mu\text{l well}^{-1}$) (1% [w/v] gelatin in PBSTG diluent [PBS, 0.05% (v/v) Tween 20, 0.1% (w/v) gelatin]). Incubate at RT for 30 minutes.
- v) Wash plate five times as above.
- vi) Work in a Class II biological safety cabinet. Dilute the control antigen (see below) in PBSTG and add to the lower right-hand corner of the plate. Add tissue homogenate samples or culture supernatant samples and control antigens at 100 $\mu\text{l/well}$. All samples and controls are added to duplicate wells. Incubate for 90 minutes at RT.

The control antigens are dilutions of a heat killed cell culture supernatant of EHNV 86/8774. The controls are expected to give the following OD, although there will be some variation from laboratory to laboratory and $\pm 10\%$ variation should therefore be allowed:

Control	Dilution in PBS*	OD (405 nm)*
A	1/5	>2.0
B	1/40	1.90
D	1/200	0.68
F	1/3000	0.16

* These dilutions and OD values are determined by the OIE Reference Laboratory for EHNV and will vary with the batch of control antigen. The values above are for batch 86/8774-4-5-01. The positive-negative cut-off for clarified tissue homogenate samples from redbfin perch and rainbow trout in this ELISA is approximated by the OD value of control D on each plate.

- vii) Wash the plate by hand to avoid contamination of the plate washer. Work in a Class II cabinet. Aspirate wells using a multichannel pipette. Rinse the plate twice.
- viii) Wash the plate five times on the plate washer, as above.
- ix) Add the second antibody sheep-anti-EHNV diluted 1/32,000 in PBSTG (100 $\mu\text{l well}^{-1}$). Incubate for 90 minutes at RT.
- x) Wash the plate five times on the plate washer.
- xi) Add the conjugate diluted 1/1500 in PBSTG (100 $\mu\text{l well}^{-1}$). Incubate for 90 minutes at RT.
- xii) Wash the plate five times on the plate washer.
- xiii) Add ABTS substrate (22 ml ABTS + 10 $\mu\text{l H}_2\text{O}_2$) (100 $\mu\text{l well}^{-1}$) and place the plate on a plate shaker. Time this step from the moment substrate is added to the first wells of plate 1. Incubate for 20 minutes.
- xiv) Immediately add ABTS stop solution (50 $\mu\text{l well}^{-1}$), shake the plate briefly and read OD at 405 nm. Calculate mean ELISA OD of duplicate wells. Calculate the coefficient of variation of the duplicates: samples with CV >15% should be retested if the mean OD lies near the positive-negative cut-off.

Normalisation of data and decision limit quality control

If it is desired to normalise data from plate to plate and over time, or to undertake decision limit quality control, the following procedure can be followed. Run control antigens in ELISA on at least five occasions over a period of 3 weeks (a total of 20 separate ELISA plates). Calculate the mean OD for each control antigen. Then, for each plate subsequently used, calculate a plate correction factor (PCF) as follows:

PCF = (mean OD control A/actual OD + mean OD control B/actual OD + mean OD control D/actual OD + mean OD control F/actual OD)/4. Multiply the actual mean OD of each sample by the PCF for that plate and report these values.

PCF is allowed to vary between 0.8 and 1.2, which approximates to a coefficient of variation of 10%. Values outside this range suggest that a plate needs to be retested. Plots of PCF over time provide a ready means for monitoring the stability of reagents, procedural variations and operator errors. This QC method has been validated for antigen capture ELISA.

Buffers and other reagents

Borate coating buffer

Boric acid	6.18 g
Disodium tetraborate (Na ₂ B ₄ O ₇ ·10H ₂ O)	9.54 g
NaCl	4.38 g
MQ water to	1 litre
Autoclave	

10 × phosphate buffered saline

NaCl	80.00 g
KCl	2.00 g
Na ₂ HPO ₄	11.50 g
KH ₂ PO ₄	2.00 g
MQ water to	900 ml
Adjust pH to 7.2 with HCl or NaOH; make up to 1 litre	
Autoclave	

For working strength dilute 1/10 and recheck pH.

For storage of powder in jars, make up twice the above quantity of powder; store; to make up add 1.8 litres MQW, pH, make up to 2 litres.

ABTS

Citrate phosphate buffer	
Citric acid	21.00 g
Na ₂ HPO ₄	14.00 g
MQ water to 800 ml; adjust pH to 4.2; make up to 1 litre	
ABTS	0.55 g
Citrate phosphate buffer to	1 litre
Dispense in 22-ml aliquots and freeze.	
Immediately prior to use add 10 µl H ₂ O ₂ per 22-ml aliquot.	

ABTS stop solution (0.01% NaN₃ in 0.1 M citric acid)

Citric acid	10.5 g
MQW to	500 ml
Add 50 mg sodium azide or 1 ml of 5% solution.	

*KPL Conjugate #14-23-06¹⁸**TSGM cryoprotectant*

10 × Tris/saline, pH 7.4	50 ml
Glycerol	250 ml
Sterile purified water to	500 ml
Autoclave	
Add 10% Merthiolate	1 ml
Store in dark bottle at 4°C.	

10 × Tris/saline (250 mM Tris, 1.5 M NaCl)

Tris	15.14 g
NaCl	43.83 g
Sterile purified water	500 ml
pH adjust to	7.4

18 Reagent Supplier: Bio-Mediq DPC Australia, P.O. Box 106, Doncaster, Victoria 3108, Australia; Tel.: (+61-3) 9840 2767; Fax: (+61-3) 9840 2767. Visit: www.kpl.com for links to worldwide network distributors

4.3.1.2.2.3. *Immunoelectron microscopy*

Gold-labelling of sections containing tissues or cell cultures

Principle of the test: cell cultures, tissues and/or tissue homogenates can be used for examination by electron microscopy. Conventional electron microscopy (examination of ultra-thin sections) will generate data on virus structure and morphogenesis. Negative contrast electron microscopy will produce images that can be used to examine the particulate structure of the virus. The use of ranavirus-specific antibodies and conjugated gold with these preparations permits both ultrastructure and antigenicity to be examined (Hyatt, 1991). These collective data enable classification to the genus Ranavirus.

Cell cultures and tissues

- i) Fix tissues or cell cultures as described in Hyatt (1991). Briefly, 2.5% (v/v) buffered glutaraldehyde (cacodylate or phosphate) is used to fix cells for 40 minutes. Following primary fixation the cells are rinsed in the same buffer (3 × 20 minutes), post-fixed in 1% (w/v) buffered osmium tetroxide (1 hour), washed (3 × 5 minutes) in double-distilled/reverse osmosis (RO) water, dehydrated through graded alcohol (70–100%) and infiltrated and embedded in an epoxy resin (e.g. Spurr's or epon). For gold labelling of ultra-thin resin sections, attention must be given to fixation and embedding regimes. For example, cells should be fixed in 0.25% (v/v) glutaraldehyde with 2–4% paraformaldehyde. No secondary fixation is used and the cells are infiltrated and embedded in an acrylic resin such as LR White.
- ii) Following fixation and embedding, cut and transfer ultrathin sections onto filmed nickel grids.
- iii) Cut sections from the appropriate blocks.
- iv) Block in 2% (w/v) skim milk powder in PBS-A (10 minutes).
- v) Block free aldehydes with 0.1 M glycine in PBS-A (20 minutes).
- vi) Wash in PBS-A (3 × 1 minutes). This is an optional step used only if there is an excess of free aldehydes (a high background may be indicative of this).
- vii) If protein A-gold is not being used then block in normal species serum – this serum should be homologous to that complexed to gold. Recommended dilution is approximately 1/40 (10 minutes).
- viii) Incubate in primary antibody. If incubation details are unknown then perform initial reactions with 1/100 to 1/2700 dilutions (with three-fold dilutions). Dilute antibodies in 1% (v/v) cold water fish gelatin in PBS-A, (60 minutes, RT).
- ix) Rinse in 1% (v/v) coldwater fish gelatin in PBS-A, (6 × 3 minutes).
- x) Incubate in gold-labelled secondary antibody or protein A-gold or protein G-gold. Suggested dilution 1/40 in a PBS-A containing 1% (w/v) bovine serum albumin (BSA), 0.1% (v/v) Tween 20 and 0.1% (v/v) Triton X, 60 minutes, RT.
- xi) Rinse in PBS-A (6 × 3 minutes, RT).
- xii) Post-fix in 2.5% (v/v) glutaraldehyde in PBS-A (5 minutes, RT).
- xiii) Rinse in water (RO) (3 × 3 minutes, RT).
- xiv) Dry on filter paper (type not critical).
- xv) Stain in uranyl acetate and lead acetate.

Interpretation of results

Viruses within the cytoplasm of infected cells will be specifically gold-labelled. Viruses will be located singularly, within assembly bodies (inclusion bodies) and within paracrystalline arrays.

Gold-labelling of virus particles (viruses adsorbed to grids)

- i) Dounce homogenise 10% (w/v) liver, kidney or spleen and clarify (5 minutes, 2500 g).
- ii) Adsorb the supernatant (from homogenate or cell cultures) to grid substrate.

- iii) Use carbon-coated 200 mesh gold grids.
- iv) Fix the sample with 0.1% (v/v) glutaraldehyde and 1% (v/v) Nonidet P40 (NP40) in PBS (2 minutes).
- v) Wash in PBS (3 × 3 minutes).
- vi) Block with 5% (v/v) cold water fish gelatin (Sigma) in PBS (10 minutes) followed with incubation buffer (PBS/0.1% cold water fish gelatin).
- vii) Incubate with antibody (affinity purified rabbit anti-EHNV, Lot No. M708; supplied by the OIE Reference Laboratory; suggested dilution 1/500) for 1 hour, at RT.
- viii) Wash grids (6 × 3 minutes) in incubation buffer.

- ix) Incubate with 10 nm protein A-gold (for dilution, refer to suppliers recommendation) for 1 hour, at RT.
- x) Wash (6 × 3 minutes).
- xi) Fix with 2.5% glutaraldehyde (5 minutes).
- xii) Wash with distilled water (3 × 3 minutes) and stain with 2% phosphotungstic acid (pH 6.8) for 1 minute.

Interpretation of results

The inclusion of NP40 will permit antibodies and protein A-gold to penetrate the outer membrane and react with the underlying capsid. Labelling should be specific for the virus. Non-EHNV affinity purified rabbit serum (1/500) should be included as a negative control.

4.3.1.2.2.4. Immunohistochemistry (immunoperoxidase stain)

Samples: formalin-fixed paraffin-embedded tissue sections.

Technical procedure

The following protocol is intended for the qualitative demonstration of ranavirus antigens in formalin-fixed paraffin-embedded tissue sections (He *et al.*, 2002). It assumes that antigens may have become cross linked and therefore includes a protease digestion step that may be omitted if unfixed samples are examined. A commercial kit (DAKO® LSAB K0679) with peroxidase-labelled streptavidin and a mixture of biotinylated anti-rabbit/anti-mouse/anti-goat immunoglobulins as link antibodies is used for staining. Other commercially supplied reagents are also used. For convenience these are also supplied by DAKO¹⁹. The primary affinity purified rabbit anti-EHNV antibody (Lot No. M708) is supplied freeze-dried by the OIE Reference Laboratory.

- i) Cut 5 µm sections and mount on SuperFrost® Plus G/Edge slides (Menzel-Glaser, HD Scientific Cat. No. HD 041300 72P3). Mark around the section with a diamond pencil to limit the spread of reagents.
- ii) De-paraffinise the section:
 - Pre-heat slides in a 60°C incubator for 30 minutes.
 - Place slides in a xylene bath and incubate for 5 minutes. Repeat once. Note that xylene replacements can be used without deleterious effects.
 - Tap off excess liquid and place slides in absolute ethanol for 3 minutes. Repeat once.
 - Tap off excess liquid and place slides in 95% ethanol for 3 minutes. Repeat once.
 - Tap off excess liquid and place slides in distilled or deionised water for 30 seconds.

¹⁹ Dako Cytomation California Inc., 6392 via Real, Carpinteria, CA 93013, USA, Tel.: (+1-805) 566 6655, Fax: (+1-805) 566 6688; Dako Cytomation Pty Ltd, Unit 4, 13 Lord Street, Botany, NSW 2019, Australia, Fax: (+61-2) 9316 4773; Visit www.dakocytomation.com for links to other countries.

- iii) Expose antigens using a protease treatment. Flood slide with proteinase K (5–7 µg ml⁻¹) and incubate for 20 minutes (ready-to-use solution, DakoCytomation Cat. No. S3020). Rinse slide by immersing three times in water. Place in a PBST bath for 5 minutes (PBS pH 7.2, 0.05% [v/v] Tween 20). Tap off the excess wash solution and carefully wipe around the section.
- iv) Perform the immunostaining reaction using the Universal DAKO LSAB[®]+ Kit, Peroxidase (DakoCytomation Cat No. K0679). Ensuring the tissue section is completely covered, add the following reagents to the slide. Avoid drying out.
- v) 3% hydrogen peroxide: cover the section and incubate for 5 minutes. Rinse gently with PBST and place in a fresh wash bath.
- vi) Primary antibody (affinity purified rabbit anti-EHNV 1:/1500 Lot No. M708) and negative control reagent (non-immune rabbit serum at a dilution of 1/1500) on a second slide. Cover the section and incubate for 15 minutes. Rinse slides.
- vii) Link: cover the section and incubate for 15 minutes. Rinse slides.
- viii) Streptavidin peroxidase: cover the section and incubate for 15 minutes. Rinse slides.
- ix) Substrate–chromogen solution: cover the section and incubate for 5 minutes. Rinse slides gently with distilled water.
- x) Counterstain by placing slides in a bath of DAKO[®] Mayer's Haematoxylin for 1 minute (Lillie's Modification, Cat. No. S3309). Rinse gently with distilled water. Immerse 10 times into a water bath. Place in distilled or deionised water for 2 minutes.
- xi) Mount and cover-slip samples with an aqueous-based mounting medium (DAKO[®] Faramount Aqueous Mounting Medium Cat. No. S3025).

Interpretation of results

Ranavirus antigen appears as a brown stain in the areas surrounding degenerate and necrotic areas in parenchymal areas. There should be no staining with negative control rabbit serum on the same section.

Availability of test and reagents: antibody reagents and test protocols are available from the OIE Reference Laboratory.

4.3.1.2.3. Molecular techniques

Identification of ranavirus at genus and species level is possible using two PCR methods based on the MCP gene. In the first method, two PCR assays using MCP primers are used with restriction analysis to detect and rapidly differentiate fish ranaviruses (EHNV, ECV) from amphibian ranaviruses (FV3, BIV) (Harp & Petranka, 2006). This can be completed in less than 24 hours at relatively low cost. In the second method, a single MCP PCR assay is used to generate a 580 bp product, which is then sequenced to identify the type of ranavirus ([refer to Chapter 2.3.1. Epizootic haematopoietic necrosis](#)).

Samples: virus from cell culture or direct analysis of tissue homogenate.

4.3.1.2.3.1. PCR and restriction endonuclease analysis (REA): technical procedure

Amplified product from PCR assay MCP-1 digested with PflM I enables differentiation of Australian iridoviruses (EHNV and BIV) from non-Australian iridoviruses (FV3, Americas; and ECV, Europe). Amplified product from PCR assay MCP-2 digested with Hinc II, Acc I and Fnu4H I (individually) enables differentiation of EHNV and BIV (Australia) from each other and from FV3 (Americas) and ECV (Europe).

Preparation of reagents

EHNV-purified DNA and BIV-purified DNA PCR control reagents are supplied by the reference laboratory in freeze-dried form. Reconstitute using 0.5 ml of Tris-EDTA (TE) buffer (10 mM Tris/HCl, 1 mM EDTA, pH 8.0) and allow the vial to stand at RT for 2 minutes. Mix the vial very gently. For routine use, as a PCR control, it is recommended that working stocks be prepared as a 1/10 dilution in

TE buffer (pH 8.0). Aliquots of 250 µl should be stored at –20°C. Each aliquot is sufficient for at least 50 reactions (1 to 5 µl added to cocktail) and has a minimum shelf life of 6 months from date of diluting.

Primers M151 and M152 (MCP-1, 321 bp), M153 and M154 (MCP-2, 625 bp) are supplied in working strength and should be stored at –20°C. Primers can also be ordered from commercial suppliers. For primer sequences, refer to Table 4.1.

Table 4.1. MCP-1 and MCP-2 primer sequences

PCR assay	Primer	Sequence	Product size	Gene location
MCP-1	M151	AAC-CCG-GCT-TTC-GGG-CAG-CA	321 bp	266–586
	M152	CGG-GGC-GGG-GTT-GAT-GAG-AT		
MCP-2	M153	ATG-ACC-GTC-GCC-CTC-ATC-AC	625 bp	842–1466
	M154	CCA-TCG-AGC-CGT-TCA-TGA-TG		

PCR cocktail

Amplification reactions in a final volume of 50 µl (including 5 µl DNA sample) contain 2.5 µl of each working primer, 200 µM of each of the nucleotides dATP, dTTP, dGTP and dCTP, 10 × PCR buffer (66.6 mM Tris/HCl, 16.6 mM (NH₄)₂SO₄, 2.5 mM MgCl₂, 1.65 mg ml⁻¹ BSA, 10 mM beta-mercaptoethanol) and 2 U Taq polymerase. Instructions on preparation of 10 × PCR buffer are included in Table 4.2.

Table 4.2. 10 × PCR buffer preparation

Ingredients	Amount	Final concentration in 50 µl PCR mix
Tris	4.050 g	66.6 mM
Ammonium sulphate	1.100 g	16.6 mM
BSA (albumin bovine fraction V fatty acid free)	0.825 g	1.65 mg ml ⁻¹
Magnesium chloride	1.25 ml	2.5 mM
TE buffer (sterile)	50 ml	

NOTE: alternative commercial buffers may also be used.

Two negative controls are included, one comprising PCR cocktail only and the second containing 5 µl TE buffer.

The MCP-1 and MCP-2 reactions have the following profile: 1 cycle of denaturation at 94°C for 3 minutes, followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing at 50°C for 30 seconds and extension at 72°C for 1 minute; a final extension of 72°C for 5 minutes, and cooling to 4°C.

NOTE: the annealing temperature may be increased to 60 or 62°C to reduce non-specific amplification when the assay is used to test fish tissues.

PCR results are assessed by electrophoresis in 2% agarose gels stained with ethidium bromide. EHNV PCR control DNA (1/10 working stock) should give a result similar in intensity to the 10–3 band in both cases.

Restriction endonuclease analysis (REA)

PCR amplicons are subjected to REA with the enzymes described in Table 4.3. All endonucleases should be used according to the manufacturers' instructions. REA reactions are prepared by adding 1–4 µl of PCR product, 2 U of the appropriate restriction endonuclease, 1.6 µl of buffer (supplied with each restriction endonuclease), 1.6 µl of 100 µg ml⁻¹ BSA (for PflM I and Hinc II) and made up to a final volume of 16 µl with sterile purified water. Restriction digests are incubated for 2–4 hours at the recommended temperatures and assessed by agarose gel electrophoresis in 3% gels. The predicted band sizes after restriction are given in Table 4.4.

Table 4.3. Restriction endonuclease analysis of ranavirus MCP amplicons

PCR Assay	Restriction enzyme	Predicted band sizes after restriction (bp)	Pattern applies to
MCP-1 (321bp)	<i>PfM I</i>	321	EHNV, BIV
		131, 190	FV3, WIV
MCP-2 (625bp)	<i>Hinc II</i>	100, 138, 387	EHNV
		100, 525	BIV, FV3
		100, 240, 285	WIV
	<i>Acc I</i>	238, 387	EHNV
		625	BIV, ESV, ECV, WIV
		164, 461	FV3, GV
	<i>Fnu4H I</i>	33, 38, 44, 239, 271	EHNV
		3, 33, 38, 44, 108, 399	BIV
		3, 38, 44, 108, 432	FV3, GV
		3, 9, 38, 44, 108, 151, 272	ESV, ECV
		3, 44, 71, 108, 399	WIV

GV: Gutapo virus (Hyatt *et al.*, 2000).

Aliquot into 500 µl volumes and store at –20°C. For a working solution, add 3.5 µl of beta-mercaptoethanol per 500 µl 10 × buffer. Any remaining buffer should be discarded after preparing the PCR cocktail.

The sensitivity of PCR in diagnostic applications directly on fish tissues is being evaluated.

Detailed protocols to enable completion of the test, worksheets and purified control EHNV DNA are available from the OIE Reference Laboratory.

4.3.1.2.3.2. Alternative PCR and sequencing for viral identification

In this assay two primers, a reverse primer (5'-AAA-GAC-CCG-TTT-TGC-AGC-AAA-C-3') and a forward primer (5'-CGC-AGT-CAA-GGC-CTT-GAT-GT-3'), are used for amplification of the target MCP sequence (580 base pairs [bp]) of EHNV DNA by PCR. This PCR procedure can be used for the specific detection of ranaviruses from redfin perch, rainbow trout, sheatfish, catfish, guppy fish (*Poecilia reticulata*), doctor fish (*Labroides dimidatus*) and a range of amphibian ranaviruses (Eaton *et al.*, 1991). Nucleic acid (1 µl) is added to Taq polymerase buffer containing 0.1 µM of each primer, 2.5 U Taq polymerase (Promega) and 2.5 mM MgCl₂. The mixture is incubated in an automatic thermal cycler programmed for 35 cycles at 95°C for 60 seconds, 55°C for 60 seconds, and 72°C for 60 seconds, and finally held at 72°C for 15 minutes. Amplified DNA (580 bp) is analysed by agarose gel electrophoresis, excised and sequenced using a range of standard technologies). Each viral species is identified by its unique DNA sequence available from GenBank. Samples can be submitted to the OIE reference laboratory for specific identification.

4.3.1.2.4. Agent purification

Purification of EHNV has been described (Drury *et al.*, 1995; Hyatt *et al.*, 2000) and a protocol is available from the reference laboratory.

4.3.2. Serological methods

Neutralising antibodies have not been detected in fish or mammals exposed to ranaviruses Indirect ELISA for detection of antibodies induced following exposure to ranavirus has been described for *Bufo marinus* Protocols and specific anti-immunoglobulin reagents required to conduct these tests are available from the reference laboratory.

5. Rating of tests against purpose of use

The methods currently available for surveillance, detection, and diagnosis of ranavirus are listed in Table 5.1. The designations used in the Table indicate: a = the method is the recommended method for reasons of availability, utility, and diagnostic specificity and sensitivity; b = the method is a standard method with good diagnostic sensitivity and specificity; c = the method has application in some situations, but cost, accuracy, or other factors severely limits its application; d = the method is presently not recommended for this purpose; and NA = not applicable. These are somewhat subjective as suitability involves issues of reliability, sensitivity, specificity and utility. Although not all of the tests listed as category a or b have undergone formal standardisation and validation (see Chapter 1.1.2 of this *Aquatic Manual*), their routine nature and the fact that they have been used widely without dubious results, makes them acceptable.

Table 5.1. Methods for targeted surveillance and diagnosis

Method	Targeted surveillance				Presumptive diagnosis	Confirmatory diagnosis
	Ova/milt	Tadpoles	Metamorphs	Adults		
Gross signs	na	d	d	d	d	d
Histopathology	na	d	d	d	b	d
Immunoperoxidase stain	na	c	c	c	b	b
Transmission EM	na	d	d	d	c	c
Immuno-EM	na	d	d	d	c	c
Cell culture	na	a	a	a	a	a
Antigen-capture ELISA	na	a	a	a	b	b
Antibody-capture ELISA	na	d	d	c	c	d
PCR-REA	na	d	a	d	c	a
PCR sequence analysis	na	d	d	d	c	a

EM = electron microscopy; ELISA = enzyme-linked immunosorbent assay; PCR = polymerase chain reaction; REA: restriction endonuclease analysis; na: not applicable

6. Test(s) recommended for targeted surveillance to declare freedom from ranavirus

Statistically valid sampling practices need to be used but these cannot presently be defined for amphibians.

Correct organs/samples need to be collected.

Standardised tests of specified sensitivity and specificity should be used. This restricts certification testing to cell culture, the gold standard test.

Serology might also play a useful role in surveys to identify infected amphibian populations. Further research is required to confirm the validity of this approach.

7. Corroborative diagnostic criteria

7.1. Definition of suspect case

Amphibian, apparently healthy, moribund or dead in which skin and or parenchymal tissues contain histological evidence of focal, multifocal or locally extensive liquefactive or coagulative necrosis with or without intracytoplasmic basophilic inclusion bodies.

7.2. Definition of confirmed case

Amphibian, apparently healthy, moribund or dead in which skin and or parenchymal tissues contain histological evidence of focal, multifocal or locally extensive liquefactive or coagulative necrosis with or without intracytoplasmic basophilic inclusion bodies and/or in which ranavirus is demonstrated by the following means:

1. Characteristic CPE in cell culture and cell culture is positive for ranavirus in immunoperoxidase test or antigen-capture ELISA or PCR,

or
2. Tissues positive in antigen-capture ELISA or immunoperoxidase stain or immunoelectron microscopy or PCR

And for both 1 and 2, where PCR is used
3. Sequence consistent with ranavirus is demonstrated by PCR-REA or PCR-sequencing.

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NB: There is an OIE Reference Laboratory for Infection with ranavirus (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).

KILLING OF FARMED FISH FOR DISEASE CONTROL PURPOSES

EU comments

The EU notes that previous EU comments concerning the prior stunning of fish were not taken into account; however, the report of the meeting of the OIE Aquatic Animal Health Standards Commission does not explain the basis for the proposed changes and for refusing the EU comments.

The EU wishes to present specific comments in the text. As foreseen in point 3 of Article 7.4.2, for the EU the alterations are not clear and we ask for a clarification on the points indicated in the text below. As the EU comments concern a general animal welfare principle, the EU would suggest that the same phrasing and requirements are used as in correspondent Chapter 7.6 in the Terrestrial Code.

Article 7.4.1.

Scope

These recommendations are based on the premise that a decision to kill the farmed fish for disease control purposes has been made, and address the need to ensure the welfare of the farmed fish until they are dead.

The stunning and killing of fish for human consumption is covered in Chapter 7.3.

The killing death of individual farmed fish, in the course of farming operations (i.e. sorting, grading, or background morbidity) is out of the scope of this chapter.

Account should also be taken of the guidance given in the following chapters in the *Aquatic Code*: Chapter 4.4. Contingency Planning, Chapter 4.6. Handling, Disposal and Treatment of Aquatic Animal Waste, Chapter 5.4. Control of Aquatic Animal Health Risks Associated with Transport, Chapter 7.2. Welfare of Farmed Fish during Transport and Chapter 7.3. Welfare Aspects of Stunning and Killing of Farmed Fish for Human Consumption.

Article 7.4.2.

General principles

1. Contingency plans for disease control should be in place at a national level and should contain details of disease control strategies, managerial structure, and operational procedures. Fish welfare considerations should be addressed within such contingency plans for disease control.
2. Depending on the situation, emergency killing of fish may be carried out on site or after fish are transported to an approved killing facility.
3. When killing fish for disease control purposes, methods used may render the fish unsuitable for human consumption (e.g. pharmacological, maceration). This should be specified in contingency plans. Fish not suitable for human consumption may be killed by specific methods other than those included in Chapter 7.3. (e.g. chemical, mechanical), all of which should be included in contingency plans.

EU comments

In point 3 of Art. 7.4.2, the EU suggests the following insertion: in the text "When killing fish for disease control purpose, methods used should result in immediate loss of consciousness lasting until death, when loss of consciousness is not immediate, induction of unconsciousness should be non-aversive or the least aversive possible and should not cause avoidable anxiety, pain, distress or suffering in fish. The methods used may render the fish unsuitable for human consumption..."

Rationale:

To avoid ambiguous text and ensure that procedures used for farmed fish are the same as those used for other animals (Chapter 7.6 of the Terrestrial Code).

4. Fish suitable for human consumption should be killed following the provisions provided in Chapter 7.3. Welfare aspects of stunning and killing of farmed fish for human consumption.

Article 7.4.3.

The following principles should apply when killing fish:

1. Operational procedures should be adapted to the specific operating circumstances on the premises and should address biosecurity and fish welfare specific to the disease of concern.
2. Killing of fish should be carried out without delay by appropriately qualified personnel with all due consideration made to increased biosecurity protocols.
3. The handling of fish should be kept to a minimum to avoid stress and minimised to prevent spread of disease. ~~and when done, it~~ This should be done in accordance with the Articles described below.
4. ~~Methods used to kill the fish should result in immediate death or loss of consciousness lasting until death.~~

EU comments

The EU wishes to have justification regarding the deletion of prior stunning.

Rationale:

Stunning of animals prior to killing is a basic animal welfare requirement. Sometimes this is not possible in an emergency, but whenever it is feasible to stun and kill this is preferable. We are not sure whether this deletion should be seen in conjunction with other amendments in the document (see below art. 7.4.6. and 7.4.7.). If the intention was to remove all recommendations concerning stunning the EU cannot support this deletion.

54. There should be continuous monitoring of the procedures to ensure they are consistently effective with regard to biosecurity and fish welfare.
65. Standard operating procedures (SOP's) should be available and followed at the premises.

Article 7.4.4.

Operational guidelines for affected premises

A protocol plan for the killing of fish on affected premises due for to disease control issues purposes should be developed by the operator and approved by the *Competent Authority*, taking into consideration welfare and biosecurity requirements as well as safety of the personnel.

The protocol Considerations should include consideration of:

1. minimising handling and movement of fish;

2. species, number, age, size of fish to be killed;
3. methods for killing the fish;
4. availability of pharmacological substances ~~chemicals/equipment needed to kill the fish;~~
5. equipment needed to kill the fish;
- ~~6. biosecurity issues;~~
6. any legal issues that may be involved, ~~for example, (e.g. the use of~~ pharmacological substances) ~~controlled drugs or chemicals;~~
7. presence of other nearby aquaculture premises;
8. disposal of killed fish (in accordance with Chapter 4.6.).

Article 7.4.5.

Competencies and responsibilities of the operational team

The operational team is responsible for the planning, implementation of, and reporting from the killing of the fish.

1. Team leader
 - a) Competencies
 - i) ability to assess fish welfare, especially relating to the effectiveness of the killing techniques selected and utilised in the fish killing operations, to detect and correct any deficiencies;

EU comments

In the point above, the EU suggests that the word stunning is inserted in the sentence: "ability to assess fish welfare, especially relating to the effectiveness of the stunning and/or killing techniques selected and utilised in the fish killing operations, to detect and correct any deficiencies."

Rationale:

In the Terrestrial Code competency on stunning is required and this should be the case here as well.

- ii) ability to assess biosecurity risks and mitigation measures being applied to prevent spread of disease;
 - iii) skills to manage all activities on premises and deliver outcome on time;
 - iv) awareness of the emotional impact on fish farmers, team members and general public;
 - v) effective communication skills.
- b) Responsibilities
 - i) determine most appropriate killing method(s) to ensure that the fish are killed without avoidable pain and distress which balance biosecurity considerations;
 - ii) plan overall operations on the affected premises;
 - iii) determine and address requirements for fish welfare, operator safety and biosecurity;
 - iv) organise, brief and manage a team of people to facilitate killing of the relevant fish in accordance with national contingency plans for disease control;

- v) determine logistics required;
- vi) monitor operations to ensure that fish welfare, operator safety and biosecurity requirements are met;
- vii) report upwards on progress and problems;
- viii) provide a written report summarising the killing, practices utilised in the operation and their effect on aquatic animal welfare and subsequent biosecurity outcomes. The report should be archived and be accessible for a period of time defined by the *Competent Authority*;
- ix) review on-site facilities in terms of their appropriateness for mass destruction.

2. On-farm personnel responsible for killing of fish

- a) Competencies
 - i) specific knowledge of fish, and their behaviour and environment;
 - ii) trained and competent in fish handling and killing procedures;

EU comments

In the above point, the EU suggests that the word "stunning" is inserted in the sentence: "trained and competent in fish handling, stunning and killing procedures".

Rationale:

In the Terrestrial Code competency on stunning is required, and this should be the case here as well.

- iii) trained and competent in the operation and maintenance of equipment.
- b) Responsibilities
 - i) ensure humane killing of fish through effective killing techniques;

EU Comments

Please consider inserting the word "*stunning*" so that the requirement reads: "ensure humane stunning and killing of fish through effective stunning and killing techniques".

Rationale:

Consistency throughout the chapter and mentioned above

- ii) assist team leader as required;
- iii) design and construct temporary fish handling facilities, when required.

Article 7.4.6.

Chemical Pharmacological killing methods

This Article refers to killing methods using an overdose of anaesthetics.

1. Use of chemicals pharmacological substances

- a) Chemicals Pharmacological substances used for killing fish should kill the fish effectively, not merely have an anaesthetic effect;

EU Comments

Point 1(a) and (c) seem to contradict one another. If it is permitted to use pharmacological substances to anaesthetise, then the EU would suggest the following words to be added to the sentence: "..., unless fish are killed by another method before they regain consciousness."

Rationale:

Allows for the situation where a substance is used to stun the fish, and ensures the welfare of the fish by compelling they promptly be killed via another method.

- b) when using such chemicals pharmacological substances, the operating personnel should ensure that the solution has the correct concentration, and that sea water is used for marine fish species and freshwater for freshwater species;
- c) fish should be kept in the pharmacological substance chemical solution until they are dead. Fish that are merely anaesthetised should be killed before they regain consciousness by another method such as bleeding, decapitation or another appropriate killing method.

EU Comments

In point c), in this instance the word "solution" should have been retained as this more accurately reflects the situation

Rationale:

Terminology. Technically, fish are not submerged in a pure substance, but a solution with a dissolved percentage of the substance.

2. Advantages

- a) Large numbers of fish may be killed in one batch;
- b) handling is not required until fish are anaesthetised or euthanized;

EU Comments

In point 2.b), the EU would suggest OIE to consider changing "euthanized" to "kill" for consistency of terminology in this chapter of the code.

Rationale:

Correct terminology.

- c) use of chemicals pharmacological substances is a non-invasive technique and thus minimises biosecurity risks.

3. Disadvantages

- a) may need to be followed by killing if fish are only anaesthetised;
- b) some chemicals pharmacological substances induce a panic reaction in the fish;
- c) care is essential in the preparation and provision of treated water, and in the disposal of water and/or fish carcasses that have been treated with anaesthetic agents pharmacological substances.

EU comments

In Articles 7.4.6. and 7.4.7, the OIE has only listed two methods for killing fish, and when using a pharmacological substance the fish will be both anaesthetised and killed by the substance so that mention of prior stunning seems irrelevant in this case. For the mechanical killing methods it is stated initially that these methods should only be used following stunning.

The EU would like a clarification on whether this Chapter still contains a general recommendation with regard to stunning of animals prior to killing, whenever this is feasible in the given situation.

Article 7.4.7.

Mechanical killing methods

The following mechanical killing methods should only be used for killing fish following stunning.

1. Decapitation

- a) Decapitation, using a sharp device such as a guillotine or knife, may be used for killing fish but only following anaesthesia;
- b) the required equipment should be kept in good working order;
- c) contamination of the working area by blood due to bleeding and body fluids and other organic material may present a biosecurity risk and is the major disadvantage of this method.

2. Maceration

- a) Maceration by a mechanical device with rotating blades or projections causes immediate fragmentation and death in newly hatched fish and embryonated eggs, as well as fertilised/unfertilised eggs of fish. It is a suitable method for the processing of such material. The procedure results in rapid death and a large number of eggs/newly hatched fry can be killed quickly;
- b) maceration requires specialised equipment which should be kept in good working order. The rate of introducing material into the device should be such that the cutting blades continue to rotate at their fully functional rate and that they do not fall below the defined critical speed defined by the manufacturer;
- ~~e) large fish should be introduced head first into the device;~~
- ~~dc)~~ contamination of the working area by blood due to bleeding and body fluids and other organic material may present a biosecurity risk and is the major disadvantage of this method.

EU Comments

The EU asks that OIE consider the insertion of a new article 7.4.8. on the use of carbon dioxide gas as a killing method with the following phrasing:

"Killing with Carbon dioxide gas

When all other methods (of killing) have been fully evaluated and have been considered to be inappropriate, carbon dioxide may be used to kill fish dependant on species. Fish are added to baths of CO2 saturated water and left in the bath till death supervenes. Care should be taken that the solution is not diluted during the course of use by inadvertent addition of water whilst fish are being added."

Rationale:

For some species there is no readily available method to kill large numbers of fish. In such cases as a last resort, carbondioxide could be used. However it is necessary to take into account that some species tolerate high levels of CO2 and that the substance is ineffective for these species and should not be used in any case. On the other hand as this method has been shown to have clear negative impact on fish welfare (EFSA opinions March and May 2008), it is also necessary to encourage the development of more humane methods for all species that are farmed commercially. For this reason the permission to use CO2 as a last resort should be reevaluated after a certain period.