



**REPORT OF THE MEETING OF THE OIE AD HOC GROUP ON  
SUSCEPTIBILITY OF FISH SPECIES TO INFECTION WITH OIE LISTED DISEASES<sup>1</sup>**

**Paris, 25–27 April 2017**

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The OIE *ad hoc* Group on susceptibility of fish species to infection with OIE listed diseases (the *ad hoc* Group) met for their second meeting at OIE Headquarters from 25–27 April 2017. (Please note, the report of the first *ad hoc* Group meeting held from 17–19 January 2017 was not published).

The list of participants and the Terms of Reference are presented in [Annex I](#) and [Annex II](#), respectively.

Dr Gillian Mylrea, Deputy Head Standards Department, welcomed members to this meeting, the second for this *ad hoc* Group and thanked them for their ongoing work on this important topic.

The Chair of the *ad hoc* Group, Dr Mark Crane, clarified that the purpose of this meeting was to finalise the assessments started at their previous meeting for infection with epizootic haematopoietic necrosis virus (EHNV), infection with *Gyrodactylus salaris* (*G. salaris*) and infection with koi herpesvirus (KHV); and to start work on the assessment for infection with infectious salmon anaemia (ISAV). He explained that the *ad hoc* Group would progressively apply the criteria to the OIE listed fish diseases, noting that it would take several meetings to complete this task. During this meeting the *ad hoc* Group finalised assessments for EHNV, ISAV and *G. salaris*.

The *ad hoc* Group applied the three-stage approach, outlined in Article 1.5.3. of the *Aquatic Code*, to assess susceptibility of a species, described as shown below:

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

Hosts that were classified as susceptible species (as described in Article 1.5.7.) were proposed for inclusion in Article 10.X.2. of the relevant *disease-specific* chapter of the *Aquatic Code*.

Hosts that were classified as species for which there is incomplete evidence for susceptibility (as described in Article 1.5.8.) were proposed for inclusion in a new Section 2.2.2. *Species with incomplete evidence for susceptibility* of the relevant chapter of the *Aquatic Manual*.

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<sup>1</sup> Note: This *ad hoc* Group report reflects the views of its members and may not necessarily reflect the views of the OIE. This report should be read in conjunction with the March 2015 report of the Aquatic Animal Health Standards Commission because this report provides its considerations and comments. It is available at <http://www.oie.int/en/international-standard-setting/specialists-commissions-groups/aquatic-animal-commission-reports/meeting-reports/>

The detailed assessments for each specific pathogenic agent assessed by the *ad hoc* Group are provided in Annexes III to V.

Disease	Annex Number
Infection with epizootic haematopoietic necrosis virus	Annex III
Infection with infectious salmon anaemia virus	Annex IV
Infection with <i>Gyrodactylus salaris</i>	Annex V

The *ad hoc* Group wished to note the following:

1. In many of the older publications accurate pathogen identification was not carried out because molecular typing techniques were not available at the time. Therefore, for many of these cases, a weight of evidence approach using combined data from relevant studies was used to assess susceptibility.
2. The *ad hoc* Group worked on the assumption that authors had correctly identified the host species on which they were reporting.
3. References that reported invasive experimental procedures as the route of transmission were not progressed past Stage 1 (i.e. Article 1.5.4.). In these cases the criteria A-D were noted as not applicable and the outcome inconclusive.
4. The *ad hoc* Group used the following outcome key when assessing the susceptibility of the species:

*1: The species meets the criteria for susceptibility and is proposed for inclusion in Article X.X.2. of the Aquatic Code;*

*2: The species meets some but not all of the criteria and is proposed for inclusion in Section 2.2.2. 'Species with incomplete evidence for susceptibility' of the Aquatic Manual;*

*3: The species does not meet the criteria (e.g. PCR- positive on gills or intestines and no other evidence; studies with questionable methodology or inconsistent results) and is not proposed for inclusion in either the Aquatic Code or Aquatic Manual;*

*4: There is evidence of non-susceptibility and the species is not proposed for inclusion in either the Aquatic Code or Aquatic Manual.*

5. Where there was conflicting evidence in the scientific literature for the same host species, or assessments differed (e.g. assessments ranging between '1' and '3'), the *ad hoc* Group provided some explanatory text in the relevant Annex as to their rationale for the final outcome.
6. For assessments that were inconsistent with known pathogen epidemiology (e.g. when a virus previously presumed as highly species-specific is shown to occur in a distant taxonomic group), the *ad hoc* Group required two or more independent studies to justify a new host species to be considered as susceptible.
7. The *ad hoc* Group separately identified hosts for which there was only evidence for criteria in Article 1.5.4. ('natural pathways for infection') and 1.5.5. ('pathogenic agent has been adequately identified'), but not 1.5.6. ('presence of the pathogenic agent constitutes an infection'), e.g. shown to be PCR positive without virus isolation, i.e. Outcome '3'.

The *ad hoc* Group recommended that these organisms not be included in Section 2.2.2. (Species with incomplete evidence for susceptibility) of the relevant chapter of the *Aquatic Manual*, as has been done in the revised crustacean disease chapters of the *Aquatic Manual* because finfish viruses can be cultured and therefore virus isolation from internal organs was required to be considered conclusive evidence of the presence of infectious virus. (Note: this differs from crustacean viruses where there are no *in-vitro* methods for virus isolation.)

The *ad hoc* Group made the following recommendations:

- The *ad hoc* Group agreed to commence work electronically on KHV, Spring viraemia of carp and infection with Salmonid alphavirus.
  
- The *ad hoc* Group requested that another physical meeting be held in 2017 to finalise these assessments and to start applying the criteria to the remaining OIE listed fish diseases.

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.../Annexes



**MEETING OF THE AD HOC GROUP ON SUSCEPTIBILITY OF FISH SPECIES TO INFECTION  
WITH OIE LISTED DISEASES**

**Paris, 25–27 April 2017**

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**List of participants**

**MEMBERS OF THE AD HOC GROUP**

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**Terms of reference**

**Background**

A new Chapter 1.5. ‘Criteria for listing species as susceptible to infection with a specific pathogen’ was introduced in the 2014 edition of the *Aquatic Code*. The purpose of this chapter is to provide criteria for determining which host species are listed as susceptible in Article X.X.2. of each disease specific chapter in the *Aquatic Code*. The criteria are to be applied progressively to each disease-specific chapter in the *Aquatic Code*.

These assessments will be undertaken by *ad hoc* Groups and the assessments will be provided to Member Countries’ for comment prior to any change in the list of susceptible species in Article X.X.2. of the disease specific chapters in the *Aquatic Code*.

For species where there is some evidence of susceptibility but insufficient evidence to demonstrate susceptibility through the approach described in Article 1.5.3, information will be included in the relevant disease-specific chapter in the *Aquatic Manual*.

**Purpose**

The *ad hoc* Group on susceptibility of fish species to infection with OIE listed diseases will undertake assessments for the ten OIE listed fish diseases.

**Terms of Reference**

1. Consider evidence required to satisfy the criteria in Chapter 1.5.
2. Review relevant literature documenting susceptibility of species for OIE listed fish diseases.
3. Propose susceptible species for OIE listed diseases for fish based on Article 1.5.7.
4. Propose susceptible species for OIE listed diseases for fish based on Article 1.5.8.

**Expected outputs of the *ad hoc* Group**

1. Develop a list of susceptible species for inclusion in the relevant Article X.X.2. of fish disease-specific chapters in the *Aquatic Code*.
  2. Develop a list of species with incomplete evidence for susceptibility for inclusion in Section which 2.2.2. of the *Aquatic Manual*.
  3. Draft a report for consideration by the Aquatic Animals Commission at their September 2017 meeting.
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### ASSESSMENT OF HOST SUSCEPTIBILITY TO INFECTION WITH EPIZOOTIC HAEMATOPOIETIC NECROSIS VIRUS (EHNV)

Criteria for susceptibility to infection with EHNV are detailed in Table 1 (as per Article 1.5.6. of the *Aquatic Code*). This table includes Replication (A), Viability/Infectivity (B), Pathology/Clinical Signs (C) and Location (D). Hosts were considered to be infected with EHNV if they fulfilled either criterion A, or at least two of criteria B, C and D (as per point 3 of Article 1.5.7. of the *Aquatic Code*).

**Table 1.** Criteria for susceptibility to infection with EHNV

A: Replication	B: Viability/Infectivity	C: Pathology/Clinical signs	D: Location
Sequential virus titration showing increase in viral titres  OR  Demonstration of increasing copy number over time by qPCR with confirmatory PCR/sequencing  OR  TEM showing virions in host cells  OR  Products (e.g. antigens) of virus replication detected	Isolation by cell culture  OR  Cohabitation with passage to a susceptible host with confirmed infection in the sentinel species by PCR and demonstrating at least one of the following: i. clinical signs, with or without associated mortality, ii. Histopathology, iii. Re-isolation of virus in cell culture.*	Tropism for vascular endothelium and haematopoietic necrosis.  Perivascular mononuclear inflammatory response in liver.	Gills, cardiovascular system, kidney, liver **

Key:

\* To demonstrate viability or infectivity of the target pathogen within the host being assessed, single passage in any known susceptible SPF host is required.

\*\* It is noted that target organs may differ from those described for existing susceptible species. Where gills are used surface contamination should be ruled out.

## ASSESSMENT FOR HOST SUSCEPTIBILITY

The assessment for host susceptibility to infection with EHNV is provided in Table 2.

**Table 2.** Outcome of assessment for host susceptibility to infection with EHNV

Genus	Species	Common name	Stage 1: Transmission*	Stage 2: Pathogen identification	Stage 3: Evidence for infection				Outcome**	References
					A	B	C	D		
<i>Oncorhynchus</i>	<i>mykiss</i>	rainbow trout	N/E	PCR/IFAT/ELISA	Y	Y	Y	Y	1	4, 3, 10, 11
<i>Perca</i>	<i>fluviatilis</i>	European perch	N/E	PCR/IFAT	Y	Y	Y	Y	1	2, 4, 9, 11
<i>Macquaria</i>	<i>australasica</i>	macquarie perch	E	PCR	Y	Y	Y	Y	1	2, 11
<i>Bidyanus</i>	<i>bidyanus</i>	silver perch	E	PCR	Y	Y	Y	Y	1	2, 11
<i>Galaxias</i>	<i>olidus</i>	mountain galaxias	E	Incomplete	Y	Y	Y	Y	1	11 (virus later characterized by 2)
<i>Gambusia</i>	<i>affinis</i>	mosquito fish	E	Incomplete	Y	Y	Y	Y	1	11 (virus later characterized by 2)
<i>Ameiurus</i>	<i>melas</i>	black bullhead	E	IFAT	N	Y	Y	Y	1	5
<i>Esox</i>	<i>lucius</i>	northern pike	E	IHC	Y	Y	Y	Y	1	6
<i>Sander</i>	<i>luciperca</i>	pike-perch	E	PCR/sequencing	N	Y	Y	Y	1	7
<i>Melanotaenia</i>	<i>fluviatilis</i>	crimson spotted rainbow fish	E	PCR	N	Y	Y	Y	1	2
<i>Gambusia</i>	<i>holbrooki</i>	eastern mosquito fish	E	PCR	N	Y	Y	Y	1	2
<i>Macquaria</i>	<i>ambigua</i>	golden perch	E	PCR	N	N	N	N	3	2

Genus	Species	Common name	Stage 1: Transmission*	Stage 2: Pathogen identification	Stage 3: Evidence for infection				Outcome**	References
					A	B	C	D		
<i>Tandanus</i>	<i>tandanus</i>	freshwater catfish	EI	PCR	NA	NA	NA	NA	3	2
<i>Mogurnda</i>	<i>adpersa</i>	purple spotted gudgeon	E	PCR	N	Y	N	N	3	2
<i>Salmo</i>	<i>salar</i>	atlantic salmon	EI	First report	NA	NA	NA	NA	3	10
<i>Maccullochella</i>	<i>peelii</i>	murray cod	E/EI	PCR	N	N	N	N	3/4	2, 11
<i>Nannoperca</i>	<i>australis</i>	southern pigmy perch	E	PCR	N	N	N	N	4	2
<i>Maccullochella</i>	<i>macquariensis</i>	trout cod	E	PCR	N	N	N	N	4	2
<i>Hypseleotris</i>	<i>species</i>		E	PCR	N	N	N	N	4	2
<i>Craterocephalus</i>	<i>stercusmuscarum fulvus</i>	unspoked Hardyhead	E	PCR	N	N	N	N	4	2
<i>Cyprinus</i>	<i>carpio</i>	common carp	E	PCR/sequencing	N	N	N	N	4	6
<i>Carassius</i>	<i>auratus</i>	goldfish	E	PCR/sequencing	N	N	N	N	4	6, 9

**Route of infection Key\***

*N:* Natural infection

*E:* Experimental (non-invasive)

*EI:* Experimental (invasive)

*NA:* Not applicable (e.g. PCR negative, no other data)

Criterion A alone is sufficient to determine infection. Otherwise at least two of criteria B/C/D.

**Outcome Key\*\***

1: Meets the criteria for susceptibility.

2: Some but not all of the criteria have been met.

3: Criteria have not been met (e.g., PCR- positive on gills or intestines and no other evidence; studies with questionable methodology or inconsistent results).

4: Evidence of non-susceptibility.

**Additional information relevant to assessments for EHNV**

Macquarie perch (*Macquaria australasica*)

The *ad hoc* Group assessed two papers resulting in outcome assessments of a ‘1’ and ‘3’ and agreed to include this species as susceptible in the *Aquatic Code*. In Becker *et al.* (2013), the only indication of infection by bath challenge was positive histopathology in one fish (of one sufficiently tested), the evidence was considered inconclusive, and the *ad hoc* Group assessed it as a ‘3’. However, the *ad hoc* Group considered the Langdon *et al.* (1989) paper to be a strong study and the outcome status of ‘1’ is based on this paper in addition to the strain being characterized (PCR sequencing) by Becker *et al.* (2013).

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  12. WHITTINGTON R.J., PHILBEY A., REDDACLIFF G.L. & MACGOWN A.R. (1994). Epidemiology of epizootic haematopoietic necrosis virus (EHNV) infection in farmed rainbow trout, *Oncorhynchus mykiss* (Walbaum): findings based on virus isolation, antigen capture ELISA and serology. *Journal of Fish Diseases*, **17**, 205–218.
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### ASSESSMENT OF HOST SUSCEPTIBILITY TO INFECTION WITH SALMON INFECTIOUS ANAEMIA VIRUS (ISAV)

Criteria for susceptibility to infection with ISAV are detailed in Table 1 (as per Article 1.5.6. of the *Aquatic Code*). This table includes Replication (A), Viability/Infectivity (B), Pathology/Clinical Signs (C) and Location (D). Hosts were considered to be infected with ISAV if they fulfilled either criterion A, or at least two of criteria B, C and D (as per point 3 of Article 1.5.7. of the *Aquatic Code*).

**Table 1.** Criteria for susceptibility to infection with ISAV

A: Replication	B: Viability / Infectivity	C: Pathology / Clinical signs	D: Location
<p>Sequential virus titration showing increase in viral titres;</p> <p>OR</p> <p>Demonstration of increasing copy number over time by qPCR with confirmatory PCR/sequencing;</p> <p>OR</p> <p>TEM showing virions in host cells;</p> <p>OR</p> <p>Products of virus replication detected e.g. demonstration of viral antigen by specific immunoassay of tissue imprints or fixed tissue sections;</p>	<p>Isolation by cell culture. This needs to be from an internal organ.</p> <p>OR</p> <p>Cohabitation with passage to a susceptible host with confirmed infection in the sentinel species by PCR and demonstrating at least one of the following: i. clinical signs with or without associated mortality, ii. Histopathology, iii. Re-isolation of virus in cell culture.</p>	<p>Yellowish or blood-tinged fluid in peritoneal and pericardial cavities.</p> <p>Oedema of the swim bladder.</p> <p>Small haemorrhages of the visceral and parietal peritoneum.</p> <p>Focal or diffusely dark red liver. A thin fibrin layer may be present on the surface.</p> <p>Swollen, dark red spleen with rounded margins.</p> <p>Dark redness of the intestinal wall mucosa in the blind sacs, mid- and hind-gut, without blood in the gut lumen of fresh specimens.</p> <p>Swollen, dark red kidney with blood and liquid effusing from cut surfaces.</p> <p>Pinpoint haemorrhages of the skeletal muscle.</p> <p>Low hematocrit (severe anemia).</p>	<p>Gill, heart, mid-kidney, spleen, liver, pancreas/intestine*</p>

\* It is noted that target organs may be differ from those described for existing susceptible species. Where gills and pancreas/intestines are used surface contamination should be ruled out.

## ASSESSMENT FOR HOST SUSCEPTIBILITY

The assessment for host susceptibility to infection with salmon anaemia virus is provided in Table 2.

**Table 2.** Outcome of assessment for host susceptibility to infection with ISAV

Genus	Species	Common name	Stage 1: Transmission	Stage 2 : Pathogen identification	Stage 3:				Outcome**	References
					A	B	C	D		
<i>Oncorhynchus</i>	<i>mykiss</i>	Rainbow trout	E and EI	RT-PCR and cell culture	N	Y	Y	Y	1	2, 22
<i>Salmo</i>	<i>salar</i>	Atlantic salmon	E	RT-PCR	Y	N	Y	Y	1	11
<i>Salmo</i>	<i>trutta</i>	Brown trout = sea trout	N	RT-PCR	N	Y	N	Y	1	17
<i>Oncorhynchus</i>	<i>masou</i>	Amago trout	I and E	RT-PCR	N	N	Y	Y	2	3
<i>Clupea</i>	<i>harengus</i>	Atlantic herring	E	RT-PCR and culture -ve	N	Y	N	N	2	14
<i>Oncorhynchus</i>	<i>kisutch</i>	Coho salmon	N	RT-PCR	N	Y	N	N	3	5, 6, 7,8, 9, 24
<i>Gadus</i>	<i>morhua</i>	Cod	I and N	Cell culture and RT-PCR	N	N	N	N	4	10, 21
<i>Pollachius</i>	<i>virens</i>	Saithe	I and E	- veRT-PCR	N	N	N	N	4	23
<i>Mytilus</i>	<i>edulis</i>	Blue mussel	N	RT-PCR	N	N	N	N	4	13, 19
<i>Oncorhynchus</i>	<i>tshawytscha</i>	Chinook salmon	I	Cell culture	N	N	N	N	4	18
<i>Cyprinus</i>	<i>carpio</i>	Common carp	I	RT-PCR	N	N	N	N	4	4
<i>Carassius</i>	<i>auratus</i>	Goldfish	I	RT-PCR	N	N	N	N	4	4



Genus	Species	Common name	Stage 1: Transmission	Stage 2 : Pathogen identification	Stage 3:				Outcome**	References
					A	B	C	D		
<i>Hippoglossus</i>	<i>hippoglossus</i>	Atlantic halibut	I	RT-PCR	N	N	N	N	4	21
<i>Caligus</i>	<i>rogercresseyi</i>	Sea lice	N	RT-PCR and cell culture	N	N	N	N	4	15
<i>Pollachius</i>	<i>virens</i>	Pollock	N	RT-PCR	N	N	N	Y	4	10, 12
<i>Cyclopterus</i>	<i>lumpus L.</i>	Lumpfish	N	RT-PCR and cell culture	N	N	N	N	4	10
<i>Salvelinus</i>	<i>alpinus</i>	Arctic charr	I	RT-PCR	N	N	N	N	N/A	22
<i>Oncorhynchus</i>	<i>keta</i>	Chum salmon	I	CELL CULTURE	N	Y	N	N	N/A	18
<i>Oncorhynchus</i>	<i>nerka</i>	Sockeye salmon	I	RT-PCR	N	N	N	N	NA	4
<i>Salvelinus</i>	<i>leucomaenis</i>	Japanese Char	I	RT-PCR	N	Y	N	N	NA	4
<i>Plecoglossus</i>	<i>altivelis</i>	Ayu sweetfish	I	RT-PCR	N	N	N	N	NA	4
<i>Gnathopogon</i>	<i>elongatus caeruleus</i>	Biwa gudgeon	I	RT-PCR	N	N	N	N	NA	4
<i>Anguilla</i>	<i>anguilla</i>	European eel							Not assessed	
<i>Alosa</i>	<i>pseudoharengus</i>	Alewife							Not assessed	

### Route of infection Key

*N:* Natural infection

*E:* Experimental (non-invasive)

*EI:* Experimental (invasive)

*NA:* Not applicable; (e.g. PCR negative, no other data)

Criterion A alone is sufficient to determine infection. Otherwise at least two of criteria B/C/D.

### Outcome Key

1: Meets the criteria for susceptibility.

2: Some but not all of the criteria have been met.

3: Criteria have not been met (e.g., PCR- positive on gills or intestines and no other evidence; studies with questionable methodology or inconsistent results).

4: Evidence of non-susceptibility.

## Annex IV (contd)

### **Additional information relevant to ISAV assessments**

In this assessment the *ad hoc* Group assumed that the susceptibility to HPR-deleted was the same as for HPR0 (EFSA Journal 2012; 10 (11):2971. [22 pp.]). Below is more detailed explanatory text for some of the assessments.

#### Amago trout (*Oncorhynchus masou*)

The *ad hoc* Group assessed *Oncorhynchus masou* as having an outcome score of a '2' because while 6 out of 20 fish were positive via PCR and one fish died with clinical signs, the virus was not transmitted to Atlantic salmon. Based on the limited evidence the *ad hoc* Group considered that there was incomplete evidence for inclusion in the *Aquatic Code* as a susceptible host because this was the first and only study on the species and the results were based on only one fish. Therefore, there is a need for corroborating evidence before indicating it to be a susceptible species.

#### Coho salmon (*Oncorhynchus kisutch*)

A natural outbreak of ISA in Coho salmon is reported in Chapter 2.3.5. of the *Aquatic Manual* based on a study published by Kibenge *et al.* (2001) where ISAV was detected by RT-PCR in tissue homogenates from animals undergoing a mortality event. Since that study, substantial evidence on the susceptibility of Coho salmon to this virus has been published indicating that this species is not a viable host for ISAV.

Given the new information from surveillance data and from other researchers, and that the original findings (Kibenge *et al.*, 2001) may have been a laboratory contamination, the *ad hoc* Group proposed to include Coho salmon (*Oncorhynchus kisutch*) in the Section\_2.2.2. 'Species with incomplete evidence for susceptibility' of the *Aquatic Manual* until more conclusive information is made available.

The following is a compilation of the information supporting the assessment that Coho salmon is not susceptible to ISAV (Original study by Kibenge *et al.* [2001]):

- The isolate detected in Chilean Coho salmon in 1999 was a perfect match for an isolate in Canada that was routinely used in the laboratory that identified the virus in Coho salmon as a positive control and in exposure studies (Kibenge *et al.*, 2002; Kibenge *et al.*, 2006).
  - Since this time, genetic analyses of field isolates from farms in close proximity are seldom a perfect match (Kibenge *et al.*, 2009; Lyngstad *et al.*, 2011) suggesting it is unlikely that we would find a perfect match on isolates from Canada and Chile.
- There were several outbreaks of ISA in Canada at the time that the original Coho salmon were tested so the laboratory had opportunity to cross-contaminate both with research and field specimens.
  - The laboratory was later inspected and deemed to have insufficient separation of samples for laboratory GOP (OIE inspection at UPEI, 2012).
- The culture of the virus in this study could only be done on one tissue homogenate sample and only on TO cell lines with trypsin. Given the opportunities for cross-contamination and the lack of replicability of the results it is likely that the finding is a false positive.

Other evidence suggesting Coho salmon is not a susceptible host for ISAV.

- There is now a well-described infectious condition in Coho salmon in Chile called “jaundice disease”, which resembles what Kibenge *et al.* (2001) described. Although data suggest Coho salmon jaundice disease is an infectious condition (Smith *et al.*, 2006), no pathogen has been isolated from affected fish, including ISAV, despite extensive investigation of this disease (Alba *et al.*, submitted for publication).
- No outbreaks of ISA occurred in Chile in 1999 when the Coho salmon were reported by Kibenge *et al.* to be positive for the virus despite millions of susceptible Atlantic salmon hosts in the area and the fact that the strain of virus detected in the Coho salmon was known to be pathogenic to Atlantic salmon. It was not until eight years later that the first case of ISA occurred in Chile.
- The Chilean ISAV isolate in 2007 associated with clinical ISA was more closely related to the Norwegian ISAV isolates than the North American isolates.
- A study by Kibenge in 2006 found that even by intraperitoneal (IP) infection of high virus titres, ISAV could not induce disease in Coho salmon, and although not reported in the paper, the virus presumably could not be detected by RT-PCR at the end of the study. The actual findings of the PCR testing were not presented in the paper, but they were presumed negative given the authors did not discuss them in the manuscript and they would have aided in the conclusion that Coho salmon are asymptomatic carriers of the ISA virus.
- Another study, in which injected Coho salmon IP with high concentrations of ISAV, was able to re-isolate the virus from 1 of 5 fish sampled 13 days post-injection, but the other 10 fish sampled later in the study were not positive. In a second trial in the same study, none of the Coho salmon injected with ISAV (n=15) were positive for virus on cell culture despite the successful infection of the Atlantic salmon in the study.
- Lastly, the Chilean government has been testing Coho salmon as part of their ISAV surveillance programme using Taqman RT-PCR as described in Snow *et al.* (2006). Between 2008 and 2012, while known cases of ISA were occurring in Chile, Sernapesca evaluated 39,214 pools of Coho salmon representing 118,864 fish samples and none were positive for the virus. During the same time period they sampled 144,472 pools of Atlantic salmon representing 414,583 fish and detected 3105 positive pools. The government of Chile also tested several pools of fish from farms rearing multiple species including Coho salmon together (n=28,873), and reported 19 positive samples. All of these positive pools were determined, based on individual fish analysis, to be from the Atlantic salmon in the pools (personal comm. M. Lara Sernapesca). The latter suggests that even on farms with positive Atlantic salmon the Coho salmon do not test positive by RT-PCR.
  - The government data were also analysed statistically to determine the probability of freedom from disease in farmed Coho salmon in Chile (Alba *et al.*, submitted for publication). The authors concluded with high certainty based on their models that Coho salmon in Chile were free of ISAV.

#### Annex IV (contd)

Given the new information from surveillance data and from other researchers, and that the original findings (Kibenge *et al.*, 2001) may have been a laboratory contamination, the *ad hoc* Group proposed to include Coho salmon (*Oncorhynchus kisutch*) in the Section 2.2.2. 'Species with incomplete evidence for susceptibility' of the *Aquatic Manual* until more conclusive information is made available.

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**ASSESSMENT OF HOST SUSCEPTIBILITY TO INFECTION WITH  
*GYRODACTYLUS SALARIS***

The *ad hoc* Group noted that for *G.salaris* the only criterion used to determine Stage 3 (as per Article 1.5.6. of the *Aquatic Code*) was (A) ‘Evidence of replication’ because attachment of the parasite occurs transiently on many species and therefore clinical signs and location of infection alone do not constitute a true infection. Therefore, Viability/Infectivity (B), Pathology/Clinical Signs (C) and Location (D) were not applicable.

Criteria for replication aimed to differentiate between replication versus maturation of existing parasites. Because *G. salaris* is hyperviviparous, adult parasites likely contain embryos when transferred to test species. Thus a limited increase in parasite numbers upon transfer may reflect maturation of existing embryos rather than new reproduction/replication. Consequently, the *ad hoc* Group defined replication as a doubling, or more, in parasite numbers that is maintained beyond the lifespan expected for *G. salaris* on a susceptible host at the given water temperature. Jensen and Bakke (1999) provide average lifespans and reproductive rates for *G. salaris* on *Salmo salar* (their preferred host) held at different water temperatures.

**Table 1. Criteria for susceptibility to infection with *G. salaris***

<b>A: Replication</b>	<b>B: Viability/Infectivity</b>	<b>C: Pathology/Clinical signs</b>	<b>D: Location</b>
Sequential examination showing at least a two-fold increase in parasite numbers beyond the expected lifespan for the given conditions.	N/A	N/A	N/A

### ASSESSMENT FOR HOST SUSCEPTIBILITY

The assessment for host susceptibility to infection with *G. salaris* is provided in Table 2.

**Table 2.** Outcome of assessment for host susceptibility to infection with *G. salaris*

Genus	Species	Common name	Stage 1: Transmission*	Stage 2: Pathogen identification	Stage 3: Evidence for infection				Outcome	References
					A	B	C	D		
<i>Salmo</i>	<i>salar</i>	Atlantic salmon	N/E	PCR/genotyping	Y	NA	NA	NA	1	7, 9, 11, 12
<i>Oncorhynchus</i>	<i>mykiss</i>	Rainbow trout	N/E	PCR/genotyping	Y	NA	NA	NA	1	10
<i>Salvelinus</i>	<i>alpinus</i>	Arctic char	N/E	PCR/genotyping	Y	NA	NA	NA	1	9, 12, 16
<i>Salvelinus</i>	<i>fontinalis</i>	North American brook trout	N	PCR/genotyping	Y	NA	NA	NA	1	4, , 15
<i>Thymallus</i>	<i>thymallus</i>	Grayling	E	PCR/genotyping	Y	NA	NA	NA	1	11
<i>Salmo</i>	<i>trutta</i>	Brown trout	N/E	PCR/genotyping	Y	NA	NA	NA	1	11
<i>Coregonus</i>	<i>lavaretus</i>	Whitefish	E	Morphology	N	NA	NA	NA	3	14
<i>Anguilla</i>	<i>anguilla</i>	European eel	E	Morphology	N	NA	NA	NA	3	3
<i>Salvelinus</i>	<i>namaycush</i>	North American lake trout	E	Morphology	N	NA	NA	NA	3	5, 6
<i>Gasterosteus</i>	<i>aculeatus</i>	3 spine stickleback	E	Morphology	N	NA	NA	NA	3	13
<i>Pungitius</i>	<i>pungitius</i>	9 spine stickleback	E	Morphology	N	NA	NA	NA	3	13
<i>Platichthys</i>	<i>flesus</i>	Flounder	E	Morphology	N	NA	NA	NA	3	13
<i>Coregonus</i>	<i>lavaretus</i>	Whitefish	E	Morphology	N	NA	NA	NA	3	14
<i>Lampetra</i>	<i>planeri</i>	Lamprey	E	Morphology	N	NA	NA	NA	3	2
<i>Rutilus</i>	<i>rutilus</i>	Roach	E	Morphology	N	NA	NA	NA	3	2
<i>Phoxinus</i>	<i>phoxinus</i>	Minnows	E	Morphology	N	NA	NA	NA	3	1
<i>Perca</i>	<i>fluviatilis</i>	Perch	E	Morphology	N	NA	NA	NA	3	2



**Route of infection Key\***

*N: Natural infection*

*E: Experimental (non-invasive)*

*EI: Experimental (invasive)*

*NA: Not applicable; (e.g. PCR negative, no other data)*

**Outcome Key\*\***

*1: Meets the criteria for susceptibility*

*2: Some but not all of the criteria have been met.*

*3: Criteria have not been met (e.g. PCR- positive on gills or intestines and no other evidence; studies with questionable methodology or inconsistent results).*

*4: Evidence of non-susceptibility.*

**Additional information relevant to *G. salaris***

The *ad hoc* Group accepted pathological identification based on morphology when assessed by a recognised expert (i.e. did not require molecular confirmation).

The *ad hoc* Group noted that many species can sustain viable populations for short durations and could thus act as temporary vectors for spread for the parasite, even though the species do not fulfil the criterion used to determine Stage 3 because there is no supporting evidence of replication as defined by the *ad hoc* Group.

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