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Species which may act as vectors or reservoirs of diseases covered by the Animal Health Law: Listed pathogens of fish

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Abstract

Vector or reservoir species of five fish diseases listed in the Animal Health Law were identified, based on evidence generated through an extensive literature review (ELR), to support a possible updating of Regulation (EU) 2018/1882. Fish species on or in which highly polymorphic region-deleted infectious salmon anaemia virus (HPRΔ ISAV), Koi herpes virus (KHV), epizootic haematopoietic necrosis virus (EHNV), infectious haematopoietic necrosis virus (IHNV) or viral haemorrhagic septicaemia virus (VHSV) were detected, in the field or during experiments, were classified as reservoir species with different levels of certainty depending on the diagnostic tests used. Where experimental evidence indicated transmission of the pathogen from a studied species to another known susceptible species, the studied species was classified as a vector species. Although the quantification of the risk of spread of the pathogens by the vectors or reservoir species was not part of the terms or reference, such risks do exist for the vector species, since transmission from infected vector species to susceptible species was proven. Where evidence for transmission from infected fish was not found, these were defined as reservoirs. Nonetheless, the risk of the spread of the pathogens from infected reservoir species cannot be excluded. Evidence identifying conditions that may prevent transmission by vectors or reservoir fish species during transport was collected from scientific literature. For VHSV, IHNV or HPRΔ ISAV, it was concluded that under transport conditions at temperatures below 25°C, it is likely (66–90%) they will remain infective. Therefore, vector or reservoir species that may have been exposed to these pathogens in an affected area in the wild, aquaculture establishments or through water supply can possibly transmit VHSV, IHNV or HPRΔ ISAV into a non-affected area when transported at a temperature below 25°C. The conclusion was the same for EHN and KHV; however, they are likely to remain infective under all transport temperatures.

Keywords: Vector, Reservoir, highly polymorphic region-deleted infectious salmon anaemia virus, Koi herpes virus, cyprinid herpesvirus-3, epizootic haematopoietic necrosis virus, infectious haematopoietic necrosis virus

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Summary

Term of Reference 1 (ToR 1) requested the European Food Safety Authority (EFSA) to assess which species or groups of species of aquatic animals pose a considerable risk for spreading pathogens causing the diseases of aquatic species listed in COMMISSION IMPLEMENTING REGULATION (EU) 2018/1882 EU Regulation 2016/429. This Opinion specifically focuses on assessing vector or reservoir species of five diseases of fish, i.e. highly polymorphic region-deleted infectious salmon anaemia virus (Δ ISAV), Koi herpes virus (KHV) (CyHV3), epizootic haematopoietic necrosis virus (EHNV), infectious haematopoietic necrosis virus (IHNV) and viral haemorrhagic septicaemia virus (VHSV). The aim of the assessments is to indicate if the Annex to Implementing Regulation (EU) 2018/1882, listing those vectors or reservoir species, needs to be updated. EFSA was not requested to update the list of susceptible species, listed in the same Implementing Regulation, as this work is already being coordinated by the Reference laboratories of the EU and the World Organisation for Animal Health (WOAH). In addition, it was agreed that a species cannot be classified as both susceptible and vector or reservoir species.

The following working definitions were agreed for the assessment: a fish species can be considered a **vector** when the pathogen has been identified in or on the fish species and it has been demonstrated to transmit the pathogen to susceptible species. To be considered a **reservoir** species, the pathogen should have been identified in or on the fish species, but evidence of transmission of the pathogen to susceptible species could not been found. It should be cautioned, however, that these are working definitions to address the term of reference. A clear separation between reservoir, vectors and susceptible species is not always easily made in the field, especially for aquatic animal diseases.

Although the quantification of the risk of spread of the pathogens by the vectors or reservoir species was not part of the terms or reference, such risks do exist for the vector species, since transmission from infected vector species to susceptible species was proven. Where evidence for transmission from infected fish was not found, these were defined as reservoirs. Nonetheless, the risk of the spread of the pathogens from infected reservoir species cannot be excluded.

An ELR has been carried out to gather all published peer-reviewed scientific evidence available on parameters needed to assess the role of aquatic species (only fish species) as vectors or reservoirs. The detailed methods for searching the literature, study selection, data collection and quality assurance are described in detail in EFSA (2023). The data, extracted from the eligible literature, were assessed in two steps. In the first step, the working group experts individually identified those studies where the target pathogens were detected with reference tests (i.e. WOAH, EURL) in or on fish species, either in experimental or field settings, with a high (> 90%) certainty. This immediately led to the classification as reservoir or vector species (the latter only for experimental studies with proven transmission of the pathogen from the vector to the susceptible species). Also, those studies were identified that led to a clear exclusion of the species as vector or reservoir (> 90% certainty) due to negative test results.

In a second step, the studies with inconclusive (< 66% certainty) results were discussed in smaller groups and then consolidated by the whole working group. The cut-off level for classifying species as vectors or reservoirs was set at a minimum certainty of 66%.

The results of the assessment indicated that:

The following species is considered a reservoir for **EHNV** with > 90% certainty: *Tandanus tandanus* (dewfish).

The following species are considered reservoir species for **EHNV** with 66–90% certainty: *Maccullochella peelii* (Murray cod), *Macquaria novemaculeata* (Australian bass) and *Macquaria ambigua* (callop).

The following species are considered vector species for **KHV** with > 90% certainty: *Carassius auratus* (goldfish), *Carassius gibelio* (Prussian carp), *Ctenopharyngodon idella* (grass carp), *Gymnocephalus cernua* (Eurasian ruffe), *Hypophthalmichthys molitrix* (silver carp), *Rutilus rutilus* (common roach) and *Tinca tinca* (tench).

The following species are considered reservoir species for **KHV** with > 90% certainty: *Acipenser gueldenstaedtii* (Russian sturgeon), *Acipenser oxyrinchus* (Atlantic sturgeon), *Acipenser ruthenus* × *Huso huso* (hybrid sterlet × beluga), *Barbatula barbatula* (stone loach), *Gasterosteus aculeatus* (three-spine stickleback), *Perca fluviatilis* (European perch) and *Scardinius erythrophthalmus* (Pearl roach).

The following species are considered reservoir species for **KHV** with 66–90% certainty: *Oreochromis niloticus* (Nile tilapia) and *Pseudorasbora parva* (topmouth gudgeon).

The following species are considered reservoir species for **HPRΔ ISAV** with > 90% certainty: *Clupea harengus* (Atlantic herring), *Oncorhynchus masou* (masu salmon) and *Oncorhynchus kisutch* (coho salmon).

The following species are considered reservoir species for **HPRΔ ISAV** with 66–90% certainty: *Gadus morhua* (Atlantic cod), *Oncorhynchus keta* (chum salmon).

The following species are considered reservoir species for **IHNV** with > 90% certainty: *Aulorhynchus flavidus* (tube-snout), *Acipenser transmontanus* (white sturgeon), *Clupea pallasii pallasii* (Pacific herring), *Cyprinus carpio* (common carp), *Danio rerio* (zebrafish), *Perca flavescens* (American yellow perch) and *Seriola quinqueradiata* (amberjack).

The following species are considered reservoir species for **IHNV** with 66–90% certainty: *Cymatogaster aggregata* (shiner perch) and *Oncorhynchus gorbuscha* (pink salmon).

The following species are considered reservoir species for **VHSV** with > 90% certainty: *Anguilla anguilla* (European eel), *Argentina sphyraena* (lesser argentine), *Belone belone* (garfish), *Cottus pollux* (Japanese fluvial sculpin), *Cyprinus carpio* (common carp), *Enchelyopus cimbrius* (fourbeard rockling), *Epinephelus akaara* (Hong Kong grouper), *Esox lucius* × *Esox masquinongy* (tiger muskellunge), *Eutrigla gurnardus* (grey gurnard); *Gadiculus argenteus* (silvery pout), *Gadus chalcogrammus* (Alaska pollock), *Hippoglossus hippoglossus* (Atlantic halibut), *Ictalurus punctatus* (channel catfish), *Merluccius productus* (Pacific hake), *Moxostoma anisurum* (silver redhorse), *Moxostoma macrolepidotum* (shorthead redhorse sucker), *Oncorhynchus mykiss* (rainbow trout) × *Salvelinus fontinalis* (American brook charr), *Oryzias latipes* (Japanese rice fish), *Pagrus major* (red sea bream), *Percopsis omiscomaycus* (trout perch), *Petromyzon marinus* (sea lamprey), *Pomoxis annularis* (white crappie), *Rhinogobius* sp. (Yoshinobori complex; Japanese goby), *Reinhardtius hippoglossoides* (Greenland halibut), *Salvelinus alpinus* (Arctic char), *Scorpaena porcus* (black scorpionfish), *Sebastes inermis* (black rockfish) and *Seriola quinqueradiata* (amberjack).

The following species are considered reservoir species for **VHSV** with 66–90% certainty: *Alosa pseudoharengus* (alewife), *Anoplopoma fimbria* (sablefish), *Carassius auratus* (goldfish), *Fundulus diaphanous* (banded killifish), *Hypomesus pretiosus* (surf smelt), *Lota lota* (burbot), *Notemigonus crysoleucas* (golden shiners), *Oncorhynchus mykiss* (rainbow trout) × *Salmo trutta* (Amu-Darya trout), *Oncorhynchus mykiss* (rainbow trout) × *Salvelinus alpinus* (Alpine Char), *Oncorhynchus mykiss* (rainbow trout) × *Salvelinus namaycush* (American Lake Charr).

In addition, a list of vector or reservoir species for which no peer reviewed studies with evidence of its role as vector or reservoir was found, are suggested to be removed from the current list Commission Implementing Regulation 1882/2018.

Term of Reference 2 (ToR 2) requested EFSA to assess the suitability of the conditions under which fish species should be regarded as vectors or reservoirs for the purposes of movements. These conditions are set out in Annex I to Commission Delegated Regulation (EU) 2020/990 and in Annex XXX to Commission Delegated Regulation (EU) 2020/692. Alternative conditions had to be proposed, if the conditions in those Regulations were considered not to prevent the transmission of the targeted pathogen via movement of vectors or reservoirs.

To provide a concise answer within the time frame of the mandate, it was decided to focus the assessment on those conditions that would prevent transmission facilitated by the movement of vectors and reservoirs, for which scientific evidence was available. In a first step, the experts in the working group carried out a narrative literature review to collect any evidence from scientific literature identifying conditions that may prevent transmission by vectors. In addition, information on the duration of the experimental studies and the water temperature were compiled during the ELS carried out for ToR 1, collecting the ranges of the different durations and temperatures for which transmission has been proven for the different pathogens by the different vector species. Then, the experts concluded by consensus if the collected evidence was sufficient to support the need to alter the conditions stipulated in Annex I to Commission Delegated Regulation (EU) 2020/990 and in Annex XXX to Commission Delegated Regulation (EU) 2020/692.

For VHSV, IHNV or HPRΔ ISAV, it was concluded that under transport conditions at temperatures below 25°C, it is likely (66–90% certainty) that VHSV, IHNV and HPRΔ ISAV will remain infective.

Therefore, vector or reservoir species that may have been exposed to VHSV, IHNV or HPRΔ ISAV in an affected area can possibly transmit VHSV, IHNV or HPRΔ ISAV when transported at a temperature below 25°C into a non-affected area. Exposure in a VHSV, IHNV or HPRΔ ISAV affected area may have occurred if the vector or reservoir originate from (a) an aquaculture establishment or group of aquaculture establishments, where susceptible species or reservoir or other vector species are kept, (b) the wild, where they may have been exposed to susceptible, reservoir or other vector species or (c) an aquaculture establishment supplied with water possibly contaminated with VHSV, IHNV or HPRΔ ISAV.

For EHN and KHV, it was concluded that both pathogens may remain infective under all transport temperatures. Therefore, vectors or reservoir species can transmit these viruses when they originate from the same sources as listed in points (a), (b) and (c) above, when transported at any temperature. It was suggested that these conclusions may be considered for amendments to Annex I of Reg 2020/990 and Annex XXX of Reg 2020/692.

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1. Introduction

1.1. Background and Terms of Reference as provided by the requestor

In accordance with Article 8 of Regulation (EU) 2016/429 (AHL), the disease-specific rules for listed diseases provided in the AHL, and the rules adopted pursuant to that Regulation, apply to listed species. In compliance with that Article, the Commission shall establish a list of animal species or groups of species, which pose a considerable risk for the spread of specific listed diseases based on the capability of those animals to carry those specific diseases. Animal species or groups of animal species shall only be added to the list if they pose a considerable risk for the spread of a specific listed disease because they are vectors or reservoirs for that disease, or scientific evidence indicates that such role is likely.

The list of vector species, which is set out in the fourth column of the table in the Annex to Implementing Regulation (EU) 2018/1882, was carried forward from the list, which was previously set out in Commission Regulation (EU) 1251/2008. The Commission now requires scientific advice to inform an amendment to that list, to ensure that only species, which comply with Article 8 of the AHL, are listed. This amendment may involve species, which are currently set out in the fourth column of the Annex to Implementing Regulation (EU) 2018/1882 being removed and/or new species being added to that list.

It should be noted that vector species of aquatic animals are not listed in the WOAH Aquatic Code[1] or in the WOAH Aquatic Manual.[2] In the disease specific chapters of the WOAH Aquatic Manual however, as well as listing susceptible species, other species which have shown incomplete evidence of susceptibility are listed, as are species in which PCR positive results have been reported, but where an active infection has not been demonstrated. In 2020, the EU Reference Laboratories (EURLs) for fish, crustaceans and molluscs, with the assistance of experts, reviewed those non-susceptible species, which are listed in the WOAH Manual, in an effort to determine whether or not, they could be considered to be vectors of specific listed diseases. The reports which have been prepared by the EURLs and which have been furnished to the Commission, may be of assistance to the risk assessor in providing the scientific advice which is currently sought. The three reports (concerning fish, molluscs and crustaceans) accompany this letter. It should, however, be noted that these reports also contain information concerning susceptible species to the listed diseases, which is not pertinent to this request for a scientific opinion.

In addition, for those species and groups of species referred to above, which should be listed in accordance with Article 8 of the AHL, scientific advice is also required concerning the conditions under which these species should be regarded as vectors or reservoirs for the purposes of movements.

The conditions under which these species should be regarded as vectors are set out in Annex I to Commission Delegated Regulation (EU) 2020/990[3] and in Annex XXX to Commission Delegated Regulation (EU) 2020/692[4]. It should be noted that the conditions set out in Annex I to Commission Delegated Regulation (EU) 2020/990 are not identical to the conditions set out in Annex XXX to Commission Delegated Regulation (EU) 2020/692, and both sets of conditions are different to those which were previously set out in columns 3 and 4 of Annex I to Commission Regulation (EC) 1251/2008.

Terms of reference

In view of the above, the Commission asks EFSA for a scientific opinion on the listing of vector species of aquatic animals in accordance with Article 8 of Regulation (EU) 2016/429, as follows:

1) For each of the aquatic diseases listed in Annex II to the AHL, an assessment concerning which species or groups of species of aquatic animals pose a considerable risk for their spread based on the fact that:

    i) they are vector species or reservoirs for that disease, or
    ii) scientific evidence indicates that such role is likely.

[1] WOAH Aquatic Animal Health Code, 2021, 23rd Edition.
[2] WOAH Aquatic Manual, 2021, 8th Edition.
[3] Commission Delegated Regulation (EU) 2020/990 of 28 April 2020 supplementing Regulation (EU) 2016/429 of the European Parliament and of the Council, as regards animal health and certification requirements for movements within the Union of aquatic animals and products of animal origin from aquatic animals (OJ L 221, 28 April 2020, p. 42).
[4] Commission Delegated Regulation (EU) 2020/692 of 30 January 2020 supplementing Regulation (EU) 2016/429 of the European Parliament and of the Council as regards rules for entry into the Union, and the movement and handling after entry of consignments of certain animals, germinal products and products of animal origin (OJ L 174, 3.6.2020, p. 379).

For each of the species or groups of species, which are assessed to be vector species or reservoirs of the listed diseases, or where scientific evidence indicates that such role is likely, they should be aquatic animals, which are not already listed as susceptible to the listed disease.

2) For each of the species or groups of species, which are assessed to fulfil the requirements for listing by virtue of being a vector or reservoir of a listed disease, or where scientific evidence indicates such a role is likely, an assessment of the suitability of the conditions under which they should be regarded as vectors or reservoirs for the purposes of movements. These conditions are set out in Annex I to Commission Delegated Regulation (EU) 2020/990 and in Annex XXX to Commission Delegated Regulation (EU) 2020/692, however, alternative conditions should be proposed, if the conditions, which are set out in those Regulations, are assessed to be unsuitable.

1.2. Interpretation of the Terms of Reference

1.2.1. Term of Reference 1: Assessment of potential vectors and reservoir species of diseases of fish, crustaceans and molluscs listed in Annex II to the AHL

Term of Reference 1 (ToR 1) requests EFSA to provide a list of vector species or reservoir species of pathogens of fish, crustaceans and molluscs, listed in Annex II to the AHL, aiming to update the fourth column of the Annex to Implementing Regulation (EU) 2018/1882.

EFSA was not requested to update the list of susceptible species, already listed in the third column of the same Implementing Regulation. In addition, it was agreed that a species cannot be classified simultaneously as susceptible as well as vector or reservoir species.

This work is complementary to the work that was coordinated by the EURL and WOAH concerning the identification of susceptible species.

This Scientific Opinion focuses on all life stages, including eggs, sperm and gametes of **fish** belonging to the superclass Agnatha and to the classes Chondrichthyes, Sarcopterygii and Actinopterygii. The pathogens listed by the AHL affecting fish are:

- Highly polymorphic region (HPR)-deleted infectious salmon anaemia virus (HPRΔ ISAV)
- Koi herpes virus (KHV) = Cyprinid herpesvirus-3 (CyHV3)
- Epizootic haematopoietic necrosis virus (EHNV)
- Infectious haematopoietic necrosis virus (IHNV)
- Viral haemorrhagic septicaemia virus (VHSV)

It was agreed that for this assessment, a fish species can be considered a **vector** when the pathogen has been identified in or on the species and it has been demonstrated to transmit the pathogen to susceptible species, or there is scientific evidence that indicates that this transmission is likely. In addition, the vector species must not already be listed as susceptible to the respective pathogen.

Vectors may transmit pathogenic agents to susceptible species in two ways: (i) the pathogenic agent can multiply within the vector's body and then be transmitted to other susceptible species; (ii) the pathogenic agent can remain in or on the vector without multiplying and be mechanically transmitted to other susceptible species.

To be considered a **reservoir** species, on the other hand, the pathogen has been identified in or on the fish species, but evidence of transmission of the pathogen to susceptible species is not available. In addition, it was agreed that the reservoir species must not already be listed as susceptible to the respective pathogen.

It should be cautioned, however, that these are working definitions to address the term of reference. A clear separation between reservoir, vectors and susceptible species is not always easily made on the basis of field observations alone, and for aquatic animal diseases in particular.

Although the quantification of the risk of spread of the pathogens by the vectors or reservoir species was not part of the terms or reference, such risks do exist for the vector species, since transmission from infected vector species to susceptible species was proven. Where evidence for transmission from infected fish was not found, these were defined as reservoirs. Nonetheless, the risk of the spread of the pathogens from infected reservoir species cannot be excluded.

1.2.2. Term of Reference 2: Conditions under which fish species shall be regarded as vectors or reservoirs of diseases of fish listed in Annex II to the AHL

The list of potential vectors and reservoir species developed in ToR 1 should be considered as vectors or reservoirs for movements in the EU, provided that certain conditions are fulfilled.

The conditions in the EC Delegated Reg 2020/990 Annex I specify that the species may be regarded as vectors when animals are present in: (a) an aquaculture establishment or group of **aquaculture establishments** where susceptible species listed in column 3 of that table in Annex 1, are kept; or (b) the **wild** where they can be **exposed to susceptible species** listed in column 3 of that table.

The conditions in EC Delegated Reg 2020/692 Annex XXX stipulate that Vectors may be regarded as the species that have been in contact with listed susceptible species listed in column 3 of the table in the Annex to Commission Implementing Regulation (EU) 2018/1882 through **co-habitation or through water supply**.

It should be noted that although these two delegated acts explicitly mention vectors, it is assumed that the same conditions apply for reservoirs. Thus, when vector species and reservoir species do not fulfil these conditions, they can be moved provided that the transport complies with the EU regulations and all the measures have been implemented which would prevent the contamination or infection of the transported species.

To address ToR 2, besides the conditions already laid down in EU Reg 2020/990 Annex I and Reg EC Delegated 2020/692 there are other conditions that need to be fulfilled by a species to be considered a vector. Evidence found in the scientific literature related to the above factors for the specific pathogens was scrutinised and summarised. If there was no proof that certain specific conditions can exclude that the fish species will act as potential vector or reservoir, there was no change in the conditions already laid down in the above-mentioned regulations.

2. Data and methodologies

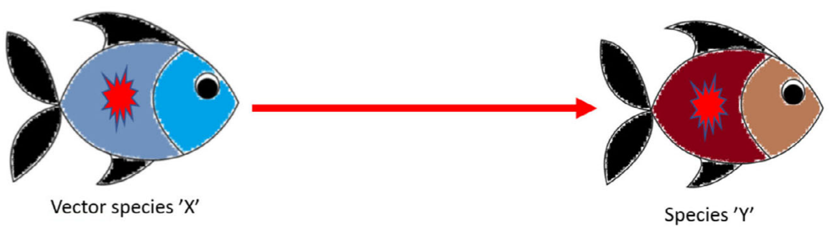
2.1. Methodologies

2.1.1. Term of Reference 1: Assessment of potential vectors and reservoir species of pathogens of fish, listed in Annex II to the AHL

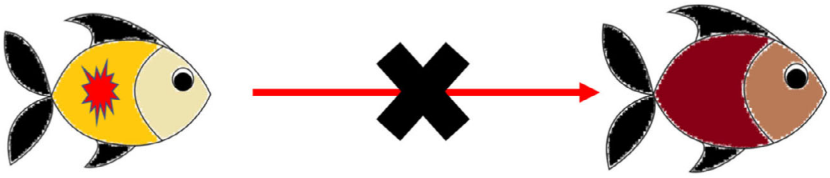
An extensive literature review (ELR) has been carried out to gather all scientific evidence available on parameters needed to assess the role of aquatic species as vectors or reservoirs species of specific pathogens of fish, listed by the AHL. To assess the evidence the following review questions were posed:

Review questions:

1) **For vector species: What is the evidence generated by experimental infection studies, demonstrating transmission of 'Pathogen A' from 'Vector species X' on- or in which Pathogen A was detected, to a 'Species Y'?**



2) **For reservoir species: What is the evidence generated by experimental infection studies or field studies, demonstrating the detection of Pathogen A on- or in Reservoir species X, without further evidence of transmission of pathogen A to a 'Species Y'?**



As agreed in the interpretation of the ToRs, to define a vector species, proof of onwards transmission from species X to species Y is needed. This proof is usually not available from field detections. Field detections imply Pathogen A was detected in species X during outbreak investigations, prevalence studies or any other study where the pathogen was detected in fish in a specific area or farm. In these situations, it cannot be definitively proven that species Y has been infected through species X and not from any other source of infection.

The detailed methods for searching the literature, the study selection, data collection and quality assurance are described in detail in EFSA (2023).

The data set was generated with the relevant information extracted from the eligible literature needed to answer the review questions. Then, the assessment methodology for deciding if the information was sufficient to classify the fish species as a potential vector or reservoir species, according to the working definition provided in Section 1.2.1, was applied in two steps.

2.1.1.1. First step: Individual assessments by the fish experts of the data extracted from specific papers assigned to them

Questions:

How certain are you that species X is a RESERVOIR species based on the evidence generated through the ELR (for field and experimental infection studies that did not investigate species Y and field studies where infection of species Y could not be proven)?

How certain are you that species X is a VECTOR species based on the evidence generated through the ELR (for experimental infection studies that have also investigated infection of species Y)?

In the first step, the experts were asked to identify the species for which a clear 'yes' or 'no' could be answered on the above questions with a high certainty **(> 90% certainty).** The experts were asked to provide the reasoning for their choice and reminded to respect the working definition of vectors and reservoirs, and not to consider other information that was not collected or extracted from the eligible peer-reviewed literature that was outside the scope of the working definition, e.g. on observed clinical signs.

As a guidance to help the decision-making, the following criteria were agreed a priori among the experts:

- **Positive results (> 90% certainty):**

Experimental infections: there is higher certainty when evidence from experimental infections is available compared to field studies because the animals are infected under controlled conditions so there is no need for sequencing or confirmatory tests. The following tests are accredited by the EU reference laboratory (EURL) and the WOAH as reference tests for the concerned pathogens:

- VHSV and IHNV (EURL for fish and crustacean diseases, 2021)
    - cell culture plus ELISA with Monoclonal antibodies or
    - qPCR assays based on Jonstrup et al. (2012) for VHSV; Purcell et al. (2013) for IHNV
- EHNV (EURL for fish and crustacean diseases, 2021)
    - Cell culture plus IFAT, ELISA or PCR, Real time PCR. Sequencing is required
- KHV (WOAH, 2023)
    - qPCR (Gilad, 2004; Engelsma et al., 2013)
- ISAV (EURL for fish and crustacean diseases, 2021)
    - HPR del- cell + IFAT; qPCR (Snow et al., 2004), plus sequencing

VECTOR: when at least one positive reference test for 'Species X' and 'Species Y' was reported.

RESERVOIR: where there is at least one positive reference test for 'detection of Pathogen A in Species X' (and negative or not tested for Species 'Y').

Field studies: as they are subject to more uncertainty, ideally two reference tests taken from the same animals or reported in the same paper are needed to conclude Pathogen A was truly present in an animal from species X:

RESERVOIR: when positive for two reference tests for 'Species X'.

- **Negative results (0–10% certainty) [this is equivalent to 90–100% certainty that species X is not a vector or reservoir].**

The review should have captured only papers where Pathogen A had been detected in Species X. However, there are some papers where negative results were recorded for Pathogen A detection in species X, e.g. when several diagnostic tests were used to detect pathogen A in species X, and not all results were positive. Depending on the specific situation (e.g. if other studies are available or not), negative results in Species X can provide more than 90% certainty that Species X is NOT a RESERVOIR species based on the evidence extracted from the literature.

In addition, in transmission experiments, where negative results for Pathogen A detection in Species Y were recorded (susceptible species), the assessment focussed on Species X as reservoir species and the same method as described above was followed.

Any positive test result that was not one of the above situations were considered as inconclusive. The inconclusive results were elaborated in the next step of the assessment (group discussion).

2.1.1.2. Second step: group discussion

- **Smaller expert working group discussion**
  - The individual judgements were presented and discussed to reach a consensus judgement between 3–4 experts on fish diseases.
  - Only inconclusive cases were discussed, and experts were asked to identify a more precise certainty range for the inconclusive assessments:
    - Likely66–90%
    - As likely as not33–66%
    - Unlikely10–33%
- **Whole working group**
  - The results of the smaller expert group were presented, discussed and consolidated by the whole working group.
  - The cut-off level for classifying species as vectors or reservoirs was set at a minimum certainty of 66%.

Since some fish species could be the subject of different studies with different study design and methodological quality, their assessment could result in different classifications. In these situations, the classification as vector prevailed above the classification as reservoir species, as evidence of transmission was present. Nonetheless, all the outcomes of all the assessments of different studies with a certainty > 66% are provided in the assessment section (Tables 1–3), but only the classification with the highest risk for transmission was taken up in the conclusions. Studies of species for which the assessments had a certainty < 66% are provided in Appendix B, Table B.1.

Finally, it should be noted that one the limitation of the assessment based ELR is that it was exclusively based on peer-reviewed evidence. Current lack of qualitative evidence or published studies on specific species does not mean the species cannot play a role as vector or reservoir. Therefore, the assessment should be updated when new evidence becomes available.

2.1.2. Term of Reference 2: Conditions under which fish species shall be regarded as vectors or reservoirs of diseases of fish listed in Annex II to the AHL

Several conditions need to be fulfilled for a fish species to be able act as a vector or reservoir of a pathogenic agent for the purposes of movements.

The conditions laid down in EC Delegated Reg 2020/990 Annex I and EC Delegated Reg 2020/692, focus on **the exposure to a pathogenic agent.** The vectors or reservoirs should have been exposed to the pathogenic agent at source. There are other conditions, however, that will influence if a potential vector species transmits the pathogenic agent to a susceptible species at the destination:

- **Contact with susceptible species:** The vectors or reservoirs should be in contact at the place of destination with uninfected susceptible/listed species.
- **Survival of the pathogen in or on the vector or reservoir:** the tenacity of the specific pathogenic agent will play a role in the probability of survival of the pathogen until the exposure and possible infection of a susceptible species.
- **Environmental conditions:** there are many different environmental conditions which could impact the persistence of a pathogen outside the vector or reservoir or within the vector or reservoir, at the source, during transport or at the destination. These conditions include temperature, pH, salinity, pollutants, turbidity, UV radiation and microbial water quality. However, it is presumed the water quality would not change significantly during the journey when vectors are moved to their destination.
- **Duration of the journey**: the shorter the duration of the journey between place of origin and destination, the more viable pathogenic organisms can be found, as decay for all pathogens is a function of time (Oidtmann et al., 2017).
- **Experimental infections**: temperature in combination with time are the most common factors, which affect persistence of aquatic animal pathogens. The conditions during experimental infection should be considered (such as the use of sterilised water, mud or suspended solids) and the effect of UV light and temperature as they can impact the time during which the pathogen can persist.
- **Testing at the origin:** Test sensitivity, test specificity and sampling protocol to determine the pathogen-free status of the consignment should be considered. Fallow periods between restocking farms following confirmed outbreaks should be considered (WOAH, 2022).

To deliver a concise and timely Scientific Opinion, it was agreed not to provide an exhaustive description of all those possible conditions. On the contrary, it was decided to focus only on those conditions that would PREVENT transmission facilitated by the movement of vectors and reservoirs, for which scientific evidence is available. In a first step, the experts in the working group carried out a narrative literature review to collect any evidence from scientific literature identifying conditions that may prevent transmission by vectors. In addition, information on the duration of the experimental studies and the water temperature were compiled during the ELS, carried out for ToR 1, collecting the ranges of the different durations and temperatures for which transmission has been proven for the different pathogens by the different vector species. Then, the experts concluded by consensus if the collected evidence was sufficient to support the need to alter the conditions stipulated in Annex I to Commission Delegated Regulation (EU) 2020/990 and in Annex XXX to Commission Delegated Regulation (EU) 2020/692.

2.2. Data

2.2.1. Term of Reference 1: Assessment of potential vectors and reservoir species of diseases of fish listed in Annex II to the AHL

The detailed data set extracted through the ELR is available in EFSA (2023) – see Annex.

2.2.2. Term of Reference 2: Conditions under which fish species shall be regarded as vectors or reservoirs of diseases of fish listed in Annex II to the AHL

The detailed data set extracted through the ELR is available in EFSA (2023) – see Annex.

3. Assessment

3.1. Term of Reference 1: Assessment of potential vectors and reservoir species of diseases of fish listed in Annex II to the AHL

Table 1 summarises the results of the assessment of potential vectors of AHL-listed pathogens of fish. The outcome of the assessment was that *Ctenopharyngodon idella* (grass carp), *Gymnocephalus cernua* (Eurasian ruffe), *Hypophthalmichthys molitrix* (silver carp), *Rutilus rutilus* (common roach) and

Tinca tinca (Tench) are considered to be vector species for **KHV** with more than 90% certainty, and *Carassius auratus* (goldfish) and *Carassius gibelio* (Prussian carp) with a certainty between 66 and 90%. It should be mentioned that all these species were also already suggested as vector species by the previous EURL report (EURL, 2022).

The assessment was based on the evidence from experimental infection studies that was generated by the ELR. The reasoning and level of certainty of the proposed classification is provided. More detailed data that were extracted from eligible studies, but that were considered as insufficient evidence, can be found in EFSA (2023).

Table 1: Proposed vectors of the AHL-listed fish pathogen Koi herpes virus based on evidence from experimental infection studies, with certainty and reasoning of classification

Vector Species X	Presence in EU*	Transmission route investigated	Species Y	Pathogen detection method with positive results in species Y	Certainty of classification as vector species	Reasoning for classification as vector species	Reference	Suggested classification by previous EURL report (2022)
AHL-listed fish pathogen: KHV								
Carassius auratus (goldfish)	Yes	Cohabitation	*Cyprinus carpio* (common carp)	PCR	66–90%	DNA demonstrated only in one individual of species Y	Radosavljevic et al. (2012)	Vector/reservoir
				Nested PCR ISH IFAT	66–90%	DNA demonstrated in Species Y	Bergmann et al. (2010)	
				RT-PCR Seq	66–90%	Two independent (RT)-PCRs detected both virus DNA and also RNA, indicating expression of genes and replication. No virus isolation.	El-Matbouli and Soliman (2011)	
Carassius gibelio (Carassius auratus gibelio in paper) (Prussian carp)	Yes	Cohabitation	*Cyprinus carpio* (common carp)	PCR	66–90%	DNA demonstrated only in one individual of species Y.	Radosavljevic et al. (2012)	Vector/reservoir
Ctenopharyngodon idella (grass carp)	Yes	Cohabitation	*Cyprinus carpio* (common carp)	PCR and nested PCR	90–100%	PCR product detected in species Y	Kempter et al. (2012)	Vector/reservoir
				PCR	66–90%	DNA demonstrated only in one individual of species Y	Radosavljevic et al. (2012)	
Gymnocephalus cernua (Eurasian ruffe)	Yes	Cohabitation	*Cyprinus carpio* (common carp)	PCR and nested PCR	90–100%	PCR product detected in species Y	Kempter et al. (2012)	Vector/reservoir
Hypophthalmichthys molitrix (silver carp)	Yes	Cohabitation	*Cyprinus carpio* (common carp)	PCR and nested PCR	90–100%	PCR product detected in species Y	Kempter et al. (2012)	Vector/reservoir
Rutilus rutilus (common roach)	Yes	Cohabitation	*Cyprinus carpio* (common carp)	PCR and nested PCR	90–100%	PCR product detected in species Y	Kempter et al. (2012)	Vector/reservoir

Vector Species X	Presence in EU*	Transmission route investigated	Species Y	Pathogen detection method with positive results in species Y	Certainty of classification as vector species	Reasoning for classification as vector species	Reference	Suggested classification by previous EURL report (2022)
Tinca tinca (tench)	Yes	Cohabitation	*Cyprinus carpio* (common carp)	PCR and nested PCR	90–100%	PCR product detected in species Y	Kempter et al. (2012)	Vector/reservoir
				PCR	66–90%	DNA demonstrated only in one individual of species Y	Radosavljevic et al. (2012)	

*Source: Gbif.org; PCR: polymerase chain reaction; Seq: sequencing; RT-PCR; reverse transcription polymerase chain reaction; IFAT: immunofluorescence antibody test; ISH; in situ hybridisation; EURL: EU Reference Laboratory.

Table 2 summarises the results of the assessment of potential reservoir species of AHL-listed pathogens of fish, based on the evidence from experimental infection studies, generated by the ELR. However, in these studies, no evidence of transmission to species Y was found or studied. The level of certainty and reasoning of the proposed classification is provided. More detailed data from studies that did not provide sufficient evidence are in EFSA (2023).

The outcome of the assessment was that *Tandanus tandanus* (dewfish) is considered a reservoir species for **EHNV** with > 90% certainty and *Maccullochella peelii* (Murray cod), *Macquaria novemaculeata* (Australian bass) and *Macquaria ambigua* (callop) with 66–90% certainty.

Clupea harengus (Atlantic herring) and *Oncorhynchus masou* (masu salmon) are considered a reservoir species for **HPRΔ ISAV** with > 90% certainty; and *Gadus morhua* (atlantic cod); *Oncorhynchus keta* (chum salmon) and *Oncorhynchus kisutch* (coho salmon) with 66–90% certainty.

Acipenser transmontanus (white sturgeon), *Clupea pallasii pallasii* (Pacific herring), *Cyprinus carpio* (common carp), *Danio rerio* (zebrafish), *Perca flavescens* (American yellow perch) and *Seriola quinqueradiata* (Japanese amberjack) are considered a reservoir species for **IHNV** with > 90% certainty.

Acipenser ruthenus × *Huso huso* (hybrid sterlet × beluga), *Barbatula barbatula* (stone loach) and *Pseudorasbora parva* (topmouth gudgeon) are considered a reservoir species for **KHV** with > 90% certainty; and *Gasterosteus aculeatus* (three-spine stickleback), *Oncorhynchus mykiss* (rainbow trout), *Perca fluviatilis* (European perch), *Rutilus rutilus* (roach), *Scardinius erythrophthalmus* (pearl roach) and *Tinca tinca* (tench) with 66–90% certainty.

Acanthopagrus schlegeli (black porgy), *Cottus pollux* (Japanese fluvial sculpin), *Cyprinus carpio* (common carp), *Epinephelus akaara (*Hong Kong grouper), *Esox lucius* × *Esox masquinongy* (tiger muskellunge), *Hippoglossus hippoglossus* (Atlantic halibut), *Ictalurus punctatus* (channel catfish), *Oryzias latipes* (Japanese rice fish), *Pagrus major* (red sea bream), *Petromyzon marinus* (sea lamprey), *Rhinogobius* sp. (Yoshinobori complex; Japanese goby), *Salvelinus fontinalis* (brook trout), *Sebastes inermis* (black rockfish) and *Seriola quinqueradiata* (Japanese amberjack) are considered reservoir species for **VHSV** with more than 90% certainty. *Carassius auratus* (goldfish), *Notemigonus crysoleucas* (golden shiners), *Oncorhynchus mykiss* (rainbow trout) × *Salvelinus alpinus* (Alpine charr). *Oncorhynchus mykiss* (rainbow trout) × *Salvelinus namaycush* (American Lake Charr) and *Oncorhynchus mykiss* (rainbow trout) × *Salmo trutta* (Amu-Darya trout) are considered a reservoir species for VHSV with 66–90% certainty.

The species that were already indicated by the previous report from EURL (EURL, 2022) as vector species are shown in the Table 2. As agreed during the interpretation of the terms of reference, in this review only pathogen detection in species Y was considered as evidence of transmission, whereas for the assessment for the previous EURL report may have included a broader range of evidence for transmission to species Y, including the demonstration of clinical signs.

Table 2: Proposed reservoirs of AHL-listed fish pathogens based on evidence from experimental infection studies, with certainty and reasoning of classification

Reservoir species X	Presence in EU*	Pathogen detection method in species X with positive results	Certainty of classification as reservoir species	Reasoning for classification as reservoir species	Reference	Suggested classification by previous EURL report (2022)
AHL-listed fish pathogen: EHNV						
Maccullochella peelii (Murray cod)	No	CellCult	66–90%	Virus isolation after bath exposure	Langdon (1989)	Vector/reservoir
Macquaria ambigua (callop)	No	CellCult	66–90%	Virus isolation and mortality after experimental infection by IP	Langdon (1989)	Vector/reservoir
Macquaria novemaculeata (Australian bass)	No	CellCult	66–90%	Virus isolation and mortality after experimental infection by IP	Langdon (1989)	Not assessed
Tandanus tandanus (dewfish)	No	PCR CellCult	90–100%	Five of 15 positive for EHNV (virus isolation) in IP injection challenge	Becker et al. (2013)	Vector/reservoir
AHL-listed fish pathogen: HPR∆ ISAV						
Clupea harengus (Atlantic herring)	Yes	RT-PCR	90–100%	One reference test was performed. No virus isolation.	Nylund et al. (2002)	Vector/reservoir
Gadus morhua (Atlantic cod)	Yes	PCR Sec	66–90%	Detected in the brain of cod by RT-PCR and qRT-PCR after 45 days IP	Grove et al. (2007)	Not assessed
Oncorhynchus keta (chum salmon)	Yes	CellCult	66–90%	Virus isolated from some individuals with low titres	Rolland and Winton (2003)	Not assessed
Oncorhynchus kisutch (coho salmon)	Yes	CellCult	66–90%	Virus isolated from some individuals with low titres	Rolland and Winton (2003)	Vector/reservoir
Oncorhynchus masou (masu salmon)	Yes	RT-PCR	90–100%	Virus detected by PCR in Masu salmon but no ISAV detected in Atlantic salmon after cohabitation with Masu salmon	Ito et al. (2014)	Vector/reservoir
AHL-listed fish pathogen: IHNV						
Acipenser transmontanus (white sturgeon)	Yes	Plaque assay	66–90%	Experimental infection using immersion prove to cause mortality in larval stages. Virus is re-isolated from larval stages.	LaPatra et al. (1995)	Vector/reservoir
Clupea pallasii pallasii (Pacific herring)	No	Plaque assay	90–100%	IHNV recovered from larvae tissue by RT-PCR after bath infection. No mortality or virus shedding.	Hart et al. (2011)	Vector/reservoir

Reservoir species X	Presence in EU*	Pathogen detection method in species X with positive results	Certainty of classification as reservoir species	Reasoning for classification as reservoir species	Reference	Suggested classification by previous EURL report (2022)
Cyprinus carpio (common carp)	Yes	PCR Plaque assay	90–100%	Cell culture and PCR positive but no active infection after immersion	Palmer and Emmenegger (2014)	Vector/reservoir
Danio rerio (zebrafish)	Yes	RT-qPCR	90–100%	One reference test was performed. Increased virus load	Briolat et al. (2014)	Not assessed
		CellCult	90–100%	Zebrafish experimentally infected- virus detected by isolation in cell culture, no shedding in water or mortality	LaPatra et al. (2000)	
Perca flavescens (American yellow perch)	Yes	PCR Plaque assay	90–100%	Cell culture and PCR positive and some clinic after immersion	Palmer and Emmenegger (2014)	Vector/reservoir
Seriola quinqueradiata (Japanese amberjack)	No	PCR CellCul	90–100%	Virus replication after IP injection only in amberjack	Ito and Kamaishi (2021)	Not assessed
AHL-listed fish pathogen: KHV						
Acipenser ruthenus × *Huso huso* (hybrid sterlet × beluga)	Yes	Nested-PCR	90–100%	Detected by PCR	Pospichal et al. (2016)	Vector/reservoir
Barbatula barbatula (stone loach)	No	Nested-PCR	90–100%	Detected by PCR	Pospichal et al. (2016)	Vector/reservoir
Gasterosteus aculeatus (three-spine stickleback)	Yes	PCR	66–90%	KHV detected by standard PCR method (relatively high number of copies)	Fabian et al. (2013)	Not assessed
Oncorhynchus mykiss (rainbow trout)	Yes	PCR	66–90%	Infection by cohabitation and immersion, PCR positive	Bergmann and Cieslak (2016)	Vector/reservoir
Perca fluviatilis (European-perch)	Yes	PCR	66–90%	KHV detected by standard PCR method (relatively high number of copies)	Fabian et al. (2013)	Vector/reservoir
Pseudorasbora parva (topmouth gudgeon)	Yes	qPCR	90–100%	One reference test was performed. Positive results in fish exposed to removal of skin mucus and in fish stressed by scaring by net.	Pospichal et al. (2018)	Not assessed
Rutilus rutilus (roach)	Yes	PCR	66–90%	KHV detected by standard PCR method (relatively high number of copies)	Fabian et al. (2013)	Vector/reservoir
Scardinius erythrophthalmus (pearl roach)	Yes	PCR	66–90%	KHV detected by standard PCR method (relatively high number of copies)	Fabian et al. (2013)	Not assessed

Reservoir species X	Presence in EU*	Pathogen detection method in species X with positive results	Certainty of classification as reservoir species	Reasoning for classification as reservoir species	Reference	Suggested classification by previous EURL report (2022)
Tinca tinca (tench)	Yes	PCR	66–90%	KHV detected by standard PCR method (relatively high number of copies)	Fabian et al. (2013)	Vector/reservoir
AHL-listed fish pathogen: VHSV						
Acanthopagrus schlegeli (black porgy)	No	IFAT	90–100%	Virus detected by isolation from dead fish	Isshiki et al. (2003)	Vector/reservoir
Carassius auratus (goldfish)	Yes	CellCult	66–90%	VHSV re-isolated after IP.	Getchell et al. (2015)	Vector/reservoir
		PCR				
		HIS				
		PCR	90–100%	PCR positive after co habitation	El-Matbouli et al. (2007)	
		Seq				
		LAMP assay				
Cottus pollux (Japanese fluvial sculpin)	No	RT-PCR	90–100%	Bath challenge produced mortality of 80%, VHSV detected by PCR and virus isolation from dead fish	Ito and Olesen (2013)	Vector/reservoir
		CellCult				
Cyprinus carpio (common carp)	Yes	RT-qPCRR	90–100%	Virus detected virus isolation in cell culture from target organs up to 28 dpe and by PCR up to 90 dpe.	Cornwell et al. (2013b)	Vector/reservoir
		CellCult				
		RT-qPCRR	90–100%	Detected by PCR	Goodwin et al. (2012)	
		Plaque assay	90–100%	Reasoning?	Emmenegger et al. (2013)	
Epinephelus akaara (Hong Kong grouper)	No	IFAT	90–100%	Virus detected by isolation from both dead and surviving fish	Isshiki et al. (2003)	Vector/reservoir
Esox lucius × *Esox masquinongy* (tiger muskellunge)	No	RT-qPCRR	90–100%	Isolated virus, cross lesions and characteristic histology detected. No transmission	Groocock et al. (2012)	Vector/reservoir
		CellCult				
		ImmHis				
		RT-qPCRR	90–100%	Oral infection 6 of 16 positive by both PCR and isolation in cell culture	Getchell et al. (2013)	
		CellCult				
		His				

Reservoir species X	Presence in EU*	Pathogen detection method in species X with positive results	Certainty of classification as reservoir species	Reasoning for classification as reservoir species	Reference	Suggested classification by previous EURL report (2022)
Hippoglossus hippoglossus (Atlantic halibut)	Yes	CellCult ELISA	90–100%	Virus re-isolated on cell culture from moribund fish and identified by ELISA.	Bowden (2003)	Vector/reservoir
		ELISA	66–90%	Virus isolated only from 1 individual	Snow et al. (2005)	Not assessed
Ictalurus punctatus (channel catfish)	Yes	RT-qPCRR CellCult	90–100%	Detected by PCR and virus Isolated and characteristic histology could be detected in 1 fish, but 28 dpe no virus was detected. No shedding of virus of transmission to other fish shown.	Groocock et al. (2012)	Vector/reservoir
		RT-qPCRR	90–100%	Detected by PCR in 20 of 20 at 15°C; in 18 of 20 at 20°C; in 20 of 20 at 25°C	Goodwin et al. (2012)	
Notemigonus crysoleucas (golden shiners)	No	CellCult RT-qPCR	90–100%	Detected by RT-qPCR, no sequencing	Cornwell et al. (2013a,b)	Vector/reservoir
Oncorhynchus mykiss (rainbow trout) × *Salvelinus alpinus* (Alpine Charr)	Yes	CellCult	66–90%	Text mentions virus isolation after experimental infection by immersion, but the data are not displayed	Dorson et al. (1991)	Vector/reservoir
Oncorhynchus mykiss (rainbow trout) × *Salvelinus namaycush* (American Lake Charr)	Yes	CellCult	66–90%	Text mentions virus isolation after experimental infection by immersion, but the data are not displayed	Dorson et al. (1991)	Vector/reservoir
Oncorhynchus mykiss (rainbow trout) × *Salmo trutta* (Amu-Darya Trout)	Yes	CellCult	66–90%	Focus is on mortality and resistance – but scarce details regarding virus detection. No virus isolated	Dorson et al. (1991)	Vector/reservoir
Oryzias latipes (Japanese rice fish)	No	RT-PCR CellCult	90–100%	Bath challenge produced mortality of 100%, VHSV detected by PCR and virus isolation from dead fish.	Ito and Olesen (2013)	Vector/reservoir
Pagrus major (red sea bream)	Yes	CellCult	90–100%	High mortality and virus isolation after IP injection	Ito et al. (2004)	Vector/reservoir
Petromyzon marinus (sea lamprey)	Yes	CellCult RT-PCR His ImmHis	90–100%	Virus detected by both PCR, cell culture and immunohistochemistry in target organs	Coffee et al. (2017)	Susceptible

Reservoir species X	Presence in EU*	Pathogen detection method in species X with positive results	Certainty of classification as reservoir species	Reasoning for classification as reservoir species	Reference	Suggested classification by previous EURL report (2022)
Rhinogobius sp. (Yoshinobori complex; Japanese goby)	No	PCR CellCult	90–100%	Bath challenge produced mortality of 10%, VHSV detected by PCR and virus isolation from dead fish	Ito and Olesen (2013)	Vector/reservoir
Sebastes inermis (black rockfish)	No	CellCult	90–100%	High mortality and virus isolation after IP injection	Ito et al. (2004)	Vector/reservoir
Seriola quinqueradiata (Japanese amberjack)	No	CellCult	90–100%	High mortality and virus isolation after IP injection	Ito et al. (2004)	Vector/reservoir

*Source: Gbif.org; PCR: polymerase chain reaction; His: histology; Seq: sequencing; RT-PCR: reverse transcription polymerase chain reaction; RT-qPCR: quantitative real-time reverse transcription PCR; IFAT: immunofluorescence antibody test; IP: intraperitoneal injection; ISH: in situ hybridisation; CellCult: cell culture; ImmHis: immune-histology; EURL: EU Reference Laboratory; dpe; days post exposure.

Table 3 summarises the results of the assessment of potential reservoir species of AHL-listed pathogens of fish, based on the evidence from field studies that was generated by the ELR. As from these studies potential transmission could not be evaluated, their role as potential reservoir species was assessed.

The outcome of the assessment was that *Oncorhynchus kisutch* (Coho salmon) is considered a reservoir species for **HPRΔ ISAV** with more than 90% certainty.

Aulorhynchus flavidus (tube-snout) is considered a reservoir species for **IHNV** with more than 90% certainty. *Clupea pallasii pallasii* (Pacific herring), *Cymatogaster aggregata* (shiner perch) and *Oncorhynchus gorbuscha* (pink salmon) are considered a reservoir species for IHNV with 66–90%.

Acipenser oxyrinchus (Russian sturgeon), *Acipenser gueldenstaedtii* (Atlantic sturgeon) and *Oreochromis niloticus* (Nile tilapia) are considered a reservoir species for **KHV** with more than 90% certainty.

The following species are considered reservoir species for **VHSV** with > 90% certainty: *Anguilla anguilla* (European eel), *Argentina sphyraena* (lesser argentine), *Belone belone* (garfish), *Cyprinus carpio* (common carp), *Enchelyopus cimbrius* (FOURBEARD rockling), *Eutrigla gurnardus* (grey gurnard), *Gadiculus argenteus* (Silvery pout), *Gadus chalcogrammus* (Alaska pollock), *Ictalurus punctatus* (channel catfish), *Merluccius productus* (Pacific hake), *Moxostoma anisurum* (silver redhorse), *Moxostoma macrolepidotum* (shorthead redhorse sucker), *Percopsis omiscomaycus* (trout perch), *Petromyzon marinus* (sea lamprey), *Pomoxis annularis* (white crappie), *Reinhardtius hippoglossoides* (Greenland halibut), *Salvelinus alpinus* (Arctic charr) and *Scorpaena porcus* (black scorpionfish).

Alosa pseudoharengus (alewife), *Anoplopoma fimbria* (sablefish), *Catostomus commersonii* (white sucker), *Fundulus diaphanous* (Banded killifish), *Hypomesus pretiosus* (surf smelt) and *Lota lota* (burbot) and *Trisopterus minutus* (poor cod) are considered reservoir species for **VHSV** with 66–90% certainty.

More details of field studies that provided insufficient evidence are included in EFSA (2023).

Finally, Table A.1 in Appendix A lists all the currently listed reservoir and/or vector species in Commission Implementing Regulation 1882/2018 for which no eligible papers were found during the ELR. It is therefore suggested to remove these species from the list.

Table 3: Proposed Reservoir of AHL-listed fish pathogens based on evidence from field studies, retrieved through extensive literature review, with certainty and reasoning of classification

Reservoir species X	Presence in the EU*	Pathogen detection method in species X with positive results	Certainty of classification as reservoir species	Reasoning for classification as reservoir species	Reference	Suggested classification by previous EURL report (2022)
AHL-listed fish pathogen: HPRΔ ISAV						
Oncorhynchus kisutch (coho salmon)	Yes	RT-PCR	90–100%	Detected both by PCR and ELISA	Kibenge et al. (2002)	Vector/reservoir
AHL-listed fish pathogen: IHNV						
Aulorhynchus flavidus (tube-snout)	No	CellCult DNA probe Neutralising antibody test	90–100%	Two out of 72 fish tested positive by DNA probe and neutralising antibody test	Kent et al. (1998)	Vector/reservoir
Clupea pallasii pallasii (Pacific herring)	No	CellCult DNA probe	66–90%	One out of 162 fish tested positive by DNA probe and neutralising antibody test	Kent et al. (1998)	Vector/reservoir
Cymatogaster aggregata (shiner perch)	No	CellCult DNA probe	66–90%	One out of 307 fish tested positive by DNA probe and neutralising antibody test	Kent et al. (1998)	Vector/reservoir
Oncorhynchus gorbuscha (Pink salmon)	Yes	CellCult RT-PCR	66–90%	1 of 72 fish positive by PCR	Breyta et al. (2017)	Vector/reservoir
AHL-listed fish pathogen: KHV						
Acipenser gueldenstaedtii (Russian sturgeon)	Yes	PCR and nested PCR Seq IFAT InSiHy	90–100%	Positive on more than two reference tests	Kempter et al. (2009)	Vector/reservoir
Acipenser oxyrinchus (Atlantic sturgeon)	Yes	PCR and nested PCR Seq IFAT InSiHy	90–100%	Positive on more than two reference tests	Kempter et al. (2009)	Vector/reservoir
Oreochromis niloticus (Nile tilapia)	Yes	PCR His TEM	90–100%	Internal organs test positive with reference method (PCR) and TEM	Wahidi et al. (2019)	Not assessed

Reservoir species X	Presence in the EU*	Pathogen detection method in species X with positive results	Certainty of classification as reservoir species	Reasoning for classification as reservoir species	Reference	Suggested classification by previous EURL report (2022)
AHL-listed fish pathogen: VHSV						
Alosa pseudoharengus (alewife)	No	PCR Seq	66–90%	Detected with both RT-PCR and sequencing	Stepien and Niner (2020)	Vector/reservoir
Anguilla anguilla (European eel)	Yes	CellCult ImmHis Seroneutralization	90–100%	Pathogenicity assay in RT-PCR plus isolation on cell and identifcation with Mabs	Castric et al. (1992)	Vector/reservoir
Anoplopoma fimbria (sablefish)	No	PCR	66–90%	Detected by PCR.	Hedrick et al. (2003)	Vector/reservoir
Argentina sphyraena (lesser argentine)	Yes	CellCult ELISA	90–100%	Detected by isolation in cell culture and ELISA in 1–3 fish	Mortensen et al. (1999)	Vector/reservoir
Belone belone (garfish)	Yes	RT-mPCR CellCult Seq	90–100%	Positive by cell culture and detection by RT-PCR and sequencing	Ogut and Altuntas (2014)	Vector/reservoir
Catostomus commersonii (white sucker)	Yes	PCR CellCult	66–90%	Detected in only 2 of 101 fish, high Ct-values, no cultivation	Cornwell et al. (2011)	Vector/reservoir
Cyprinus carpio (common carp)	Yes	RT-PCR CellCult Seq	90–100%	Positive by RT-PCR, cell culture and sequencing	Thompson et al. (2011)	Vector/reservoir
Enchelyopus cimbrius (fourbeard rockling)	Yes	CellCult ELISA	90–100%	Two reference tests were performed with positive results	Mortensen et al. (1999)	Vector/reservoir
Eutrigla gurnardus (grey gurnard)	Yes	CellCult ELISA PCR Seq	90–100%	Internal organs positive on cell culture in 1 of 3 pools	Wallace et al. (2016)	Vector/reservoir
Fundulus diaphanous (banded killifish)	No	CellCult RT-qPCRR	66–90%	Positive by PCR and no virus isolation by cell culture	Bain et al. (2010)	Vector/reservoir

Reservoir species X	Presence in the EU*	Pathogen detection method in species X with positive results	Certainty of classification as reservoir species	Reasoning for classification as reservoir species	Reference	Suggested classification by previous EURL report (2022)
Gadiculus argenteus (silvery pout)	Yes	rRT-PCR Seq	90–100%	Only one reference test was performed, but only PCR positive in 1 pool. However, confirmation by sequence analysis.	Sandlund et al. (2014)	Vector/reservoir
Gadus chalcogrammus (Alaska pollock)	No	PCR CellCult TEM	90–100%	Positive by cell culture and RT-PCR	Meyers et al. (1999)	Vector/reservoir
Hypomesus pretiosus (surf smelt)	No	PCR CellCult Plaque assay Seq	66–90%	Detected in 60 of 60 dead fish by virus isolation and PCR/sequencing	Hedrick et al. (2003)	Vector/reservoir
Ictalurus punctatus (channel catfish)	Yes	RT-PCR CellCult Seq	90–100%	Positive by RT-PCR, cell culture and sequencing	Thompson et al. (2011)	Not assessed
Lota lota (burbot)	Yes	nRT-PCR CellCult	66–90%	Virus isolation positive, confirmed by nRT-PCR. 1 fish positive.	Al-Hussinee et al. (2011)	Vector/reservoir
Merluccius productus (Pacific hake)	No	CellCult PCR TEM Nested PCR Seq	90–100%	Seventeen of 19 fish positive by cell culture and RT-PCR	Meyers et al. (1999)	Vector/reservoir
Moxostoma anisurum (silver redhorse)	No	RT-PCR CellCult Seq	90–100%	Detected by both isolation in cell culture and PCR, also pathology	Faisal et al. (2012)	Vector/reservoir
Moxostoma macrolepidotum (shorthead redhorse sucker)	No	RT-PCR CellCult Seq	90–100%	Detected by both isolation in cell culture and PCR, also pathology	Faisal et al. (2012)	Vector/reservoir
		RT-PCR CellCult Seq	90–100%	Positive by RT-PCR, cell culture and sequencing	Thompson et al. (2011)	Vector/reservoir

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Reservoir species X	Presence in the EU*	Pathogen detection method in species X with positive results	Certainty of classification as reservoir species	Reasoning for classification as reservoir species	Reference	Suggested classification by previous EURL report (2022)
Percopsis omiscomaycus (trout perch)	No	RT-PCR CellCult Seq	90–100%	Positive by RT-PCR, cell culture and sequencing	Thompson et al. (2011)	Vector/reservoir
Petromyzon marinus (sea lamprey)	Yes	RT-PCR CellCult Seq	90–100%	Positive by RT-PCR, cell culture and sequencing	Thompson et al. (2011)	Susceptible
Pomoxis annularis (white crappie)	No	nRT-PCR Seq CellCult ImmHis	90–100%	Positive by cell culture, IHC and RT-PCR, further confirmed by sequencing	Al-Hussinee et al. (2011)	Vector/reservoir
Reinhardtius hippoglossoides (Greenland halibut)	Yes	RT-PCR Seq CellCult Immunodot Seroneutralisation TEM	90–100%	Positive by cell culture, IHC and RT-PCR	Lopez-Vazquez et al. (2006) and Dopazo et al. (2002)	Vector/reservoir
Salvelinus alpinus (Arctic char)	Yes	CellCult IFAT	90–100%	Detected in 5 fish by isolation in cell culture	Knuesel et al. (2003)	Vector/reservoir
Scorpaena porcus (black scorpionfish)	No	RT-mPCR CellCult Seq	90–100%	Both CPE and detection by RT-PCR and sequencing	Ogut and Altuntas (2014)	Vector/reservoir
Trisopterus minutus (poor cod)	Yes	CellCult ELISA	66–90%	Only one pool WAS positive but a reference method was used	King et al. (2001)	Vector/reservoir

*Source: Gbif.org; PCR: polymerase chain reaction; His: histology; TEM: transmission electron microscope; Seq: sequencing; RT-PCR: reverse transcription polymerase chain reaction; RT-qPCR: quantitative real-time reverse transcription PCR; IFAT: immunofluorescence antibody test; ISH: In situ hybridisation; CellCult: cell culture; ImmHis: immune-histology; EURL: EU Reference Laboratory; nRT-PCR: nested reverse transcription PCR; RT-mPCR: reverse transcription multiplex PCR.

3.2. Term of Reference 2: Conditions under which fish species shall be regarded as vectors or reservoirs of pathogens listed in Annex II to the AHL

For a species to act as a vector there should be prior exposure to the pathogen of interest at the place of origin. That is, contact with susceptible species, other vector species or reservoir species or a pathogen-contaminated environment in the period before movement should have happened.

The type of aquaculture establishment from where the vector species is moved will influence the probability of exposure to the pathogen at the place of origin, going from low risk in closed systems to increasing risk in semi-closed and open water aquaculture systems. Nonetheless, it should be mentioned that even in very high biosecurity, closed, aquaculture systems in affected areas, introductions of listed pathogens can occur and high biosecurity conditions of the establishment, when in an affected area, cannot provide 100% assurance of pathogen freedom before movement of the vector species.

Potential survival of the pathogen during the journey will mainly depend on the duration of the journey, the tenacity of the pathogen and temperatures and water quality during transport. The duration between exposure to the potential source of infection and then exposure to naïve stocks of farmed aquatic animals should take account of the incubation period, any latent period and pre-movement testing. The temperature and water quality can reduce the persistence of pathogen that may be present in the carrying water/matrix. However, the impact of these parameters is specific to each pathogen.

- Epizootic haematopoietic necrosis
  EHNV is extremely resistant to drying and can survive for months in water. For these reasons, it is presumed that EHNV would persist for months to years in a fish farm in water and sediment as well as on equipment. EHNV will only be inactivated after 24 h at 40°C and within 15 min at 60°C (Langdon, 1989). There is a lack of information on possible effect of salinity; however, only freshwater species were shown to be susceptible. **Considering the available evidence, it is very likely to almost certain (90–100% certainty) that EHNV will remain infective at any possible transport condition.**

- Viral haemorrhagic septicaemia
  VHSV is sensitive to enzymatic degradation, environments with high bacterial load and high temperatures (above 28°C). VHSV can survive outside the host tissue in fresh water and sea water, but is affected by temperature, ultraviolet (UV) exposure, microbial community and suspended sediments. VHSV is tolerant of high salt concentrations such as in brine-treated fish (Skall et al., 2015). The viral glycoprotein of VHSV is misfolded at temperatures above 22–24°C (Lorenzen, 1997, Lorenzen and Olesen, 1995) and aquatic animals kept at temperatures above this temperature should not pose a risk as vectors. VHSV is inactivated at 30°C within 24 h (Bovo et al., 2005)**. Under transport conditions at temperatures below 25°C, it is likely (66–90% certainty) that VHSV will remain infective.**

- Infectious haematopoietic necrosis
  IHNV stability in host tissues during storage and processing is largely influenced by temperature. IHNV can survive outside the host tissue in fresh water and sea water, but is affected by temperature, UV exposure, microbial community and suspended sediments. When exposed to sunlight (UV-A and UV-B), IHNV at the water surface is rapidly inactivated with six orders of magnitude of virus rendered non-infectious within 3 h (Garver et al., 2013). In addition, infectious virus is inactivated by the microbial community within the water source and with increased amounts of suspended sediments (Garver et al., 2013; Kamei et al., 1987). IHNV is inactivated within 90 min at 32°C. (Bovo et al., 2005), and as VHSV G-protein is misfolded at temperatures above 22–24°C (Cain et al., 1999). **Under transport conditions at temperatures below 25°C, it is likely (66–90% certainty) that IHNV will remain infective.**

- Koi herpes virus disease
  KHV can remain infectious in water up to 21 h at water temperatures between 23 and 25°C (Perelberg et al., 2003). Other studies in Japan have displayed a significant reduction in the infectious titre of KHV within 3 days in environmental water or sediment samples at 15°C, while KHV remained for more than 7 days infective after being added to sterilised water samples

(Shimizu et al., 2006). In Japan, KHV DNA was detected in river water samples at temperatures of between 9 and 11°C, 4 months prior to a KHV disease outbreak being observed (Haramoto et al., 2007). Using PCR, KHV DNA was detected in high quantities in water samples collected at eight sites along the Yura river system during 3 months after a KHV disease outbreak at water temperatures ranging from 28.4 to 14.5°C (Minamoto et al., 2009a). In Lake Biwa, Japan, KHV was found to be widely distributed at different sites in the lake, 5 years after the first observed KHV outbreak. Mean concentrations of KHV in the lake water showed annual variation, with a peak in the summer and a decline in winter, and the virus was most prevalent in turbid, eutrophic water found in the lake margins (Minamoto et al., 2009b).
Considering the available evidence, it is very likely to almost certain (90–100%) that KHV will remain infective at any possible transport condition.

- Infection with HPR-deleted infectious salmon anaemia virus
  ISAV has been shown to be readily inactivated by UV exposure and ozone treatment (Liltved et al., 2006). It is also sensitive to low-pH values (e.g. pH 4, 30 min) and to temperatures above 37°C. Exposure to 56°C for 30 min will inactivate the virus (Falk et al., 1997). In natural seawater at 10°C, declining virus titres were shown during a period of 72 h (Vike et al., 2014). Results from an intraperitoneal challenge experiment indicated a loss of infectivity between 3 and 6 h (Vike et al., 2014). However, the infectivity will also be affected by the presence of particles and organic material in the water, which may potentially aid or reduce the stability of the virus.
  Under transport conditions at temperatures below 25°C, it is likely (66–90% certainty) that HPR-deleted infectious salmon anaemia virus will remain infective.

Table 4 summarises the conditions under which fish species should be regarded as vectors or reservoirs and should be considered to amend Annex I of Reg 2020/990 and Annex XXX of Reg 2020/692.

Table 4: Proposed conditions under which fish species listed shall be regarded as vectors or reservoirs

Name of listed pathogen	Conditions to be considered to amend Annex I of Reg 2020/990 and Annex XXX of Reg 2020/692
EHNV, KHV	Vector or reservoir species that were exposed to EHNV in an affected area can possibly transmit EHNV when transported into a non-affected area. Exposure in the affected area may have occurred if they originate from: (a) an aquaculture establishment or group of aquaculture establishments, where susceptible species, reservoir species or other vector species are kept; or (b) the wild, where they may have been exposed to susceptible, reservoir or other vector species; (c) an aquaculture supplied with water possibly contaminated with EHNV.
VHS, IHNV and HPRΔ ISAV	Vector or reservoir species that were exposed to VHSV in an affected area can possibly transmit VHSV when transported at a temperate below 25°C into a non-affected area. Exposure in the affected area may have occurred if the vector/reservoir originate from: (a) an aquaculture establishment or group of aquaculture establishments, where susceptible species, reservoir species or other vector species are kept; (b) the wild in an affected area, where they may have been exposed to susceptible species, reservoir species or other vector species; (c) an aquaculture supplied with water possibly contaminated with VHSV.

4. Conclusions

4.1. Term of Reference 1: Assessment of potential vectors and reservoir species of diseases of fish listed in Annex II to the AHL

EHNV

Reservoir

- *Tandanus tandanus* (dewfish) is considered a reservoir species for EHNV with > 90% certainty and *Maccullochella peelii* (Murray cod), *Macquaria novemaculeata* (Australian bass) and *Macquaria ambigua* (callop) with 66–90% certainty.

- No evidence or insufficient evidence was generated by the ELR for the following species currently listed Vectors/reservoirs in Commission Implementing Regulation 1882/2018: *Aristichthys nobilis*, *Carassius auratus*, *Carassius carassius*, *Cyprinus carpio*, *Hypophthalmichthys molitrix*, *Leuciscus* spp., *Rutilus rutilus*, *Scardinius erythrophthalmus* and *Tinca tinca*.

KHV

Vector

- The following species are considered vector species for KHV with > 90% certainty: *Carassius auratus* (goldfish), *Carassius gibelio* (Prussian carp), *Ctenopharyngodon idella* (grass carp), *Gymnocephalus cernua* (Eurasian ruffe), *Hypophthalmichthys molitrix* (silver carp), *Rutilus rutilus* (common roach) and *Tinca tinca* (tench).

Reservoir

The following species are considered reservoir species for KHV with > 90% certainty: *Acipenser gueldenstaedtii* (Russian sturgeon), *Acipenser oxyrinchus* (Atlantic sturgeon), *Acipenser ruthenus* × *Huso huso* (hybrid sterlet × beluga), *Barbatula barbatula* (stone loach), *Gasterosteus aculeatus* (three-spine stickleback), *Perca fluviatilis* (European perch) and *Scardinius erythrophthalmus* (pearl roach).

- The following species are considered reservoir species for KHV with 66–90% certainty: *Oreochromis niloticus* (Nile tilapia) and *Pseudorasbora parva* (topmouth gudgeon).

HPRΔ ISAV

Reservoir

- *Clupea harengus* (Atlantic herring), *Oncorhynchus masou* (masu salmon) and *Oncorhynchus kisutch* (coho salmon) are considered reservoir species for HPRΔ ISAV with > 90% certainty.
- *Gadus morhua* (Atlantic cod), *Oncorhynchus keta* (chum salmon), *Oncorhynchus kisutch* (coho salmon) are considered reservoir species for HPRΔ ISAV with 66–90% certainty.

IHNV

Reservoir

- The following species are considered reservoir species for IHNV with > 90% certainty: *Aulorhynchus flavidus* (tube-snout), *Acipenser transmontanus* (white sturgeon), *Clupea pallasii pallasii* (Pacific herring), *Cyprinus carpio* (common carp), *Danio rerio* (zebrafish), *Perca flavescens* (American yellow perch) and *Seriola quinqueradiata* (Japanese amberjack).
- *Cymatogaster aggregata* (shiner perch) and *Oncorhynchus gorbuscha* (pink salmon) are considered reservoir species for IHNV with 66–90% certainty.
- No evidence or insufficient evidence was generated by the ELR for the following species (including animals other than fish) currently listed Vectors/reservoirs in Commission Implementing Regulation 1882/2018: *Acipenser baerii*, *Acipenser gueldenstaedtii, Acipenser ruthenus, Acipenser stellatus, Acipenser sturio, Ameiurus melas, Aristichthys nobilis, Astacus astacus, Carassius auratus, Carassius carassius, Clarias gariepinus, Gadus morhua, Hippoglossus hippoglossus, Hypophthalmichthys molitrix, Huso huso, Ictalurus punctatus, Ictalurus* spp., *Leuciscus* spp., *Melanogrammus aeglefinus, Platichthys flesus, Pacifastacus leniusculus, Procambarus clarkii, Pangasius pangasius, Rutilus rutilus, Sander lucioperca, Scardinius erythrophthalmus, Silurus glanis, Tinca tinca*.

VHSV

Reservoir

- The following species are considered reservoir species for VHSV with > 90% certainty:
  Anguilla anguilla (European eel), *Argentina sphyraena* (lesser argentine), *Belone belone* (garfish), *Cottus pollux* (Japanese fluvial sculpin), *Cyprinus carpio* (common carp), *Enchelyopus cimbrius* (fourbeard rockling), *Epinephelus akaara* (Hong Kong grouper), *Esox lucius* × *Esox masquinongy* (tiger muskellunge), *Eutrigla gurnardus* (grey gurnard); *Gadiculus argenteus* (silvery pout), *Gadus chalcogrammus* (Alaska pollock), *Hippoglossus hippoglossus* (Atlantic

halibut), *Ictalurus punctatus* (channel catfish), *Merluccius productus* (Pacific hake), *Moxostoma anisurum* (silver redhorse), *Moxostoma macrolepidotum* (shorthead redhorse sucker), *Oncorhynchus mykiss* (rainbow trout) × *Salvelinus fontinalis* (American Brook Charr), *Oryzias latipes* (Japanese rice fish), *Pagrus major* (red sea bream), *Percopsis omiscomaycus* (trout perch), *Petromyzon marinus* (sea lamprey), *Pomoxis annularis* (white crappie), *Rhinogobius* sp. (Yoshinobori complex; Japanese goby), *Reinhardtius hippoglossoides* (Greenland halibut), *Salvelinus alpinus* (Arctic charr), *Scorpaena porcus* (black scorpionfish), *Sebastes inermis* (black rockfish) and *Seriola quinqueradiata* (Japanese amberjack).

- The following species are considered reservoir species for **VHSV** with 66–90% certainty: *Alosa pseudoharengus* (alewife), *Anoplopoma fimbria* (sablefish), *Carassius auratus* (goldfish), *Fundulus diaphanous* (banded killifish), *Hypomesus pretiosus* (surf smelt), *Lota lota* (burbot), *Notemigonus crysoleucas* (golden shiners), *Oncorhynchus mykiss* (rainbow trout) × *Salmo trutta* (Amu-Darya trout), *Oncorhynchus mykiss* (rainbow trout) × *Salvelinus alpinus* (Alpine Char), *Oncorhynchus mykiss* (rainbow trout) × *Salvelinus namaycush* (American Lake Charr).
- No evidence or insufficient evidence was generated by the ELR for the following species currently listed Vectors/reservoirs in Commission Implementing Regulation 1882/2018: *Acipenser baerii*; *Acipenser gueldenstaedtii; Acipenser ruthenus; Acipenser stellatus; Acipenser sturio, Ameiurus melas, Argyrosomus regius, Aristichthys nobilis, Clarias gariepinus, Dentex dentex, Dicentrarchus labrax, Diplodus puntazzo, Diplodus vulgaris, Epinephelus aeneus, Epinephelus marginatus, Huso huso, Hypophthalmichthys molitrix, Ictalurus* spp.*, Leuciscus* spp., *Morone chrysops x, Morone saxatilis, Mugil cephalus, Oreochromis* sp.*, Pagellus bogaraveo, Pagellus erythrinus, Pangasius pangasius, Rutilus rutilus, Salvelinus fontinalis, Sander lucioperca, Scardinius erythrophthalmus, Sciaenops ocellatus, Silurus glanis, Solea senegalensis, Solea solea, Sparus aurata, Thunnus* spp., *Thunnus thynnus, Tinca tinca, Umbrina cirrose*.
- The assessment was exclusively based on peer reviewed evidence and should be updated when new evidence becomes available.

4.2. Term of Reference 2: Conditions under which fish species shall be regarded as vectors or reservoirs of diseases of fish listed in Annex II to the AHL

- **VHSV, IHNV or HPR-deleted infectious salmon anaemia virus**
  - Under transport conditions at temperatures below 25°C, it is likely (66–90% certainty) that VHSV, IHNV and HPRΔ ISAV will remain infective.
  - Vector or reservoir species can transmit VHSV, IHNV or HPRΔ ISAV when transported at a **temperature below 25°C** into a non-affected area. Exposure in an VHSV, IHNV or HPRΔ ISAV affected area may have occurred if the vector or reservoir originate from:

    a) an aquaculture establishment where susceptible species or reservoir or other vector species of are kept;
    b) the wild, where they may have been exposed to susceptible, reservoir or other vector species;
    c) an aquaculture establishment supplied with water possibly contaminated with VHSV, IHNV or HPRΔ ISAV.

- **EHN and KHV**
  - It is very likely to almost certain (90–100% certainty) that EHNV and KHV will remain infective at **any possible transport condition.**
  - Vector or reservoir species can transmit EHNV when transported into a non-affected area. Exposure in the affected area may have occurred if they originate from:

    a) an aquaculture establishment, where susceptible species, reservoir species or other vector species are kept; or
    b) the wild, where they may have been exposed to susceptible, reservoir or other vector species;
    c) an aquaculture supplied with water possibly contaminated with EHNV.

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Abbreviations

AHL	Animal Health Law
EHNV	epizootic haematopoietic necrosis virus
ELR	extensive literature review
EURL	EU Reference Laboratory
His	histology
HPRΔ	ISAV highly polymorphic region-deleted infectious salmon anaemia virus
IHNV	infectious haematopoietic necrosis virus
ISH	*in situ* hybridisation
ITS	internal transcribed spacer
KHV	Koi herpes virus
PCR	polymerase chain reaction
RFLP	restriction fragment length polymorphism RFTM Ray's fluid thioglycollate medium
ToR	Term of Reference
Seq	sequencing
VHSV	viral haemorrhagic septicaemia virus
WOAH	World Organisation for Animal Health

Appendix A – Currently listed vector or reservoir species without sufficient evidence in peer-reviewed papers.

Table A.1: Currently listed Vectors/reservoirs in Commission Implementing Regulation 1882/2018 for which no evidence or insufficient evidence was generated by the extensive literature review

Infection with EHNV				
VECTOR/ RESERVOIRS* SPECIES Scientific name	**VECTOR/ RESERVOIRS* SPECIES Common name**	**Reference**	**Reasoning**	**Certainty**
Aristichthys nobilis	Bighead carp	No eligible paper found	NA	NA
Carassius auratus	Goldfish	Jensen et al. (2011)	In the study it was not possible to re-isolate virus from any of the samples from goldfish experimentally challenged with EHNV.	0–10%
Carassius carassius	Crucian carp	No eligible paper found	NA	NA
Cyprinus carpio	Common carp	Jensen et al. (2011)	In the study, it was not possible to re-isolate virus from any of the samples from carp experimentally challenged with EHNV.	NA
Hypophthalmichthys molitrix	Silver carp	No eligible paper found	NA	NA
Leuciscus spp.	Chub spp.	No eligible paper found	NA	NA
Rutilus rutilus	Roach	No eligible paper found	NA	NA
Scardinius erythrophthalmus	Rudd	No eligible paper found	NA	NA
Tinca tinca	Tench	No eligible paper found	NA	NA
Infection with VHSV				
VECTOR/ RESERVOIRS* Scientific name	**VECTOR/ RESERVOIRS* Common name**	**Reference**	**Reasoning**	**Certainty**
Acipenser baerii	Siberian sturgeon	No eligible paper found	NA	NA
Acipenser gueldenstaedtii	Danube sturgeon	No eligible paper found	NA	NA
Acipenser ruthenus	Sterlet sturgeon	No eligible paper found	NA	NA
Acipenser stellatus	Starry sturgeon	No eligible paper found	NA	NA
Acipenser sturio	Sturgeon	No eligible paper found	NA	NA
Ameiurus melas	Black bullhead	No eligible paper found	NA	NA
Argyrosomus regius	Meagre	No eligible paper found	NA	NA
Aristichthys nobilis	Bighead carp	No eligible paper found	NA	NA
Clarias gariepinus	North African catfish	No eligible paper found	NA	NA
Dentex dentex	Common dentex	No eligible paper found	NA	NA
Dicentrarchus labrax	European seabass	No eligible paper found	NA	NA
Diplodus puntazzo	Sharpsnout seabream	No eligible paper found	NA	NA
Diplodus vulgaris	Common two-banded seabream	No eligible paper found	NA	NA
Epinephelus aeneus	White grouper	No eligible paper found	NA	NA
Epinephelus marginatus	Dusky grouper	No eligible paper found	NA	NA
Huso huso	Beluga	No eligible paper found	NA	NA
Hypophthalmichthys molitrix	Silver carp	No eligible paper found	NA	NA

Ictalurus spp.	Catfish spp.	No eligible paper found	NA	NA
Leuciscus spp.	Chub spp.	No eligible paper found	NA	NA
Morone chrysops × *Morone saxatilis*	White bass × Striped bass hybrids	No eligible paper found	NA	NA
Mugil cephalus	Flathead grey mullet	No eligible paper found	NA	NA
Oreochromis	Tilapia	No eligible paper found	NA	NA
Pagellus bogaraveo	Blackspot seabream	No eligible paper found	NA	NA
Pagellus erythrinus	Common pandora	No eligible paper found	NA	NA
Pangasius pangasius	Pangas catfish	No eligible paper found	NA	NA
Rutilus rutilus	Roach	No eligible paper found	NA	NA
Salvelinus fontinalis	Brook trout	No eligible paper found	NA	NA
Sander lucioperca	pikeperch	No eligible paper found	NA	NA
Scardinius erythrophthalmus	Rudd	No eligible paper found	NA	NA
Sciaenops ocellatus	Red drum	No eligible paper found	NA	NA
Silurus glanis	Wels catfish	No eligible paper found	NA	NA
Solea senegalensis	Senegalese sole	No eligible paper found	NA	NA
Solea solea	Common sole	No eligible paper found	NA	NA
Sparus aurata	Gilthead seabream	No eligible paper found	NA	NA
Thunnus spp.	True tuna spp.	No eligible paper found	NA	NA
Thunnus thynnus	Atlantic bluefin tuna	No eligible paper found	NA	NA
Tinca tinca	Tench	No eligible paper found	NA	NA
Umbrina cirrosa	Shi drum	No eligible paper found	NA	NA
Infection with IHNV				
VECTOR/ RESERVOIRS* Scientific name	**VECTOR/ RESERVOIRS* Common name**	**Reference**	**Reasoning**	**Certainty**
Acipenser baerii	Siberian sturgeon	No eligible paper found	NA	NA
Acipenser gueldenstaedtii	Danube sturgeon	No eligible paper found	NA	NA
Acipenser ruthenus	Sterlet sturgeon	No eligible paper found	NA	NA
Acipenser stellatus	Starry sturgeon	No eligible paper found	NA	NA
Acipenser sturio	Sturgeon	No eligible paper found	NA	NA
Ameiurus melas	Black bullhead	No eligible paper found	NA	NA
Aristichthys nobilis	Bighead carp	No eligible paper found	NA	NA
Astacus astacus	Noble Crayfish	No eligible paper found and not a fish species	NA	NA
Carassius auratus	Goldfish	No eligible paper found	NA	NA
Carassius carassius	Crucian carp	No eligible paper found	NA	NA
Clarias gariepinus	North African catfish	No eligible paper found	NA	NA
Gadus morhua	Atlantic cod	No eligible paper found	NA	NA
Hippoglossus hippoglossus	Atlantic halibut	No eligible paper found	NA	NA
Hypophthalmichthys molitrix	Silver carp	No eligible paper found	NA	NA
Huso huso	Beluga	No eligible paper found	NA	NA
Ictalurus punctatus	Channel catfish	No eligible paper found	NA	NA
Ictalurus spp.	Catfish spp.	No eligible paper found	NA	NA
Leuciscus spp.	Chub spp.	No eligible paper found	NA	NA
Melanogrammus aeglefinus	Haddock	No eligible paper found	NA	NA
Pacifastacus leniusculus	Signal Crayfish	No eligible paper found	NA	NA

Procambarus clarkii	Red Swamp crawfish	No eligible paper found and not a fish species	NA	NA
Pangasius pangasius	Pangas catfish	No eligible paper found	NA	NA
Platichthys flesus	European flounder	No eligible paper found	NA	NA
Rutilus rutilus	Roach	No eligible paper found	NA	NA
Sander lucioperca	Pikeperch	No eligible paper found	NA	NA
Scardinius erythrophthalmus	Rudd	No eligible paper found	NA	NA
Silurus glanis	Welsh catfish	No eligible paper found	NA	NA
Tinca tinca	Tench	No eligible paper found	NA	NA

*Regulation 1882/2018 does not differentiate between vectors or reservoir species.

Appendix B – Vector or reservoir species mentioned in peer-reviewed papers that were excluded in the ELR

Table B.1 lists species for which studies were retrieved through searching the electronic databases, but that were excluded because they did not pass the eligibility criteria; or species for which evidence was extracted from relevant studies, but the certainty of the assessment was too low for classification as vector or reservoir.

Table B.1: Species that were excluded during the eligibility screening and assessment

Infection with EHNV					
Scientific name	**Reference**	**Certainty**	**Reasoning**	**Conclusion WG and AHAW Panel**	**Suggested classification in previous EURL report (2022)**
Craterocephalus fulvus	Becker et al. (2013)	0–10%	Bath challenge and IP challenge. Same or slightly increased mortality as control fish depending on the species. No virus detected.	Not classified	Not assessed
Hypseleotris klunzingeri					
Maccullochella macquariensis					
Maccullochella peelii					
Macquaria ambigua					Vector/Reservoir
Mogurnda adspersa					Vector/Reservoir
Nannoperca australis					Not assessed
Salmo salar	Langdon (1989)	NA	The study was excluded in the first level of screening because it investigates the presence of the pathogen in rainbow trout, *Salmo gairdneri*, which is an already known susceptible species.	Not assessed	Vector/Reservoir
Infection with HPRΔ ISAV					
Scientific name	**Reference**	**Certainty**	**Reasoning**	**Conclusion WG and AHAW Panel**	**Suggested classification in previous EURL report (2022)**
Salvelinus alpinus	Snow et al. (2001)	33–66%	According to the paper, ISAV could only be detected by RT-PC following IP-injection of ISAV. No positives by cell cultivation. 9/10 fish positive at 28 dpi and no fish positive at 40 dpi.	Not classified	Not assessed

Infection with VHSV					
Scientific name	**Reference**	**Certainty**	**Reasoning**	**Conclusion WG and AHAW Panel**	**Suggested classification in previous EURL report (2022)**
Acanthopagrus schlegeli	EFSA. Aquatic species susceptible to diseases listed in Directive 2006/88/EC 1 Scientific Opinion of the Panel on Animal Health and Welfare (AHAW) Adopted on the 11th of September 2008. The EFSA Journal. 2008; September: 1–144.	NA	Excluded because it's not a primary research study	Not assessed	Vector/Reservoir
Ammodytes personatus	Skall et al. (2005)	NA	Excluded because it's not a primary research study	Not assessed	Vector/Reservoir
Gasterosteus mustelus	Skall et al. (2005)	NA	Excluded because it's not a primary research study	Not assessed	Vector/Reservoir
Glyptocephalus stelleri	Lee et al. (2007)	NA	Excluded because the full text is not written in any EU language	Not assessed	Vector/Reservoir
Hoplobrotula armata	Lee et al. (2007)	NA	Excluded because the full text is not written in any EU language	Not assessed	Vector/Reservoir
Larimichthys polyactis	Lee et al. (2007)	NA	Excluded because the full text is not written in any EU language	Not assessed	Vector/Reservoir
Liparis tessellatus	Lee et al. (2007)	NA	Excluded because the full text is not written in any EU language	Not assessed	Vector/Reservoir
Moina macrocopa	Ito and Olesen (2007)	NA	Excluded at the first level of screening because the study does not investigate any not known susceptible species	Not assessed	Vector/Reservoir
Oncorhynchus aguabonita	Wolf (1988)	NA	Excluded because the full text is not written in any EU language	Not assessed	Vector/Reservoir
Oryzias dancena	Kim et al. (2013)	NA	Excluded because the full text is not written in any EU language	Not assessed	Vector/Reservoir

Pampus argenteus	Lee et al. (2007)	NA	Excluded because the full text is not written in any EU language	Not assessed	Vector/Reservoir
Oryzias dancena	kim et al. (2013)	NA	Excluded because the full text is not written in any EU language	Not assessed	Vector/Reservoir
Sebastes schlegeli	Isshiki et al. (2003)	10–33%	Unclear %, antibodies detected by VNT.	Not classified	Not assessed
Scorpaena izensis	Lee et al. (2007)	NA	Excluded because the full text is not written in any EU language	Not assessed	Vector/Reservoir
Scyliorhinus torazame	Lee et al. (2007)	NA	Excluded because the full text is not written in any EU language	Not assessed	Vector/Reservoir
Semotilus corporalis	Cornwell et al. (2015)	NA	Excluded because the full text wasn't available	Not assessed	Vector/Reservoir
Seriola dumerili	OIE. Viral Haemorrhagic Septicaemia. OIE Aquatic Animal Disease Cards. 2017	NA	Excluded because it's not a primary research study	Not assessed	Vector/Reservoir
Trichiurus lepturus	Lee et al. (2007)	NA	Excluded because the full text is not written in any EU language	Not assessed	Vector/Reservoir
Infection with IHNV					
Scientific name	**Reference**	**Certainty**	**Reasoning**		**Suggested classification in previous EURL report (2022)**
Anguilla anguilla	Bergmann et al. (2003)	NA	Excluded at the second level of screening because the study investigates the susceptibility of rainbow trout, which is an already known susceptible species (the origin of the isolates was not taken into account)	Not assessed	Vector/Reservoir
	Jørgensen et al. (1994)	NA	Excluded at the second level of screening because IHNV was not identified in/on species X.	Not assessed	Vector/Reservoir

Lota maxima	Polinski et al. (2010)	33–66%	Reduced survival and virus re-isolation. Mortality in negative control not explained.	Not classified	Vector/Reservoir
Plecoglossus altivelis	Nishizawa et al. (2006)	NA	Excluded at the first level of screening	Not assessed	Vector/Reservoir
Schopthalmus maximus	EFSA (2008) Scientific Opinion of the Panel on AHAW on a request from the European Commission on aquatic animal species	NA	Excluded because it's not a primary research study	Not assessed	Vector/Reservoir
	Liu et al. (2008)	10–33%	Lack of details on diagnostic methods	Not classified	Vector/Reservoir
Thymallus thymallus	Follett et al. (1997)	NA	According to the study, Arctic grayling was refractory to experimental infection with IHNV	Not assessed	Vector/Reservoir
Infection with KHV					
Scientific name	**Reference**	**Certainty**	**Reasoning**	**Conclusion WG and AHAW Panel**	**Suggested classification in previous EURL report (2022)**
Ameiurus nebulosus	Matras et al. (2019)	10–33%	Detection of KHV DNA up to 4 weeks.	Not classified	Not assessed
Ancistrus spp.	Bergmann et al. (2009)	33–66%	Non-validated method used to detect KHV and a few positive found at 2nd round of nested PCR in species X. Conclusion is not supported by data.	Not classified	Not assessed
Ameiurus nebulosus	Fabian et al. (2013)	0–10%	KHV detected by standard PCR method, at VERY low copy number (< 3 in mean for all positive species). Only DNA is detected in exposed carp (which were KHV free) no transfer of disease. Possibly water contamination with DNA.	0–10%	Not assessed

Carassius auratus	Lievens et al. (2011)	0–10%	PCR positive, but only gills were tested. Paper does not focus on evidence collection on vectors or reservoirs	Not classified	Not assessed
	Meyer et al. (2012)	NA	Not relevant: pathogen injection to study immune response	Not classified	Not assessed
Esox lucius	Fabian et al. (2013)	0–10%	KHV detected by standard PCR method, at VERY low copy number (< 3 in mean for all positive species). Only DNA is detected in exposed carp (which were KHV free) no transfer of disease. Possibly water contamination with DNA.	0–10%	Not assessed
Gasterosteus aculeatus	Matras et al. (2019)	10–33%	Detection of KHV DNA only 2 weeks after infection.	Not classified	Not assessed
Gobio gobio	Fabian et al. (2013)	0–10%	KHV detected by standard PCR method, at VERY low copy number (< 3 in mean for all positive species). Only DNA is detected in exposed carp (which were KHV free) no transfer of disease. Possibly water contamination with DNA.	0–10%	Not assessed
Hyphessobrycon eques	Maganha et al. (2022)	10–33%	Only one detection by PCR and sequencing	Not classified	Not assessed
Leuciscus idus	Bergmann et al. (2009)	33–66%	Non-validated method used to detect KHV and a few positive found at 2nd round of nested PCR in species X. Conclusion is not supported by data.	Not classified	Vector/Reservoir

Leuciscus leuciscus	Fabian et al. (2013)	0–10%	KHV detected by standard PCR method, at VERY low copy number (< 3 in mean for all positive species). Only DNA is detected in exposed carp (which were KHV free) no transfer of disease. Possibly water contamination with DNA.	0–10%	Not assessed
Misgurnus anguilicaudatus	Maganha et al. (2022)	10–33%	Only one detection by PCR and sequencing	Not classified	Not assessed
Neogobius melanostomus	Jin et al. (2020)	0–10%	Only states tissue, not clear if it is gills included. Could be environmental contamination.	Not classified	Not assessed
Oncorhynchus mykiss	Bergmann et al. (2021)	33–66%	According to the findings in the paper, a portion of rainbow trout can be experimentally (immersion/ cohabitant) infected with KHV-E (no disease), produce antibodies against the virus and transfer the virus to naive carp. No isolation of virus in cell culture from either species.	Not classified	Not assessed
Oreochromis niloticus	Rianto et al. (2019)	10–33%	Only gills were tested and lack of details of pathogen confirmation by reference methods	Not classified	
Pygocentrus nattereri	Maganha et al. (2022)	10–33%	Only one detection by PCR and sequencing	Not classified	Not assessed
Xiphophorus maculatus	Maganha et al. (2022)	10–33%	Only one detection by PCR and no sequencing	Not classified	Not assessed

PCR: polymerase chain reaction; IP: intraperitoneal injection; EURL: EU Reference Laboratory; IP: intraperitoneal challenge; dpi: days post infection; VNT: virus neutralisation test.

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Annex A – Protocol

Annex A is available under the Supporting Information section on the online version of the scientific output.