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

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Abstract

Vector or reservoir species of three diseases of crustaceans listed in the Animal Health Law were identified based on evidence generated through an extensive literature review, to support a possible updating of Regulation (EU) 2018/1882. Crustacean species on or in which Taura syndrome virus (TSV), Yellow head virus (YHV) or White spot syndrome virus (WSSV) were identified, 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 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 of 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 crustaceans 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 during transport was collected from scientific literature. It was concluded that it is very likely to almost certain (90–100%) that WSSV, TSV and YHV will remain infective at any possible transport condition. Therefore, vector or reservoir species that may have been exposed to these pathogens in an affected area in the wild or aquaculture establishments or by water supply can possibly transmit WSSV, TSV and YHV.

Keywords: Vectors, Reservoir, Taura syndrome virus (TSV), yellow head virus (YHV) or White spot syndrome virus (WSSV), transport conditions

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Summary

Term of Reference 1 (ToR1) requested European Food Safety Authority (EFSA) to assess which species or groups of species of aquatic animals pose a considerable risk for spreading pathogen causing the diseases of aquatic species listed in EU Regulation 2016/429. This Opinion specifically focuses on assessing vector or reservoir species of the three diseases of crustaceans, i.e. Taura syndrome virus (TSV), Yellow head virus (YHV) or White spot syndrome virus (WSSV). The aim of the assessments is to indicate if the Annex to Implementing Regulation (EU) 2018/1882, listing those reservoir or pathogen 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 simultaneously as susceptible and vector or reservoir species.

The following working definitions were agreed for the assessment: A crustacean species can be considered a **vector** when the pathogen has been identified in or on the crustacean 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 crustacean 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 so easy to be made in the field, 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.

An extensive literature review (ERL) has been carried out to gather all published peer-reviewed scientific evidence available on parameters needed to assess the role of aquatic 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 pathogens were identified with reference tests in or on crustacean species, either in experimental or field settings, with a high certainty (> 90%). This immediately led to the classification as reservoir or vector species (the latter only for experimental studies with proven transmission of the pathogen to the susceptible species from the vector species). Also, those studies that led to a clear exclusion of the species as vector or reservoir (> 90% certainty) due to negative test results were identified individually.

In a second step, the studies with more doubtful test 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%.

In addition to the list of vector and pathogen species, a list of vector or reservoir species that are currently listed in the Commission Implementing Regulation 1882/2018, for which no evidence was found was provided for each pathogen, with the suggestion to remove them from the list.

The results of the assessment indicated that the genus *Nitocra* (a genus of copepod) and the species *Octolasmis neptuni* (Pedunculate barnacle) are considered to be vectors for infection with **WSSV** with > 90% certainty.

Apocyclops royi (a species of copepod) and *Ergasilus manicatus* (Gill louse) are considered to be reservoir of **WSSV**, with a certainty between 90% and 100% and *Artemia* (Brine shrimp), Pseudodiaptomus annandalei and *Squilla mantis (*Spottail mantis shrimp*)* with a certainty between 66% and 90%.

Episesarma mederi (Mangrove crab) and *Macrobrachium lanchesteri* (Riceland prawn) are considered to be vectors for infection with **TSV** with > 90% certainty.

Chelonibia testudinaria, Ergasilus manicatus (Gill louse), *Penaeus japonicus* (Japanese tiger prawn)*, Octolasmis lowei, Scylla serrata* (Mud crab/Mangrove crab) and *Gelasimus vocans* are considered to be reservoir species for infection with Taura syndrome virus with a certainty between 90% and 100%. *Penaeus chinensis* (Chinese white shrimp), *Macrobrachium rosenbergii* (Giant river prawn), *Penaeus indicus* (Indian prawn) and *Penaeus japonicus* (Kuruma prawn) are all considered to be reservoir species for infection with **TSV** with a certainty between 66–90%.

For **YHV,** *Metapenaeus* brevicornis (Yellow prawn) is considered to be a reservoir with 90–100% certainty. *Callinectes sapidus* (Blue crab), *Penaeus chinensis* (Chinese white shrimp) *and Palaemon kadiakensis* (Mississippi grass shrimp) are all considered to be reservoir species for infection with **YHV** with a certainty between 66 and 90.

In addition to the list of vector and pathogen species, a list of vector or reservoir species that are currently listed in the Commission Implementing Regulation 1882/2018, for which no evidence was found was provided for each pathogen, with the suggestion to remove them from the current published list.

Term of Reference 2 (ToR2) requested EFSA to assess the suitability of the conditions under which crustacean 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, which are set out in those Regulations, were assessed to be unsuitable.

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 TOR1, 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.

It was concluded to be very likely to almost certain (90–100%) that WSSV, TSV and YHV will remain infective at any possible transport condition. Therefore, vector or reservoir species that were exposed to WSSV, TSV and YHV in an affected area can possibly transmit WSSV, TSV and YHV 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; (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 WSSV, TSV and YHV.

Table of contents

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.

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[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, 28.4.2020, 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 (if appropriate)

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 (ToR1) requests EFSA to provide a list of vector species or reservoirs 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 the **subphylum Crustacea**. The pathogens listed by the AHL affecting crustaceans are:

- Taura syndrome virus (TSV)
- Yellow head virus (YHV)
- White spot syndrome virus (WSSV)

It was agreed that for this assessment, a crustacean 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 crustacean 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 crustacean species shall be regarded as vectors or reservoirs of diseases of crustaceans listed in Annex II to the AHL

The list of potential vectors and reservoir species developed in ToR1 should be considered as vectors or reservoirs for movements in the EU, provided that certain conditions are fulfilled.

The conditions in the EU legislation EC Delegated Reg 2020/990 Annex I specify that the species may be regarded as vectors if the 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, or vectors or reservoirs are kept; or (b) the **wild,** where they may have been **exposed to susceptible species** listed in column 3 of that table, or vectors or reservoirs.

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 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 EC Delegated Reg 2020/990 Annex I and EC Delegated Reg 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 will be scrutinised and summarised. If there is no proof that certain specific conditions can exclude that the crustacean species will act as potential vector or reservoir, there will be no change in the conditions already laid down in the above-mentioned regulations.

2. Data and methodologies

2.1. Methodologies

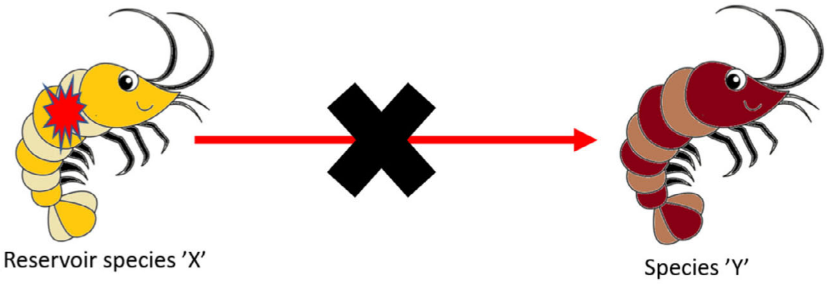
2.1.1. Term of reference 1: Assessment of potential vectors and reservoir species of pathogens of crustaceans, listed in Annex II to the AHL

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 of specific pathogens of crustaceans, 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 is detected in crustaceans 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 crustacean 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 crustacean 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. 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, and therefore, a second reference test is superfluous. The following tests are accredited by the EU reference laboratory (EURL) as reference tests for the concerned pathogens:

- WSSV: Nested PCR according to Lo et al. (1996a,b) or qPCR according to Durand & Lightner (2002).
- TSV: PCR according to Nunan et al. (1998) or qPCR according to Tang et al. (2004).
- YHV: PCR according to Mohr et al. (2015) or nested PCR according to Wijegoonawardane et al. (2008).

VECTOR: When at least one positive reference test for detection of pathogen A in 'Species X' and 'Species Y' was reported.

RESERVOIR: When 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 and therefore, ideally two reference tests taken from the same animals or reported in the same paper are needed to conclude pathogen X 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 ELR 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 (EFSA, 2023).

In addition, in transmission experiments where negative results for Pathogen A detection in Species Y were recorded (susceptible species), the assessment focussed on the assessment of 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 was considered as doubtful. The doubtful 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 three and four experts on diseases of crustaceans.
  - Only doubtful cases were discussed, and experts were asked to identify a more precise certainty range for the doubtful assessments:

○ Likely	66–90%
○ As likely as not	33–66%
○ Unlikely	10–33%

- **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 crustacean 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 of more than 66% were provided in the assessment section (Table 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 below 66% were provided in Appendix B, Table B.1.

Finally, it should be noted that One 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 crustacean species shall be regarded as vectors or reservoirs of diseases of crustaceans listed in annex II to the AHL

Several conditions need to be fulfilled for a crustacean species to be able to 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/listed 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 method used for experimental infection should be considered, such as use of sterilised water, mud or suspended solids, the effect of UV light and temperature that 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 TOR1, 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 crustaceans listed in Annex II to the AHL

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

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

Table 1 summarises the results of the assessment of **potential vectors species** of AHL-listed pathogens of crustaceans. The genus *Nitocra* (genus of copepods) and the species *Octolasmis neptuni* (Pedunculate barnacle) were assessed as vectors of **WSSV** with more than 90% certainty. *Nitocra* were also identified as vector/reservoir species of **WSSV** in the EURL report (EURL, 2022). *Sesarma mederi* (Mangrove crab) and *Macrobrachium lanchesteri* (Riceland prawn) were assessed as vector of **TSV** with more than 90% certainty.

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 can be found in EFSA (2023).

Table 1: Proposed vectors of AHL-listed crustacean pathogens 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**	**Reference**	**Suggested classification by previous EURL report (2022)**
AHL-listed crustacean pathogen: WSSV								
Nitocra (genus of copepodes)	Yes	Ingestion	*Penaeus japonicus* (Japanese tiger prawn)	PCR	90–100%	Ingestion of infected larvae or adults of *Nitocra* sp. Virus was detectable by PCR.	Zhang et al. (2008)	Vector/reservoir
Octolasmis neptuni (Pedunculate barnacle)	No	Feeding	*Penaeus vannamei*	PCR	90–100%	Evidence of transmission from species X to Y; verified with PCR (reference method).	Priyangha et al. (2020)	Vector

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	Reference	Suggested classification by previous EURL report (2022)
AHL-listed crustacean pathogen: TSV								
Episesarma mederi (*Sesarma mederi in paper*) (Mangrove crab)	No	Feeding	*Penaeus vannamei* (White leg shrimp)	PCR	90–100%	Transmission of TSV by feeding leads to 100% mortality and positive RT-PCR	Kiatpathomchai et al. (2008)	Not assessed
Macrobrachium lanchesteri (Riceland prawn)	No	Feeding	*Penaeus vannamei* (White leg shrimp)	PCR	90–100%	Transmission of TSV by feeding leads to 100% mortality and positive RT-PCR	Kiatpathomchai et al. (2008)	Not assessed

*: Source: Gbif.org. PCR, Polymerase chain reaction; RT-PCR, Reverse transcription polymerase chain reaction; EURL, EU Reference Laboratory.

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

Artemia (Brine shrimp), *Apocyclops royi* (a species of copepods), *Ergasilus manicatus* (Gill louse) and *Squilla mantis* (Spottail mantis squillid) were considered to be reservoirs of **WSSV** with 66–90%, 90–100%, 90–100% and 66–90% certainty, respectively.

Chelonibia testudinaria, Ergasilus manicatus (Gill louse), *Penaeus japonicus* (Japanese tiger prawn)*, Octolasmis lowei* (a species of barnacles)*, Scylla serrata* (Mud crab/Mangrove crab) and *Gelasimus vocans* (a species of fiddler crab) were considered to be reservoirs of **TSV** with 90–100% certainty, and *Penaeus chinensis* (Chinese white shrimp) with 66–90% certainty.

Metapenaeus brevicornis was considered to be a reservoir of **YHV** with 90–100% certainty, while *Callinectes sapidus* (Blue crab), *Penaeus chinensis* (Chinese white shrimp) and *Palaemon kadiakensis* (Mississippi grass shrimp) were all considered to be reservoirs of **YHV** with 66–90% certainty.

It should be noted that *Callinectes sapidus,* assessed as fulfilling the criteria for being categorised as a reservoir species for **YHV** (Table 2), was already indicated in the previous report by the EURL (EURL, 2022) as vector species. As agreed during the interpretation of the terms of reference, in this Opinion, pathogen detection in species Y was the only criterion required to be fulfilled to be considered evidence of transmission, whereas for the assessment for the EURL previous 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 crustacean 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**	**Certainty of classification as reservoir species**	**Reasoning for classification**	**Reference**	**Suggested classification by previous report EURL (2022)**
AHL-listed crustacean pathogen: WSSV						
Apocyclops royi (a species of copepod)	No	PCR	90–100%	No evidence of transmission; evidence of replication in species X	Chang et al. (2011)	Vector/reservoir
Artemia (Brine shrimp)	Yes	PCR	66–90%	No evidence for replication in species X. Does not lead to increased mortality in species Y, but is detectable by PCR in both species X and Y. PCR used is not a reference method	Zhang et al. (2010)	Not assessed
Ergasilus manicatus (Gill louse)	No	PCR	90–100%	No evidence of transmission; evidence of replication in species X	Overstreet et al. (2009)	Vector/reservoir
AHL-listed crustacean pathogen: TSV						
Chelonibia testudinaria (*Chelonibia patula* in paper) (Turtle barnacle)	Yes	PCR	90–100%	No evidence of transmission; evidence of replication in species.	Overstreet et al. (2009)	Vector
Ergasilus manicatus (Gill louse)	No	PCR	90–100%	No evidence of transmission; evidence of replication in species X	Overstreet et al. (2009)	Vector/reservoir
Penaeus japonicus *(Marsupenaeus* japonicus in paper) *(*Japanese tiger prawn*)*	Yes	PCR	90–100%	Detection of TSV RNA in multiple organs using recommended real-time RT-qPCR and conventional RT-PCR after an intramuscular injection	Chang et al. (2004)	Not assessed
Octolasmis lowei (*Octolasmis muelleri* in paper) (common name not found)	No	PCR	90–100%	No evidence of transmission; evidence of replication in species.	Overstreet et al. (2009)	Vector
Penaeus chinensis (Chinese white shrimp) (*P. orientalis* in the paper)	No	PCR	66–90%	Detection using WOAH recommended RT-PCR assay, but no information on sampling approach. No confirmation by sequence analysis.	Yin et al. (2014)	Vector/reservoir
Scylla serrata (Mud crab/ Mangrove crab)	No	PCR	90–100%	Molecular detection using the IQ2000 kit, and the signal increases between day 5 and day 52. No transmission undertaken.	Kiatpathomchai et al. (2008)	Vector
Gelasimus vocans (*Uca vocans* in paper) (Fiddler crab)	No	PCR	90–100%	Molecular detection using the IQ2000 kit, and the signal increases between day 5 and day 51. No transmission undertaken.	Kiatpathomchai et al. (2008)	Vector

Reservoir species X	**Presence in EU***	**Pathogen detection method in species X**	**Certainty of classification as reservoir species**	**Reasoning for classification**	**Reference**	**Suggested classification by previous report EURL (2022)**
AHL-listed crustacean pathogen: YHV						
Callinectes sapidus (Blue crab)	Yes	1-set PCR Semi-nested PCR	66–90%	No evidence of transmission; YHV detectable in tissue 1 week after inoculation.	Ma et al. (2009)	Vector/reservoir
Metapenaeus brevicornis (Yellow prawn)		PCR	90–100%	*Metapenaeus brevicornis* infected by injection; limited evidence of the pathogen by RT-PCR after 30 days; limited detection at 30 days by IHC. No transmission attempted.	Longyant et al. (2006)	
Palaemon kadiakensis (*Palaeomentes kadiakensis* in paper) (Mississippi grass shrimp)	No	1-set PCR Semi-nested PCR	66–90%	No evidence of transmission; YHV detectable in tissue >2 weeks after inoculation; fresh water species.	Ma et al. (2009)	Not assessed
Penaeus chinensis (Chinese white shrimp) (*P. orientalis* in the paper)	No	PCR	66–90%	Detection using WOAH recommended RT-PCR assay, but no information on sampling approach. No confirmation by sequence analysis	Yin et al. (2014)	Not assessed

*: Source: Gbif.org. PCR, Polymerase Chain Reaction; RT-PCR, Reverse transcription polymerase chain reaction; EURL, EU Reference Laboratory; RT-qPCR, Reverse transcription quantitative polymerase chain reaction.

Table 3 summarises the results of the assessment of **potential reservoir** species of AHL-listed pathogens of crustaceans, 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 outcomes of the assessment and the level of certainty for the classification are provided. More details of field studies that provided insufficient evidence are provided in EFSA (2023).

Macrobrachium rosenbergii (Giant river prawn), *Penaeus indicus* (Indian prawn) and *Penaeus japonicus* (Kuruma prawn) were all considered to be reservoirs of **TSV** with 66–90% certainty. *Pseudodiaptomus annandalei* (a species of copepods, *Schmackeria dubia* in publication) and *Squilla mantis* (Spottail mantis shrimp) were considered to be reservoirs of **WSSV** with 90–100% and 66–90% certainty, respectively.

Table 3: Proposed reservoir of AHL-listed crustacean pathogens based on evidence from field studies, with certainty and reasoning of classification

Reservoir species X	**Presence in EU***	**Pathogen detection method in species X with positive results**	**Certainty for classification**	**Reason for classification**	**Reference**	**Suggested classification by previous report EURL (2022)**
AHL-listed crustacean pathogen: TSV						
Macrobrachium rosenbergii (Giant river prawn)	No	PCR Seq	66–90%	Only one individual tested; although not tested with a reference test, it was confirmed with sequencing	Nielsen et al. (2005)	Vector/reservoir
		PCR ISH	33–66%	No transmission experiments. Detection methods not well described. Number of PCR and ISH positive individuals not reported.	Chuchird and Limsuwan (2007)	
Penaeus indicus (Indian prawn)	No	PCR Seq	66–90%	Each sample consisted of pleopods, or gills, or whole animal. Unsure if infected or the virus is in the gut or on surface.	Tang et al. (2012)	Vector/reservoir
Penaeus japonicus (Kuruma prawn)	Yes (farmed)	PCR Seq	66–90%	Only one individual tested; although not tested with a reference test, it was confirmed with sequencing	Nielsen et al. (2005)	Vector/reservoir
AHL-listed crustacean pathogen: WSSV						
Pseudodiaptomus annandalei (named as *Schmackeria dubia* in the publication) (a species of copepods)	No	PCR	66–90%	Detection in small numbers by WOAH recommended assay, but no sequence confirmation. Unsure if infected or the virus is in the gut or on surface.	Lo et al. (1996a,b)	Not assessed
Squilla mantis (Spottail mantis shrimp)	Yes	PCR	66–90%	PCR results reference method not shown; 5 positive individuals mentioned in text	Hossain et al. (2001)	Vector/reservoir

*: Source: Gbif.org. PCR, polymerase chain reaction; His, histology; TEM, transmission electron microscope; Seq, sequencing; EURL, EU Reference Laboratory; Seq, Sequencing; ISH, In situ hybridisation.

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 extensive literature review. These species are therefore suggested to be removed from the list.

Table B.1, in Appendix B, lists all species that were suggested in the EURL report (2022) that were excluded during the eligibility screening. In addition, it included those species for which studies were identified and passed the eligibility screening during the ELS for which data were extracted, but the certainty was too low to classify the species as reservoir or vector.

3.2. Term of reference 2: Conditions under which crustacean 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 in the place of origin. That is, there should be contact with susceptible species, other vector species or reservoir species or a pathogen-contaminated environment in the period before movement.

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 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:

- Infection with Taura syndrome virus

There is a lack of information on the optimal temperature for replication of TSV in the natural host and on the survival of TSV outside of the host. Shrimps have been shown to be resistant to some viral isolates at high temperatures (32°C) but not to other viral isolates (Côté & Lightner, 2010). Other viruses in the same family (Dicistroviridae) have been shown to be resistant to low pH (pH 3) (Christian & Scotti, 2008).

Considering the available evidence, it is very likely to almost certain (90–100%) that Taura syndrome virus will remain infective at any possible transport condition.

- Infection with yellow head virus

There is a lack of information on the optimal temperature for replication of YHV in the natural host and on the survival of YHV outside of the host. YHV has been shown to be viable for up to 72 h in aerated seawater (Flegel et al., 1995). It can be inactivated by heating at 60°C for 15 min (Flegel et al., 1997). Considering the above, it is unlikely that yellow head virus will be eliminated from the vector species during transport.

Considering the available evidence, it is very likely to almost certain (90–100%) that YHV will remain infective at any possible transport condition.

- Infection with white spot syndrome virus

Outside of the host, WSSV is viable for at least 30 days at 30°C in seawater (Momoyama et al., 1998) and is viable in ponds for at least 3–4 days (Nakano et al., 1998). In laboratory studies, the virus is inactivated by exposure to UV light at 9.30×10^5 μWs/cm^2 and is no longer infective after 21 days of sun drying, in drained ponds (Chang et al., 1998). The virus is also non-viable after 40 days in waterlogged pond sediment (Satheesh Kumar et al., 2013).

Inactivation can be achieved by heating to 55°C for 90 min; 70°C for 5 min (Chang et al., 1998); 50°C for 60 min; 60°C for 1 min; 70°C for 0.2 min (Nakano et al., 1998), desiccation for 1 h at 30°C or 3 h at 26°C (Maeda et al., 1998; Nakano et al., 1998) or pH 3 for 60 min; pH 12 for 10 min (Chang et al., 1998; Balasubramanian et al., 2006).

Only one transmission experiment was found (Zang et al., 2008) demonstrating transmission from WSSV infected *Nicotra* sp. to susceptible species. The temperatures at which the transmission was examined and demonstrated was 20°C.

Considering the available evidence, it is very likely to almost certain (90–100%) that WSSV will remain infective at any possible transport condition.

Table 4 summarises the conditions under which crustacean 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: Conditions under which crustacean species should 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
TSV, WSSV, YHV	Vector or reservoir species that were exposed to Taura syndrome virus in an affected area can possibly transmit TSV, WSSV and YHV 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 Taura syndrome virus

4. Conclusions

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

WSSV

- **Vectors**
  - The genus *Nitocra* (a genus of copepods) and the species *Octolasmis neptuni* (Pedunculate barnacle) are considered to be vectors for infection with WSSV with more than 90% certainty.
- **Reservoirs**
  - *Apocyclops royi* (a species of copepods) and *Ergasilus manicatus* (Gill louse) are considered to be reservoirs of white spot syndrome virus, with a certainty between 90% and 100%.
  - *Artemia* (Brine shrimp), Pseudodiaptomus annandalei (a species of copepods) and *Squilla mantis (*Spottail mantis shrimp*)* are considered to be reservoirs of white spot syndrome virus, with a certainty between 66% and 90%.
- No evidence or insufficient evidence was generated by the extensive literature review for the following species currently listed vectors/reservoirs in Commission Implementing Regulation 1882/2018: *Atrina* spp., *Buccinum undatum, Crassostrea angulata, Cerastoderma edule, Magallana gigas (Crassostrea gigas), Crassostrea virginica, Donax trunculus, Haliotis discus hannai, Haliotis tuberculata, Littorina littorea, Mercenaria mercenaria, Meretrix lusoria, Mya arenaria, Mytilus edulis, Mytilus galloprovincialis, Octopus vulgaris, Ostrea edulis, Pecten maximus, Ruditapes decussatus, Ruditapes philippinarum, Sepia officinalis, Strombus* spp., *Polititapes aureus (Venerupis aurea), Venerupis corrugata (Venerupis pullastra) and Venus verrucosa*. In addition, they are all mollusc species, and therefore, they have not been part of the scope of this assessment.

TSV

- **Vectors**
  - *Episesarma mederi* (Mangrove crab) and *Macrobrachium lanchesteri* (Riceland prawn) are considered to be vectors for infection with TSV with more than 90% certainty.
- **Reservoirs**
  - *Chelonibia testudinaria, Ergasilus manicatus* (Gill louse), *Penaeus japonicus* (Japanese tiger prawn), *Octolasmis lowei* (a species of barnacles), *Scylla serrata* (Mud crab/ Mangrove

crab) and *Gelasimus vocans* (a species of fiddler crab) are considered to be reservoir species for infection with TSV with a certainty between 90% and 100%.

  - *Penaeus chinensis* (Chinese white shrimp), *Macrobrachium rosenbergii* (Giant river prawn), *Penaeus indicus* (Indian prawn) and *Penaeus japonicus* (Kuruma prawn) are all considered to be reservoir species for infection with TSV with a certainty between 66% and 90%.

- No evidence or insufficient evidence was generated by the extensive literature review for the currently listed crustacean vectors/reservoirs species in Commission Implementing Regulation 1882/2018: *Atrina* spp., *Buccinum undatum, Brachyura* spp., *Cherax destructor, Homarus Gammarus, Necora puber (Portunus puber), Palinurus* spp., *Penaeus kerathurus*.
- No evidence or insufficient evidence was generated by the extensive literature review for the currently listed mollusc vectors/reservoirs species in Commission Implementing Regulation 1882/2018 *Crassostrea angulate, Cerastoderma edule, Magallana gigas (Crassostrea gigas), Crassostrea virginica, Donax trunculus, Haliotis discus hannai, Haliotis tuberculate, Littorina littorea, Mercenaria mercenaria, Meretrix lusoria, Mya arenaria, Mytilus edulis, Mytilus galloprovincialis, Octopus vulgaris, Ostrea edulis, Pecten maximus, Ruditapes decussatus, Ruditapes philippinarum, Sepia officinalis, Strombus* spp., *Polititapes aureus (Venerupis aurea), Venerupis corrugata (Venerupis pullastra) and Venus verrucosa*. In addition, as they were mollusc species, they were not part of the assessment.

YHV

Reservoirs

- *Metapenaeus* brevicornis (Yellow prawn) is considered to be a reservoir for infection with YHV with 90–100% certainty.
  - *Callinectes sapidus* (Blue crab), *Penaeus chinensis* (Chinese white shrimp) and *Palaemon kadiakensis* (Mississippi grass shrimp) are all considered to be reservoir species for infection with YHV with a certainty between 66% and 90%.
  - No evidence or insufficient evidence was generated by the extensive literature review for the following crustacean species currently listed as vectors/reservoirs in Commission Implementing Regulation 1882/2018: *Atrina* spp., *Penaeus aztecus, Penaeus duorarum, Penaeus setiferus*.

- No evidence or insufficient evidence was generated by the extensive literature review for the following mollusc species currently listed vectors/reservoirs in Commission Implementing Regulation 1882/2018: *Buccinum undatum, Crassostrea angulate, Cerastoderma edule, Magallana gigas (Crassostrea gigas), Crassostrea virginica, Donax trunculus, Haliotis discus hannai, Haliotis tuberculate, Littorina littorea, Mercenaria mercenaria, Meretrix lusoria, Mya arenaria, Mytilus edulis, Mytilus galloprovincialis, Octopus vulgaris, Ostrea edulis, Pecten maximus, Ruditapes decussatus, Ruditapes philippinarum, Sepia officinalis, Strombus* spp., *Polititapes aureus (Venerupis aurea), Venerupis corrugata (Venerupis pullastra)* and *Venus verrucosa*. In addition, as they were mollusc species, they were not part of the assessment.
- 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 crustacean species shall be regarded as vectors or reservoirs of diseases of crustaceans listed in Annex II to the AHL

- It is very likely to almost certain (90–100%) that **WSSV, TSV** and **YHV** will remain infective at **any possible transport condition**.
- Therefore, vector or reservoir species that were exposed to **WSSV, TSV** and **YHV** in an affected area can possibly transmit **WSSV, TSV** and **YHV** 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 establishment supplied with water** possibly contaminated with **WSSV, TSV** and **YHV.**

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Abbreviations

AHL	Animal Health Law
ERL	extensive literature review
PCR	polymerase chain reaction
RT-PCR	reverse transcription polymerase chain reaction
ToR	Term of Reference
TSV	Taura syndrome virus
WOAH	World Organisation for Animal Health
WSSV	White spot syndrome virus
YHV	Yellow head virus

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

Table A.1: Currently listed vectors in Commission Implementing Regulation 1882/2018 for which no paper was found by the extensive literature review to be categorised as vector or reservoir species

Infection with Taura syndrome virus

Vector species Scientific names	Vector species Common names	Reference	Reasoning	Certainty
Atrina spp.	Pen shells spp.	No eligible paper found	NA	NA
Buccinum undatum	Whelk	No eligible paper found	NA	NA
Brachyura spp.	Marine crabs spp.	No eligible paper found	NA	NA
Cherax destructor	Yabby crayfish	No eligible paper found	NA	NA
Crassostrea angulata	Portuguese cupped oyster	No eligible paper found	NA	NA
Cerastoderma edule	Common edible cockle	No eligible paper found	NA	NA
Crassostrea virginica	American cupped oyster	No eligible paper found	NA	NA
Donax trunculus	Truncate donax	No eligible paper found	NA	NA
Haliotis discus hannai	Japanese abalone	No eligible paper found	NA	NA
Haliotis tuberculate	Tuberculate abalone	No eligible paper found	NA	NA
Homarus gammarus	European lobster	No eligible paper found	NA	NA
Littorina littorea	Common periwinkle	No eligible paper found	NA	NA
Mgallana gigas (Crassostrea gigas)	Pacific cupped oyster	No eligible paper found	NA	NA
Mercenaria mercenaria	Northern quahog	No eligible paper found	NA	NA
Meretrix lusoria	Japanese hard clam	No eligible paper found	NA	NA
Mya arenaria	Sand gaper	No eligible paper found	NA	NA
Mytilus edulis	Blue mussel	No eligible paper found	NA	NA
Mytilus galloprovincialis	Mediterranean mussel	No eligible paper found	NA	NA
Necora puber (Portunus puber in legislation)	Swimming crab	No eligible paper found	NA	NA
Octopus vulgaris	Common octopus	No eligible paper found	NA	NA
Ostrea edulis	European flat oyster	No eligible paper found	NA	NA
Palinurus spp.	Palinurid spiny lobsters spp.	No eligible paper found	NA	NA
Polititapes aureus (Venerupis aurea)	Golden carpet shell	No eligible paper found	NA	NA
Pecten maximus	Great Atlantic scallop	No eligible paper found	NA	NA
Penaeus kerathurus	Caramote prawn	No eligible paper found	NA	NA
Ruditapes decussatus	Grooved carpet shell	No eligible paper found	NA	NA
Ruditapes philippinarum	Japanese carpet shell	No eligible paper found	NA	NA
Sepia officinalis	Common cuttlefish	No eligible paper found	NA	NA
Strombus spp.	Stromboid conchs spp.	No eligible paper found	NA	NA
(Venerupis corrugate) Venerupis pullastra	Pullet carpet shell	No eligible paper found	NA	NA
Venus verrucosa	Warty venus	No eligible paper found	NA	NA

Infection with yellow head virus

Vector species Scientific names	***Vector species Common names***			
Atrina spp.	Pen shells spp.	No eligible paper found	NA	NA
Buccinum undatum	Whelk	No eligible paper found	NA	NA
Crassostrea angulata	Portuguese cupped oyster	No eligible paper found	NA	NA
Cerastoderma edule	Common edible cockle	No eligible paper found	NA	NA
Crassostrea gigas	Pacific cupped oyster	No eligible paper found	NA	NA

Crassostrea virginica	American cupped oyster	No eligible paper found	NA	NA
Donax trunculus	Truncate donax	No eligible paper found	NA	NA
Haliotis discus hannai	Japanese abalone	No eligible paper found	NA	NA
Haliotis tuberculata	Tuberculate abalone	No eligible paper found	NA	NA
Littorina littorea	Common periwinkle	No eligible paper found	NA	NA
Mercenaria mercenaria	Northern quahog	No eligible paper found	NA	NA
Meretrix lusoria	Japanese hard clam	No eligible paper found	NA	NA
Mya arenaria	Sand gaper	No eligible paper found	NA	NA
Mytilus edulis	Blue mussel	No eligible paper found	NA	NA
Mytilus galloprovincialis	Mediterranean mussel	No eligible paper found	NA	NA
Octopus vulgaris	Common octopus	No eligible paper found	NA	NA
Ostrea edulis	European flat oyster	No eligible paper found	NA	NA
Pecten maximus	Great Atlantic scallop	No eligible paper found	NA	NA
Polititapes aureus (Venerupis aurea)	Golden carpet shell	No eligible paper found	NA	NA
Ruditapes decussatus	Grooved carpet shell	No eligible paper found	NA	NA
Ruditapes philippinarum	Japanese carpet shell	No eligible paper found	NA	NA
Sepia officinalis	Common cuttlefish	No eligible paper found	NA	NA
Strombus spp.	Stromboid conchs spp.	No eligible paper found	NA	NA
Venerupis corrugata (Venerupis pullastra)	Pullet carpet shell	No eligible paper found	NA	NA
Venus verrucosa	Warty venus	No eligible paper found	NA	NA
Infection with white spot syndrome virus				
Vector species Scientific names	Vector species Common names			
Atrina spp.	Pen shells spp.	No eligible paper found	NA	NA
Buccinum undatum	Whelk	No eligible paper found	NA	NA
Crassostrea angulata	Portuguese cupped oyster	No eligible paper found	NA	NA
Cerastoderma edule	Common edible cockle	No eligible paper found	NA	NA
Crassostrea gigas	Pacific cupped oyster	No eligible paper found	NA	NA
Crassostrea virginica	American cupped oyster	No eligible paper found	NA	NA
Donax trunculus	Truncate donax	No eligible paper found	NA	NA
Haliotis discus hannai	Japanese abalone	No eligible paper found	NA	NA
Haliotis tuberculata	Tuberculate abalone	No eligible paper found	NA	NA
Littorina littorea	Common periwinkle	No eligible paper found	NA	NA
Mercenaria mercenaria	Northern quahog	No eligible paper found	NA	NA
Meretrix lusoria	Japanese hard clam	No eligible paper found	NA	NA
Mya arenaria	Sand gaper	No eligible paper found	NA	NA
Mytilus edulis	Blue mussel	No eligible paper found	NA	NA
Mytilus galloprovincialis	Mediterranean mussel	No eligible paper found	NA	NA
Octopus vulgaris	Common octopus	No eligible paper found	NA	NA
Ostrea edulis	European flat oyster	No eligible paper found	NA	NA
Pecten maximus	Great Atlantic scallop	No eligible paper found	NA	NA
Polititapes aureus (Venerupis aurea)	Golden carpet shell	No eligible paper found	NA	NA
Ruditapes decussatus	Grooved carpet shell	No eligible paper found	NA	NA
Ruditapes philippinarum	Japanese carpet shell	No eligible paper found	NA	NA
Sepia officinalis	Common cuttlefish	No eligible paper found	NA	NA
Strombus spp.	Stromboid conchs spp.	No eligible paper found	NA	NA
Venerupis corrugata (Venerupis pullastra)	Pullet carpet shell	No eligible paper found	NA	NA
Venus verrucosa	Warty venus	No eligible paper found	NA	NA

Appendix B – Studies excluded during the extensive literature review

Table B.1 lists species for which studies were retrieved in Distiller, but that were excluded because the studies did not pass the eligibility criteria; or species for which evidence was extracted during the ELS, but the certainty was too low for classification as vector or reservoir species.

Table B.1: Species from excluded studies during the ELS and during the assessment due to lack of certainty

Infection with WSSV					
Scientific name	**Reference**	**Certainty**	**Reasoning**	**Conclusion WG and AHAW Panel**	**Suggested classification by EURL (2022), not the outcome of this current assessment**
Acartia clausi	Mang et al. (2007)	33–66%	No evidence of transmission; infection confirmed with conventional PCR, no reference method, but only one replicate	Not classified	Not assessed
Acetes sp.	Supamattaya et al (1998)	NA	Already listed in EU legislation as susceptible species	Not assessed	Vector/reservoir
Alpheus lobidens	Takahashi et al. (2003)	NA	Already listed in EU legislation as susceptible species	Not assessed	Vector/reservoir
Artemesia longinaris	Martorelli et al. (2010)	NA	Already listed in EU legislation as susceptible species	Not assessed	Vector/reservoir
Artemia	Feng et al. (2013)	10–33%	No evidence of transmission, no indication that the virus can amplify in Species X	Not classified	Not assessed
	Jiang (2012)	10–33%	The Artemia were shown to have ingested phytoplankton shown to be positive for WSSV by in situ hybridisation. The Artemia was positive by dot blot (results not shown) and vector transmission was demonstrated by mortality of species Y. No PCR or sequence analysis.	Not classified	Not assessed
Artemia nauplii	Otta et al. (1999)	10–33%	PCR (reference test used) but only one sample; unsure if infected or the virus is in the gut or on surface; other organisms might have been present in sample; not able to infect *P. monodon* present in same water	Not classified	Not assessed
Ashtoret miersii	Sahul Hameed et al. (2003)	NA	Already listed in EU legislation as susceptible species	Not assessed	Vector/reservoir

Astacus astacus	Jiravanichpaisal et al. (2004)	NA	Already listed in EU legislation as susceptible species	Not assessed	Vector/reservoir
Atergatis integerrimus	Sahul Hameed et al. (2003)	NA	Already listed in EU legislation as susceptible species	Not assessed	Vector/reservoir
Austinogebia edulis	Zhu et al. (2019)	NA	Already listed in EU legislation as susceptible species	Not assessed	Vector/reservoir
Calanus pacificus	Mendoza-Cano et al. (2014)	33–66%	Very little information regarding sample size, inoculation method; only one replicate; but low Ct value	Not classified	Not assessed
Calanus pacificus californicus	Mendoza-Cano et al. (2014)	33–66%	Not a natural challenge Potential evidence of replication, but detection by SYBR not from WOAH manual, and no sequence confirmation. Only tested 84 h post challenge.	Not classified	Vector/reservoir
Calappa lophos	Wang et al. (1998)	NA	Already listed in EU legislation as susceptible species	Not assessed	Vector/reservoir
Calappa philarigus	Sahul Hameed et al. (2003)	NA	Already listed in EU legislation as susceptible species	Not assessed	Vector/reservoir
Callinectes arcuatus	Macías-Rodríguez et al. (2014)	NA	Already listed in EU legislation as susceptible species	Not assessed	Vector/reservoir
Callinectes sapidus	Powel et al. (2015)	NA	Already listed in EU legislation as susceptible species	Not assessed	Vector/reservoir
	Blaylock et al. (2019)				
Charybdis annulata	Sahul Hameed et al. (2003)	NA	Already listed in EU legislation as susceptible species	Not assessed	Vector/reservoir
Charybdis cruciata	Hossain et al. (2001)	NA	Already listed in EU legislation as susceptible species	Not assessed	Vector/reservoir
Charybdis feriata	Kou et al. (1998)	NA	Already listed in EU legislation as susceptible species	Not assessed	Vector/reservoir
	Lo et al. (1996b)				
	Wang et al. (1998)				
Charybdis natator	Kou et al. (1998)	NA	Already listed in EU legislation as susceptible species	Not assessed	Vector/reservoir
	Sahul Hameed et al. (2003)				
Charybdis lucifera	Sahul Hameed et al. (2003)	NA	Already listed in EU legislation as susceptible species	Not assessed	Vector/reservoir
Charybdis japonica	Takahashi et al. (2003)	NA	Already listed in EU legislation as susceptible species	Not assessed	Vector/reservoir
Chelonibia testudinaria	Overstreet et al. (2009)	10–33%	No evidence of transmission; no evidence of replication in species X	Not classified	Not assessed

Copepoda *(species name not provided)*	Porchas-Cornejo et al. (2018)	33–66%	Real-time PCR detection of samples collected in a single trawl. Ct values not provided to help interpret whether this is an active infection or contamination from other species in the same trawl	Not classified	Not assessed
Cyrtograpsus angulatus	Martorelli et al. (2010)	NA	Already listed in EU legislation as susceptible species	Not assessed	Vector/reservoir
Demania splendida	Sahul Hameed et al. (2003)	NA	Already listed in EU legislation as susceptible species	Not assessed	Vector/reservoir
Diogenes nitidimanus	Chang et al. (2012)	NA	Already listed in EU legislation as susceptible species	Not assessed	Vector/reservoir
Doclea muricata	Sahul Hameed et al. (2003)	NA	Already listed in EU legislation as susceptible species	Not assessed	Vector/reservoir
Grapsus albolineatus	Sahul Hameed et al. (2003)	NA	Already listed in EU legislation as susceptible species	Not assessed	Vector/reservoir
Halimede ochtodes	Sahul Hameed et al. (2003)	NA	Already listed in EU legislation as susceptible species	Not assessed	Vector/reservoir
Hemigrapsus takanoi	Kawato et al. (2019)	NA	Already listed in EU legislation as susceptible species	Not assessed	Vector/reservoir
Liagore rubronaculata	Sahul Hameed et al. (2003)	NA	Already listed in EU legislation as susceptible species	Not assessed	Vector/reservoir
Lithodes maja	Sahul Hameed et al. (2003)	NA	Already listed in EU legislation as susceptible species	Not assessed	Vector/reservoir
Macrobrachium idella	Rajendran et al. (1999) Sahul Hameed et al. (2003)	NA	Already listed in EU legislation as susceptible species	Not assessed	Vector/reservoir
Macrobrachium lamerrae	Sahul Hameed et al. (2000)	NA	Already listed in EU legislation as susceptible species	Not assessed	Vector/reservoir
Macrobrachium sulcatus	Hossain et al. (2001)	NA	Already listed in EU legislation as susceptible species	Not assessed	Vector/reservoir
Marphysa gravelyi	Vijayan et al. (2005)	NA	It is not a crustacean. In addition, it is already listed in EU legislation as susceptible species	Not assessed	Vector/reservoir
Matuta planipes	Otta et al. (1999)	NA	Already listed in EU legislation as susceptible species	Not assessed	Vector/reservoir
Menippe rumphii	Sahul Hameed et al. (2003)	NA	Already listed in EU legislation as susceptible species	Not assessed	Vector/reservoir
Metapaulius depressus	Rozenberg et al. (2015)	NA	Already listed in EU legislation as susceptible species	Not assessed	Vector/reservoir

Metapenaeus brevicornis	Hossain et al. (2001)	NA	Already listed in EU legislation as susceptible species	Not assessed	Vector/reservoir
Metapenaeus affinis	Gholamhoseini et al. (2013)	NA	Already listed in EU legislation as susceptible species	Not assessed	Vector/reservoir
Metopograpsus messor	Hossain et al. (2001)	NA	Already listed in EU legislation as susceptible species	Not assessed	Vector/reservoir
Neomysis awatschensis	Mang et al. (2007)	33–66%	No evidence of transmission; infection confirmed with conventional PCR, no reference method, but only one replicate	Not classified	Not assessed
Octolasmis lowei	Overstreet et al. (2009)	10–33%	No evidence of transmission; no evidence of replication in species X	Not classified	Not assessed
Orconectes punctimanus	Lo et al. (1996a, b)	NA	Already listed in EU legislation as susceptible species	Not assessed	Vector/reservoir
Pagurus angustus	Chang et al. (2012)	NA	Already listed in EU legislation as susceptible species	Not assessed	Vector/reservoir
Palaemon macrodactylus	Martorelli et al. (2010)	NA	Already listed in EU legislation as susceptible species	Not assessed	Vector/reservoir
Palaemonetes pugio	Muhammad et al. (2015)	NA	Already listed in EU legislation as susceptible species	Not assessed	Vector/reservoir
Panulirus homarus	Rajendran et al. (1999)	NA	Already listed in EU legislation as susceptible species	Not assessed	Vector/reservoir
Panulirus longipes	Wang et al. (1998)	NA	Already listed in EU legislation as susceptible species	Not assessed	Vector/reservoir
Panulirus polyphagus	Rajendran et al. (1999)	NA	Already listed in EU legislation as susceptible species	Not assessed	Vector/reservoir
Paradorippe granulata	Sahul Hameed et al. (2003)	NA	Already listed in EU legislation as susceptible species	Not assessed	Vector/reservoir
Parapenaeopsis stylifera	Gholamhoseini et al. (2013) Hossain et al. (2001)	NA	Already listed in EU legislation as susceptible species	Not assessed	Vector/reservoir
Paratelphusa hydrodomous	Raj et al. (2012) Sahul Hameed et al. (2001)	NA	Already listed in EU legislation as susceptible species	Not assessed	Vector/reservoir
Parthenope prensor	Sahul Hameed et al. (2003)	NA	Already listed in EU legislation as susceptible species	Not assessed	Vector/reservoir
Penaeus aztecus	Lightner et al. (1998)	33–66%	No transmission experiments. No PCR results – only histology data.	Not classified	Not assessed
Penaeus californiensis	Macías-Rodríguez et al. (2014)	NA	Already listed in EU legislation as susceptible species	Not assessed	Vector/reservoir

Penaeus duorarum	Lightner et al. (1998)	NA	Already listed in EU legislation as susceptible species	Not assessed	Vector/reservoir
Penaeus penicillatus	Chou et al. (1998) Lo et al. (1996a) Wang et al. (1998)	NA	Already listed in EU legislation as susceptible species	Not assessed	Vector/reservoir
Penaeus setiferus	Lightner et al. (1998)	NA	Already listed in EU legislation as susceptible species	Not assessed	Vector/reservoir
Penaeus stylirostris	Lightner et al. (1998)	NA	Already listed in EU legislation as susceptible species	Not assessed	Vector/reservoir
Philyra syndactyla	Sahul Hameed et al. (2003)	NA	Already listed in EU legislation as susceptible species	Not assessed	Vector/reservoir
Podophthalmus vigil	Sahul Hameed et al. (2003)	NA	Already listed in EU legislation as susceptible species	Not assessed	Vector/reservoir
Scylla paramamosain	Rajan et al. (2000) Lightner et al. (1998) Ma et al. (2009)	NA	Already listed in EU legislation as susceptible species	Not assessed	Vector/reservoir
Sesarmops intermedius	Kawato et al. (2019)	NA	Already listed in EU legislation as susceptible species	Not assessed	Vector/reservoir
Squilla sp.	Anirban et al. (2002)	10–33%	Small numbers of positive sample; some by first round others by nested PCR. Nothing detected using the WOAH primers but not unexpected detection given product of 1.5 kb product and this was formalin fixed material. Assays used not in WOAH manual, and confirmation of specificity by hydridisation (Southern blot) rather than sequencing.	Not classified	Not assessed
Thalamita danae	Sahul Hameed et al. (2003)	NA	Already listed in EU legislation as susceptible species	Not assessed	Vector/reservoir
Gelasimus vocans	Hossain et al. (2001)	NA	Already listed in EU legislation as susceptible species	Not assessed	Vector/reservoir

Infection with YHV

Scientific name	**Reference**	**Certainty**	**Reasoning**	**Conclusion AHAW Panel**	**Suggested classification by EURL (2022), not the outcome of this current assessment**
Acetes sp.	Flegel et al. (1995)	NA	Excluded because it is not a primary research study	Not assessed	Vector/reservoir

Chelonibia testudinaria	Overstreet et al. (2009)	10–33%	No evidence of transmission; no evidence of replication in species X	Not classified	Vector/reservoir
Cherax quadricarinatus	Soowannayan et al. (2015)	33–66%	Experimental challenge rather than detection in wild animals. PCR only using primer set outside of the WOAH manual and no evidence of sequence confirmation. No evidence of infection by immunohistochemistry Evidence for transmission confirmed using the same RT-PCR approach.	Not classified	Not assessed
Clibanarius vittatus	Ma et al. (2009)	0–10%	No evidence of transmission; very weak signal that disappears after few days. PCR that is in literature but no reference test	Not classified	Not assessed
Ergasilus manicatus	Overstreet et al. (2009)	10–33%	No evidence of transmission; no evidence of replication in species X	Not classified	Vector/reservoir
Fundulus grandis	Overstreet et al. (2009)	NA	Data not extracted for this species (only crustaceans were considered)	Not assessed	Vector/reservoir
Macrobrachium lanchesteri	Longyant et al. (2005)	0–10%	No natural infections. Detection by RT-PCR after injection only. Limited mortality after injection.	Not classified	Not assessed
Macrobrachium rosenbergii	See et al. (2021)	33–66%	Detected during an evaluation of a new multiplex assay. Not a WOAH assay but sequence data confirm product as YHV in origin	Not classified	Vector/reservoir
	Chuchird et al. (2007)		No transmission experiments. Detection methods not well described. Number of PCR and ISH-positive individuals not reported.		
Macrobrachium sintangense	Longyant et al. (2005)	33–66%	No natural infections. Detection by RT-PCR after injection only. Limited mortality after injection.	Not classified	Vector/reservoir
Metapenaeus bennettae	Walker et al. (2001)	NA	Excluded because the full text was not available	Not assessed	Vector/reservoir
Metapenaeus ensis	Chantanachookin et al. (1993)	NA	Excluded at the first level of screening because the study does not investigate any not known susceptible species	Not assessed	Vector/reservoir

Octolasmis lowei	Overstreet et al. (2009)	10–33%	No evidence of transmission; no evidence of replication in species X	Not classified	Vector/reservoir
Palaemon serrifer	Longyant et al. (2005)	33–66%	No natural infections. Detection by RT-PCR after injection only. Limited mortality after injection.	Not classified	Vector/reservoir
Penaeus esculentus	Walker et al. (2001)	NA	Excluded because the full text wasn't available	Not assessed	Vector/reservoir
	Spann et al. (2003)		The study investigates the gill-associated virus (GAV)		
	Spann et al. (2003)		The study investigates the gill-associated virus (GAV)		
Penaeus japonicus	Wang et al. (1996)	NA	No molecular identification of pathogen.	Not assessed	Vector/reservoir
Penaeus merguiensis	Flegel et al. (1997)	NA	Excluded because it is not a primary research study	Not assessed	Vector/reservoir
	Chantanachookin et al. (1993)		Excluded at the first level of screening because the study does not investigate any not known susceptible species		
Penaeus setiferus	Lightner et al. (1998)	NA	Species not investigated in this study	Not assessed	Vector/reservoir
Penaeus indicus	East et al. (2005)	NA	Excluded in the second level of screening because the pathogen was not identified in/on species X	Not assessed	Vector/reservoir
Scylla serrata	East et al. (2005)	NA	Excluded in the second level of screening because the pathogen was not identified in/on species X	Not assessed	Vector/reservoir
Leptuca spinicarpa	Ma et al. (2009)	0–10%	No evidence of transmission; very weak signal that disappears after few days	Not classified	Vector/reservoir
Infection with TSV					
Scientific name	**Reference**	**Certainty**	**Reasoning**	**Conclusion AHAW Panel**	**Suggested classification by EURL (2022), not the outcome of this current assessment**
Artemia	Overstreet et al. (1997)	10–33%	Artemia (brine shrimp) were used as a vehicle to set the susceptibility of *Penaeus setiferus* (Northern white shrimp) to TSV. No indication of how the TSV was	Not classified	Not assessed

			incorporated in to the brine shrimp and there was no analysis to show if the animals were infected or just contaminated with virus.		
Fundulus grandis	Overstreet et al. (2009)	NA	Data not extracted for this species (only crustaceans were considered)	Not assessed	Vector/reservoir
Penaeus schmitti	Fajardo et al. (2010)	33–66%	Translation required. Positive by nested PCR only and therefore potential low level carrier. Not able to determine if confirmed by sequence analysis was undertaken but cannot see any evidence	Not classified	Not assessed
Macrobrachium lanchesteri	Kiatpathomchai et al. (2008)	10–33%	Molecular detection using the IQ2000 kit, but the signal is lost by day 10 post injection. Transmission experiments performed by feeding, and high virus loads TSV was detected in species Y by RT-PCR and the were high mortality rates. No sequence confirmation	Not classified	Not assessed
Palaemon styliferus	Kiatpathomchai et al. (2008)	0–10%	Molecular detection using the IQ2000 kit, but the signal is lost by day 10 post injection. No transmission undertaken	Not classified	Not assessed

PCR, polymerase chain reaction; RT-PCR, reverse transcription polymerase chain reaction; WOAH, World Organisation for Animal Health.