SCIENTIFIC OPINION

efsa JOURNAL

ADOPTED: 21 June 2023

doi: 10.2903/j.efsa.2023.8173

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

EFSA Panel on Animal Health and Welfare (AHAW),
Søren Saxmose Nielsen, Julio Alvarez, Dominique Bicout, Paolo Calistri, Elisabetta Canali, Julian Ashley Drewe, Bruno Garin-Bastuji, José Louis Gonzales Rojas, Christian Gortazar Smith, Mette Herskin, Virginie Michel, Miguel Angel Miranda Chueca, Barbara Padalino, Helen Roberts, Hans Spoolder, Karl Ståhl, Antonio Velarde, Arvo Viltrop, Christoph Winckler, Isabelle Arzul, Shetty Dharmaveer, Niels Jørgen Olesen, Morten Schiøtt, Hilde Sindre, David Stone, Niccoló Vendramin, Sotiria-Eleni Antoniou, Sofie Dhollander, Anna Eleonora Karagianni, Linnea Lindgren Kero, Marzia Gnocchi, Inma Aznar, Fulvio Barizzone, Irene Pilar Munoz Guajardo and Helen Roberts

Abstract

Vector or reservoir species of five mollusc diseases 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. Mollusc species on or in which *Mikrocytos mackini*, *Perkinsus marinus*, *Bonamia exitiosa*, *Bonamia ostreae* and *Marteilia refringens* 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, this 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 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 molluscs was not found, these were defined as reservoir. 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 mollusc species during transport was collected from scientific literature. It was concluded that it is very likely to almost certain (90–100%) that *M. mackini, P. marinus, B. exitiosa B. ostreae* and *M. refringens* 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 at aquaculture establishments or through contaminated water supply can possibly transmit these pathogens. For transmission of *M. refringens,* the presence of an intermediate host, a copepod, is necessary.

Keywords: Vector, Reservoir, *Mikrocytos mackini*, *Perkinsus marinus*, *Bonamia exitiosa*, *Bonamia ostreae*, *Marteilia refringens*, transport conditions

Requestor: European Commission

Question number: EFSA-Q-2023-00060

Correspondence: ahaw@efsa.europa.eu

Panel members: Søren Saxmose Nielsen, Julio Alvarez, Dominique Joseph Bicout, Paolo Calistri, Elisabetta Canali, Julian Ashley Drewe, Bruno Garin-Bastuji, José Luis Gonzales Rojas, Christian Gortázar, Mette S Herskin, Virginie Michel, Miguel Ángel Miranda, Barbara Padalino, Paolo Pasquali (active until 5 April 2023), Helen Clare Roberts, Hans Spoolder, Karl Ståhl, Antonio Velarde, Arvo Viltrop and Christoph Winckler.

Declaration of interest: If you wish to access the declaration of interests of any expert contributing to an EFSA scientific assessment, please contact interestmanagement@efsa.europa.eu.

Acknowledgements: The Panel wishes to thank the following for the support provided to this scientific output: Marc Engelsma, Deborah Cheslett, Ana Roque and, Dolors Furones for carrying out the extensive literature review that formed the basis of the assessment.

Suggested citation: EFSA AHAW Panel (EFSA Panel on Animal Health and Welfare), Nielsen, S. S., Alvarez, J., Bicout, D., Calistri, P., Canali, E., Drewe, J. A., Garin-Bastuji, B., Gonzales Rojas, J. L., Smith, C. G., Herskin, M., Michel, V., Miranda Chueca, M. A., Padalino, B., Spoolder, H., Ståhl, K., Velarde, A., Viltrop, A., Winckler, C., … Roberts, H. (2023). Species which may act as vectors or reservoirs of diseases covered by the Animal Health Law: Listed pathogens of molluscs. *EFSA Journal, 21*(8), 1–34. https://doi.org/10.2903/j.efsa.2023.8173

ISSN: 1831-4732

The EFSA Journal is a publication of the European Food Safety Authority, a European agency funded by the European Union.

Summary

Term of Reference 1 (ToR 1) requested 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 COMMISSION IMPLEMENTING REGULATION (EU) 2018/1882 EU Regulation 2016/429. This Opinion specifically focuses on assessing vector or reservoir species of the five diseases of molluscs, i.e. *Mikrocytos mackini*, *Perkinsus marinus*, *Bonamia exitiosa*, *Bonamia ostreae* and *Marteilia refringens*. The aim of the assessments is to indicate if the Annex to Implementing Regulation (EU) 2018/1882, listing those vector 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 simultaneously as susceptible and vector or reservoir species.

The following working definitions were agreed for the assessment: a mollusc species can be considered a vector when the pathogen has been identified in or on the mollusc 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 mollusc 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 (already said previously). Nonetheless, the risk of the spread of the pathogens from infected reservoir species cannot be excluded.

An extensive literature review (ELR) 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 Engelsma et al. (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 mollusc 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 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 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 for ***M. mackini*** the species *Crassostrea virginica* (American cupped oyster) is considered a vector species with > 90% certainty.

For ***B. exitiosa*** and ***B. ostreae***, no species were considered to be vectors. *Ostrea stentina* (dwarf oyster) and *Ostrea angasi* (angasi oyster) are considered to be reservoir species with > 90% certainty for *B. exitosa* and *B. ostreae,* respectively.

For ***M. refringens*****,** the following species are considered to be reservoir species with > 90% certainty: *Mytilus galloprovincialis* (Galician mussel)*, Chamelea gallina* (chicken venus) and *O. stentina* (dwarf oyster).

For ***P. marinus*****,** the species *Boonea impressa* is considered to be a vector species with > 90% certainty, while *Macoma balthica* (Baltic macoma), *Modiolus capax* (fat horsemussel), *Mya arenaria* (large-neck clam)*, Crassostrea ariakensi* (Suminoe oyster)*, Crassostrea brasiliana* (Brazilian oyster)*, Crassostrea corteziensis* (Cortez oyster)*, Crassostrea rhizophorae* (Pacific cupped oyster) and *Saccostrea palmula* (palmate oyster) are considered to be reservoir species with > 90% certainty and *Mercenaria mercenaria* (American hard-shelled clam) and *Crassostrea* sp. with 66–90% certainty.

In addition, a list of vector or reservoir species for which no 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 molluscs 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 assessed to 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 ELR, 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.

It was concluded that it is very likely to almost certain (90–100%) that *M. mackini, P. marinus, B. exitiosa, B. ostreae* and *M. refringens* will remain infective at any possible transport condition. Therefore, vector or reservoir species that were exposed to these pathogens can possibly transmit them 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 for *M. mackini, P. marinus, B. exitiosa, B. ostreae;* or the intermediate host for *M. refringens*; (b) the **wild**, where they may have been exposed to susceptible, reservoir or other vector species for *M. mackini, P. marinus, B. exitiosa, B. ostreae; or* the intermediate host for *M. refringens; and* (c) an **aquaculture establishment supplied with water** possibly contaminated with *M. mackini, P. marinus, B. exitiosa* or *B. exitiosa* or with the intermediate hosts infected with *M. refringens*. It was suggested that these conclusions may be considered as or for amendments to Annex I of Reg 2020/990 and Annex XXX of Reg 2020/692.

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.

[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, 28.5.2020, 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 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 and 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 belonging to aquatic molluscs belonging to the **phylum Mollusca**. The pathogens listed by the AHL affecting molluscs are:

- *B. exitiosa* (Cercozoa; Haplosporidiidae)
- *B. ostreae* (Cercozoa; Haplosporidiidae)
- *M. refringens* (Cercozoa; Marteiliidae)
- *M. mackini* (Cercozoa; Mikrocytiidae)
- *P. marinus* (Mysozoa; Perkinsidae).

For assessment *M. refringens* included *M. refringens* genotype O only *Marteilia pararefringens* (corresponding to *M. refringens* genotype M and previously named *Marteilia maurini)*. was not included in the assessment.

It was agreed that for this assessment, a mollusc 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 alive 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 mollusc species, but evidence of transmission of the pathogen to susceptible species is not available. In addition, 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 ToR. 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 mollusc species shall be regarded as vectors or reservoirs of diseases of molluscs 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 EU legislation EU Reg 2020/990 Annex I specify that the species may be regarded as vectors if they 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 EU Reg 2020/692 Annex XXX stipulate that vectors may be regarded as the species that have been in contact with listed susceptible species 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, namely, to identify that, besides the conditions already laid down in EU Reg 2020/990 Annex I and Reg 2020/692, related to the exposure to the pathogenic agent at the source, 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 mollusc species will act as potential vector or reservoir, there will be no change in the conditions already laid down in EU Reg 2020/990 Annex I and Reg 2020/692 proposed.

2. Data and methodologies

2.1. Methodologies

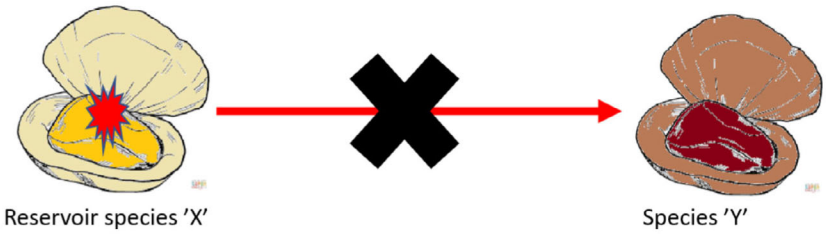
2.1.1. Term of Reference 1: Assessment of potential vectors and reservoir species of pathogens of molluscs, listed in Annex II to the AHL

An ELR has been carried out to gather all scientific evidence available of parameters needed to assess the role of aquatic species as vectors or reservoirs of specific pathogens of molluscs, 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 be defined 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 Engelsma et al. (2023) – see the Annex.

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 mollusc 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, and therefore a second reference test is superfluous.

Diagnostic tests considered accurate for the diagnosis of the listed molluscs pathogens in the ELR, with one test being sufficient to confirm pathogen detection in the vector or reservoir in experimental infections, and two tests for field detections are available on https://www.eurl-mollusc.eu/Diagnostic-manual. An infection with a pathogen is confirmed if positive results are obtained by:

- histopathology or tissue imprints or *in situ* hybridisation (ISH) **AND** PCR & sequencing, or,
- if no material for histopathology, or tissue imprints or ISH available: two PCR assays targeting different fragments of the genome and sequencing.

To identify potential vector and reservoir species, however, the identity of the pathogen rather than the infection is needed to be confirmed. While histology, cytology and some PCR or ISH are not considered species specific, in the frame of this work the following methods, alone or in combination with other methods, were considered to be sufficient to correctly identify pathogen species:

- For ***M. refringens:*** PCR combined with sequencing targeting the IGS fragment (Lopez-Flores et al., 2004, 2008a,b).
- For ***B. exitiosa:*** PCR combined with sequencing targeting the 18S or the ITS region or ISH (Hill et al., 2010,b).
- ***For P. marinus:*** PCR combined with sequencing of a long fragment (3.2-kb fragment of *P. marinus* DNA) (Marsh et al., 1995); or, PCR combined with sequencing targeting the ITS fragment (Casas et al., 2002; Park et al., 2005, 2006; Escobedo-Fregoso et al., 2017; Robledo et al., 1999; Da Silva et al., 2014) or the LSU gene (Lenaers et al., 1989; Reece et al., 2008b); or PCR-RFLP (Abollo et al., 2006) or ISH (Reece et al., 2008b).

Histology as a single test for confirmation of the pathogen was not considered accurate enough.

- **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 more than one Species X or Pathogen A was tested. Depending on the specific situation (e.g. if other studies are available or not), negative results in Species X can provide less than 10% certainty that Species X is a RESERVOIR species based on the evidence extracted from the literature (external report).

In addition, in transmission experiments, where negative results for Pathogen A detection in Species Y (susceptible species) after transmission from Species X (potential vector) were recorded, 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 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 five experts on mollusc diseases.
  - Only inconclusive cases were discussed, and experts were asked to identify a more precise certainty range for the inconclusive 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 mollusc 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 > 66% were 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% 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 a vector or reservoir. Therefore, the assessment should be updated when new evidence becomes available.

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

Several conditions need to be fulfilled for a mollusc species to be able act as a vector 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 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 vector/reservoir/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, 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 mollusc listed in Annex II to the AHL

The detailed data set extracted through the ELR is available in the EJ Supporting publications Annex to External Scientific report molluscs_20230511.xlsx (detailed Excel table with all variables).

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

- The detailed data set extracted through the ELR is available in the EJ Supporting publications Annex to External Scientific report molluscs_20230511.xlsx (detailed Excel table with all variables)

3. Assessment

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

Table 1 summarises the results of the assessment of potential vectors of AHL-listed pathogens of molluscs. The outcome of the assessment was that only two species fulfilled the criteria for categorisation as a vector: *Crassostrea virginica* (American cupped oyster) and *Boonea impressa* (impressed odostome) were assessed as vectors of *Mikrocytos mackini* and *Perkinsus marinus* respectively, with more than 90% certainty.

The assessment was based on the evidence from experimental infection studies that was generated by the extensive literature review (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 the Engelsma et al. (2023).

Table 1: Proposed vectors of AHL-listed mollusc 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 as vector species	Reference	Suggested classification by previous EURL report (2022)
AHL-listed mollusc pathogen: *Mikrocytos mackini*								
Crassostrea virginica (American Cupped Oyster)	Yes	Injection	*Crassostrea gigas (accepted name Magallana gigas)*	Tissue imprints	90–100%	Positive for at least one reference test	Bower et al. (1997)	Vector/Reservoir
AHL-listed mollusc pathogen: *Perkinsus marinus*								
Boonea impressa (Impressed odostome)	No	Cohabitation	*Crassostrea virginica*	RFTM culture medium	90–100%	Positive for at least one reference test	White et al. (1987)	Not assessed

*: Source: Gbif.org; RFTM: Ray's fluid thioglycollate medium.

Table 2 summarises the results of the assessment of potential reservoir species of AHL-listed pathogens of molluscs, 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 reasoning and level of certainty of the proposed classification is provided. More detailed data from studies that did not provide sufficient evidence are in Engelsma et al. (2023).

Magallana ariakensis (Suminoe oyster), *C. corteziensis* (Cortez oyster), *Macoma balthica* (Baltic macoma) and *Mya arenaria* (large-neck oyster) were assessed as reservoir species of *P. marinus* with more than 90% certainty.

It should be noted that *M. ariakensis* and *C. corteziensis* were already indicated by the EURL report as susceptible species but are currently not listed in the EU regulation. 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 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 mollusc 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 for classification as RESERVOIR species	Reasoning for classification as reservoir species	Reference	Suggested classification by previous EURL report (2022)
AHL-listed mollusc pathogen: *Perkinsus marinus*						
Magalla ariakensis (*Crassostrea ariakensis* in paper) (Suminoe oyster)	No	PCR PCR-RFLP ISH	90–100%	Species specific *Perkinsus marinus* ITS primers were used	Moss et al. (2006,b)	Susceptible species
Crassostrea corteziensis (Cortez oyster)	No	PCR Seq	90–100%	Not clear if the PCR is species specific, however *Perkinsus* ITS sequenced	Escobedo-Fregoso et al. (2017)	Susceptible species
		PCR Seq RFTM assay ISH	90–100%	Not clear from text if PCR is species specific NTS fragment 98% similarity to *P. marinus*, ITS fragment 100% similar to *P. marinus*	Gutierrez-Rivera et al. (2015)	
Macoma balthica (Baltic macoma)	Yes	RFTM assay PCR His	90–100%	Pathogen could be re-isolated by RFTM. *P. marinus* species specific PCR was used. Histology is not specific for pathogen identification, but additional methods were used for pathogen species identification	Dungan et al., 2007	Not assessed
Mya arenaria (large-neck clam)	Yes	RFTM assay PCR His	90–100%	Pathogen could be re-isolated by RFTM. *P. marinus* species specific PCR was used. Histology is not specific for pathogen identification but additional methods were used for pathogen species identification	Dungan et al., 2007	Vector/Reservoir

*: Source: Gbif.org. PCR, polymerase chain reaction; His, histology; RFTM, Ray's fluid thioglycollate medium; RFLP, restriction fragment length polymorphism; Seq, sequencing; ISH, *in situ* hybridisation; EURL, EU Reference Laboratory; ITS, internal transcribed spacer.

Table 3 summarises the results of the assessment of potential reservoir species of AHL-listed pathogens of molluscs, 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 Engelsma et al. (2023).

The outcome of the assessment was that *Ostrea stentina* (dwarf oyster) is considered to be a reservoir species for *Bonamia exitiosa* with > 90% certainty. *O. stentina* was already indicated by the previous EURL report (REF) as susceptible species.

Ostrea angasi (angasi oyster) is considered to be a reservoir species for *Bonamia ostreae* with 66–90% certainty.

Chamelea gallina (chicken venus), *Mytilus galloprovincialis* (Galician mussel) and *O. stentina* (dwarf oyster) are considered to be reservoir species for *M. refringens* with > 90% certainty. *C. gallina* and *O. stentina* were already indicated by the previous EURL report as susceptible species.

C. corteziensis (Cortez oyster), *Crassostrea brasiliana* (mangrove oyster), *C. rhizophorae* (Pacific cupped oyster), *Mya arenaria* (large-neck clam), *Modiolus capax* (FAT horsemussel) and *Saccostrea palmula* (Palmate oyster) were considered to be reservoir species for *P. marinus* with > 90% certainty. *M. mercenaria* (American hard-shelled clam) and *Crassostrea* sp. are considered to be reservoir species for *P. marinus* with 66–90% certainty. *C. corteziensis, Crassostrea tulipa, Crassostrea* sp., *C. rhizophorae* and *S. palmula* were already indicated by the previous EURL report as susceptible species.

Table 3: Proposed reservoirs of AHL-listed mollusc 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 of classification as reservoir species	Reasoning for categorisation as reservoir species	Bibliography	Suggested classification in previous EURL report (2022)
AHL-listed mollusc pathogen: *Bonamia exitiosa*						
Ostrea stentina (dwarf oyster)	Yes	PCR-RFLP	90–100%	Only single assay used but evidence supported by multiple publications for same species	Elgharsalli et al. (2018)	Susceptible species
		PCR	90–100%	At least two assays were used with positive results for the same species	Hill et al. (2010)	Susceptible species
		Seq				
		His				
		ISHy				
AHL-listed mollusc pathogen: *Bonamia ostreae*						
Ostrea angasi (angasi oyster)	No	His	66–90%	Doubtful pathogen identification with histology only, but within *B. ostreae* endemic area	Bougrier et al. (1986)	Doubtful
		Smear				
AHL-listed mollusc pathogen: *Marteilia refringens*						
Chamellea gallina (chicken venus)	Yes	His	90–100%	At least two assays were used with positive results for the same species	Lopez-Flores et al. (2008b)	Susceptible species
		PCR				
		InSiHy				
		Seq				
Mytilus galloprovincialis (Galician mussel)	Yes	PCR	90–100%	At least two assays were used with positive results for the same species	Lattos et al. (2022)	Doubtful
		Seq				
		Seq	90–100%	Only single assay used but evidence supported by multiple publications for same species	Novoa et al. (2005)	
		His	90–100%	At least two assays were used with positive results for the same species	Arzul et al. (2014)	
		PCR				
		PCR-RFLP				
		Seq				
		PCR-RFLP	90–100%	Only single assay used but evidence supported by multiple publications for same species	Balseiro et al. (2007)	

Reservoir species X	Presence in EU*	Pathogen detection method in species X with positive results	Certainty of classification as reservoir species	Reasoning for categorisation as reservoir species	Bibliography	Suggested classification in previous EURL report (2022)
Ostrea stentina (dwarf oyster)	Yes	PCR-RFLP	90–100%	Only single assay used but evidence supported by multiple publications for same species	Elgharsalli et al. (2018)	Susceptible species
		PCR-RFLP Seq His TEM	90–100%	At least two assays were used with positive results for the same species	Elgharsalli et al. (2013)	Susceptible species
		His InSiHy PCR PCR-RFLP Seq	90–100%	At least two assays were used with positive results for the same species	Lopez-Sanmartin et al. (2015)	Susceptible species
Perkinsus marinus						
Crassostrea brasiliana (mangrove oyster) (identified as *Crassostrea gasar* in the publication; West Africa mangrove oyster)	No	RTFM Culture His PCR Seq	66–90%	Pathogen ID clear, host species most likely *C. brasiliana*	Cunha et al. (2019)	Susceptible species
		His RTFM Culture PCR-RFLP Seq	90–100%	Occurrence of the African species, *C. gasar,* in Brazil has not been confirmed. Most likely, it has been misidentified and is *C. brasiliana* (Armal & Simone 2014). At least two assays were used with positive results for the same species	Queiroga et al. (2015)	Susceptible species
		RTFM Culture His PCR Seq InSiHy	90–100%	Occurrence of the African species, *C. gasar,* in Brazil has not been confirmed. Most likely, it has been misidentified and is *C. brasiliana* (Armal & Simone 2014). At least two assays were used with positive results for the same species	Da Silva et al. (2014)	Susceptible species
		RTFM Culture PCR-RFLP	90–100%	Occurrence of the African species, *C. gasar,* in Brazil has not been confirmed. Most likely,	Scardua et al. (2017)	Susceptible species

Reservoir species X	Presence in EU*	Pathogen detection method in species X with positive results	Certainty of classification as reservoir species	Reasoning for categorisation as reservoir species	Bibliography	Suggested classification in previous EURL report (2022)
		Seq		it has been misidentified and is *C. brasiliana* (Armal & Simone 2014). At least two assays were used with positive results for the same species		
Crassostrea sp., most likely *C. brasiliana* (mangrove oyster)	*C. brasiliana* does not occur in the EU	PCR	66–90%	Results on pathogen ID clear. The oyster species is stated as *Crassostrea* sp., most likely *Crassostrea brasiliana*	Leibowitz et al. (2019)	Susceptible species
		Seq				
			66–90%			
		PCR				
		Seq				
		His	66–90%	Slight difference between observed sequence and *P. marinus* sequence in Genbank, but closest relative	Caceres-Martinez et al. (2016)	Susceptible species
		PCR	90–100%	At least two reference tests with positive results for same species	Escobedo-Fregoso et al. (2015)	Susceptible species
		PCR-RFLP				
		Seq				
		RTFM Culture	90–100%	At least two assays were used with positive results for the same species	Escobedo-Fregoso et al. (2017)	Susceptible species
		PCR				
		Seq				
		RTFM Culture	66–90%	Doubtful path ID, specificity of primers not clear from manuscript	Villanueva-Fonseca et al. (2020)	Susceptible species
		PCR				
Crassostrea rhizophorae (Pacific cupped oyster)	No	Seq	66–90%	PCR and sequencing only but convincing data set	Lohan et al. (2016)	Susceptible species
		RTFM Culture	90–100%	At least two assays were used with positive results for the same species	Da Silva et al. (2013)	Susceptible species
		PCR				
		PCR-RFLP				
		Seq				
		RTFM Culture	90–100%	At least two assays were used with positive results for the same species	Scardua et al. (2017)	Susceptible species
		PCR-RFLP				
		Seq				

Reservoir species X	Presence in EU*	Pathogen detection method in species X with positive results	Certainty of classification as reservoir species	Reasoning for categorisation as reservoir species	Bibliography	Suggested classification in previous EURL report (2022)
Crassostrea sp., most likely *C. brasiliana* (Mangrove oyster)	*C. brasiliana* does not occur in the EU	PCR Seq	66–90%	Results on pathogen ID clear. The oyster species is stated as *Crassostrea* sp., most likely *Crassostrea brasiliana*	Leibowitz et al. (2019)	Susceptible species
Mercenaria mercenaria (American Hard-Shelled Clam)	Yes	PCR Seq		Species specific PCR used In addition to presence of other *Perkinsus* species, 14 of the obtained sequences were highly similar or identical to the sequence of *P. marinus*	Pecher et al. (2008)	Vector/Reservoir
Mya arenaria (Large-Neck Clam)	Yes	PCR InSiHy RTFM Culture	90–100%	At least two assays were used with positive results for the same species	Reece et al. (2008)	Vector/Reservoir
Modiolus capax (fat horsemussel)	No	RTFM Culture PCR	90–100%	At least two assays were used with positive results for the same species	Góngora-Gómez et al. (2021)	Not assessed
Saccostrea palmula (palmate oyster)	Yes	RTFM Culture PCR Seq InSiHy	90–100%	At least two assays were used with positive results for the same species	Cáceres-Martínez et al. (2012)	Susceptible species

*: Source: Gbif.org. PCR, polymerase chain reaction; His, histology; RFTM, Ray's fluid thioglycollate medium; RFLP, restriction fragment length polymorphism; Seq, sequencing; InSiHy, *in situ* hybridisation; EURL, EU Reference Laboratory; ITS, internal transcribed spacer.

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. Also, all the references provided in the EURL were scrutinised in the extensive literature review. It is therefore suggested to remove them 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.

3.1.1. Term of Reference 2: Conditions under which mollusc species shall be regarded as vectors or reservoirs of diseases of mollusc 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, 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, 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:

- Infection with *M. mackini*

  From publications on susceptible host species M*. gigas* (name in legislation *Crassostrea gigas*) and *O. edulis* as well as the identified vector species *C. virginica* infection with *M. mackini* appears to be restricted to colder water temperatures. *M. mackini* does not develop disease in oysters held at 17°C (Bower et al., 1997). However, the parasite can survive at warmer temperatures for prolonged periods as exposed *M. gigas* incubated at 17°C develop disease on returned to 10°C (Bower et al., 1997; Hervio et al., 1996).
  The disease appears more severe in oysters older than 2 years and after a period of 3–4 months at temperatures less than 10°C (Hervio et al., 1996). However, juvenile of *C. rhizophorae* are also susceptible to infection and resulting disease (Bower et al., 2005).
  Considering the available evidence, it is very likely to almost certain (90–100%) that *M. mackini* will remain infective at any possible transport condition.

- Infection with *P. marinus*

  Intensity and prevalence of infection with *P. marinus* appear correlated with salinity. Although the parasite can persist at salinity below 9 psu, prevalence and intensity of *P. marinus* infections are greatest at salinities greater than 12 psu (Mackin, 1955). Temperature also influences infection with *P. marinus*, with maximum prevalence and intensity observed 1–2 months after maximum summer water temperatures (Burreson & Ragone Calvo, 1996). However, this impact might depend on parasite strains and oyster susceptibility.
  Under experimental conditions, infection with *P. marinus* was established at 22°C in susceptible species *C. virginica* and identified reservoir species *M. arenaria* and *M. balthica* (Dungan et al., 2007). In *M. ariakensis* this could be established at 20–22°C and 25 psu (Moss et al., 2006). Escobedo-Fregoso and co-authors (2017) recorded an increase in infection intensity in *C. corteziensis* when temperatures were experimentally increased to 26°C. Considering the available evidence, it is very likely to almost certain (90–100%) that *P. marinus* will remain infective at any possible transport condition.

- Infection with *B. exitiosa*

  Considering the broad global distribution of *B. exitiosa* a wide tolerance to environmental conditions could be expected. However, actual data on the physiological parameters are lacking from most scientific papers. In the identified reservoir species *O. stentina*, *B. exitiosa* was present in Tunisia within the temperature range of 17.1–30.0°C and a salinity of 36.2 psu to 37.8 psu (Elgharsalli et al., 2016).
  Available data suggest that stressing oysters using warm water (25–26°C) or hypersaline (39–40 psu) water favour *B. exitiosa* development (Hine et al., 2002). Considering the available evidence, it is very likely to almost certain (90–100%) that *B. exitiosa* will remain infective at any possible transport condition.

- Infection with *B. ostreae*

  Although infection with *B. ostreae* occurs throughout the year, prevalence generally peaks in late winter early spring when temperature is low. *In vitro* low temperatures (between 4°C and 10°C) and high salinity (above 35 psu) favour *B. ostreae* survival. In the field, lower summer temperatures and higher summer salinities are associated with higher prevalence the following winter (Arzul et al., 2006). Considering the available evidence, it is very likely to almost certain (90–100%) that *B. ostreae* will remain infective at any possible transport condition.

- Infection with *M. refringens**

  Transmission of the parasite is not direct and needs an intermediate host which is probably a copepod, *Paracartia grani* or *P. latisetosa* (Arzul et al., 2014). The transmission occurs when the water temperature is above 17°C (Grizel, 1987). Warm water temperature is not only correlated with higher prevalence but also higher infection intensity levels (Audemard et al., 2001). Although salinity and hydrodynamics have less impact on the infection, low salinity and low water renewal seem to be favourable for the parasite (Audemard et al., 2001). Considering the available evidence, it is very likely to almost certain (90–100%) that *M. refringens* will remain infective at any possible transport condition.

Table 4 summarises the conditions under which mollusc 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 mollusc 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
Infection with *Mikrocytos mackini, Perkinsus marinus, Bonamia exitiosa, Bonamia ostreae and Marteilia refringens*.	Vector or reservoir species that were exposed to *Mikrocytos mackini, Perkinsus marinus, Bonamia exitiosa, Bonamia ostreae* and *Marteilia refringens* in an affected area can possibly transmit any of these pathogens 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 for *Mikrocytos mackini, Perkinsus marinus, Bonamia exitiosa, Bonamia ostreae; or* the intermediate host for *Marteilia refringens*. b) the **wild**, where they may have been exposed to susceptible, reservoir or other vector species for *Mikrocytos mackini, Perkinsus marinus, Bonamia exitiosa, Bonamia ostreae*; or to the intermediate host for *Marteilia refringens*. c) an **aquaculture establishment supplied with water** possibly contaminated with *Mikrocytos mackini, Perkinsus marinus, Bonamia ostreae* and/or *Bonamia exitiosa or with the intermediate hosts infected with Marteilia refringens*.

4. Conclusions

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

Bonamia exitiosa

- **Vectors**
  - No evidence was found to identify any vectors for *B. exitiosa*.
- **Reservoirs**
  - *O. stentina* (dwarf oyster) is considered to be a reservoir species for *B. exitiosa* with more than 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: *Crassostrea angulate, Crassostrea* and *C. virginica*

Bonamia ostreae

- **Vectors**
  - No evidence was found to identify any vectors for *B. ostreae*.
- **Reservoirs**
  - *Ostrea angasi* (angasi oyster) is considered to be a reservoir species for *B. ostreae* 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: *Cerastoderma edule, Donax trunculus, Mya arenaria, M. mercenaria, Meretrix lusoria, Pecten maximus, Ruditapes decussatus, Ruditapes philippinarum, Polititapes aureus (Venerupis aurea), Venerupis corrugata (Venerupis pullastra), Venus verrucosa*.

Marteilia refringens

- **Vectors**
  - No evidence was found to identify any vectors for *M. refringens*.
- **Reservoirs**
  - *Mytilus galloprovincialis* (Galician mussel), *Chamellea gallina* (chicken venus), *O. stentina* (dwarf oyster) are considered to be reservoir species for *M. refringens* with more than 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: *Cerastoderma edule, Donax trunculus, Mya arenaria, M. mercenaria, Meretrix lusoria, Ruditapes decussatus, Ruditapes philippinarum, Polititapes aureus (Venerupis aurea), Venerupis corrugata (Venerupis pullastra)* and *Venus verrucosa*.

Mikrocytos mackini

- **Vectors**
  - *C. virginica* (American cupped OYSTER) is considered to be a vector species for *M. mackini* with more than 90% certainty.
- **Reservoirs**
  - No evidence was found to identify any reservoirs for *M. mackini*.

Perkinsus marinus

- **Vectors**
  - *B. impressa* (impressed odostome) is considered to be a vector species for *P. marinus* with more than 90% certainty.
- **Reservoirs**
  - *Macoma balthica* (Baltic macoma), *Modiolus capax* (fat horsemussel), *Mya arenaria* (large-neck clam), *M. ariakensis* (Suminoe oyster), *Crassostrea brasiliana* (mangrove oyster), *C. corteziensis* (cortez oyster), *Crassostrea rhizophorae* (Pacific cupped oyster) and *Saccostrea palmula* (palmate oyster) are considered to be reservoir species *for P. marinus* with more than 90% certainty.
  - *M. mercenaria* (American hard-shelled clam) and *Crassostrea* sp. are considered to be reservoir species *for P. marinus* 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: *Brachyura* spp., *Cherax destructor, Homarus gammarus, Macrobrachium rosenbergii, Palinurus* spp., *Penaeus indicus, Penaeus japonicus, Penaeus kerathurus, Penaeus stylirostris, Penaeus vannamei, Portunus puber* and *Scylla serrata*. In addition, they are all crustacean species and therefore they have not been part of the scope of this assessment.
- The assessment was exclusively based on peer reviewed evidence and should be updated when new evidence becomes available.

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

- Conditions that may be considered to amend Annex I of Reg 2020/990 and Annex XXX of Reg 2020/692 are:
- It is very likely to almost certain (90–100%) that *M. mackini, P. marinus, B. exitiosa, B. ostreae* or *M. refringens* will remain infective at any possible transport condition.
- Vector or reservoir species that were exposed to *M. mackini, P. marinus, B. exitiosa, B. ostreae* and *M. refringens* in an affected area can possibly transmit any of these pathogens 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 for *M. mackini, P. marinus, B. exitiosa, B. ostreae;* or the intermediate host for *M. refringens*.
  b) the **wild**, where they may have been exposed to susceptible, reservoir or other vector species for *M. mackini, P. marinus, B. exitiosa, B. ostreae*; or to the intermediate host for *M. refringens*.
  c) an **aquaculture establishment supplied with water** possibly contaminated with *M. mackini, P. marinus, B. ostreae* or *B. exitiosa* or with the intermediate hosts infected with *M. refringens*.

References

Abollo E, Casas SM, Ceschia G and Villalba A, 2006. Differential diagnosis of Perkinsus species by polymerase chain reaction-restriction fragment length polymorphism assay. Molecular and Cellular Probes, 20, 323–329.

Abdel-Baki AAS, Al-Quraishy S, Dkhil MA, Oliveira E, Casal G and Azevedo C, 2014. Perkinsus sp. (Alveolata, Perkinsidae) a parasite of the clam Meretrix meretrix (Veneridae) from Arabian Gulf: Ultrastructural observations of the trophozoites and the cellular response of the host. Acta Protozoologica, 53, 215–221.

Arzul I, Miossec L, Blanchet E, Garcia C, François C and Joly J-P, 2006. Bonamia ostreae and Ostrea edulis: a stable host-parasite system in France? ISVEE Conference XI, Cairns, Australia, 6–11 August 2006, Symposia Proceedings, 5 p.

Arzul I, Chollet B, Boyer S, Bonnet D, Gaillard J, Baldi Y, Robert M, Joly J, Garcia C and Bouchoucha M, 2014. Contribution to the understanding of the cycle of the protozoan parasite Marteilia refringens. Parasitology, 141, 227–240.

Audemard C, Barnaud A, Collins CM, Le Roux F, Sauriau P-G, Coustau C, Blachier P and Berthe FCJ, 2001. Claire ponds as an experimental model for Marteilia refringens life-cycle studies: new perspectives. Journal of Experimental Marine Biology and Ecology, 257, 87–108.

Audemard C, Le Roux F, Barnaud A, Collins C, Sautour B, Sauriau PG, de Montaudouin X, Coustau C, Combes C and Berthe F, 2002. Needle in a haystack: involvement of the copepod Paracartia grani, in the life-cycle of the oyster pathogen Marteilia refringens. Parasitology, 124, 315–323.

Balseiro P, Montes A, Ceschia G, Gestal C, Novoa B and Figueras A, 2007. Molecular epizootiology of the European Marteilia spp., infecting mussels (Mytilus galloprovincialis and M. edulis) and oysters (Ostrea edulis): an update. Bulletin of the European Association of Fish Pathologists, 27, 148–156.

Bougrier S, Tigé G, Bachère E and Grizel H, 1986. Ostrea angasi acclimatization to French coasts. Aquaculture, 58, 151–154.

Bower S, Hervio MD and Meyer GR, 1997. Infectivity of Mikrocytos mackini, the causative agent of Denman Island disease in Pacific oysters Crassostrea gigas, to various species of oysters. Diseases of Aquatic Organism, 29, 111–116.

Bower SM, Bate K and Meyer GR, 2005. Susceptibility of juvenile Crassostrea gigas and resistance of Panope abrupta to Mikrocytos mackini. Journal of Invertebrate Pathology, 88, 95–99.

Boyer S, Chollet B, Bonnet D and Arzul I, 2013. New evidence for the involvement of Paracartia grani (Copepoda, Calanoida) in the life cycle of Marteilia refringens (Paramyxea). International Journal for Parasitology, 43, 1089–1099.

Burreson EM and Ragone Calvo LM, 1996. Epizootiology of Perkinsus marinus disease of oysters in Chesapeake Bay, with emphasis on data since 1985. Journal of Shellfish Research, 15, 17–34.

Cáceres-Martínez J, Ortega MG, Vásquez-Yeomans R, García TDJP, Stokes NA and Carnegie RB, 2012. Natural and cultured populations of the mangrove oyster Saccostrea palmula from Sinaloa, Mexico, infected by *Perkinsus marinus*. Journal of Invertebrate Pathology, 110, 321–325.

Caceres-Martinez J, Madero-Lopez LH, Padilla-Lardizabal G and Vasquez-Yeomans R, 2016. Epizootiology of Perkinsus marinus, parasite of the pleasure oyster Crassostrea corteziensis, in the Pacific coast of Mexico. Journal of Invertebrate Pathology, 139, 12–18.

Cahour A, 1979. Marteilia refringens and Crassostrea gigas. Marine Fishers Review, 41, 19–20.

Calvo GW, Luckenbach MW, Allen SK and Jr B, 2001. A comparative field study of Crassostrea ariakensis (Fujita 1913) and Crassostrea virginica (Gmelin 1791) in relation to salinity in Virginia. Journal of Shellfish Research, 20, 221–230.

Carrasco N, Lopez-Flores I, Alcaraz M, Furones MD, Berthe FCJ and Arzul I, 2007. Dynamics of the parasite Marteilia refringens (Paramyxea) in Mytilus galloprovincialis and zooplankton populations in Alfacs Bay (Catalonia, Spain). Parasitology, 134, 1541–1550.

Carrasco N, Arzul I, Berthe FCJ and Furones MD, 2008. In situ hybridization detection of initial infective stages of Marteilia refringens (Paramyxea) in its host Mytilus galloprovincialis. Journal of Fish Diseases, 31, 153–157.

Carrasco N, Arzul I, Chollet B, Robert M, Joly JP, Furones MD and Berthe FCJ, 2008. Comparative experimental infection of the copepod Paracartia grani with Marteilia refringens and Marteilia maurini. Journal of Fish Diseases, 31, 497–504.

Casas SM, Villalba A and Reece KS, 2002. Study of perkinsosis in the carpet shell clam Tapes decussatus in Galicia (NW Spain). I. Identification of the aetiological agent and in vitro modulation of zoosporulation by temperature and salinity. Diseases of Aquatic Organism, 50, 1–65.

Culloty SC, Novoa B, Pernas M, Longshaw M, Mulcahy MF, Feist SW and Figueras A, 1999. Susceptibility of a number of bivalve species to the protozoan parasite Bonamia ostreae and their ability to act as vectors for this parasite. Diseases of Aquatic Organisms, 37, 73–80.

Cunha ACL, Pontinha VD, MAM C, Suhnel S, Medeiros SC, AMM L, Harakava R, Tachibana L, Mello DF, Danielli NM, Dafre AL, ARM M and JLP M, 2019. Two epizootic Perkinsus spp. events in commercial oyster farms at Santa Catarina. Brazil. Journal of Fish Diseases, 42, 455–463.

Da Ros L and Canzonier WJ, 1985. Perkinsus, a protistan threat to bivalve culture in the Mediterranean basin. Bulletin of the European Association of Fish Pathologists, 5, 23–27.

Da Silva PM, Scardua MP, Vianna RT, Mendonca RC, Vieira CB, Dungan CF, Scott GP and Reece KS, 2014. Two Perkinsus spp. infect Crassostrea gasar oysters from cultured and wild populations of the Rio Sao Francisco estuary, Sergipe, northeastern Brazil. Journal of Invertebrate Pathology, 119, 62–71.

Dungan CF, Reece KS, Hamilton RM, Stokes NA and Burreson EM, 2007. Experimental cross-infections by Perkinsus marinus and P. chesapeaki in three sympatric species of Chesapeake Bay oysters and clams. Diseases of Aquatic Organism, 76, 67–75.

Dungan CF, Carnegie RB, Hill KM, McCollough CB, Laramore SE, Kelly CJ, Stokes NA and Scarpa J, 2012. Diseases of oysters Crassostrea ariakensis and C. virginica reared in ambient waters from the Choptank River, Maryland and the Indian River Lagoon, Florida. Diseases of Aquatic Organisms, 101, 173–183.

Elgharsalli R, Aloui-Bejaoui N, Salah H, Chollet B, Joly JP, Robert M, Couraleau Y and Arzul I, 2013. Characterization of the protozoan parasite Marteilia refringens infecting the dwarf oyster Ostrea stentina in Tunisia. Journal of Invertebrate Pathology, 112, 175–183.

Elgharsalli R, Seguineau C, Arzul I, Aloui-Bejaoui N, Quere C and Moal J, 2016. Effect of infection by the protistan parasite Marteilia refringens on the enzyme activity and energy reserves of oyster Ostrea stentina (Payraudeau, 1826) in Tunisia. Journal of Marine Biology Association UK, 98, 161–170.

Elgharsalli R, Seguineau C, Arzul I, Aloui-Bejaoui N, Quere C and Moal J, 2018. Effect of infection by the protistan parasite Marteilia refringens on the enzyme activity and energy reserves of oyster Ostrea stentina (Payraudeau, 1826) in Tunisia. Journal of the Marine Biological Association of the United Kingdom, 98, 161–170.

Engelsma M, Cheslett D, Roque A and Furones D, 2023. Extensive literature review on vectors and reservoirs of AHL-listed pathogens of molluscs. EFSA supporting publication 2023;EN-8124, 47 pp. https://doi.org/10.2903/sp.efsa.2023.EN-8124

Enríquez-Espinoza TL, Castro-Longoria R, Mendoza-Cano F and Grijalva-Chon JM, 2015. Perkinsus marinus in Crassostrea gigas and Chione fluctifraga from Kino bay, Sonora, Mexico. Biotecnia, 17, 10–13.

Escobedo-Fregoso C, Arzul I, Carrasco N, Gutierrez-Rivera JN, Llera-Herrera R and Vazquez-Juarez R, 2015. Polymorphism at the ITS and NTS loci of Perkinsus marinus isolated from cultivated oyster Crassostrea corteziensis in Nayarit, Mexico and phylogentic relationship to P. marinus along the Atlantic Coast. Transboundary and Emerging Diseases, 62, 137–147.

Escobedo-Fregoso C, Ramirez-Salcedo J and Vázquez-Juárez R, 2017. Host response when Perkinsus marinus infection intensities increase in the Oyster Crassostrea corteziensis. Journal of Shellfish Research, 36, 717–727.

le Roux F, Lorenzo G, Peyret P, Audemard C, Figueras A, Vivares C, Gouy M and Berthe F, 2001. Molecular evidence for the existence of two species of Marteilia in Europe. Journal of Eukaryotic Microbiology, 48, 449–454.

Farley CA, Wolff PH and Elston RA, 1988. A longterm study of "microcell" disease in oysters with a description of a new genus, Mikrocytos (g.n.), and two new species, Mikrocytos mackini (sp.n.) and Mikrocytos roughleyi (sp.n.). Fish Bull, 86, 581–593.

Fuentes J, Villalba A, Zapata C and Alvarez G, 1995. Effects of stock and culture environment on infections by Marteilia refringens and Mytilicola intestinalis in the mussel Mytilus galloprovincialis cultured in Galicia (NW Spain). Diseases of Aquatic Organisms, 21, 221–226.

Góngora-Gómez AM, Rubio-Zepeda F, Villanueva-Fonseca LC, Alvarez-Dagnino E, Muñoz-Sevilla NP, Hernández-Sepúlveda JA and García-Ulloa M, 2016. Primer registro de Perkinsus sp. (Protozoa, Apicomplexa) en el callo de hacha Atrina maura en Sinaloa, México. Revista de biología marina y oceanografía, 51, 689–694.

Góngora-Gómez AM, López-Meyer M, Villanueva-Fonseca LC, Navarro-Chávez MF, Acosta-Campos MJ, Hernández-Sepúlveda JA and García-Ulloa M, 2021. Detection of Perkinsus marinus in a wild population of the horsemussel Modiolus capax from the southwestern Gulf of California. Hidrobiologica, 31, 253–261.

Grijalva-Chon JM, Castro-Longoria R, Enriquez-Espinoza TL, Maeda-Martinez AN and Mendoza-Cano F, 2015. Molecular evidence of the protozoan parasite Marteilia refringens in Crassostrea gigas and Crassostrea corteziensis from the Gulf of California. Latin American Journal of Aquatic Research, 43, 776–780.

Grizel H, 1987. Les maladies des mollusques: étiologie et progrès récents des recherches. Oceanis, 13, 357–370.

Gutierrez-Rivera JN, Arcos-Ortega GF, Luna-Gonzalez A, Rodriguez-Jaramillo MC, Arechiga-Carvajal ET and Vazquez-Juarez R, 2015. Differential expression of serine protease inhibitors 1 and 2 in Crassostrea corteziensis and C. virginica infected with Perkinsus marinus. Diseases of Aquatic Organisms, 112, 185–197.

Hervio D, Bower SM and Meyer GR, 1996. Detection, isolation and experimental transmission of Mikrocytos mackini, a microcell parasite of Pacific oysters Crassostrea gigas (Thunberg). Journal of Invertebrate Pathology, 67, 72–79.

Hill KM, Carnegie RB, Aloui-Bejaoui N, El Gharsalli R, White DM, Stokes NA and Burreson EM, 2010. Observation of a Bonamia sp. infecting the oyster Ostrea stentina in Tunisia, and a consideration of its phylogenetic affinities. Journal of Invertebrate Pathology, 103, 179–185.

Hine PM, Diggles BK, Parsons MJD, Pringle A and Bull B, 2002. The effects of stressors on the dynamics of Bonamia exitiosus Hine, Cochennec-Laureau & Berthe, infections in flat oysters Ostrea chilensis (Philippi). Journal of Fish Diseases, 25, 545–554.

Kendall LR, Ewart JW, Ulrich PN and Marsh AG, 2007. Low incidence and limited effect of the oyster pathogen dermo (Perkinsus marinus) on an artificial reef in Delaware's Inland Bays. Estuaries and Coasts, 30, 154–162.

Kingsley-Smith PR, Harwell HD, Kellogg ML, Allen SM, Allen SK, Meritt D, Paynter K and Luckenbach MW, 2009. Survival and growth of triploid Crassostrea virginica (Gmelin, 1791) and C. ariakensis (Fujita, 1913) in bottom environments of Chesapeake Bay: implications for an introduction. Journal of Shellfish Research, 28, 169–184.

Laramore SE, Krebs W, Lave AL and Gallagher K, 2017. Survey of bivalve molluscs for Bonamia spp. and other parasitic pathogens in Florida east coast lagoons. Journal of Shellfish Research, 36, 379–390.

Lattos A, Papadopoulos DK, Feidantsis K, Karagiannis D, Giantsis IA and Michaelidis B, 2022. Are marine heatwaves responsible for mortalities of farmed Mytilus galloprovincialis? A pathophysiological analysis of Marteilia infected mussels from thermaikos gulf, Greece. Animals, 12.

Leibowitz MP, Pereira FL, Leal CAG, Cunha EAP, Azevedo VAC and Figueiredo HCP, 2019. Molecular detection of the pathogenic protist Perkinsus marinus in farmed native and introduced oysters (Crassostrea spp.) in southern Brazil. Genetics and Molecular Research, 18, unpaginated.

Lenaers G, Maroteaux L, Michot B and Herzog M, 1989. Dinoflagellates in evolution. A molecular phylogenetic analysis of large subunit ribosomal RNA. Journal of Molecular Ecology, 29, 40–51.

Lohan KMP, Hill-Spanik KM, Torchin ME, Aguirre-Macedo L, Fleischer RC and Ruiz GM, 2016. Richness and distribution of tropical oyster parasites in two oceans. Parasitology, 143, 1119–1132.
Lombardi SA, Harlan NP and Paynter KT, 2013. Survival, acid-base balance, and gaping responses of the Asian oysler Crassostrea ariakensis and the eastern oyster Crassostrea virginica during clamped emersion and hypoxic immersion. Journal of Shellfish Research, 32, 409–415.
Lopez-Flores I, de la Herran R, Garrido-Ramos MA, Navas JI, Ruiz-Rejon C and Ruiz Rejon M, 2004. The molecular diagnosis of Marteilia refringens and differentiation between Marteilia strains infecting oysters and mussels based on the rDNA IGS sequence. Parasitology, 12, 411–419.
Lopez-Flores I, Garrido-Ramos MA, e la Herran R, Ruiz-Rejon C, Ruiz-Rejon M and Navas JI, 2008a. Identification of Marteilia refringens infecting the razor clam Solen marginatus by PCR and in situ hybridization. Molecular and Cellular Probes, 22, 151–155.
Lopez-Flores I, Robles F, Valencia JM, Grau A, Villalba A, Rdl H, Garrido-Ramos MA, Ruiz-Rejon C, Ruiz-Rejon M and Navas JI, 2008b. Detection of Marteilia refringens using nested PCR and in situ hybridisation in Chamelea gallina from the Balearic Islands (Spain). Diseases of Aquatic Organisms, 82, 79–87.
Lopez-Sanmartin M, Batista FM, Marin MC, Garrido I, Quintero D, Grade A, Ruano F, Rdl H and Navas JI, 2015. Detection of Marteilia refringens infecting the European flat oyster Ostrea edulis and the dwarf oyster Ostrea stentina in southern Portugal and Spain. Journal of Invertebrate Pathology, 130, 52–55.
Lynch SA, Armitage DV, Coughlan J, Mulcahy MF and Culloty SC, 2007. Investigating the possible role of benthic macroinvertebrates and zooplankton in the life cycle of the haplosporidian Bonamia ostreae. Experimental Parasitology, 115, 359–368.
Lynch SA, Abollo E, Ramilo A, Cao A, Culloty SC and Villalba A, 2010. Observations raise the question if the Pacific oyster, Crassostrea gigas, can act as either a carrier or a reservoir for Bonamia ostreae or Bonamia exitiosa. Parasitology, 137, 1515–1526.
Mackin JG, 1955. Dermocystidium marinum and salinity. Proceedings of the National Shellfisheries Association, 46, 116–128.
Marsh AG, Gauthier JD and Vasta GR, 1995. A semiquantitative PCR assay for assessing Perkinsus marinus infections in the eastern oyster, Crassostrea virginica. Journal of Parasitology, 81, 577–583.
McLean RI and Abbe GR, 2008. Characteristics of Crassostrea ariakensis (Fujita 1913) and Crassostrea virginica (Gmelin 1791) in the discharge area of a nuclear power plant in Central Chesapeake Bay. Journal of Shellfish Research, 27, 517–524.
Meyer GR, Bower SM, Lowe G and Davies S, 2008. Resistance of the Manila clam (Venerupis philippinarum) to infection with Mikrocytos mackini. Journal of Invertebrate Pathology, 98, 54–57.
Moss JA, Burreson EM and Reece KS, 2006. Advanced Perkinsus marinus infections in Crassostrea Ariakensis maintained under laboratory conditions. Journal of Shellfish Research, 25, 65–72.
Navarro E, Iglesias JIP, Camacho AP and Labarta U, 1996. The effect of diets of phytoplankton and suspended bottom material on feeding and absorption of raft mussels (Mytilus galloprovincialis Lmk). Journal of Experimental Marine Biology and Ecology, 198, 175–189.
Novoa B, Posada D and Figueras A, 2005. Polymorphisms in the sequences of Marteilia internal transcribed spacer region of the ribosomal RNA genes (ITS-1) in Spain: genetic types are not related with bivalve hosts. Journal of Fish Diseases, 28, 331–338.
Oidtmann B, Dixon P, Way K, Joiner C and Bayley AE, 2017. Risk of waterborne virus spread – review of survival of relevant fish and crustacean viruses in the aquatic environment and implications for control measures. Reviews in aquaculture, 10, 641–669.
Pascual M, Martin A G, Zampatti E, Coatanea D, Defossez J and Robert R, 1991. Testing of the Argentina oyster, Ostrea puelchana, in several French oyster farming sites. ICES CM, 1991/K:30, No pages found
Park KI, Park JK, Lee J and Choi KS, 2005. Use of molecular markers for species identification of Korean Perkinsus sp. isolated from Manila clams Ruditapes philippinarum. Diseases of Aquatic Organism, 66, 255–263.
Park KI, Ngo TTT, Choi SD, Cho M and Choi KS, 2006. Occurrence of Perkinsus olseni in the Venus clam Protothaca jedoensis in Korean waters. Journal of Invertebrates Pathology, 93, 81–87.
Paynter KT, Goodwin JD, Chen ME, Ward NJ, Sherman MW, Meritt DW and Allen SK, 2008. Crassostrea ariakensis in Chesapeake Bay: growth, disease and mortality in shallow subtidal environments. Journal of Shellfish Research, 27, 509–516.
Pecher WT, Alavi MR, Schott EJ, Fernandez-Robledo JA, Roth L, Berg ST and Vasta GR, 2008. Assessment of the northern distribution range of selected Perkinsus species in eastern oysters (Crassostrea virginica) and hard clams (Mercenaria mercenaria) with the use of PCR-based detection assays. Journal of Parasitology, 94, 410–422.
Pinto TR, Boehs G, Pessoa WFB, Luz MSA and Costa H, 2016. Detection of Perkinsus marinus in the oyster Crassostrea rhizophorae in southern Bahia by proteomic analysis. Brazilian Journal of Veterinary Research and Animal Science, 53, 1.
Queiroga FR, Vianna RT, Vieira CB, Farias ND and Silva PM, 2015. Parasites infecting the cultured oyster Crassostrea gasar (Adanson, 1757) in Northeast Brazil. Parasitology, 142, 756–766.

Reece KS, Dungan CF and Burreson EM, 2008. Molecular epizootiology of Perkinsus marinus and P. chesapeaki infections among wild oysters and clams in Chesapeake Bay, USA. Diseases of Aquatic Organisms, 82, 237–248.

Robledo JAF, Wright AC, Marsh AG and Vasta GR, 1999. Nucleotide sequence variability in the nontranscribed spacer of the rRNA locus in the oyster parasite Perkinsus marinus. Journal of Parasitology, 85, 650–656.

Scardua MP, Vianna RT, Duarte SS, Farias ND, Correia MLD, Santos HTA and Silva PM, 2017. Growth, mortality and susceptibility of oyster Crassostrea spp. to Perkinsus spp. infection during on growing in northeast Brazil. Revista Brasileira De Parasitologia Veterinaria, 26, 401–410.

Serracca L, Prearo M, Rossini I, Battistini R, Corsi M and Ercolini C, 2014. Four-Year Surveillance of Marteilia refringens in Shellfish Farms in the Gulf of La Spezia (Liguria, Italy). Turkish Journal of Fisheries and Aquatic Sciences, 14, 893–896.

Silva PM, Vianna RT, Guertler C, Ferreira LP, Santana LN, Fernandez-Boo S, Ramilo A, Cao A and Villalba A, 2013. First report of the protozoan parasite Perkinsus marinus in South America, infecting mangrove oysters Crassostrea rhizophorae from the Paraiba River (NE, Brazil). Journal of Invertebrate Pathology, 113, 96–103.

Spiers ZB, Gabor M, Fell SA, Carnegie RB, Dove M, O'Connor W, Frances J, Go J, Marsh IB and Jenkins C, 2014. Longitudinal study of winter mortality disease in Sydney rock oysters Saccostrea glomerata. Diseases of Aquatic Organisms, 110, 151–164.

Vazquez N, Aranguren R, Dungan CF and Cremonte F, 2018. Parasites in two coexisting bivalves of the Patagonia coast, southwestern Atlantic Ocean: The Puelche oyster (Ostrea puelchana) and false oyster (Pododesmus rudis). Journal of Invertebrate Pathology, 158, 6–15.

Villanueva-Fonseca LC, Garcia-Ulloa M, Lopez-Meyer M, Villanueva-Fonseca BP, Hernandez-Sepulveda JA, Munoz-Sevilla NP and Gongora-Gomez AM, 2020. Perkinsus marinus in the pleasure oyster Crassostrea corteziensis cultivated on the southeast coast of the gulf of California, Mexico. Latin American Journal of Aquatic Research, 48, 529–537.

White ME, Powell EN, Ray SM and Wilson EA, 1987. Host-to-host transmission of Perkinsus marinus in oyster (Crassostrea virginica) populations by the ectoparasitic snail Boonea impressa (Pyramidellidae).

WOAH, 2022. Aquatic Animal Health Code. Chapter 4.7 Fallowing in aquaculture. Available online: https://www.woah.org/fileadmin/Home/eng/Health_standards/aahc/current/chapitre_fallowing_aquaculture.pdf

Abbreviations

AHL	Animal Health Law
ELR	extensive literature review
EURL	EU Reference Laboratory
His	histology
ISH	*in situ* hybridisation
ITS	internal transcribed spacer
PCR	polymerase chain reaction
RFLP	restriction fragment length polymorphism RFTM Ray's fluid thioglycollate medium
Seq	sequencing
WOAH	World Organisation for Animal Health
ToR	Term of Reference

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

Table A.1: Currently listed Vectors in Commission Implementing Regulation 1882/2018 for which no evidence, or insufficient evidence was generated by the extensive literature review to be categorised as vector or reservoir species

Mikrocytos mackini	***VECTOR SPECIES Scientific names***	***VECTOR SPECIES Common names***
	NA	**NA**
Perkinsus marinus	***VECTOR SPECIES Scientific names***	***VECTOR SPECIES Common names***
	Brachyura spp.	Marine crabs
	Cherax destructor	Yabi crayfish
	Homarus gammarus	European lobster
	Macrobrachium rosenbergii	Giant river prawn
	Palinurus spp.	Spiny lobster
	Penaeus indicus	Indian white prawn
	Penaeus japonicus	Kuruma prawn
	Penaeus kerathurus	Caramote prawn
	Penaeus stylirostris	Blue shrimp
	Penaeus vannamei	Whiteleg shrimp
	Portunus puber	Swimming crab
	Scylla serrata	Indopacific swamp crab
Bonamia exitiosa	***VECTOR SPECIES Scientific names***	***VECTOR SPECIES Common names***
	Crassostrea angulata	Portuguese oyster
	Crassostrea virginica	American Cupped oyster
	Magallana gigas (Crassotrea gigas in legislation)	Pacific giant oyster
Bonamia ostreae	***VECTOR SPECIES Scientific names***	***VECTOR SPECIES Common names***
	Cerastoderma edule	Common edible cockle
	Donax trunculus	Wedge shell
	Mercenaria mercenaria	Northern quahog
	Meretrix lusoria	Japanese hard clam
	Mya arenaria	Sand gaper
	Pecten maximus	Atlantic scallop
	Polititapes aureus (Venerupis aurea in legislation)	European aurora venus clam
	Ruditapes decussatus	Grooved carpet shell
	Ruditapes philippinarum	Japanese carpet shell
	Venerupis corrugata (Venerupis pullastra in legislation)	Pullet carpet shell
	Venus verrucosa	Warty venus
Infection with Marteilia refringens	***VECTOR SPECIES Scientific names***	***VECTOR SPECIES Common names***
	Cerastoderma edule	Common edible cockle
	Donax trunculus	Wedge shell
	Mercenaria mercenaria	Northern quahog
	Meretrix lusoria	Japanese hard clam
	Mya arenaria	Sand gaper
	Ruditapes decussatus	Grooved carpet shell

	Ruditapes philippinarum	Japanese carpet shell
	Polititapes aureus (Venerupis aurea in legislation)	European aurora venus clam
	Venerupis corrugata (Venerupis pullastra in legislation)	Pullet carpet shell
	Venus verrucosa	Warty venus

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 of literature, 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 excluded during the ELS and during the assessment due to lack of certainty

Scientific name	Reference	Certainty	Reasoning	Conclusion WG and AHAW Panel	Suggested classification by EURL (2022), not the outcome of this current assessment
Infection with *Mikrocytos mackini*					
Ostrea lurida	Farley (1988)	NA	Excluded because already susceptible species	Not classified	Vector/Reservoir
Ruditapes philippinarum	Meyer et al. (2008)	0–10%	The parasite is detected up to 48 h post challenge, this is however most likely the challenge material as stated by the authors	Not classified	Not assessed
Infection with *Perkinsus marinus*					
Atrina maura	Góngora-Gómez et al. (2016)	NA	Excluded in Distiller because pathogen not identified	Not classified	Doubtful results
Atrina rigida	Laramore et al. (2017)	33–66%	Species specific *Bonamia* primers used but authors state that positive signal could be of positive *C. virginica* in close proximity	Not classified	Vector/Reservoir
Brachidontes exustus	Laramore et al. (2017)	33–66%	Species specific *Bonamia* primers used but authors state that positive signal could be of positive *C. virginica* in close proximity	Not classified	Vector/Reservoir
Cerastodema edule	Da Ros et al. (1985)	33–66%	RFTM assay used for detection, typed only to genus level	Not classified	Not assessed
Chionista fluctifraga	Enriquez-Espinoza et al. (2015)	10–33%	Doubtful path ID, RTFM method itself not suitable for species identification, could not be confirmed by PCR	Not classified	Doubtful results
Magallana ariakensis	Calvo et al. (2001)	33–66%	Pathogen detection by RFTM assay, species not further identified	Reservoir (see Table 2: Moss et al. (2006)	Susceptible species
	Kingsley-Smith et al. (2009)	33–66%	Diagnose based on RFTM culture only		
	Dungan et al. (2012)	33–66%	Identified to genus *Perkinsus*		

	Lombardi et al. (2013)	33–66%	No specific data available on infection -- > '*C. virginica* had higher *P. marinus* scores than *Crassostrea ariakensis*,...'		
	McLean et al. (2008)	33–66%	10% prevalence based on detection in 2 oysters at the level of one cell per oyster		
	Paynter et al. (2008)	33–66%	Oysters deployed in *P. marinus* area but only RFTM assay used as detection method		
Crassostrea columbiensis	Lohan et al. (2016)	33–66%	ITS1 sequences PerkITS85/PerkITS750 (Casas et al. 2002). No confirmation of infection status for these hosts, documents occurrence and association with particular host species. Percentage and total number of detections: 40% (6)	Not classified	Not assessed
Crassostrea rhizophorae	Pinto et al. (2016)	33–66%	Proteins identified as belonging to *C. rhizophorae* and *Perkinsus marinus*. Pathogen identification method is non-standard test. Specificity of test and identification of pathogen not known.	Not classified	Not assessed
Geukensia demissa	Laramore et al. (2017)	33–66%	Species specific *Bonamia* primers used but authors state that positive signal could be of positive *C. virginica* in close proximity	Not classified	Vector/Reservoir
Ischadium recurvum	Laramore et al. (2017)	33–66%	Species specific *Bonamia* primers used but authors state that positive signal could be of positive *C. virginica* in close proximity	Not classified	Vector/Reservoir
Isognomon alatus	Laramore et al. (2017)	33–66%	Species specific *Bonamia* primers used but authors state that positive signal could be of positive *C. virginica* in close proximity	Not classified	Vector/Reservoir
Isognomon bicolor	Laramore et al. (2017)	33–66%	Species specific *Bonamia* primers used but authors state that positive signal could be of positive *C. virginica* in close proximity	Not classified	Vector/Reservoir
Mercenaria mercenaria	Kendall et al. (2007)	33–66%	Slight detection with *P. marinus* specific primers in PCR in the hard clams	Not classified	Not assessed
Meretrix meretrix	Abdel-Baki et al. (2014)	NA	Excluded because species for which pathogen ID doubtful	Not classified	Doubtful results
Ostrea stentina	Laramore et al. (2017)	33–66%	Species specific *Bonamia* primers used but authors state that positive signal could be of positive *C. virginica* in close proximity	Not classified	Vector/Reservoir
Pododesmus rudis	Vázquez et al. (2018)	NA	Species for which pathogen ID doubtful	Not classified	Doubtful results

Polititapes aureus	DaS Ros et al. (1985)	33–66%	RFTM assay used for detection, typed only to genus level	Not classified	Not assessed
Ruditapes decussatus	Da Ros et al. (1985)	33–66%	RFTM assay used for detection, typed only to genus level	Not classified	Not assessed
Striostrea prismatica	Lohan et al. (2016)	33–66%	ITS1 sequences PerkITS85/PerkITS750 (Casas et al. 2002). No confirmation of infection status for these hosts, documents occurrence and association with particular host species. percentage and total number of detections: 6.7% (1)	Not classified	Not assessed
Venus verrucosa	Da Ros et al. (1985)	33–66%	RFTM assay used for detection, typed only to genus level	Not classified	Not assessed
Infection with Bonamia exitiosa					
Crassostrea gigas	Lynch et al. (2010)	33–66%	Contradictory results within the same paper	Not classified	Vector/Reservoir
Crassostrea virginica	Laramore et al. (2017)	33–66%	Species specific *Bonamia* primers used but authors state that positive signal could be of positive *C. virginica* in close proximity	Not classified	Vector/Reservoir
Geukensia demissa	Laramore et al. (2017)	33–66%	Species specific *Bonamia* primers used but authors state that positive signal could be of positive *C. virginica* in close proximity	Not classified	Vector/Reservoir
Isognomon alatus	Laramore et al. (2017)	33–66%	Species specific *Bonamia* primers used but authors state that positive signal could be of positive *C. virginica* in close proximity	Not classified	Vector/Reservoir
Isognomon bicolor	Laramore et al. (2017)	33–66%	Species specific *Bonamia* primers used but authors state that positive signal could be of positive *C. virginica* in close proximity	Not classified	Vector/Reservoir
Ostrea lurida	Hill et al. (2014)	NA	This study investigates *O. stentina*	Not classified	Vector/Reservoir
Saccostrea glomerata	Spiers et al. (2014)	NA	Excluded because pathogen not identified	Not classified	Vector/Reservoir
Infection with *Bonamia ostreae*					
Ascidiella aspersa	Lynch et al. (2007)	NA	Excluded because species is not a mollusc	Not classified	Vector/Reservoir
Actina equina	Lynch et al. (2007)	NA	Excluded because species is not a mollusc	Not classified	Vector/Reservoir
Crassostrea gigas	Culloty et al. (1999)	NA	Excluded because pathogen not identified	Not classified	Doubtful
	Lynch et al. (2010)	33–66%	Contradictory results within the same paper, doubt on positive results		Not assessed
Ophiothrix fragilis	Lynch et al. (2007)	NA	Excluded because species is not a mollusc	Not classified	Vector/Reservoir
Ostrea lurida	Farley (1988)	NA	Excluded because pathogen not identified	Not classified	Doubtful
Ostrea puelchana	Pascual et al. (1991)	NA	Not peer-reviewed paper	Not classified	Vector/Reservoir

Infection with *Marteilia refringens*					
Acartia clausi	Carrasco et al. (2007)	10–33%	The ID of the pathogen is unlikely to be correct. New species	Not classified	Vector/Reservoir
Acartia discaudata	Carrasco et al. (2007)	10–33%	The ID of the pathogen is unlikely to be correct. New species	Not classified	Vector/Reservoir
Acartia grani	Audemard et al. (2002, 2004), Boyer et al. (2013), Carrasco et al. (2008, 2015)	NA	Species not a mollusc	Not classified	Vector/Reservoir
Acartia italica	Carrasco et al. (2007)	10–33%	Species not a mollusc	Not classified	Doubtful
Brachyura larvae	Carrasco et al. (2007)	10–33%	Species not a mollusc	Not classified	Vector/Reservoir
Crassostrea corteziensis	Grijalva-Chon et al. (2015)	33–66%	Doubt on pathogen ID and methods, conventional PCR used in an area previously not known for *M. refringens*. Confirmation needed. Without further data not possible to reach a conclusion.	Not classified	Vector/Reservoir
Crassostrea gigas	Cahour (1979)	NA	Excluded because no pathogen ID	Not classified	Vector/Reservoir
	Grijalva-Chon et al. (2015)		Doubt on pathogen ID and methods, conventional PCR used in an area previously not known for *M. refringens*. Confirmation needed. Without further data not possible to reach a conclusion.		
Euterpina acutifroms	Carrasco et al. (2007)	10–33%	The ID of the pathogen is unlikely to be correct. New species	Not classified	Vector/Reservoir
Mytilus edulis	Le Roux et al. (2001)	33–66%	*M. refringens* O-type detected in single individual mussel in combination with M-type	Not classified	Doubtful
Mytilus galloprovincialis	Fuentes et al. (1995)	10–33%	Doubtful path ID, considering the host species most likely *M. refringens* M-type/M. pararefringens	Not classified	Doubtful
	Navarro et al. (1996)	10–33%	Doubtful path ID, considering the host species most likely *M. refringens* M-type/M. pararefringens	Not classified	Doubtful
	Serracca et al. (2014)	10–33%	Doubtful path ID, considering the host species most likely *M. refringens* M-type/M. pararefringens	Not classified	Doubtful
Oithona sp.	Carrasco et al. (2007)	10–33%	The ID of the pathogen is unlikely to be correct. New species	Not classified	Vector/Reservoir
Paracartia latisetosa	Arzul et al. (2014)	NA	Species not a mollusc	Not classified	Vector/Reservoir
Ruditapes phillippinarum	Boyer et al. (2013)	NA	Not mentioned in the paper	Not classified	Vector/Reservoir

PCR, polymerase chain reaction; RFTM, Ray's fluid thioglycollate medium.