External Scientific Report

APPROVED: 30 June 2023
doi: 10.2903/sp.efsa.2023.EN-8124

Extensive literature review on vectors and reservoirs of AHL-listed pathogens of molluscs

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Abstract

On request of the EU Commission the EFSA carried out a Extensive Literature Review (ELR) 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 Annex of Implementing Regulation (EU) 2018/1882. In this External Scientific Report, the ELR and assessment of potential vector and reservoir species is described of the mollusc pathogens listed in Annex II to the AHL: *Marteilia refringens*, *Bonamia exitiosa*, *Bonamia ostreae*, *Mikrocytos mackini* and *Perkinsus marinus*. In total 923 research publications were collected for abstract screening and from these 153 were selected for further full text analysis. In the final data collection and assessment 67 relevant research publications were used for extracting information on vector en reservoir species of the above mollusc pathogens. The results for mollusc species for which scientific evidence indicates that a role as vector species or reservoir species is likely are presented as tables in this report. In addition, a brief assessment has been carried out of the conditions under which mollusc species shall be regarded as vectors or reservoirs of diseases of mollusc listed in Annex II.

Key words: (Mollusc, vector, reservoir, *Marteilia refringens*, *Bonamia ostreae*, *Bonamia exitiosa*, *Mikrocytos mackini*, *Perkinsus marinus*)

Question number: EFSA-Q-2023-00257
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Acknowledgements: The team at EFSA (Sofie Dhollander, Marzia Gnocchi and others) and Isabelle Arzul of the EURL mollusc diseases are greatly acknowledged for their support and fruitful discussions.

Suggested citation: 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. doi:10.2903/sp.efsa.2023.EN-8124

ISSN: 2397-8325

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1 Introduction

1.1 Background and terms of reference as provided by the requestor

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

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

It should be noted that vector species of aquatic animals are not listed in the OIE Aquatic Code[1] or in the OIE Aquatic Manual[2]. In the disease specific chapters of the OIE 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 OIE 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.

[1] WOAH Aquatic Animal Health Code, 2021, 23rd Edition.
[2] WOAH Aquatic Manual, 2021, 8th Edition.

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 a role is likely.

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 Contract OC/EFSA/BIOHAW/2022/04 - Lot 2

The contract OC/EFSA/BIOHAW/2022/04 - Lot 2 (molluscs) was awarded by EFSA to:

Contractor: Wageningen Bioveterinary Research (The Netherlands) with partners Marine Institute (Ireland) and Institute of Agrifood Research and Technology (Spain)

[3] Commission Delegated Regulation (EU) 2020/990 of 28 April 2020 supplementing Regulation (EU) 2016/429 of the European Parliament and of the Council, as regards animal health and certification requirements for movements within the Union of aquatic animals and products of animal origin from aquatic animals (OJ L 221, 28 April 2020, p.42).

[4] Commission Delegated Regulation (EU) 2020/692 of 30 January 2020 supplementing Regulation (EU) 2016/429 of the European Parliament and of the Council as regards rules for entry into the Union, and the movement and handling after entry of consignments of certain animals, germinal products and products of animal origin (OJ L 174, 3.6.2020, p.379).

Contract/Grant title: Support in the assessment of species or groups of species of aquatic animals that pose a considerable risk of spread because they are vectors or reservoirs for some diseases and to investigate the conditions under which they should be regarded as vectors or reservoirs.

Contract number: OC/EFSA/BIOHAW/2022/04 – Lot 2

1.3 Interpretation of the Terms of Reference

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

Term of Reference 1 (ToR1) requests EFSA to provide a list of vector species or reservoirs species of pathogens of fish, crustaceans, and molluscs, listed in Annex II to the AHL, aiming to update the fourth column of the Annex to Implementing Regulation (EU) 2018/1882.

EFSA was not requested to update the list of susceptible species, already listed in the third column of the same Implementing regulation (See also annex A of this report). In addition, it was agreed that a species cannot be classified simultaneously as susceptible and vector or reservoir species.

This External Scientific Report 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:

- *Mikrocytos mackini*
- *Perkinsus marinus*
- *Bonamia exitiosa*
- *Bonamia ostreae*
- *Marteilia refringens*

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.

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.

In **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.

1.3.2 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

In addition to the assessment of potential vector or reservoir species identified under ToR1 the EFSA is requested to provide under Term of Reference 2 (ToR2) an assessment 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.

2 Data and Methodologies

2.1 Methodologies ToR 1: Assessment of potential vectors and reservoir species of pathogens of molluscs, listed in Annex II to the AHL

This Extensive Literature Review (ELR) aims to assess the role of mollusc species as vector or reservoir species of specific mollusc pathogens listed by the AHL, by gathering all scientific evidence available regarding the parameters presented in Annex D.

Review question:

1. **What is the evidence generated by experimental infection studies or field studies, demonstrating transmission of pathogen 'A' from vector species 'X' on or in which Pathogen A was detected, to a species 'Y'**
2. **What is the evidence generated by experimental infection studies or field studies, demonstrating the detection of Pathogen A on or in species X, without further evidence of transmission of pathogen A to a species 'Y'**

Pathogen A refers to pathogens listed by the AHL, affecting molluscs, that are listed in Table 1 (see also Annex A).

Table 1. Listed pathogens affecting mollusc species to be assessed

Listed pathogen affecting molluscs
Mikrocytos mackini
Bonamia exitiosa
Marteilia refringens
Perkinsus marinus
Bonamia ostreae

2.2 Eligibility criteria

The eligibility criteria for experimental and field studies are listed in Table 2 and 3. Detailed forms for eligibility screening per level of screening are provided in Annex C.

Table 2. Study eligibility criteria for experimental infection studies

Element	Criteria	Level of screening
Publication type	Primary research publications (peer reviewed)	Title and abstract
		Full-text
Language	EU language	Title and abstract
		Full-text

Study type	Experimental infections	Title and abstract
Study design	Experimental study design	Title and abstract
	Only one single species X can be included per time per experimental unit	Full-text
Population	Species X and Y are aquatic animal species belonging to molluscs	Title and abstract
	Species X should not be a known susceptible species listed in Commission implementing regulation (EU) 2018/1882	Full text
Exposure	Exposure to pathogen A (AHL listed pathogens listed in Table 1)	Title and abstract
		Full-text
Outcome	Demonstration of pathogen A present in or on species X	Full-text
	Species Y must be experimentally exposed to pathogen A, either by direct or indirect contact with species X (cohabitation, immersion, ingestion)	
	Remark: these are outcomes used as eligibility criteria. Detailed eligibility screening forms and lists of parameters to be extracted are provided in Annex C and D	

Table 3. Study eligibility criteria for field studies

Element	Criteria	Level of screening
Publication type	Primary research publications (peer reviewed)	Title and abstract
		Full-text
Language	EU language	Title and abstract
		Full-text
Study type	Field studies	Title and abstract
Study design	Case studies, prevalence studies, surveillance reports, etc.	Title and abstract
Population	Species X is aquatic animal species belonging to molluscs	Title and abstract
	Species X should not be a known susceptible species listed in Commission implementing regulation (EU) 2018/1882	Full-text
Exposure	Exposure to pathogen A (AHL listed pathogens listed in Table 1)	Title and abstract
		Full-text
Outcome	Demonstration of pathogen A present in or on species X	Full-text
	Remark: these are outcomes used as eligibility criteria. Detailed eligibility screening forms and lists of parameters to be extracted are provided in Annex C and D	

2.3 Methods for searching the results

2.3.1 Information sources

Information sources used in the review are listed in table 4

Table 4: Information sources consulted

Information source	Platform
Scopus	Scopus.com (Elsevier)
CAB Abstracts	Web of Science (Clarivate)
Web of Science Core Collection - Science Citation Index Expanded - Emerging Sources Citation Index	Web of Science (Clarivate)

2.3.2 Reference management

Full references and abstracts were downloaded from Endnote 20 and uploaded into the Literature Review software DistillerSR ® (Evidence Partners) by EFSA.

2.3.3 Search strategy

For each of the aquatic animal group specific combinations of search terms was applied. The use of Boolean operators (AND, OR, NOT), truncation ($) and wildcard (*) symbols assured that search terms account for synonyms, abbreviations and spelling variants, enhancing thus the sensitivity of the search strategy. The used search strings are provided in Annex B.

The search strings will be a combination of 2 elements:

String I: name of the pathogens and diseases listed in Table 1, with alternative names when relevant, and

String II: selection of experimental studies (e*xperiment* OR transmission*) or field detections (detect*)

2.4 Methods for study selection

The reviews were carried out in Distiller®, under the license owned by EFSA. EFSA was responsible to set up the project in Distiller®, run the search in Endnote and upload the references. Thereafter the consortium WBVR-MI-IRTA carried out the reviews. An overview of the extensive literature review process is provided in Figure 1.

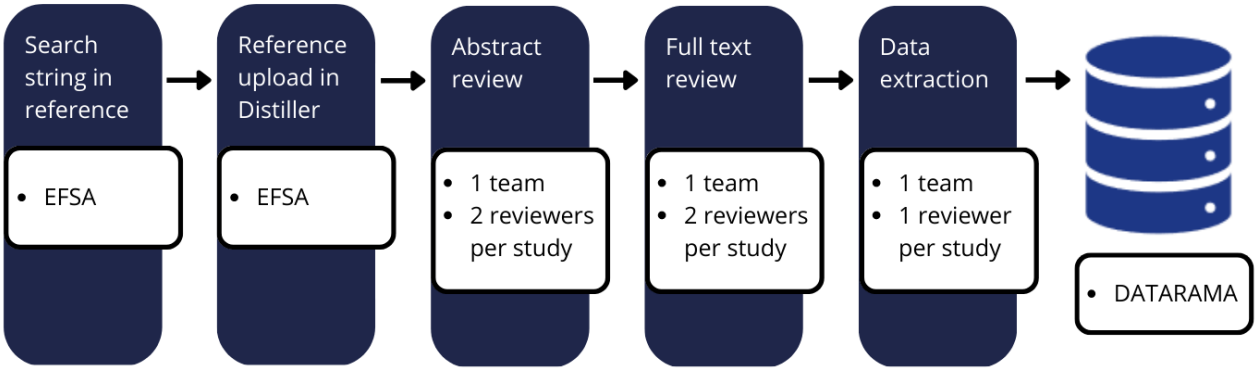

Figure 1. Overview of the extensive literature review process. Datarama is DistillerSR®'s database, from which data can be exported for manipulation.

After the specific search strings were applied in the reference manager (e.g., Endnote in EFSA), duplicates were removed in Endnote, whereafter a reference output file containing all results was uploaded to Distiller.

The review flow is visualised in Figure 2. Level 1 screening for eligibility was performed jointly for both experimental and field studies, while level two screening and data extraction were specific for either experimental infections or field studies.

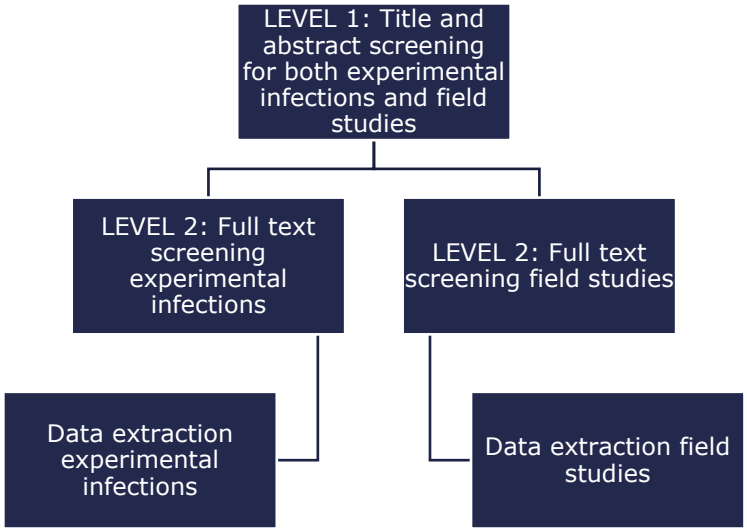

Figure 2. Review workflow applied for the output references for each of the aquatic species' groups of interest.

Title and abstract review

The level 1 selection process involved the screening of title and abstract using a screening check list developed according to the eligibility criteria defined in section 1 above. If the information contained in the title or abstract was not relevant for the research objectives (any of the eligibility criteria is not met), the article was not selected for full text assessment. The first level of screening was performed independently and blindly by two researchers (i.e., two reviewers per study). Conflicts were resolved before going to the second level. To solve

conflicts, reviewers discussed their reasoning and reached a consensus decision. Reasons for exclusion were recorded by Distiller.

Full text review

The level 2 selection process involved the screening of full text using a screening checklist developed according to the eligibility criteria defined section 1 above. This step was carried out by two reviewers. Conflicts were resolved before going to the data extraction level. To solve conflicts, reviewers discussed their reasoning and reached a consensus decision. Reasons for exclusion were recorded by Distiller®.

Both level 1 and level 2 screenings involved an initial phase of harmonisation and training regarding the assessment of study eligibility criteria, across all screeners of each objective. During the selection process, Distiller recorded automatically:

- Total number of unique records (title/abstracts) identified through electronic search
- Number of records excluded after level 1 screening
- Records (full text) potentially eligible
- Number of records excluded after level 2 screening (by reason for exclusion)
- Final number of studies included in the review

2.5 Methods for data collection

From those studies that passed both selection levels, data collection was performed using forms set up in Distiller® provided in Annex D. These forms ensured that data validity checks were performed during data collection, in particular compliance to the data types specified in the forms.

Changes to the existing forms during the review were avoided to ensure that all data collected were compatible with the data already collected in previous ELRs and were easy to be summarised.

One reviewer per study individually extracted data from studies that have passed screening for relevance, but a quality assurance process was applied, as detailed below.

2.6 Methods for assessment of the collected data

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 mollusc species as a potential vector or reservoir species, according to the working definition provided in Section 1.2.1., was applied in two steps:

2.6.1 **First step:** Individual assessments by the consortium of the data extracted from papers

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)?

The possible answers are:

90-100% certain (yes)
10-90% certain (doubtful)
0-10% certain (no)

The reasoning of choice was provided with to respect the working definition of vectors and reservoirs, and not to consider other information that was not collected extracted from the eligible peer reviewed literature.

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 test is not necessary, so:

  VECTOR: Yes, where at least 1 positive test for X species and Y species is reported

  RESERVOIR: Yes, where there is at least 1 positive test for X species (and negative for Y)

  Field studies there is more uncertainty and therefore, ideally 2 tests taken from the same animals or reported in the same paper are needed:

  RESERVOIR: Yes, when positive for 2 tests for X species or supported by multiple publications. When the latter is the case this has been substantiated in the tables under the "Reasons for choice".

- **Doubtful results (10-90% certainty)**
  Any positive test result that is not one of the above situations. Experts are asked to describe the reason why the result is doubtful. The doubtful results will be elaborated in the next step of the assessment.

- **Negative results: < 10% certainty**
  The review should have captured only papers where pathogen A has 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 1 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 presented in the table.

  Also, where negative results for Pathogen A detection in species Y (susceptible species) after transmission from species X (potential vector) were recorded, the assessment only focused on the assessment of species X as reservoir species and the same method as above can be followed.

2.6.2 **Second step:** Consensus assessment with focus on doubtful cases

- Judgements were discussed within the consortium and changes made if needed, based on consensus
- focus was placed on the doubtful cases and if a more precise certainty could be chosen based on some criteria:

Likely	66-90%
As likely as not	33-66%

Unlikely	10-33%

- Vector/reservoir classification: In the tables the cases with positive results (>90% certainty) and doubtful results judged as likely (66-90%) were included.

The results of the Extensive Literature Review and assessment of mollusc species as vector or reservoir species of specific mollusc pathogens listed by the AHL is shown in table format in paragraph 3.1 and in comprehensive form as separate annex to this report in a Excel document.

2.7 Methodologies ToR 2: Conditions under which mollusc species shall be regarded as vectors or reservoirs of diseases of mollusc listed in Annex II to the AHL

As described in the interpretation of the ToRs (Section 1.2.2.), several conditions need to be fulfilled for a mollusc species to be able act as a vector of a pathogenic agent. Besides the exposure of the potential vector species to the pathogen at the origin, factors such as the water quality and temperature, the duration of the journey and the potential exposure at the place of destination with the susceptible species will determine if effective transmission takes place.

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 was recorded during the ELS, carried out for ToR1, collecting the ranges of the different duration and temperature 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.

3 Assessment/Results

3.1 PRISMA FLOWCHART

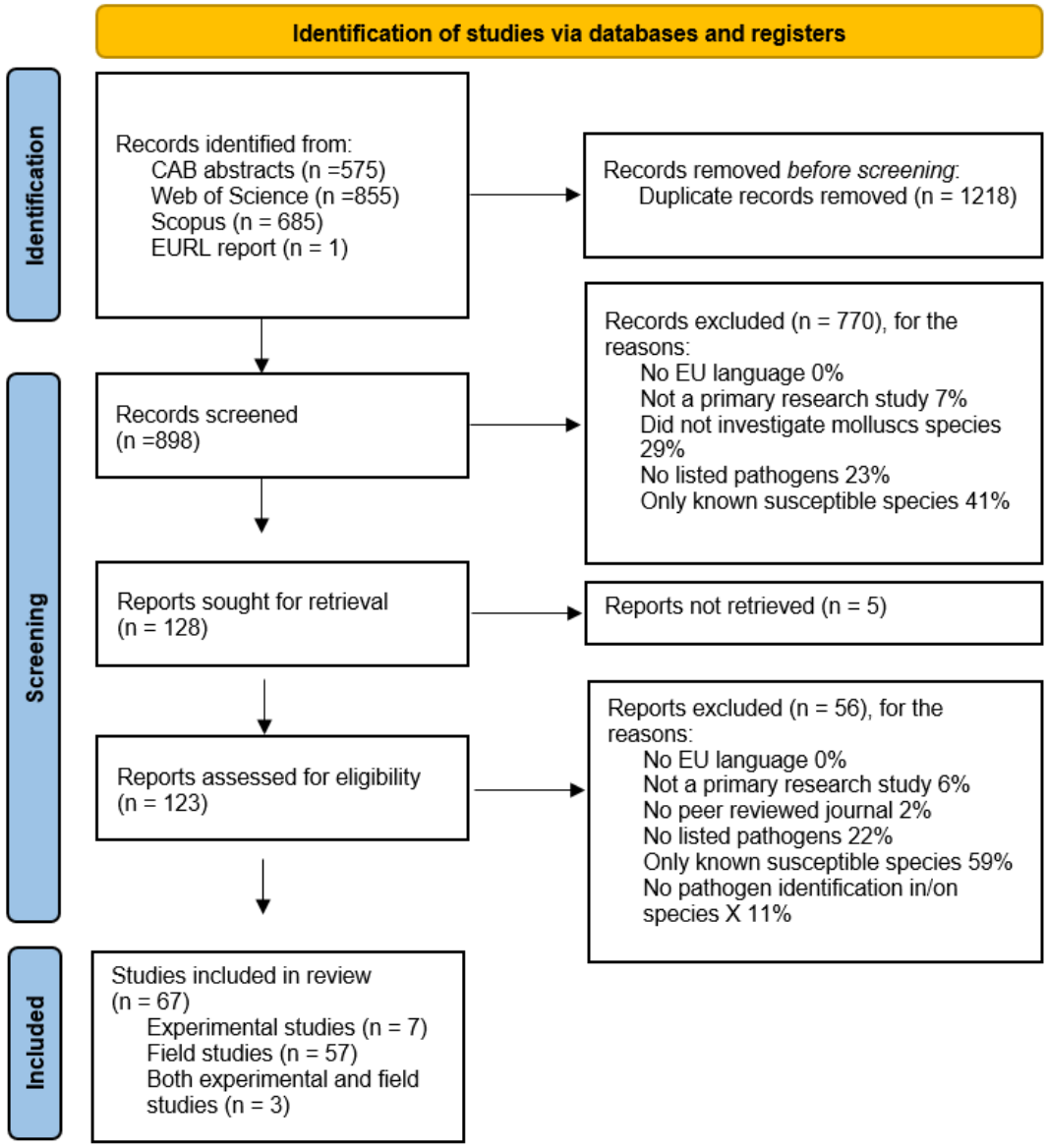

Figure 3. Prisma flowchart documenting the review process

3.2 Assessment ToR 1: Assessment of potential vectors and reservoir species of pathogens of molluscs, listed in Annex II to the AHL

The comprehensive results from the assessment of potential vectors and reservoir species of pathogens of molluscs is available as tables in a separate Excel document to this External Scientific Report. The tables 1a, 1b and 2 are extracts from these tables.

Table 1a: Transmission of pathogen 'A' from an infected vector species 'X' to a species 'Y' evidenced in experimental infection studies

Pathogen A	**Species X**	**Transmission route investigated**	**Species Y**	**Pathogen detection method in species Y**	**Result**	**Bibliography**	Certainty **VECTOR** species	**Decision[1)]**
Mikrocytos mackini	Crassostrea virginica	Injection	Crassostrea gigas	Tissue imprints	Pos.	Bower et al. 1997	90-100% (yes)	**vector**
Perkinsus marinus	Boonea impressa	Cohabitation	Crassostrea virginica	RFTM culture medium	Pos.	White et al. 1989	90-100% (yes)	**vector**

1) Decision made based on the methodology and definitions for vector and reservoir in the current ELR.

Table 1b: Infection of reservoir species X with pathogen 'A', from experimental infection studies

Pathogen A	**Species X**	**Pathogen detection method in species X**	**Result**	**Bibliography**	Certainty **RESERVOIR** species	**Decision[1)]**	**Classification EURL report[2)]**
Perkinsus marinus	Macoma balthica	RFTM assay	Pos.	Dungan et al. 2007	90-100% (yes)	**reservoir**	
Perkinsus marinus	Macoma balthica	PCR	Pos.	Dungan et al. 2007	90-100% (yes)	**reservoir**	
Perkinsus marinus	Macoma balthica	His	Pos.	Dungan et al. 2007	90-100% (yes)	**reservoir**	

Perkinsus marinus	Mya arenaria	RFTM assay	Pos.	Dungan et al. 2007	90-100% (yes)	**reservoir**	
Perkinsus marinus	Mya arenaria	PCR	Pos.	Dungan et al. 2007	90-100% (yes)	**reservoir**	
Perkinsus marinus	Mya arenaria	His	Pos.	Dungan et al. 2007	90-100% (yes)	**reservoir**	
Perkinsus marinus	Crassostrea ariakensis	PCR	Pos.	Moss et al. 2006	90-100% (yes)	**reservoir***	**susceptible species***
Perkinsus marinus	Crassostrea ariakensis	PCR-RFLP	Pos.	Moss et al. 2006	90-100% (yes)	**reservoir***	**susceptible species***
Perkinsus marinus	Crassostrea ariakensis	InSiHy	Pos.	Moss et al. 2006	90-100% (yes)	**reservoir***	**susceptible species***
Perkinsus marinus	Crassostrea corteziensis	PCR	Pos.	Escobedo-Fregoso et al. 2017	90-100% (yes)	**reservoir***	**susceptible species***
Perkinsus marinus	Crassostrea corteziensis	Seq	Pos.	Escobedo-Fregoso et al. 2017	90-100% (yes)	**reservoir***	**susceptible species***
Perkinsus marinus	Crassostrea corteziensis	PCR	Pos.	Gutierrez-Rivera et al. 2015	90-100% (yes)	**reservoir***	**susceptible species***
Perkinsus marinus	Crassostrea corteziensis	Seq	Pos.	Gutierrez-Rivera et al. 2015	90-100% (yes)	**reservoir***	**susceptible species***
Perkinsus marinus	Crassostrea corteziensis	RFTM assay	Pos.	Gutierrez-Rivera et al. 2015	90-100% (yes)	**reservoir***	**susceptible species***
Perkinsus marinus	Crassostrea corteziensis	InSiHy	Pos.	Gutierrez-Rivera et al. 2015	90-100% (yes)	**reservoir***	**susceptible species***

1) Decision made based on the methodology and definitions for vector and reservoir in the current ELR.
2) Conclusion from the scientific advice based on the EURL report 2020 "Expert group on susceptibility of mollusc species to EU listed diseases"

*Identified as susceptible species in the EURL report 2020 "Expert group on susceptibility of mollusc species to EU listed diseases".

Table 2: Infection of reservoir species X with pathogen 'A', from field studies

Pathogen A	**Species X**	**Pathogen detection method in species X**	**Result**	**Bibliography**	Certainty **RESERVOIR** species	**Decision**	

Bonamia exitiosa	Ostrea stentina	PCR-RFLP	Pos.	Elgharsalli et al. 2018	90-100% (yes)	**reservoir***	**susceptible species***
Bonamia exitiosa	Ostrea stentina	Seq	Pos.	Hill et al. 2014	10-90% (doubtful) likely (66-90%)	**reservoir***	**susceptible species***
Bonamia exitiosa	Ostrea stentina	PCR	Pos.	Hill et al. 2010	90-100% (yes)	**reservoir***	**susceptible species***
Bonamia exitiosa	Ostrea stentina	Seq	Pos.	Hill et al. 2010	90-100% (yes)	**reservoir***	**susceptible species***
Bonamia exitiosa	Ostrea stentina	His	Pos.	Hill et al. 2010	90-100% (yes)	**reservoir***	**susceptible species***
Bonamia exitiosa	Ostrea stentina	InSiHy	Pos.	Hill et al. 2010	90-100% (yes)	**reservoir***	**susceptible species***
Bonamia ostreae	Ostrea angasi	His	Pos.	Bougrier et al. 1986	10-90% (doubtful) likely (66-90%)	**reservoir**	
Bonamia ostreae	Ostrea angasi	Smear	Pos.	Bougrier et al. 1986	10-90% (doubtful) likely (66-90%)	**reservoir**	
Marteilia refringens[3)]	Mytilus galloprovincialis	PCR	Pos.	Lattos et al. 2022	90-100% (yes)	**reservoir**	
Marteilia refringens[3)]	Mytilus galloprovincialis	Seq	Pos.	Lattos et al. 2022	90-100% (yes)	**reservoir**	
Marteilia refringens[3)]	Mytilus galloprovincialis	Seq	Pos.	Novoa et al. 2005	90-100% (yes)	**reservoir**	
Marteilia refringens[3)]	Mytilus galloprovincialis	His	Pos.	Arzul et al. 2014	90-100% (yes)	**reservoir**	
Marteilia refringens[3)]	Mytilus galloprovincialis	PCR	Pos.	Arzul et al. 2014	90-100% (yes)	**reservoir**	
Marteilia refringens[3)]	Mytilus galloprovincialis	PCR-RFLP	Pos.	Arzul et al. 2014	90-100% (yes)	**reservoir**	
Marteilia refringens[3)]	Mytilus galloprovincialis	Seq	Pos.	Arzul et al. 2014	90-100% (yes)	**reservoir**	
Marteilia refringens[3)]	Mytilus galloprovincialis	PCR-RFLP	Pos.	Balseiro et al. 2007	90-100% (yes)	**reservoir**	
Marteilia refringens[3)]	Chamellea gallina	His	Pos.	Lopez-Flores et al. 2008b	90-100% (yes)	**reservoir***	**susceptible species***

Marteilia refringens[3)]	Chamellea gallina	PCR	Pos.	Lopez-Flores et al. 2008b	90-100% (yes)	**reservoir***	**susceptible species***
Marteilia refringens[3)]	Chamellea gallina	InSiHy	Pos.	Lopez-Flores et al. 2008b	90-100% (yes)	**reservoir***	**susceptible species***
Marteilia refringens[3)]	Chamellea gallina	Seq	Pos.	Lopez-Flores et al. 2008b	90-100% (yes)	**reservoir***	**susceptible species***
Marteilia refringens[3)]	Ostrea stentina	PCR-RFLP	Pos.	Elgharsalli et al. 2018	90-100% (yes)	**reservoir***	**susceptible species***
Marteilia refringens[3)]	Ostrea stentina	PCR-RFLP	Pos.	Elgharsalli et al. 2013	90-100% (yes)	**reservoir***	**susceptible species***
Marteilia refringens[3)]	Ostrea stentina	Seq	Pos.	Elgharsalli et al. 2013	90-100% (yes)	**reservoir***	**susceptible species***
Marteilia refringens[3)]	Ostrea stentina	His	Pos.	Elgharsalli et al. 2013	90-100% (yes)	**reservoir***	**susceptible species***
Marteilia refringens[3)]	Ostrea stentina	TEM	Pos.	Elgharsalli et al. 2013	90-100% (yes)	**reservoir***	**susceptible species***
Marteilia refringens[3)]	Ostrea stentina	His	Pos.	Lopez-Sanmartin et al. 2015	90-100% (yes)	**reservoir***	**susceptible species***
Marteilia refringens[3)]	Ostrea stentina	InSiHy	Pos.	Lopez-Sanmartin et al. 2015	90-100% (yes)	**reservoir***	**susceptible species***
Marteilia refringens[3)]	Ostrea stentina	PCR	Pos.	Lopez-Sanmartin et al. 2015	90-100% (yes)	**reservoir***	**susceptible species***
Marteilia refringens[3)]	Ostrea stentina	PCR-RFLP	Pos.	Lopez-Sanmartin et al. 2015	90-100% (yes)	**reservoir***	**susceptible species***
Marteilia refringens[3)]	Ostrea stentina	Seq	Pos.	Lopez-Sanmartin et al. 2015	90-100% (yes)	**reservoir***	**susceptible species***

Mikrocytos mackini	Crassostrea virginica	Tissue imprints	Pos.	Bower et al. 1997	10-90% (doubtful) likely (66-90%)	**Reservoir[4)]**	
Perkinsus marinus	Mercenaria mercenaria	PCR	Pos.	Pecher et al. 2008	10-90% (doubtful) likely (66-90%)	**reservoir**	
Perkinsus marinus	Mercenaria mercenaria	Seq	Pos.	Pecher et al. 2008	10-90% (doubtful) likely (66-90%)	**reservoir**	
Perkinsus marinus	Mya arenaria	PCR	Pos.	Reece et al. 2008	90-100% (yes)	**reservoir**	
Perkinsus marinus	Mya arenaria	InSiHy	Pos.	Reece et al. 2008	90-100% (yes)	**reservoir**	
Perkinsus marinus	Mya arenaria	RTFM Culture	Pos.	Reece et al. 2008	90-100% (yes)	**reservoir**	
Perkinsus marinus	Modiolus capax	RTFM Culture	Pos.	Góngora-Gómez et al. 2021	90-100% (yes)	**reservoir**	
Perkinsus marinus	Modiolus capax	PCR	Pos.	Góngora-Gómez et al. 2021	90-100% (yes)	**reservoir**	
Perkinsus marinus	Crassostrea corteziensis	His	Pos.	Caceres-Martinez et al. 2008	10-90% (doubtful) likely (66-90%)	**reservoir***	**susceptible species***
Perkinsus marinus	Crassostrea corteziensis	PCR	Pos.	Caceres-Martinez et al. 2008	10-90% (doubtful) likely (66-90%)	**reservoir***	**susceptible species***
Perkinsus marinus	Crassostrea corteziensis	Seq	Pos.	Caceres-Martinez et al. 2008	10-90% (doubtful) likely (66-90%)	**reservoir***	**susceptible species***
Perkinsus marinus	Crassostrea corteziensis	His	Pos.	Caceres-Martinez et al. 2016	10-90% (doubtful) likely (66-90%)	**reservoir***	**susceptible species***
Perkinsus marinus	Crassostrea corteziensis	PCR	Pos.	Escobedo-Fregoso et al. 2015	90-100% (yes)	**reservoir***	**susceptible species***
Perkinsus marinus	Crassostrea corteziensis	PCR-RFLP	Pos.	Escobedo-Fregoso et al. 2015	90-100% (yes)	**reservoir***	**susceptible species***
Perkinsus marinus	Crassostrea corteziensis	Seq	Pos.	Escobedo-Fregoso et al. 2015	90-100% (yes)	**reservoir***	**susceptible species***

Perkinsus marinus	Crassostrea corteziensis	RTFM Culture	Pos.	Escobedo-Fregoso et al. 2017	90-100% (yes)	**reservoir***	**susceptible species***
Perkinsus marinus	Crassostrea corteziensis	PCR	Pos.	Escobedo-Fregoso et al. 2017	90-100% (yes)	**reservoir***	**susceptible species***
Perkinsus marinus	Crassostrea corteziensis	Seq	Pos.	Escobedo-Fregoso et al. 2017	90-100% (yes)	**reservoir***	**susceptible species***
Perkinsus marinus	Crassostrea corteziensis	RTFM Culture	Pos.	Villanueva-Fonseca et al. 2020	10-90% (doubtful) likely (66-90%)	**reservoir***	**susceptible species***
Perkinsus marinus	Crassostrea corteziensis	PCR	Pos.	Villanueva-Fonseca et al. 2020	10-90% (doubtful) likely (66-90%)	**reservoir***	**susceptible species***
Perkinsus marinus	Crassostrea gasar[5)]	RTFM Culture	Pos.	Cunha et al. 2019	10-90% (doubtful) likely (66-90%)	**reservoir***	**susceptible species***
Perkinsus marinus	Crassostrea gasar[5)]	His	Pos.	Cunha et al. 2019	10-90% (doubtful) likely (66-90%)	**reservoir***	**susceptible species***
Perkinsus marinus	Crassostrea gasar[5)]	PCR	Pos.	Cunha et al. 2019	10-90% (doubtful) likely (66-90%)	**reservoir***	**susceptible species***
Perkinsus marinus	Crassostrea gasar[5)]	Seq	Pos.	Cunha et al. 2019	10-90% (doubtful) likely (66-90%)	**reservoir***	**susceptible species***
Perkinsus marinus	Crassostrea gasar[5)]	His	Neg.	Queiroga et al. 2015	90-100% (yes)	**reservoir***	**susceptible species***
Perkinsus marinus	Crassostrea gasar[5)]	RTFM Culture	Neg.	Queiroga et al. 2015	90-100% (yes)	**reservoir***	**susceptible species***
Perkinsus marinus	Crassostrea gasar[5)]	PCR-RFLP	Pos.	Queiroga et al. 2015	90-100% (yes)	**reservoir***	**susceptible species***
Perkinsus marinus	Crassostrea gasar[5)]	Seq	Pos.	Queiroga et al. 2015	90-100% (yes)	**reservoir***	**susceptible species***

Perkinsus marinus	Crassostrea gasar[5)]	RTFM Culture	Neg.	Da Silva et al. 2014	90-100% (yes)	**reservoir***	**susceptible species***
Perkinsus marinus	Crassostrea gasar[5)]	His	Neg.	Da Silva et al. 2014	90-100% (yes)	**reservoir***	**susceptible species***
Perkinsus marinus	Crassostrea gasar[5)]	PCR	Neg.	Da Silva et al. 2014	90-100% (yes)	**reservoir***	**susceptible species***
Perkinsus marinus	Crassostrea gasar[5)]	Seq	Pos.	Da Silva et al. 2014	90-100% (yes)	**reservoir***	**susceptible species***
Perkinsus marinus	Crassostrea gasar[5)]	InSiHy	Pos.	Da Silva et al. 2014	90-100% (yes)	**reservoir***	**susceptible species***
Perkinsus marinus	Crassostrea gasar[5)]	RTFM Culture	Pos.	Scardua et al. 2017	90-100% (yes)	**reservoir***	**susceptible species***
Perkinsus marinus	Crassostrea gasar[5)]	PCR-RFLP	Pos.	Scardua et al. 2017	90-100% (yes)	**reservoir***	**susceptible species***
Perkinsus marinus	Crassostrea gasar[5)]	Seq	Pos.	Scardua et al. 2017	90-100% (yes)	**reservoir***	**susceptible species***
Perkinsus marinus	Crassostreae sp. (Brazilian native oyster)	PCR	Pos.	Leibowitz et al. 2019	10-90% (doubtful) likely (66-90%)	**reservoir***	**susceptible species***
Perkinsus marinus	Crassostreae sp. (Brazilian native oyster)	Seq	Pos.	Leibowitz et al. 2019	10-90% (doubtful) likely (66-90%)	**reservoir***	**susceptible species***
Perkinsus marinus	Crassostrea rhizophorae	Seq	Pos.	Lohan et al. 2016	10-90% (doubtful) likely (66-90%)	**reservoir***	**susceptible species***
Perkinsus marinus	Crassostrea rhizophorae	RTFM Culture	Pos.	Da Silva et al. 2013	90-100% (yes)	**reservoir***	**susceptible species***
Perkinsus marinus	Crassostrea rhizophorae	PCR	Pos.	Da Silva et al. 2013	90-100% (yes)	**reservoir***	**susceptible species***

Perkinsus marinus	Crassostrea rhizophorae	PCR-RFLP	Pos.	Da Silva et al. 2013	90-100% (yes)	**reservoir***	**susceptible species***
Perkinsus marinus	Crassostrea rhizophorae	Seq	Pos.	Da Silva et al. 2013	90-100% (yes)	**reservoir***	**susceptible species***
Perkinsus marinus	Crassostrea rhizophorae	RTFM Culture	Pos.	Scardua et al. 2017	90-100% (yes)	**reservoir***	**susceptible species***
Perkinsus marinus	Crassostrea rhizophorae	PCR-RFLP	Pos.	Scardua et al. 2017	90-100% (yes)	**reservoir***	**susceptible species***
Perkinsus marinus	Crassostrea rhizophorae	Seq	Pos.	Scardua et al. 2017	90-100% (yes)	**reservoir***	**susceptible species***
Perkinsus marinus	Saccostrea palmula	RTFM Culture	Pos.	Cáceres-Martínez et al. 2012	90-100% (yes)	**reservoir***	**susceptible species***
Perkinsus marinus	Saccostrea palmula	PCR	Pos.	Cáceres-Martínez et al. 2012	90-100% (yes)	**reservoir***	**susceptible species***
Perkinsus marinus	Saccostrea palmula	Seq	Pos.	Cáceres-Martínez et al. 2012	90-100% (yes)	**reservoir***	**susceptible species***
Perkinsus marinus	Saccostrea palmula	InSiHy	Pos.	Cáceres-Martínez et al. 2012	90-100% (yes)	**reservoir***	**susceptible species***

1) Decision made based on the methodology and definitions for vector and reservoir in the current ELR.
2) Conclusion from the scientific advice based on the EURL report 2020 "Expert group on susceptibility of mollusc species to EU listed diseases"
3) For the purpose of this assessment *Marteilia refringens* is defined as the species infecting oysters in some papers defined as genotype O. *Marteilia refringens* type M/ *Marteilia maurini* / *Marteilia pararefringens* was not assessed.
4) *Crassostrea virginica* was already identified as vector species of *Mikrocytos mackini* in table 1a.
5) Type species misidentified in Brazil, should be considered as the native Brazilian oyster species *Crassostrea brasiliana.*

*Identified as susceptible species in the EURL report 2020 "Expert group on susceptibility of mollusc species to EU listed diseases".

3.3 Assessment ToR 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 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.

Several conditions need to be fulfilled for a mollusc species to be able act as a vector of a pathogenic agent. Besides the exposure of the potential vector species to the pathogen at the origin, factors such as the water quality and temperature, the duration of the journey and the potential exposure at the place of destination with the susceptible species will determine if effective transmission takes place.

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 was recorded during the ELS, carried out for ToR1, collecting the ranges of the different duration and temperature for which transmission has been proven for the different pathogens by the different 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 which may be present in the carrying water/matrix. However, the impact of these parameters is specific to each pathogen:

3.3.1 Infection with *Mikrocytos mackini*

From publications on susceptible host species *C. 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. *Mikrocytos mackini* does not develop into disease in oysters held at 17°C (Bower et al. 1997). However, the parasite can survive at warmer temperatures for prolonged periods as exposed *C. gigas* incubated at 17°C develop disease when 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 Pacific oysters are also susceptible to infection and resulting disease (Bower et al. 2005).

Bower, S.M., K. Bate et G.R. Meyer. 2005. Susceptibility of juvenile Crassostrea gigas and resistance of Panope abrupta to Mikrocytos mackini. Journal of Invertebrate Pathology 88: 95-99

Bower S. M., D. Hervio and G. R. Meyer 1997. Infectivity of Mikrocytos mackini, the causative agent of Denman Island disease in Pacific oysters Crassostrea gigas, to various species of oysters. Dis Aquat Org 1997 Vol. 29 Pages 111-116

Hervio, D., S.M. Bower and G.R. Meyer. 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

3.3.2 Infection with *Perkinsus marinus*

Intensity and prevalence of infection with *P. marinus* appears to be 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 impacts infection with *P. marinus*, with maximum prevalence and intensity observed 1-2 months after maximum summer water temperatures are reached (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 *C. ariakensis* this could be established at 20-22°C and 25 ppt (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.

Burreson, E.M. and L.M. Ragone Calvo. 1996. Epizootiology of Perkinsus marinus disease of oysters in Chesapeake Bay, with emphasis on data since 1985. Journal of Shellfish Research 15: 17-34.

Dungan C. F., K. S. Reece, R. M. Hamilton, N. A. Stokes and E. M. Burreson 2007. Experimental cross-infections by Perkinsus marinus and P. chesapeaki in three sympatric species of Chesapeake Bay oysters and clams. Dis Aquat Org Vol. 76 Issue 1 Pages 67-75

C. Escobedo-Fregoso, J. Ramirez-Salcedo and R. Vázquez-Juárez 2017. Host Response when Perkinsus marinus Infection Intensities Increase in the Oyster Crassostrea corteziensis. Journal of Shellfish Research Vol. 36 Issue 3 Pages 717-727

Mackin, J.G. 1955. Dermocystidium marinum and salinity. Proceedings of the National Shellfisheries Association 46: 116-128

Moss J. A., E. M. Burreson and K. S. Reece 2006. Advanced Perkinsus Marinus Infections in Crassostrea Ariakensis Maintained under Laboratory Conditions. Journal of Shellfish Research Vol. 25 Issue 1 Pages 65-72

3.3.3 Infection with *Bonamia exitiosa*

Considering the broad global distribution of *B. exitiosa* a wide tolerance to a range of environmental conditions would be expected. Actual data on the physiological parameters is, however, lacking from most of the scientific papers. In the identified reservoir species *Ostrea stentina B. exitiosa* was present in Tunisia within the temperature range of 17.1°C – 30.0°C and a salinity of 36.2‰ to 37.80‰ (Elgharsalli et al. 2016).

Available data suggest that stressing oysters using warm water (25–26°C) or hypersaline (39–40%) water favour *B. exitiosa* development (Hine et al., 2002).

R. Elgharsalli, C. Seguineau, I. Arzul, N. Aloui-Bejaoui, C. Quere and J. Moal. 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. J Marine Biol Assoc UK Vol. 98 Issue 1 Pages 161-170

Hine P.M. (2002). Severe apicomplexan infection in the oyster Ostrea chilensis: a predisposing factor in bonamiosis. Dis. Aquat. Org., 51, 49–60

3.3.4 Infection with *Bonamia 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 temperature (4°C and 10°C) and high salinity (above 35°C) 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).

Arzul, I., L. Miossec, E. Blanchet, C. Garcia, C. Francois and J.P. Joly. 2006. Bonamia ostreae *and* Ostrea edulis*: a stable host-parasite system in France? In: Proceedings of the 11th Symposium of the International Society for Veterinary Epidemiology and Economics (ISVEE), Cairns, Queensland, Australia, 6 - 11 August 2006, Theme 1 - Aquatic animal epidemiology: Crustacean and shellfish disease session, Volume T1-2.4.4: pp. 869-873.*

Arzul I., Gagnaire B., Bond C., Chollet B., Morga B., Ferrand S., Robert M. & Renault T (2009). Effects of temperature and salinity on the survival of Bonamia ostreae, a parasite infecting flat oysters Ostrea edulis. Dis.Aquat. Org., 85, 67–75

3.3.5 Infection with *Marteilia refringens*

Available data suggests that transmission of the parasite 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 (Audemard et al. 2001). Although salinity and hydrodynamics have less impact on the infection, low salinity and low water renewal seem to be favorable for the parasite (Audemard *et al.* 2001).

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

Audemard, C., A. Barnaud, C.M. Collins, F. Le Roux, P.-G. Sauriau, C. Coustau, P. Blachier and F.C.J. Berthe. 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.

4 Conclusion

4.1 Conclusions ToR 1: Assessment of potential vectors and reservoir species of pathogens of molluscs, listed in Annex II to the AHL

For the assessment of potential vector and reservoir species of pathogens of mollusc relevant scientific publications were collected and assessed according to the SRL protocol described above. The results are presented in table format in the Result section and a more comprehensive overview is added as annex to this External Scientific Report. For the concerning pathogens both a number of mollusc vectors as well as reservoirs could be identified. For correct interpretation of the results the reader should be aware of the considerations below:

- In 2020 on requests of European Commission an assessment on the susceptible species for pathogens of molluscs listed in Annex II of the AHL was carried out by an expert group and described in a scientific report from the EURL "Expert group on susceptibility of mollusc species to EU listed diseases" . The current list of Annex II is however only partly updated with the results from this assessment. This has a number of consequences for the interpretation of the results from the current assessment:
    - A number of mollusc species identified as susceptible in the 2020 EURL report are in the current assessment under review with regard to their vector/reservoir status. However, the current methodology is aimed for identifying vector/reservoir status and not suitable for assessing susceptibility of species. In the result tables these species are marked as *susceptible species** in the column “Classification EURL report”.
    - On the other hand, there are a number of species currently listed in Annex II which should be delisted as susceptible species according to the 2020 EURL report. Hence, they should be assessed as vector or reservoir species but were not included in the current assessment.
        - For *Marteilia refringens* this concerns *O. angasi*, *O. chilensis* and *O. puelchana*.
        - For *B. exitiosa* this concerns *C. virginica* and *O. lurida*. *Crassostrea virginica* is currently listed in Annex II of (EU) 2018/1882 both as susceptible species as well as vector species.
- In light of the current scientific information available the exact composition of the species *Marteilia refringens* is not fully resolved: *sensu lato* the species consist of all genotypes infecting both oysters and mussels. *Sensu stricto* this could be considered the species infecting oysters (type O) only. For the purpose of this assessment *Marteilia refringens* is defined as the species infecting oysters in some papers defined as genotype O. *Marteilia refringens* type M/ *Marteilia maurini* / *Marteilia pararefringens* was not assessed.

- The species *Crassostrea gasar* in Brazil is in this study identified as a reservoir species of *Perkinsus marinus* based on the available publications. However, occurrence of the African species *Crassostrea gasar* in Brazil is based on a misidentification of voucher specimens of *Crassostrea brasiliana* (Amaral & Simone, 2014). Hence, the native Brazilian oyster species *C. brasiliana* should be considered as reservoir species for *Perkinsus marinus*.

VS do Amaral, LRL Simone (2014) Revision of genus Crassostrea (Bivalvia: Ostreidae) of Brazil. Journal of the Marine Biological Association of the United Kingdom doi:10.1017/S0025315414000058

4.2 Conclusions ToR 2: Conditions under which mollusc species shall be regarded as vectors or reservoirs of diseases of mollusc listed in Annex II to the AHL

Salinity and temperature are the main identified drivers of infection intensity, prevalence and disease progression for the listed pathogens of molluscs. However, it is not clear whether less favourable conditions are likely to impact on the transmission of the disease through vector species. However, *Martielia refringens* requires temperatures above 17°C for sporulation to occur. Transmission of *Marteilia refringens* is therefore only possible if conditions are favourable for sporulation. Temperatures at the source and destination site may prevent transmission of the parasite.

In contrast to *Marteilia refringens* development of disease with *Mikrocytos mackini* occurs most effectively when the temperature is below 10°C for long periods. The parasite can, however, survive for long periods at 17°C and the disease has been shown to progress when oysters are returned to low temperatures it is therefore unlikely that high temperature at the source or destination will prevent spread of the parasite.

Intensification of *Perkinsus marinus* infections are favoured by high temperatures and higher salinities, however the salinity tolerance of the parasite is broad and the parasite will survive even at a salinity of 9psu. There is no information regarding how long it will persist in vector species and hence the possibility of reactivation of the parasite in a vector species when conditions become more favourable cannot be ruled out.

Infection with both *Bonamia ostrea* and *Bonamia exitiosa* occur over a wide geographic range suggesting that environmental conditions are unlikely to impact significantly on their transmission.

In conclusion, there is little conclusive evidence that environmental factors will prove a significant impediment to spread of any of the listed mollusc diseases, although certain conditions are more likely to favour disease development and assist in transmission.

References

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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. Bull Eur Ass Fish Pathol, 27:148-156

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Annex A – List of known susceptible species for mollusc pathogens

Listed susceptible species for each of the listed mollusc diseases according to the Annex to Implementing Regulation 2018/1882 as amended by Implementing Regulation (EU) 2022/925.

Listed diseases	SUSCEPTIBLE SPECIES Scientific names	SUSCEPTIBLE SPECIES Common names
Infection with Mikrocytos mackini	*Crassostrea gigas*	Pacific oyster
	Crassostrea sikamea	Kumamoto oyster
	Ostrea edulis	European flat oyster
Infection with Perkinsus marinus	*Crassostrea gigas*	Pacific oyster
	Crassostrea virginica	Eastern oyster
Infection with Bonamia exitiosa	*Crassostrea ariakensis*	Suminoe oyster
	Crassostrea virginica	Eastern oyster
	Ostrea puelchana	Argentinean oyster
	Ostrea angasi	Australian mud oyster
	Ostrea chilensis	Chilean flat oyster
	Ostrea equestris	Crested oyster
	Ostrea edulis	European flat oyster
	Ostrea lurida	Olympia oyster
Infection with Bonamia ostreae	*Crassostrea ariakensis*	Suminoe oyster
	Ostrea chilensis	Chilean flat oyster
	Ostrea edulis	European flat oyster
Infection with Marteilia refringens	*Ostrea angasi*	Australian mud oyster
	Ostrea chilensis	Chilean flat oyster
	Ostrea edulis	European flat oyster
	Ostrea puelchana	Argentinean oyster

Annex B – Search strings

Summary of the results

	CAB Abstracts	Web of Science	Scopus	After de-dup (estimation)
Infection with Mikrocytos mackini	26	38	29	45
Infection with Bonamia exitiosa	80	75	78	94
Infection with Marteilia refringens	65	83	69	109
Infection with Perkinsus marinus	261	503	378	584
Infection with Bonamia ostreae	143	156	131	210
			After de-duplication:	883
			After adding the EURL report:	884

Infection with Mikrocytos mackini

Terms
Mikrocytos mackini M mackini Mikrocytoses Mikrocytosis

CAB Abstracts

Set	Query	Results
#1	TS=("Mikrocytos mackini" OR "M mackini" OR Mikrocytoses OR Mikrocytosis)	34
#2	TS=(detect* OR experiment* OR isolat* OR occurren* OR susceptib* OR transmit* OR transmiss* OR inoculat* OR immersion OR ingestion OR cohabitation OR "co habitation")	3,854,967
#3	#1 AND #2	26

Web of Science Core Collection

Set	Query	Results
#1	TS=("Mikrocytos mackini" OR "M mackini" OR Mikrocytoses OR Mikrocytosis)	565
#2	TS=(detect* OR experiment* OR isolat* OR occurren* OR susceptib* OR transmit* OR transmiss* OR inoculat* OR immersion OR ingestion OR cohabitation OR "co habitation")	12,014,499
#3	#1 AND #2 and Meeting Abstract (Exclude – Document Types) and English (Languages)	38

Scopus

Set	Query	Results
#1	TITLE-ABS-KEY("Mikrocytos mackini" OR "M mackini" OR Mikrocytoses OR Mikrocytosis)	34

#2	TITLE-ABS-KEY (detect* OR experiment* OR isolat* OR occurren* OR susceptib* OR transmit* OR transmiss* OR inoculat* OR immersion OR ingestion OR cohabitation OR "co habitation")	22,552,050
#3	#1 AND #2	29

Infection with Bonamia exitiosa

Terms
Bonamia exitiosa B exitiosa Bonamia exitiosus B exitiosus Bonamiosis bonamioses

CAB Abstracts

Set	Query	Results
#1	TS=("Bonamia exitiosa" OR "B exitiosa" OR "Bonamia exitiosus" OR "B exitiosus" OR Bonamiosis OR bonamioses)	1101
#2	TS=(detect* OR experiment* OR isolat* OR occurren* OR susceptib* OR transmit* OR transmiss* OR inoculat* OR immersion OR ingestion OR cohabitation OR "co habitation")	3,854,967
#3	#1 AND #2 and English or Norwegian or Polish or Unspecified or Slovenian (Languages)	80

Web of Science Core Collection

Set	Query	Results
#1	TS=("Bonamia exitiosa" OR "B exitiosa" OR "Bonamia exitiosus" OR "B exitiosus" OR Bonamiosis OR bonamioses)	117
#2	TS=(detect* OR experiment* OR isolat* OR occurren* OR susceptib* OR transmit* OR transmiss* OR inoculat* OR immersion OR ingestion OR cohabitation OR "co habitation")	12,014,499
#3	#1 AND #2	75

Scopus

Set	Query	Results
#1	TITLE-ABS-KEY("Bonamia exitiosa" OR "B exitiosa" OR "Bonamia exitiosus" OR "B exitiosus" OR Bonamiosis OR bonamioses)	100
#2	TITLE-ABS-KEY (detect* OR experiment* OR isolat* OR occurren* OR susceptib* OR transmit* OR transmiss* OR inoculat* OR immersion OR ingestion OR cohabitation OR "co habitation")	22,522,050
#3	(TITLE-ABS-KEY (detect* OR experiment* OR isolat* OR occurren* OR susceptib* OR transmit* OR transmiss* OR inoculat* OR immersion OR ingestion OR cohabitation OR "co habitation")) AND (TITLE-ABS-KEY ("Bonamia exitiosa" OR "B exitiosa" OR "Bonamia exitiosus" OR "B exitiosus" OR bonamiosis OR bonamioses)) AND (LIMIT-TO (LANGUAGE , "English") OR LIMIT-TO (LANGUAGE , "Polish") OR LIMIT-TO (LANGUAGE , "Spanish"))	78

Infection with Marteilia refringens

Terms
Marteilia maurini M maurini Marteilia refringens M refringens Marteiliosis marteilioses

CAB Abstracts

Set	Query	Results
#1	TS=("Marteilia maurini" OR "M maurini" OR "Marteilia refringens" OR "M refringens" OR Marteiliosis OR marteilioses)	133
#2	TS=(detect* OR experiment* OR isolat* OR occurren* OR susceptib* OR transmit* OR transmiss* OR inoculat* OR immersion OR ingestion OR cohabitation OR "co habitation")	3,854,967

#3	#1 AND #2 and Unspecified or Spanish or Slovenian or Polish or French or Norwegian or English (Languages)	65

Web of Science Core Collection

Set	Query	Results
#1	TS=("Marteilia maurini" OR "M maurini" OR "Marteilia refringens" OR "M refringens" OR Marteiliosis OR marteilioses) AND LA=English	120
#2	TS=(detect* OR experiment* OR isolat* OR occurren* OR susceptib* OR transmit* OR transmiss* OR inoculat* OR immersion OR ingestion OR cohabitation OR "co habitation")	12,014,499
#3	#1 AND #2 and Meeting Abstract (Exclude – Document Types) and English or French or Polish or Spanish (Languages)	83

Scopus

Set	Query	Results
#1	TITLE-ABS-KEY ("Marteilia maurini" OR "M maurini" OR "Marteilia refringens" OR "M refringens" OR Marteiliosis OR marteilioses)	95
#2	TITLE-ABS-KEY (detect* OR experiment* OR isolat* OR occurren* OR susceptib* OR transmit* OR transmiss* OR inoculat* OR immersion OR ingestion OR cohabitation OR "co habitation")	22,552,050
#3	(TITLE-ABS-KEY ("Marteilia maurini" OR "M maurini" OR "Marteilia refringens" OR "M refringens" OR marteiliosis OR marteilioses)) AND (TITLE-ABS-KEY (detect* OR experiment* OR isolat* OR occurren* OR susceptib* OR transmit* OR transmiss* OR inoculat* OR immersion OR ingestion OR cohabitation OR "co habitation"))	69

Infection with Perkinsus marinus

Terms
Perkinsus marinus P marinus Dermocystidium marinum D marinum Perkinoses Perkinosis

CAB Abstracts

Set	Query	Results
#1	TS=("Perkinsus marinus" OR "P marinus" OR "Dermocystidium marinum" OR "D marinum" OR Perkinoses OR Perkinosis)	447
#2	TS=(detect* OR experiment* OR isolat* OR occurren* OR susceptib* OR transmit* OR transmiss* OR inoculat* OR immersion OR ingestion OR cohabitation OR "co habitation")	3,854,967
#3	#1 AND #2 and English or French or Portuguese (Languages)	261

Web of Science Core Collection

Set	Query	Results
#1	TS=("Perkinsus marinus" OR "P marinus" OR "Dermocystidium marinum" OR "D marinum" OR Perkinoses OR Perkinosis)	1,004
#2	TS=(detect* OR experiment* OR isolat* OR occurren* OR susceptib* OR transmit* OR transmiss* OR inoculat* OR immersion OR ingestion OR cohabitation OR "co habitation")	12,014,499
#3	#1 AND #2 and Meeting Abstract (Exclude – Document Types) and English or Spanish or French (Languages)	503

Scopus

Set	Query	Results
#1	TITLE-ABS-KEY ("Perkinsus marinus" OR "P marinus" OR "Dermocystidium marinum" OR "D marinum" OR perkinoses OR perkinosis)	679
#2	TITLE-ABS-KEY (detect* OR experiment* OR isolat* OR occurren* OR susceptib* OR tra	22,552,050

	nsmit* OR transmiss* OR inoculat* OR immersion OR ingestion OR coh abitation OR "co habitation")	
#3	(TITLE-ABS-KEY (detect* OR experiment* OR isolat* OR occurren* OR susceptib* OR tra nsmit* OR transmiss* OR inoculat* OR immersion OR ingestion OR coh abitation OR "co habitation")) AND (TITLE-ABS-KEY ("Perkinsus marinus" OR "P marinus" OR "Dermocystidium marinum" OR "D marinum" OR perkinoses OR perkinosis))	378

Infection with Bonamia ostreae

Terms
Bonamia ostreae B ostreae Bonamiosis bonamioses

CAB Abstracts

Set	Query	Results
#1	TS=("Bonamia ostreae" OR "B ostreae" OR Bonamiosis OR bonamioses)	211
#2	TS=(detect* OR experiment* OR isolat* OR occurren* OR susceptib* OR transmit* OR transmiss* OR inoculat* OR immersion OR ingestion OR cohabitation OR "co habitation")	3,854,967
#3	#1 AND #2 and Slovenian or Polish or Italian or Unspecified or French or Norwegian or English (Languages)	143

Web of Science Core Collection

Set	Query	Results
#1	TS=("Bonamia ostreae" OR "B ostreae" OR Bonamiosis OR bonamioses)	2534
#2	TS=(detect* OR experiment* OR isolat* OR occurren* OR susceptib* OR transmit* OR transmiss* OR inoculat* OR immersion OR ingestion OR cohabitation OR "co habitation")	12,014,499
#3	#1 AND #2 and Meeting Abstract (Exclude – Document Types)	156

Scopus

Set	Query	Results
#1	TITLE-ABS-KEY ("Bonamia ostreae" OR "B ostreae" OR Bonamiosis OR bonamioses)	190
#2	TITLE-ABS-KEY (detect* OR experiment* OR isolat* OR occurren* OR susceptib* OR tra nsmit* OR transmiss* OR inoculat* OR immersion OR ingestion OR coh abitation OR "co habitation")	22,552,050
#3	(TITLE-ABS-KEY ("Bonamia ostreae" OR "B ostreae" OR bonamiosis OR bonamioses)) AND (TITLE-ABS-KEY (detect* OR experiment* OR isolat* OR occurren* OR susceptib* OR tra nsmit* OR transmiss* OR inoculat* OR immersion OR ingestion OR coh abitation OR "co habitation"))	131

Annex C – Eligibility Screening forms

REMARK: Exclusion of papers will be on form level, meaning, if one question is leading to exclusion, it is not needed to answer the remaining questions in the form. The order of answering the questions is arbitrary, meaning, if the reviewing sees that the answer to one of the questions is obviously "no", he or she can address that question first

Table AVI-1: Level 1 screening (tittle and abstract)

Question	*Type*	*Answer*	*Action*
is the abstract written in an EU language?	*Radio**	*Yes* *No* *Not sure*	*Include* *Exclude* *Include*
Is this a primary research study?	*Radio*	*Yes* *No* *Not sure*	*Include* *Exclude* *Include*
Species X belonging to either fish, crustaceans or molluscs?	*Radio*	*Yes* *No* *Not sure*	*Include* *Exclude* *Include*
Does the paper report a study that included one or more pathogens listed in Table 1 of the Review Protocol?	*Radio*	*Yes* *No* *Not sure*	*Include* *Exclude* *Include*
Does this study investigate at least one Species X, which is NOT a known susceptible species?	*Radio*	*Yes* *No* *Not sure*	*Include* *Exclude* *Include*
Does the paper describe an	*Radio*	*Experimental infection* *Field detection* *Experimental infection and Field detection* *None of the above* *Not sure*	*Include and continue with experimental infection eligibility of full text* *Include and continue with field detection eligibility of full text* *Include in both experimental and field studies eligibility screening* *Exclude* *Include in both experimental and field studies eligibility screening*

*Radio Button questions *allow respondents to select a single choice from a list*.

Table AVI-2: Level 2 screening (full text)-experimental infections

Question	*Type*	*Answer*	*Action*
Is the full text available?	*Radio*	*Yes* *No*	*Include* *Exclude*
Is this primary research?	*Radio*	*Yes* *No*	*Include* *Exclude*
Is the full text in any EU language?	*Radio*	*Yes* *No*	*Include* *Exclude*
Is the research published in a peer reviewed journal?	*Radio*	*Yes* *No*	*Include* *Exclude*
Does the paper report a study that included one or more pathogens listed in Table 1 of the Review Protocol?	*Radio*	*Yes* *No* *Not identified*	*Include* *Exclude* *Exclude*

Does this study investigate at least one Species X, which is NOT a known susceptible species?	*Radio*	*Yes* *No* *Not identifie*	*Include* *Exclude* *Exclude*
Is pathogen A identified in/on species X?	*Radio*	*Yes* *No*	*Include* *Exclude*
Has the transmission from species X to Y been investigated in the study?	*Radio*	*Yes* *No*	*Include* *Exclude*
Was only single species X be assessed at the time per study group in the same tank (same experimental unit)	*Radio*	*Yes* *No*	*Include* *Exclude*
Is species Y experimentally exposed to pathogen A from Species X, either by immersion, cohabitation, ingestion or any other direct or indirect transmission?	*Radio*	*Yes* *No*	*Include* *Exclude*

*Radio Button questions *allow respondents to select a single choice from a list*.

Table AVI-3: Level 2 screening (full text)-field studies

Question	*Type*	*Answer*	*Action*
Is the full text available	*Radio*	*Yes* *No*	*Include* *Exclude*
Is the full text written in any EU language	*Radio*	*Yes* *No*	*Include* *Exclude*
Is this primary research?	*Radio*	*Yes* *No*	*Include* *Exclude*
Is the research published in a peer reviewed journal?	*Radio*	*Yes* *No*	*Include* *Exclude*
Does the paper report a study that included one or more pathogens listed in Table 1 of the Review Protocol?	*Radio*	*Yes* *No*	*Include* *Exclude*
Does this study investigate at least one Species X, which is NOT a known susceptible species?	*Radio*	*Yes* *No*	*Include* *Exclude*
Is pathogen A identified in/on species X	*Radio*	*Yes* *No*	*Include* *Exclude*

*Radio Button questions *allow respondents to select a single choice from a list*.

Annex D – Data extraction forms

Table AVI-4: Data extraction form- Experimental infections

Field name	Data type	Data format notes	Parameter number
refID	Numerical	Unique identification of a reference.	automatic
FullReference	Free-text	Full reference in the format: "all authors, YEAR, publication title, journal, issue, pages".	Generated by Distiller
groupID	Numerical	This field identifies UNIQUE STUDY GROUPS within each paper. It identifies experimental groups within the paper, so that during data analyses, it is possible to recognize multiple data forms that were filled to document different outcomes for the same group of animals.	This is a parameter generated by the reviewer for assessment purposes
Agent	Categorical	Disease agent.	0
Data extracted pertaining Species X			
Species	Categorical	Collected in the form as a RADIO LIST.	Not in Annex 5
SpeciesTestUsed	Categorical	Identification method of the aquatic animal species • Morphological characteristics • Laboratory Methods • others	1x
ageCat	categorical	Life stage of X (update categories) • Egg • Larvae • Fry • Juvenile • Adult	2x

labTestUsed1	Categorical	The specific test used to confirm infection. • Cell Culture • PCR • Sequencing • Histology • Immunohistochemistry (IHC) • In Situ Hybridisation (ISH) • Other: please specify	4x
labTestUsedC1	Text	Any additional comments about the laboratory test above.	4bx (more description, e.g., of cell culture used…) To be decided up to which detail info is needed, CT values, etc.
labTestUsed2	Categorical	The specific test used to confirm infection. • Cell Culture • PCR • Sequencing • Histology • Immunohistochemistry (IHC) • In Situ Hybridisation (ISH) • Other: please specify	4x
labTestUsedC2	Text	Any additional comments about the laboratory test above.	4bx (more description, e.g., of cell culture used…) To be decided up to which detail info is needed, CT values, etc.
labTestUsed3	Categorical	The specific test used to confirm infection. • Cell Culture • PCR • Sequencing • Histology • Immunohistochemistry (IHC) • In Situ Hybridisation (ISH) • Other: please specify	4x
labTestUsedC3	Text	Any additional comments about the laboratory test above.	4bx (more description, e.g. of cell culture used…) To be decided up to which detail info is needed, CT values, etc.
Matrix	Categorical	Matrix sampled to test for the presence of the agent. This field is categorical, but "checkbox", not radio, to allow multiple to be checked. Therefore, in the datasets each value becomes its own column. In the data cleaning process, we have	5x

durationPI	Numerical	Duration of the experiment (start to end of experiment). See data interpretation comments further below.	Not in Annex 5, but needed in case you want to see if the experiment was truncated
minDetect	Numerical	First day in which the agent was detected in the specific listed matrix in any animal in the group (in days post exposure)	10x
maxDetect	Numerical	Last day in which the agent was detected in the specific listed matrix in any animal in the group (In days post exposure)	10x
temp	Numerical	Average degrees Celsius in which Species X is kept	
Data extracted pertaining Species Y			
Species	Categorical	Collected in the form as a RADIO LIST.	Not in Annex 5 -to be included
SpeciesTestUsed	Categorical	Identification method of the aquatic animal species • Morphological characteristics • Laboratory Methods • others	1y (generate newly)
ageCat	categorical	Life stage of X (update categories) • Egg • Larvae • Fry • Juvenile • Adult	2y
sampUnitSize	Numerical	Number of animals in the study group.	Not in Annex 5
labTestUsed1	Categorical	The specific test used to confirm infection. • Cell Culture • PCR • Sequencing • Histology • Immunohistochemistry (IHC) • In Situ Hybridisation (ISH)	4y
labTestUsedC1	Text	Any additional comments about the laboratory test above.	4by (more description, e.g. of cell culture used…) To be decided up to which detail info is needed, CT values, etc.

labTestUsed2	Categorical	The specific test used to confirm infection. • Cell Culture • PCR • Sequencing • Histology • Immunohistochemistry (IHC) • In Situ Hybridisation (ISH)	4y
labTestUsedC2	Text	Any additional comments about the laboratory test above.	4by (more description, e.g. of cell culture used…) To be decided up to which detail info is needed, CT values, etc.
labTestUsed3	Categorical	The specific test used to confirm infection. • Cell Culture • PCR • Sequencing • Histology • Immunohistochemistry (IHC) • In Situ Hybridisation (ISH)	4y
labTestUsedC3	Text	Any additional comments about the laboratory test above.	4by (more description, e.g. of cell culture used…) To be decided up to which detail info is needed, CT values, etc.
Matrix	Categorical	Matrix sampled to test for the presence of the agent. This field is categorical, but "checkbox", not radio, to allow multiple to be checked. Therefore, in the datasets each value becomes its own column. In the data cleaning process, we will concatenate all values using the separator "//". That is, multiple routes will show, as, for example,	5y

Table AVI-5: Data extraction form-Field infections

Field name	Data type	Data format notes	Parameter number
refID	Numerical	Unique identification of a reference.	automatic
FullReference	Free-text	Full reference in the format: "all authors, YEAR, publication title, journal, issue, pages".	Generated by Distiller

groupID	Numerical	This field identifies UNIQUE STUDY GROUPS within each paper. It identifies experimental groups within the paper, so that during data analyses, it is possible to recognize multiple data forms that were filled to document different outcomes for the same group of animals.	This is a parameter generated by the reviewer for assessment purposes
agent	Categorical	Disease agent. Collected in the form as a RADIO LIST.	0
Data extracted pertaining Species X			
Species	Categorical	Collected in the form as a RADIO LIST.	Not in Annex 5
SpeciesTestUsed	Categorical	Identification method of the aquatic animal species • Morphological characteristics • Laboratory Methods • others	1x
ageCat	categorical	Life stage of X (update categories) • Egg • Larvae • Fry • Juvenile • Adult	2x
sampUnitSize	Numerical	Number of animals in the study group.	Not in Annex 5
Country X		Country of origin where species X was caught, when not bred in laboratory	
Waterbody X		Name of waterbody where species X was caught, when not bred in laboratory	
labTestUsed1	Categorical	The specific test used to confirm infection. • Cell Culture • PCR • Sequencing • Histology • Immunohistochemistry (IHC) • In Situ Hybridisation (ISH)	4x
labTestUsedC1	Text	Any additional comments about the laboratory test above.	4bx (more description, e.g., of cell culture used…) To be decided up to which detail info is needed, CT values, etc.

labTestUsed2	Categorical	The specific test used to confirm infection. • Cell Culture • PCR • Sequencing • Histology • Immunohistochemistry (IHC) • In Situ Hybridisation (ISH)	4x
labTestUsedC2	Text	Any additional comments about the laboratory test above.	4bx (more description, e.g., of cell culture used…) To be decided up to which detail info is needed, CT values, etc.
labTestUsed3	Categorical	The specific test used to confirm infection. • Cell Culture • PCR • Sequencing • Histology • Immunohistochemistry (IHC) • In Situ Hybridisation (ISH)	4x
labTestUsedC3	Text	Any additional comments about the laboratory test above.	4bx (more description, e.g., of cell culture used…) To be decided up to which detail info is needed, CT values, etc.
Matrix	Categorical	Matrix sampled to test for the presence of the agent. This field is categorical, but "checkbox", not radio, to allow multiple to be checked. Therefore, in the datasets each value becomes its own column. In the data cleaning process, we have concatenated all	5x