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Actividades de las comisiones especializadas

COMISIÓN DE NORMAS SANITARIAS PARA LOS ANIMALES ACUÁTICOS

Enmiendas propuestas al
Código Sanitario para los Animales Acuáticos y al Manual de las Pruebas de Diagnóstico para los Animales Acuáticos

Organización Mundial de Sanidad Animal

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Introducción

1. Desde la 92.ª Sesión General de mayo de 2025, la Comisión de Normas Sanitarias para los Animales Acuáticos (Comisión para los Animales Acuáticos) se reunió en dos oportunidades, del 17 al 24 de septiembre de 2025 y del 11 al 18 de febrero de 2026. Entre sus actividades, y de conformidad con su plan de trabajo, la Comisión avanzó en la elaboración de textos nuevos y revisados del *Código Sanitario para los Animales Acuáticos* (*Código Acuático*) y del *Manual de las Pruebas de Diagnóstico para los Animales Acuáticos* (*Manual Acuático*). Los detalles de las reuniones de la Comisión para los Animales Acuáticos están disponible en el sitio web de los Delegados y en el sitio web de la OMSA.

2. El presente documento brinda información de contexto para cada uno de los textos nuevos y revisados del *Código Acuático* y del *Manual Acuático* que se propondrán para adopción en la 93.ª Sesión General, en mayo de 2026. En la revisión de estos textos, la Comisión tuvo en cuenta los comentarios enviados por los Miembros y las organizaciones internacionales con un acuerdo de cooperación con la OMSA, las recomendaciones de diversos informes de grupos *ad hoc*, así como las observaciones de los expertos. Igualmente, la Comisión para los Animales Acuáticos trabajó en estrecha colaboración con la Comisión de Normas Sanitarias para los Animales Terrestres y con la Comisión de Normas Biológicas en actividades relevantes.

3. Las más recientes consideraciones de la Comisión a los comentarios recibidos sobre los proyectos de texto distribuidos para comentario se facilitan en los informes de la Comisión de septiembre de 2025 y de febrero de 2026. **La Comisión insta a los Miembros a consultar dichos informes para obtener más detalles sobre los textos enmendados que se propondrán para adopción**.

4. Las enmiendas del *Código Acuático* y del *Manual Acuático* que figuran en los Anexos 5 a 10, y 18 a 22 se propondrán para adopción en la 93.ª Sesión General. Los números de anexos utilizados en este documento coinciden con los indicados en el informe de febrero de 2026 de la Comisión. Las enmiendas propuestas se señalan del modo habitual con “subrayado doble” y “texto tachado”.

1. Textos del *Código Acuático* que se propondrán para adopción

1.1. Definición del glosario de ‘animales acuáticos ornamentales’ (Anexo 5)

5. Esta definición del glosario aclara la distinción entre los animales acuáticos ornamentales que se mantienen como animales de compañía y aquellos que se comercializan para su venta con este propósito. La definición se ha revisado para ajustarla a la redacción propuesta en el informe de febrero de 2025. Esta definición actualizada también es coherente con la decisión adoptada en el informe de septiembre de 2025, en el que se concluyó que los animales acuáticos mantenidos como animales de compañía no suponen un riesgo significativo de transmisión de enfermedades y, por lo tanto, no deben incluirse en el ámbito de aplicación de la definición. Además, teniendo en cuenta que el capítulo 5.12, «Movimientos de animales acuáticos ornamentales», se centra en los desplazamientos de este tipo de animales, la exclusión actual de los animales de compañía dentro de los hogares es adecuada.

6. La definición revisada del Glosario ‘animales acuáticos ornamentales’ circuló dos veces para comentario, la primera vez en el informe de la Comisión de septiembre de 2025.

7. La definición revisada del Glosario de ‘animales acuáticos ornamentales se presenta en el **Anexo 5** y se propondrá para adopción en la 93.ª Sesión General en mayo de 2026.

1.2. Capítulo 4.7. ‘Vacío sanitario en acuicultura’ (Anexo 6)

8. El Capítulo 4.7. se revisó con el fin de armonizarlo con los nuevos proyectos de Capítulo 4.10. ‘Preparación ante emergencias sanitarias’ y 4.11. ‘Gestión ante brote de enfermedad’ adoptados en la 92.ª Sesión General, y con miras a mejorar la legibilidad y claridad.

9. El Capítulo revisado 4.7. ‘Vacío sanitario en acuicultura’ circuló tres veces para comentario, la primera vez en el informe de la Comisión de febrero de 2025.

10. El Capítulo revisado 4.7. 'Vacío sanitario en acuicultura' se presenta en el **Anexo 6** y se propondrá para adopción en la 93.ª Sesión General en mayo de 2026.

1.3. Artículo 9.2.2. del Capítulo 9.2. 'Infección por Aphanomyces astaci (plaga del cangrejo de río)' (Anexo 7)

11. El Artículo revisado 9.2.2. del Capítulo 9.2. 'Infección por *Aphanomyces astaci* (plaga del cangrejo de río)' incluye especies susceptibles adicionales. Las referencias se remitieron a examen de un experto en la materia que proporcionó puntuaciones actualizadas. En el caso de *Austropotamobius torrentium*, se consideró que las pruebas eran suficientes para asignarle una puntuación de 1, y la Comisión acordó que se consideraría especie susceptible. Una vez clasificada *A. torrentium* como especie susceptible, la Comisión estimó la posibilidad de aplicar el artículo 1.5.9. 4Inclusión de especies susceptibles con un rango taxonómico de género o superior4, utilizando los criterios esbozados en su informe de septiembre de 2024 (punto 6.5.1., página 18). Tras aplicar el Artículo 1.5.9., incluyó *Austropotamobius spp*. a nivel de género. En el caso de *Cherax destructor*, las pruebas no fueron suficientes para asignarle una puntuación suficiente de susceptibilidad.

12. El Artículo revisado 9.2.2. del Capítulo 9.2. 'Infección por *Aphanomyces astaci* (plaga del cangrejo de río)' circuló dos veces para comentario, la primera vez en el informe de la Comisión de septiembre de 2025.

13. El Artículo revisado 9.2.2. del Capítulo 9.2. 'Infección por *A. astaci* (plaga del cangrejo de río) se presenta en el **Anexo 7** y se propondrá para adopción en la 93.ª Sesión General en mayo de 2026.

1.4. Artículos 10.3.5. y 10.3.6. del Capítulo 10.3. 'Infección por Gyrodactylus salaris' (Anexo 8)

14. Se revisaron los artículos 10.3.5. y 10.3.6. del Capítulo 10.3. 'Infección por *Gyrodactylus salaris*' para mejorar su claridad, a tenor de las observaciones de los Miembros. Los artículos revisados aclaran que el texto añadido relativo al uso del procedimiento 2 (ausencia histórica) para declarar la ausencia de *Gyrodactylus salaris* no busca restringir su aplicación a monocultivos de una sola especie, sino más bien hacer hincapié en que las especies capaces de presentar signos clínicos de la enfermedad deben incluirse cuando se recurre a la vigilancia pasiva. Se recordó a los Miembros que tuvieran en cuenta el Capítulo 1.4. del *Código Acuático* —en particular el Artículo 1.4.8., que exige que las condiciones permitan la manifestación clínica, que los signos de la enfermedad den lugar a una investigación, que las especies de cultivo susceptibles se sometan a una observación suficiente y que las poblaciones silvestres sean objeto de seguimiento o estén vinculadas a las poblaciones de cría. Por consiguiente, el procedimiento 2 sólo es válido cuando hay especies susceptibles que manifiestan signos clínicos y que se incluyen en el diseño de la vigilancia. En respuesta a las preocupaciones sobre la naturaleza de alto riesgo del parásito, la Comisión revisó los Artículos 10.3.5. y 10.3.6. a efectos de aclarar el uso adecuado del procedimiento 2 y destacar las orientaciones de la sección 2.2.5. del *Manual Acuático* de este capítulo específico de la enfermedad; en lo que respecta a los reservorios de infección en animales acuáticos, señaló que algunas cepas de peces no muestran signos visibles de la enfermedad, lo que hace que la vigilancia pasiva por sí sola sea insuficiente a menos que se incluyan especies clínicamente susceptibles. La Comisión también examinó los comentarios de los Miembros sobre el período exigido para las condiciones elementales de bioseguridad del procedimiento 2. Teniendo en cuenta los factores biológicos, ecológicos y de producción específicos de *G. salaris* que reducen la probabilidad anual de detección, la Comisión concluyó que un período de observación más largo de 15 años está científicamente justificado para alcanzar una confianza ≥95 % en la ausencia de la enfermedad. Esto se ajusta a lo dispuesto en el Artículo 1.4.9, que establece que el período mínimo de las condiciones elementales de bioseguridad debe ampliarse más allá del período predeterminado de diez años (basado en una probabilidad anual de detección del 30 %) cuando lo justifiquen factores específicos de la enfermedad.

15. Los Artículos revisados 10.3.5. y 10.3.6. del Capítulo 10.3. 'Infección por *G. salaris*' circularon dos veces para comentario, la primera vez en el informe de la Comisión de septiembre de 2025.

16. Los Artículos revisados 10.3.5. y 10.3.6. del Capítulo 10.3. 'Infección por *G. salaris'* are presented in **Anexo 8** y se propondrá para adopción en la 93.ª Sesión General en mayo de 2026.

1.5. Artículo 10.4.9. del Capítulo 10.4. 'Infección por la anemia infecciosa del salmón' (Anexo 9)

17. Las revisiones propuestas en el Artículo 10.4.9 del Capítulo 10.4, 'Infección por la anemia infecciosa del salmón' responden a las inquietudes de los Miembros en relación con el período de las condiciones elementales de bioseguridad y de vigilancia específica en el capítulo correspondiente de enfermedad del *Código Acuático*, tras la revisión del capítulo 1.4. 'Vigilancia de las enfermedades de los animales acuáticos'. Se señaló que, si bien el virus de la anemia infecciosa del salmón con supresión en la HPR (ISAV HPR0) se detecta transitoriamente en poblaciones estables de salmón del Atlántico y, más fácilmente, durante acontecimientos estresantes como el desove, puede no detectarse en un año. Asimismo, la epidemiología del virus de la anemia infecciosa del salmón con supresión en la HPR en instalaciones en tierra es limitada y, debido a esta incertidumbre, se requiere un período más largo para considerar un compartimento libre de infección por el virus de la anemia infecciosa del salmón con supresión en la HPR. Se revisó esta información y se consultó a los expertos de los laboratorios de referencia, que apoyaron la baja probabilidad de detección precoz del virus de la anemia infecciosa del salmón con supresión en la HPR. En base a esta información, la Comisión convino en que un período mínimo de dos años era apropiado para la vigilancia específica de un compartimento y se actualizó la evaluación de los períodos en consecuencia (disponible en el sitio web).

18. El Artículo 10.4.9. del Capítulo 10.4. 'Infección por la anemia infecciosa del salmón' circuló dos veces para comentario, la primera vez en el informe de la Comisión de septiembre de 2025.

19. El Artículo 10.4.9. del Capítulo 10.4. 'Infección por la anemia infecciosa del salmón' se presenta en el **Anexo 9** y se propondrá para adopción en la 93.ª Sesión General en mayo de 2026.

1.6. Actualización de los nombres de virus de los agentes patógenos de las enfermedades de la lista de la OMSA (Anexo 10)

20. Tal y como lo consignase en su informe de febrero de 2025, la Comisión decidió armonizar el *Código Acuático* y el *Manual Acuático* con la nomenclatura y la taxonomía actualizada del Comité Internacional de Taxonomía de Virus (ICTV por sus siglas en inglés). En su reunión de septiembre de 2025, la Comisión revisó las clasificaciones actuales del ICTV comparándolas con los nombres utilizados en las publicaciones de la OMSA y determinó que 12 de los 18 agentes patógenos virales incluidos en la lista de enfermedades de la OMSA requerían actualizarse en los capítulos de enfermedades de crustáceos, peces y moluscos. La Comisión indicó que la referencia a la infección por el virus de la cabeza amarilla genotipo 1 debía modificarse para adoptar el mismo formato que las demás enfermedades virales.

21. Los nombres científicos revisados para los virus de los agentes patógenos de las enfermedades de la lista de la OMSA se difundieron dos veces para comentario, la primera vez en el informe de la Comisión de septiembre de 2025.

22. Los nombres científicos revisados para los nombres de virus de los agentes patógenos de las enfermedades de la lista de la OMSA se presentan en el **Anexo 10** y se propondrán para adopción en la 93.ª Sesión General en mayo de 2026.

2. Textos del *Manual Acuático* que se propondrán para adopción

2.1. Secciones 2.2.1. y 2.2.2. del Capítulo 2.2.2. 'Infección por Aphanomyces astaci *(plaga del cangrejo de río)' (Anexo 18)*

23. Se proponen revisiones de la lista de especies susceptibles de las Secciones 2.2.1. y 2.2.2. del Capítulo 2.2.2. 'Infección por *Aphanomyces astaci* (plaga del cangrejo de río)'. El Grupo *ad hoc* sobre la susceptibilidad de las especies de crustáceos a la infección por las enfermedades de la lista de la OMSA se reunió en abril de 2025 con el fin de seguir aplicando los criterios del Capítulo 1.5. y determinar las especies susceptibles, centrando su labor en poner al día la evaluación de 2015 para *Aphanomyces astaci* (plaga del cangrejo del río). En su reunión de septiembre de 2025., la Comisión revisó el extenso informe del grupo *ad hoc* y acordó aplicar el Artículo 1.5.9. 'Inclusión de especies susceptibles con un rango taxonómico de género o superior' y modificar la lista de especies susceptibles del Artículo 9.2.2. de acuerdo con las recomendaciones del grupo *ad hoc*. Decidió que *Faxonius* spp. y *Procambarus* spp. debían figurar en la lista a nivel de género y confirmó la susceptibilidad de *Faxonius* spp. En el *Código Acuático* se introdujeron los cambios pertinentes en el Capítulo 9.2. 'Infección por *A. astaci'.*

24. El informe del Grupo *ad hoc* sobre la susceptibilidad de las especies de crustáceos a la infección por las enfermedades de la lista de la OMSA reunido en abril de 2025 ofrece detalles sobre las evaluaciones efectuadas con miras a determinar las especies susceptibles propuestas para inclusión.

25. Las secciones revisadas circularon dos veces para comentario, la primera vez en el informe de la Comisión de septiembre de 2025.

26. Las secciones revisadas 2.2.1. y 2.2.2. del Capítulo 2.2.2. 'Infección por *A. astaci* (plaga del cangrejo de río)' se presentan en el **Anexo 18** y se propondrán para adopción en la 93.ª Sesión General en mayo de 2026.

2.2. Capítulo 2.4.5. 'Infección por Perkinsus marinus' *(Anexo 19)*

27. Se propone una revisión exhaustiva del Capítulo 2.4.5. 'Infección por *Perkinsus marinus'* reformateado utilizando el nuevo modelo de capítulo específico de enfermedad.

28. El Capítulo revisado circuló dos veces para comentario, la primera vez en el informe de la Comisión de septiembre de 2025.

29. El Capítulo revisado 2.4.5. 'Infección por *Perkinsus marinus*' se presenta en el **Anexo 19** y se propondrá para adopción en la 93.ª Sesión General en mayo de 2026.

2.3. Capítulo 2.4.6. 'Infección por Perkinsus olseni *(Anexo 19)*

30. Se propone una revisión exhaustiva del Capítulo 2.4.6. 'Infección por *Perkinsus olseni'* reformateado utilizando el nuevo modelo de capítulo específico de enfermedad.

31. El Capítulo revisado circuló tres veces para comentario, la primera vez en el informe de la Comisión de febrero de 2025.

32. El Capítulo revisado 2.4.6. 'Infección por *Perkinsus olensi*' se presenta en el **Anexo 20** y se propondrá para adopción en la 93.ª Sesión General en mayo de 2026.

2.4. Capítulo 2.4.7. 'Infección por Xenohaliotis californiensis' *(Anexo 21)*

33. Se propone una revisión exhaustiva del Capítulo 2.4.7. 'Infección por *Xenohaliotis californiensis'* reformateado utilizando el nuevo modelo de capítulo específico de enfermedad.

34. El Capítulo revisado circuló tres veces para comentario, la primera vez en el informe de la Comisión de febrero de 2025.

35. El Capítulo revisado 2.4.7. 'Infección por *Xenohaliotis californiensis'* se presenta en el **Anexo 21** y se propondrá para adopción en la 93.ª Sesión General en mayo de 2026.

2.5. Revisión de los nombres científicos de los virus de los agentes patógenos de las enfermedades de la lista de la OMSA (Anexo 22)

36. Se revisaron los nombres actuales de los agentes patógenos de las enfermedades de la lista de la OMSA utilizados en el *Código Acuático* y en el Manual *Acuático* con respecto a la taxonomía empelada por el Comité Internacional de Taxonomía de Virus (ICTV).

37. De los 18 agentes patógenos virales incluidos en la lista de enfermedades de la OMSA, 12 requerían actualizarse en los Capítulos 2.2.3., 2.2.5., 2.2.6., 2.2.7. y 2.2.8. para los crustáceos; Capítulos 2.3.4., 2.3.5., 2.3.6., 2.3.8., 2.3.9. y 2.3.10. para los peces y Capítulo 2.4.1 para los moluscos.

38. Los nombres científicos revisados para los agentes patógenos de las enfermedades de la lista de la OMSA circularon dos veces para comentario, la primera vez en el informe de la Comisión de septiembre de 2025.

39. Los nombres científicos revisados para los agentes patógenes de las enfermedades de la lista de la OMSA se presentan en el **Anexo 22** y se propondrán para adopción en la 93.ª Sesión General en mayo de 2026.

…/… Anexos

3. Anexos

Anexo 5. – ‘Glosario’

GLOSARIO

[...]

ANIMAL ACUÁTICO ORNAMENTAL

designa un *animal acuático* destinado a ser exhibido, expuesto o a la competición, o propuesto a ~~para~~ la venta como animal de compañía.

[…]

Anexo 6. – Capítulo 4.7. 'Vacío sanitario en acuicultura'

[Seguida de una versión limpia]

SECCIÓN 4

PREVENCIÓN Y CONTROL DE LAS ENFERMEDADES

CAPÍTULO 4.7.

VACÍO SANITARIO EN ACUICULTURA

Artículo 4.7.1.

Introducción

~~Las interrupciones de la producción en los sitios de *acuicultura* son consideradas generalmente muy útiles para dejar reposar o restaurar el medio ambiente. En el marco de esta estrategia, el *vacío sanitario* interrumpe los ciclos de reinfección eliminando las fuentes de una *enfermedad* en un criadero. Por consiguiente, la utilización d~~

En *acuicultura*, el *vacío sanitario* ~~como~~ constituye una medida ~~de rutina~~ de gestión de enfermedad empleada ya sea: ~~regular de control de *enfermedades* es frecuente en *acuicultura*, ya sea como una mejor practica especialmente antes de volver a introducir poblaciones de *animales acuáticos* en un sitio previamente de repoblación ya utilizado~~

1) de forma voluntaria u obligatoria, antes de la introducción de ~~ciertas~~ poblaciones nuevas de *animales acuáticos* en un *establecimiento de acuicultura* previamente repoblado~~,~~ como parte de un *plan de bioseguridad* elaborado conforme a lo dispuesto en el Capítulo 4.1., o según las normas de la industria o los códigos de práctica;

2) de forma obligatoria, siguiendo las instrucciones de la *autoridad competente*, a raíz de un brote de una *enfermedad* que es objeto de medidas de gestión de emergencia según lo descrito en el Capítulo 4.11~~Y~~.

~~En el caso del~~ El *vacío sanitario* voluntario~~, los objetivos son~~ busca prevenir la transmisión de *agentes patógenos* entre ciclos sucesivos de producción y suprimir la presión de *infección* por parte de los *agentes patógenos*.

~~En el caso del~~ El *vacío sanitario* obligatorio~~, el objetivo es~~ busca erradicar un *agente patógeno* de un *establecimiento de acuicultura*.

Es posible proceder al *vacío sanitario* en un único *establecimiento de acuicultura* o, ~~en el caso del *vacío sanitario*~~ de manera sincronizada~~s~~, de un grupo de *establecimientos de acuicultura* relacionados epidemiológicamente que se vacían el mismo tiempo y durante la misma duración.

Artículo 4.7.2.

Consideraciones relativas al vacío sanitario

El *vacío sanitario* se utiliza para efectuar una interrupción temporal en la transmisión de ~~en los tiempos de los ciclos de transmisión de los~~ *agentes patógenos* entre ~~cohortes~~ ciclos de producción sucesiva~~s~~os de *especies susceptibles* o, cuando proceda, de especies de *vectores* de *animales acuáticos*. Deberá implementarse teniendo en cuenta los siguientes factores ~~elementos~~:

~~1) el objetivo del *vacío sanitario*, como la prevención de la transmisión entre ciclos de producción sucesivos, la supresión de la presión de *infección* por *agentes patógenos* o la erradicación de un *agente patógeno* en un *establecimiento de acuicultura*;~~

1~~2~~) las posibles fuentes de *infección* en el *establecimiento de acuicultura* ~~lugar de producción~~, como las poblaciones de *especies susceptibles* ~~*animales acuáticos*~~ de cría o silvestres ~~susceptibles~~, los *vectores*, los fómites o los *agentes patógenos* presentes en el entorno (por ejemplo, el agua o los sedimentos);

2~~3~~) las características del *agente patógeno* relevante, incluida su supervivencia y estabilidad fuera del hospedador y su período de infecciosidad ~~si el *agente patógeno* es obligado u optativo~~;

4) para los *agentes patógenos* obligados, el periodo durante el cual pueden permanecer viables en el medio ambiente;

53) la necesidad de coordinación espacial para el *vacío sanitario* sincronizado de *establecimientos de acuicultura* conectados epidemiológicamente;

43) el tipo de sistema de producción de *acuicultura*, teniendo en cuenta su incluyendo el diseño del sistema, el alcance y la aplicación de las medidas de *bioseguridad*.

56) el o los *establecimientos de acuicultura* deberán cuando no se conozca el periodo infeccioso, la granja puede proceder a un *vacío sanitario* cuya la duración del periodo del *vacío sanitario* que se aplica a un *establecimiento de acuicultura* basado en una *evaluación del riesgo*;.

6) el periodo del *vacío sanitario* no puede iniciarse hasta que se hayan retirado todos los *animales acuáticos* y el *establecimiento de acuicultura* se haya limpiado y desinfectado a fondo.

Artículo 4.7.3.

Vacío sanitario voluntario

Con el fin de promover la mejora de la situación sanitaria de la *acuicultura*, el *Servicio de Sanidad de los Animales Acuáticos* de un país podrá fomentar el uso voluntario del *vacío sanitario* como parte del *plan de bioseguridad* establecido en el Capítulo 4.1. como medida de bioseguridad para un *establecimiento de acuicultura* individual o como medida de *bioseguridad* común a todos los *establecimientos de acuicultura* que se consideren epidemiológicamente relacionados en un área determinada. Al impulsar a los operadores acuícolas a aplicar un *vacío sanitario* en sus instalaciones, la *autoridad competente* deberá hacer hincapié en los probables efectos beneficiosos del *vacío sanitario* en proporción a los costos económicos que conlleva.

El *Servicio de Sanidad de los Animales Acuáticos* también deberá tener en cuenta el nivel de *riesgo* que una determinada *enfermedad* supone para las operaciones acuícolas locales, el periodo de infecciosidad de la *enfermedad* en cuestión, así como las condiciones socioeconómicas cuando se evalúan los beneficios potenciales para los recursos acuáticos del área.

Al evaluar los posibles beneficios de recomendar el *vacío* sanitario voluntario, los *Servicios de Sanidad de los Animales Acuáticos* de un país deberán tener en cuenta, además de las consideraciones enumeradas en el Artículo 4.7.2., los siguientes factores:

1) el nivel de *riesgo* que un determinado *agente patógeno* supone para las operaciones de *acuicultura* locales, y para otros recursos acuícolas del área;

2) las condiciones socioeconómicas relevantes y los posibles efectos favorables del *vacío sanitario* en proporción con los costes que implica. Para ello, se podrá recurrir a un análisis coste-beneficio.

Artículo 4.7.4.

Vacío sanitario obligatorio

Se podrá exigir el *vacío sanitario* obligatorio ordenado por de acuerdo con las instrucciones de la *autoridad competente* tras un brote de una *enfermedad* importante que haya sido objeto de las medidas descritas en los Capítulos 4.10X. y 4.11Y. La *autoridad competente* podrá exigir que un *establecimiento de acuicultura* infectado y otros *establecimientos de acuicultura* epidemiológicamente vinculados pertinentes situados en una *zona* oficialmente declarada infectada se sometan a un período obligatorio de *vacío sanitario*. Este La *autoridad competente* prescribirá la duración y la necesidad de proceder a un *vacío sanitario* en un *establecimiento de acuicultura* y a un *vacío sanitario* sincronizado en *establecimientos de acuicultura* epidemiológicamente vinculados dentro de una *zona infectada* se llevará a cabo durante un período de tiempo prescrito por la *autoridad competente*, previa a partir de una *evaluación del riesgo*.

~~La *evaluación del riesgo* también se utilizará para determinar si se requiere Podrá exigirse un período de *vacío sanitario* sincronizado en los establecimientos de acuicultura epidemiológicamente vinculados pertinentes de en la *zona infectada* así como la duración de dicho *vacío sanitario* si así lo indica la *evaluación del riesgo*.~~

La *autoridad competente* deberá tener autoridad legal a efectos de garantizar que el *vacío* obligatorio se base en disposiciones legales que establezcan las siguientes disposiciones:

~~Artículo 4.7.52.~~

~~Disposiciones legales~~

~~En los casos referidos en el Artículo 4.7.1. en los que el *vacío sanitario* sea una medida obligatoria, prescrita por la *autoridad competente*, por ejemplo para establecer o restaurar una *zona libre* de *enfermedad*, los países deberán establecer deberá implementarse un marco legal con el fin de necesario para: para la aplicación de los procedimientos de *vacío sanitario* en los *establecimientos de acuicultura*. Las disposiciones legales podrían incluir:~~

1) ~~definición de~~ ~~definir~~ circunstancias ~~sanitarias~~ que requieren la aplicación del *vacío sanitario* o del *vacío sanitario* sincronizado, incluidas las etapas específicas de implementación para cada uno ~~simultáneo~~;

2) momento específico en el que debe comenzar el *vacío sanitario* ~~definición de definir mecanismos basados en la *evaluación de riesgo* que permitan determinar medidas específicas contra cada *enfermedad*, tales como el inicio del *vacío sanitario* entre ellas la *desinfección* y su la duración antes de la reintroducción de *especies susceptibles*.~~;

~~3) una vez obtenida la autorización de la *autoridad competente* de repoblar con *especies susceptibles*, definición de un período de *vigilancia* y *diagnóstico* para comprobar la ausencia de la *enfermedad* considerada.~~

3) duración del periodo de *vacío sanitario*;

4) circunstancias bajo las cuales se permitirá la reintroducción de especies de *acuicultura*, una vez que haya finalizado el período de *vació sanitario*.

Artículo 4.7.5~~63~~.

Parámetros técnicos para la ejecución de un plan obligatorio ~~reglamentario~~ de vacío sanitario

Teniendo en cuenta las categorías de los sistemas de producción de *acuicultura* mencionadas en el Artículo 4.1.5., así como las medidas descritas en el quinto párrafo del Artículo 4.10.7., el periodo de *vacío sanitario* de un *establecimiento de acuicultura* ~~deberá considerar el quinto párrafo del Artículo 4.X.7. y~~ El *vacío sanitario* deberá comenzar inmediatamente después de que se hayan llevado a cabo las siguientes acciones en el *establecimiento de acuicultura* o grupo de *establecimientos de acuicultura*, en el caso de un *vacío sanitario* sincronizado:

1) la ~~destrucción y~~ retirada y eliminación en condiciones de *bioseguridad* de los siguientes *animales acuáticos* de los *establecimiento de acuicultura*:

   a) ~~haber evacuado~~ todas las *especies* ~~de *animales acuáticos*~~ *susceptibles* a la *enfermedad* ~~considerada~~ de preocupación, y

   b~~2~~) ~~haber evacuado~~ todas las especies ~~de animales de *acuicultura*~~ que pueden ser *vectores* de la *enfermedad* ~~considerada~~ de preocupación, si así lo indica la *evaluación del riesgo*, y

   c~~3~~) ~~haber evacuado, si aplica, de~~ otras especies, si así lo indica la *evaluación del riesgo*; ~~y~~

2~~4~~) la evacuación del agua en la que se encontraban las poblaciones infectadas, cuando sea posible, y

3) la eliminación segura de todo residuo sólido tales como heces, piensos o animales muertos de los *establecimientos de acuicultura*, si aplica, y

3~~5~~) la finalización de las medidas de *desinfección* apropiadas (incluyendo limpieza) de los equipos y de otros materiales contaminados, de conformidad con el Artículo 4.7.4., bajo la supervisión de la *autoridad competente* ~~los Servicios de *Sanidad de los Animales Acuáticos*. haber evacuado, o desinfectado según procedimientos aprobados por el *Servicio de Sanidad de los Animales Acuáticos*, los equipos y el material contaminados o que pueden contener el agente infeccioso~~.

~~La duración~~ Además de las consideraciones expuestas en el Artículo 4.7.2., la *autoridad competente* deberá ~~determinar la duración de un período obligatorio reglamentario de *vacío sanitario* deberá basarse en a partir de~~ considerar pruebas científicas de la infecciosidad del ~~probabilidad de que un~~ *agente patógeno* ~~siga siendo infeccioso fuera de su(s) huésped(es) del medio de *acuicultura*,~~ en el medio ambiente local para determinar la duración del periodo obligatorio del *vacío sanitario* con el fin de limitar al máximo posible el~~, de manera que suponga un *riesgo* inaceptable~~ de reinfección del *establecimiento de acuicultura*. ~~Deberá tenerse en cuenta~~ Los factores que se deberán considerar incluyen la extensión del *brote* de *enfermedad*, la distribución de *especies susceptibles* y *vectores* eventuales ~~la existencia de huéspedes alternativos en el medio ambiente local~~, la capacidad de supervivencia y de infecciosidad del *agente patógeno*, y l~~o~~as ~~factores~~ condiciones climatológic~~o~~as, geográfic~~o~~as e hidrográfic~~o~~as pertinentes ~~locales~~. ~~A estos elementos, se puede agregar el nivel de *riesgo* para la *acuicultura* local y para los recursos acuáticos a mayor escala. Se utilizará un procedimiento de *evaluación de riesgo* con base científica para determinar la duración del período de *vacío sanitario*.~~

Artículo 4.6~~7~~4.

~~Instrucciones~~ Desinfección previa al inicio del periodo del vacío sanitario

~~Los países~~ ~~Las *autoridades competentes*~~ Los *Servicios de Sanidad de los Animales Acuáticos* que establezcan procedimientos de *vacío sanitario* deberán preparar una serie de instrucciones detalladas respecto a la *desinfección* de los *establecimientos de acuicultura* ~~antes del *vacío sanitario*,~~ para aprobación de la *autoridad competente*. Estas instrucciones deberán adaptarse en función del tipo de sistema de producción y de las circunstancias. La *desinfección* deberá realizarse antes del inicio del periodo del *vacío sanitario* de conformidad con el Capítulo 4.4. y, para el *vacío sanitario* obligatorio, de los Capítulos 4.10~~X~~. y 4.11~~Y~~.

~~A tales efectos, las instrucciones que figuran en el Capítulo 4.4. del *Código Acuático* y en el Capítulo 1.1.3. del *Manual Acuático* servirán de directrices,~~ teniendo siempre en cuenta los últimos conocimientos científicos sobre la eficacia de los ~~tratamientos~~ desinfectantes contra el *agente patógeno* considerado.

Artículo 4.7.7~~8~~5.

Repoblación tras el vacío sanitario

~~Todos los~~ Un *establecimiento~~s~~ de acuicultura* que haya~~n~~ sido sometido~~s~~ a un *vacío sanitario* obligatorio no deberá ser repoblado hasta que se haya completado el período obligatorio de *vacío sanitario* y se haya~~n~~ recibido ~~la autorización~~ el permiso de la *autoridad competente*.

Cuando se proceda a la repoblación, se velará por no utilizar poblaciones de *animales acuáticos* que comprometan los objetivos del procedimiento de *vacío sanitario*. Para aumentar la confianza en la eficacia de los procedimientos de *vacío sanitario*, todos los *establecimientos de acuicultura* ~~criaderos~~ sometidos a un *vacío sanitario* obligatorio deberán ser sometidos a un período de rigurosa *vigilancia* oficial después de su repoblación con *especies susceptibles*. La duración y la intensidad de la *vigilancia* se ajustarán a la *enfermedad* en cuestión ~~considerada~~ y ser conformes con los requisitos establecidos en el Capítulo 1.4. y, las *enfermedades de la lista*, con el capítulo específico de enfermedad ~~a las condiciones locales~~.

SECCIÓN 4

PREVENCIÓN Y CONTROL DE LAS ENFERMEDADES

CAPÍTULO 4.7.

VACÍO SANITARIO EN ACUICULTURA

Artículo 4.7.1.

Introducción

En *acuicultura*, el *vacío sanitario* constituye una medida de gestión de enfermedad empleada ya sea:

1) de forma voluntaria u obligatoria, antes de la introducción de poblaciones nuevas de *animales acuáticos* en un *establecimiento de acuicultura* previamente repoblado como parte de un *plan de bioseguridad* elaborado conforme a lo dispuesto en el Capítulo 4.1., o según las normas de la industria o los códigos de práctica;

2) de forma obligatoria, siguiendo las instrucciones de la *autoridad competente*, a raíz de un brote de una *enfermedad* que es objeto de medidas de gestión de emergencia según lo descrito en el Capítulo 4.11. El *vacío sanitario* voluntario busca prevenir la transmisión de *agentes patógenos* entre ciclos sucesivos de producción y suprimir la presión de *infección* por parte de los *agentes patógenos*.

El *vacío sanitario* obligatorio busca erradicar un *agente patógeno* de un *establecimiento de acuicultura*.

Es posible proceder al *vacío sanitario* en un único *establecimiento de acuicultura* o, de manera sincronizada, de un grupo de *establecimientos de acuicultura* relacionados epidemiológicamente, que se vacían el mismo tiempo y durante la misma duración.

Artículo 4.7.2.

Consideraciones relativas al vacío sanitario

El *vacío sanitario* se utiliza para efectuar una interrupción temporal en la transmisión de *agentes patógenos* entre ciclos de producción sucesivos de *especies susceptibles* o, cuando proceda, de especies de *vectores* de *animales acuáticos*. Deberá implementarse teniendo en cuenta los siguientes factores:

1) las posibles fuentes de *infección* en el *establecimiento de acuicultura*, como las poblaciones de *especies susceptibles* de cría o silvestres, los *vectores*, los fómites o los *agentes patógenos* presentes en el entorno (por ejemplo, el agua o los sedimentos);

2) las características del *agente patógeno* relevante, incluida su supervivencia y estabilidad fuera del hospedador y su período de infecciosidad;

3) la necesidad de coordinación espacial para el *vacío sanitario* sincronizado de *establecimientos de acuicultura* conectados epidemiológicamente;

4) el tipo de sistema de producción de *acuicultura*, incluyendo el diseño del sistema, el alcance y la aplicación de las medidas de *bioseguridad*.

5) la duración del periodo del *vacío sanitario* que se aplica a un *establecimiento de acuicultura* basado en una *evaluación del riesgo*;

6) el periodo del *vacío sanitario* no puede iniciarse hasta que se hayan retirado todos los *animales acuáticos* y el *establecimiento de acuicultura* se haya limpiado y desinfectado a fondo.

Artículo 4.7.3.

Vacío sanitario voluntario

Al evaluar los posibles beneficios de recomendar el *vacío sanitario* voluntario, los *Servicios de Sanidad de los Animales Acuáticos* de un país deberán tener en cuenta, además de las consideraciones enumeradas en el Artículo 4.7.2., los siguientes factores:

1) el nivel de *riesgo* que un determinado *agente patógeno* supone para las operaciones de *acuicultura* locales, y para otros recursos acuícolas del área;

2) las condiciones socioeconómicas relevantes y los posibles efectos favorables del *vacío sanitario* en proporción con los costes que implica. Para ello, se podrá recurrir a un análisis coste-beneficio.

Artículo 4.7.4.

Vacío sanitario obligatorio

Se podrá exigir el *vacío sanitario* obligatorio ordenado por la *autoridad competente* tras un brote de una *enfermedad* que haya sido objeto de las medidas descritas en los Capítulos 4.10. y 4.11. La *autoridad competente* podrá exigir que un *establecimiento de acuicultura* infectado y otros *establecimientos de acuicultura* epidemiológicamente vinculados situados en una *zona* oficialmente declarada infectada se sometan a un período de *vacío sanitario*. La *autoridad competente* prescribirá la duración y la necesidad de proceder a un *vacío sanitario* en un *establecimiento de acuicultura* y a un *vacío sanitario* sincronizado en *establecimientos de acuicultura* epidemiológicamente vinculados dentro de una *zona infectada*, a partir de una *evaluación del riesgo*.

La *autoridad competente* deberá tener autoridad legal a efectos de garantizar que el *vacío* obligatorio se base en disposiciones legales que establezcan las siguientes disposiciones:

1) circunstancias que requieren la aplicación del *vacío sanitario* o del *vacío sanitario* sincronizado, incluidas las etapas específicas de implementación para cada uno ;

2) momento específico en el que debe comenzar el *vacío sanitario* ;

3) duración del periodo de *vacío sanitario*;

4) circunstancias bajo las cuales se permitirá la reintroducción de especies de *acuicultura*, una vez que haya finalizado el período de *vació sanitario*.

Artículo 4.7.5.

Parámetros técnicos para la ejecución de un plan obligatorio de vacío sanitario

Teniendo en cuenta las categorías de los sistemas de producción de *acuicultura* mencionadas en el Artículo 4.1.5., así como las medidas descritas en el quinto párrafo del Artículo 4.10.7., el periodo de *vacío sanitario* de un *establecimiento de acuicultura* deberá comenzar inmediatamente después de que se hayan llevado a cabo las siguientes acciones en el *establecimiento de acuicultura* o grupo de *establecimientos de acuicultura*, en el caso de un *vacío sanitario* sincronizado:

1) la retirada y eliminación en condiciones de *bioseguridad* de los siguientes *animales acuáticos* de los *establecimientos de acuicultura*:

   a) todas las *especies susceptibles* a la *enfermedad* de preocupación, y

   b) todas las especies que pueden ser *vectores* de la *enfermedad* de preocupación, si así lo indica la *evaluación del riesgo,* y

   c) otras especies, si así lo indica la *evaluación del riesgo*;

2) la evacuación del agua en la que se encontraban las poblaciones infectadas, cuando sea posible, y

3) la eliminación segura de todo residuo sólido tales como heces, piensos o animales muertos de los *establecimientos de acuicultura*, si aplica, y

4) la finalización de las medidas de *desinfección* apropiadas (incluyendo limpieza) de los equipos y de otros materiales contaminados, de conformidad con el Artículo 4.7.4., bajo la supervisión de la *autoridad competente*.

Además de las consideraciones expuestas en el Artículo 4.7.2., la *autoridad competente* deberá considerar pruebas científicas de la infecciosidad del *agente patógeno* en el medio ambiente local para determinar la duración del periodo obligatorio del *vacío sanitario* con el fin de limitar al máximo posible el *riesgo* de reinfección del *establecimiento de acuicultura*. Los factores que se deberán considerar incluyen la extensión del *brote* de *enfermedad*, la distribución de *especies susceptibles* y de *vectores* eventuales, la capacidad de supervivencia y de infecciosidad del *agente patógeno*, y las condiciones climatológicas, geográficas e hidrográficas pertinentes.

Artículo 4.7.6.

Desinfección previa al inicio del periodo del vacío sanitario

Los *Servicios de Sanidad de los Animales Acuáticos* que establezcan procedimientos de *vacío sanitario* deberán preparar una serie de instrucciones detalladas respecto a la *desinfección* de los *establecimientos de acuicultura,* para aprobación de la *autoridad competente*. Estas instrucciones deberán adaptarse en función del tipo de sistema de producción y de las circunstancias. La *desinfección* deberá realizarse antes del inicio del periodo del *vacío sanitario* de conformidad con el Capítulo 4.4. y, para el *vacío sanitario* obligatorio, de los Capítulos 4.10. y 4.11. teniendo siempre en cuenta los últimos conocimientos científicos sobre la eficacia de los desinfectantes contra el *agente patógeno* considerado.

Artículo 4.7.7.

Repoblación tras el vacío sanitario

Un *establecimiento de acuicultura* que haya sido sometido a un *vacío sanitario* obligatorio no deberá ser repoblado hasta que se haya completado el período obligatorio de *vacío sanitario* y se haya recibido el permiso de la *autoridad competente*.

Cuando se proceda a la repoblación, se velará por no utilizar poblaciones de *animales acuáticos* que comprometan los objetivos del procedimiento de *vacío sanitario*. Para aumentar la confianza en la eficacia de los procedimientos de *vacío sanitario*, todos los *establecimientos de acuicultura* sometidos a un *vacío sanitario* obligatorio deberán ser sometidos a un período de rigurosa *vigilancia* oficial después de su repoblación con *especies susceptibles*. La duración y la intensidad de la *vigilancia* se ajustarán a la *enfermedad* en cuestión y ser conformes con los requisitos establecidos en el Capítulo 1.4. y, para las *enfermedades de la lista*, con el capítulo específico de enfermedad.

Anexo 7. – Artículo 9.2.2. del Capítulo 9.2. 'Infección por *Aphanomyces astaci* (plaga del cangrejo del río)'

CAPÍTULO 9.2.

INFECCIÓN POR *APHANOMYCES ASTACI* (PLAGA DEL CANGREJO DEL RÍO)

[…]

Artículo 9.2.2.

Ámbito de aplicación

Las recomendaciones de este capítulo se aplican a las siguientes especies que cumplen los criterios para ser incluidas como susceptibles de conformidad con el Capítulo 1.5. ~~a todas las especies de cangrejos que pertenecen a las tres familias Cambaridae, Astacidae y Parastacidae. Estas recomendaciones se aplican también a todas las demás especies susceptibles mencionadas en el *Manual Acuático* que sean objeto de *comercio internacional*.~~

Familia	**Nombre científico**	**Nombre común**
Astacidae	*Astacus astacus*	cangrejo de río de patas rojas
	Austropotamobius spp. (todas las especies) ~~*pallipes*~~	N/A ~~sin nombre común~~
	Pacifastacus leniusculus	cangrejo señal o cangrejo del Pacífico
	Pontastacus leptodactylus	cangrejo de patas punteadas
Cambaridae	*Faxonius spp.* (todas las especies)	N/A
	Procambarus spp. (todas las especies)	N/A
Cambaroididae	*Cambaroides japonicus*	sin nombre común
Parastacidae	*Cherax quadricarinatus*	langosta de agua dulce
Potamidae	*Potamon potamios*	sin nombre común
Varunidae	*Eriocheir sinensis*	cangrejo chino

[…]

Anexo 8. – Artículos 10.3.5. y 10.3.6. del Capítulo 10.3. ‘Infección por *Gyrodactylus salaris*’

CAPÍTULO 10.3.

INFECCIÓN POR *GYRODACTYLUS SALARIS*

[…]

Artículo 10.3.5.

País libre de infección por *G. salaris*

Si el país comparte cuerpos de agua con otros países, solo podrá hacer una autodeclaración de ausencia de infección por *G. salaris* si todos los cuerpos de agua compartidos están situados en países o *zonas* declarados libres de infección por *G. salaris* (véase el Artículo 10.3.6.).

Como se describe en el Artículo 1.4.4., un País Miembro puede hacer una autodeclaración de ausencia de infección por *G. salaris* en la totalidad de su *territorio* si puede demostrar que:

1) ninguna *especie susceptible* de las mencionadas en el Artículo 10.3.2. está presente en el país y se han reunido ininterrumpidamente las *condiciones elementales de bioseguridad* durante al menos los últimos seis meses;

O

2) ~~procedimiento 2 (ausencia histórica) no adecuado para la infección por *G. salaris*;~~ no ha ocurrido ninguna infección por *G. salaris* durante al menos los 15 últimos años, y:

   a) el País Miembro puede demostrar que las condiciones son propicias para la manifestación clínica de la infección por *G. salaris*, de acuerdo con lo descrito en el Artículo 1.4.8. del Capítulo 1.4. y teniendo en cuenta la información pertinente relativa a la manifestación clínica que figura en la Sección 2.2.5 del *Manual Acuático;* y

   b) se han reunido ininterrumpidamente las *condiciones elementales de bioseguridad* descritas en el Capítulo 1.4. durante al menos los 15 últimos años;

   El procedimiento 2 (ausencia histórica) sólo se aplica para proceder a una *autodeclaración de ausencia de enfermedad* para la *infección* por *G. salaris* ~~para el salmón Atlántico (*Salmo salar*). Si~~ si el estudio de la población abarca otras cepas de *especies susceptibles* que no manifiestan signos clínicos~~, el procedimiento 2 no es apropiado~~. En todos los otros casos, la *vigilancia pasiva* será insuficientemente sensible para sustentar una *autodeclaración de ausencia de enfermedad*;

O

3) se ha aplicado una *vigilancia específica*, de acuerdo con lo descrito en el Capítulo 1.4., durante al menos los tres últimos años sin que se haya detectado la presencia de *G. salaris*, y se han reunido ininterrumpidamente e implementado las *condiciones elementales de bioseguridad* durante al menos dos años antes del inicio de la *vigilancia específica*;

O

4) había hecho previamente una autodeclaración de ausencia de infección por *G. salaris* y perdió posteriormente su estatus libre por haberse detectado *G. salaris*, pero se han cumplido las condiciones siguientes:

   a) nada más haberse detectado *G. salaris*, el área afectada ha sido declarada *zona infectada* y se ha establecido una *zona de protección*; y

   b) las poblaciones infectadas dentro de la *zona infectada* se han sacrificado y eliminado con medios que reducen al mínimo la probabilidad de una mayor transmisión de *G. salaris*, y se han completado los

procedimientos de *desinfección* apropiados (descritos en el Capítulo 4.4.), seguidos de un periodo de *vacío sanitario* según se describe en el Capítulo 4.7.; y

c) las *condiciones elementales de bioseguridad* vigentes anteriormente han sido debidamente revisadas y modificadas y se han mantenido ininterrumpidamente desde la erradicación de la infección por *G. salaris*; y

d) se ha aplicado una *vigilancia específica*, de acuerdo con lo descrito en el Capítulo 1.4., durante:

i) por lo menos los tres últimos años en las *especies susceptibles* silvestres y de cría sin que se haya detectado la presencia de *G. salaris*, o

ii) por lo menos en el último año sin que se haya detectado la presencia de *G. salaris*, si los *establecimientos de acuicultura* afectados no tenían vínculos epidemiológicos con las poblaciones silvestres de *especies susceptibles*.

Mientras tanto, la parte del país ubicada fuera de la *zona infectada* y de la *zona de protección* podrá ser declarada *zona libre*, según se describe en el Artículo 1.4.4.

Artículo 10.3.6.

Zona libre de infección por *G. salaris*

Si una *zona* se extiende por el *territorio* de más un país, solo podrá ser declarada *zona* libre de infección por *G. salaris* si todas las *autoridades competentes* confirman que se han reunido el conjunto de condiciones exigidas.

Como se describe en el Artículo 1.4.4., un País Miembro puede hacer una autodeclaración de ausencia de infección por *G. salaris* para una *zona* dentro de su *territorio* si puede demostrar que:

1) ninguna *especie susceptible* de las mencionadas en el Artículo 10.3.2. está presente y se han reunido ininterrumpidamente las *condiciones elementales de bioseguridad* durante al menos los últimos seis meses;

O

2) ~~procedimiento 2 (ausencia histórica) no adecuado para la infección por *G. salaris*~~; no ha ocurrido ninguna infección por *G. salaris* durante al menos los 15 últimos años, y;

a) el País Miembro puede demostrar que las condiciones son propicias para la manifestación clínica de la infección por *G. salaris*, de acuerdo con lo descrito en el Artículo 1.4.8. del Capítulo 1.4. y teniendo en cuenta la información pertinente relativa a la manifestación clínica que figura en la Sección 2.2.5 del *Manual Acuático;* y

b) se han reunido ininterrumpidamente las *condiciones elementales de bioseguridad* descritas en el Capítulo 1.4. durante al menos los 15 últimos años;

El procedimiento 2 (ausencia histórica) sólo se aplica para proceder a una *autodeclaración de ausencia de enfermedad* para la *infección* por *G. salaris* ~~para el salmón Atlántico (*Salmo salar*). Si~~ si el estudio de la población abarca otras cepas de *especies susceptibles* que no manifiestan signos clínicos~~, el procedimiento 2 no es apropiado~~. En todos los otros casos, la *vigilancia pasiva* será insuficientemente sensible para sustentar una *autodeclaración de ausencia de enfermedad*;

O

3) se ha aplicado una *vigilancia específica* en la *zona*, de acuerdo con lo descrito en el Capítulo 1.4., durante al menos los tres últimos años sin que se haya detectado la presencia de *G. salaris*, y se han reunido ininterrumpidamente e implementado las *condiciones elementales de bioseguridad* durante al menos dos años antes del inicio de la *vigilancia específica*;

O

4) había hecho previamente una autodeclaración de ausencia de infección por *G. salaris* para una *zona* y perdió posteriormente su estatus libre por haberse detectado *G. salaris* en la *zona*, pero se han cumplido las condiciones siguientes:

a) nada más haberse detectado *G. salaris*, el área afectada fue declarada *zona infectada* y se ha establecido una *zona de protección*; y

b) las poblaciones infectadas dentro de la *zona infectada* se han sacrificado y eliminado con medios que reducen al mínimo la probabilidad de una mayor transmisión de *G. salaris*, y se han completado los procedimientos de *desinfección* apropiados (descritos en el Capítulo 4.4.), seguidos de un periodo de *vacío sanitario* según se describe en el Capítulo 4.7.; y

c) las *condiciones elementales de bioseguridad* vigentes anteriormente han sido debidamente revisadas y modificadas y se han mantenido ininterrumpidamente desde la erradicación de la infección por *G. salaris*; y

d) se ha aplicado una *vigilancia específica*, de acuerdo con lo descrito en el Capítulo 1.4., durante:

   i) por lo menos los tres últimos años en las *especies susceptibles* silvestres y de cría sin que se haya detectado la presencia de *G. salaris*, o

   ii) por lo menos en el último año sin que se haya detectado la presencia de *G. salaris*, si los *establecimientos de acuicultura* afectados no tenían vínculos epidemiológicos con las poblaciones silvestres de *especies susceptibles*.

Mientras tanto, una parte de la *zona* ubicada fuera de la *zona infectada* y de la *zona de protección* podrá ser declarada una nueva *zona libre*, según se describe en el Artículo 1.4.4.

[…]

Anexo 9. – Artículo 10.4.9. del Capítulo 10.4. 'Infección por el virus de la anemia infecciosa del salmón'

CAPÍTULO 10.4.

INFECCIÓN POR VIRUS DE LA ANEMIA INFECCIOSA DEL SALMÓN

[…]

Artículo 10.4.9.

Compartimento libre de infección por el virus de la anemia infecciosa del salmón

En este artículo, todas las consideraciones se entenderán hechas a cualquier virus detectable de la anemia infecciosa del salmón, incluido el virus de la anemia infecciosa del salmón HPR0.

Según se describe en el Artículo 1.4.4., un País Miembro podrá hacer una autodeclaración de ausencia de infección por el virus de la anemia infecciosa del salmón en un *compartimento* dentro de su *territorio* si puede demostrar que:

1) se ha aplicado una *vigilancia específica* en el *compartimento*, de acuerdo con lo descrito en el Capítulo 1.4., durante al menos en los dos ~~[en estudio]~~ últimos años sin que se haya detectado el virus de la anemia infecciosa del salmón, y se han reunido ininterrumpidamente e implementado las *condiciones elementales de bioseguridad* durante al menos un año antes del inicio de la *vigilancia específica*;

O

2) había hecho previamente una autodeclaración de ausencia de infección por el virus de la anemia infecciosa del salmón en un *compartimento* y perdió posteriormente su estatus libre por haberse detectado el virus de la anemia infecciosa del salmón en el *compartimento*, pero se han cumplido las condiciones siguientes:

   a) todos los *animales acuáticos* dentro del *compartimento* se han sacrificado y eliminado con medios que reducen al mínimo la probabilidad de una mayor transmisión del virus de la anemia infecciosa del salmón, se han completado los procedimientos de *desinfección* apropiados (descritos en el Capítulo 4.4.) y el *compartimento* se ha sometido a un periodo de *vacío sanitario* según se describe en el Capítulo 4.7.; y

   b) las *condiciones elementales de bioseguridad* vigentes anteriormente, incluido el *plan de bioseguridad* en el *compartimento*, han sido debidamente revisados y modificados y se han mantenido ininterrumpidamente desde la repoblación con *animales acuáticos* procedentes de una fuente aprobada libre de patógenos conforme a los requisitos de los Artículos 10.4.13. y 10.4.14. si procede; y

   c) se ha realizado una encuesta para determinar la infección por el virus de la anemia infecciosa del salmón al menos seis meses luego de la repoblación (tal como se describe en el Artículo 1.4.14.) sin que se haya detectado la presencia del *agente patógeno*.

[…]

Anexo 10. – Actualización de la taxonomía en el Artículo X.X.1. de los Capítulos 9.3., 9.5., 9.6., 9.8., 9.10., 10.4., 10.5., 10.6., 10.7., 10.9., 10.10., 10.11. y 11.1.

SECCIÓN 9

ENFERMEDADES DE LOS CRUSTÁCEOS

CAPÍTULO 9.3.

INFECCIÓN POR EL VIRUS IRIDISCENTE DE LOS DECÁPODOS TIPO 1

Artículo.9.3.1

A efectos del *Código Acuático*, la *infección* por el virus iridiscente de los decápodos tipo 1 (DIV1) designa una infección causada por el *agente patógeno* *Decapodiridovirus litopenaeus1* ~~*Decapod* del virus iridiscente de los decápodos tipo 1 (DIV1), del género Decapodiridovirus, y~~ de la familia Iridoviridae.

La información sobre los métodos de *diagnóstico* figura en el *Manual Acuático*.

[…]

CAPÍTULO 9.5.

INFECCIÓN POR EL VIRUS DE LA NECROSIS HIPODÉRMICA Y HEMATOPOYÉTICA INFECCIOSA

Artículo.9.5.1

A efectos del *Código Acuático*, la *infección* por el virus de la necrosis hipodérmica y hematopoyética infecciosa es la *infección* causada por el *agente patógeno* *Penstylhamaparvovirus decapod1*, ~~de los decápodos penstylhamaparvovirus 1 del género *Penstylhamaparvovirus* y~~ de la familia Parvoviridae.

La información sobre los métodos de *diagnóstico* figura en el *Manual Acuático*.

[…]

CAPÍTULO 9.6.

INFECCIÓN POR EL VIRUS DE LA MIONECROSIS INFECCIOSA

Artículo.9.6.1.

A efectos del *Código Acuático*, la *infección* por el virus de la mionecrosis infecciosa designa una *infección* causada por el *agente patógeno* *Artivirus ichi,* ~~del virus de la mionecrosis infecciosa~~, ~~del género *Artivirus* y~~ ~~que se asemeja a miembros~~ de la familia Artiviridae ~~Totivíridos (clasificación provisional).~~

La información sobre los métodos de *diagnóstico* figura en el *Manual Acuático*.

[…]

CAPÍTULO 9.8.

INFECCIÓN POR EL VIRUS DEL SÍNDROME DE TAURA

Artículo 9.8.1.

A efectos del *Código Acuático*, la *infección* por el virus del síndrome de Taura designa una *infección* causada por el ~~virus del síndrome de Taura, un~~ *agente patógeno* *Aparavirus tauraense* ~~del género Aparavirus,~~ de la familia Dicistroviridae ~~y del orden Picornavirales~~.

La información sobre los métodos de *diagnóstico* figura en el *Manual Acuático*.

[…]

CAPÍTULO 9.10.

INFECCIÓN POR EL VIRUS DE LA CABEZA AMARILLA GENOTIPO 1

Artículo 9.10.1.

A efectos del *Código Acuático*, la *infección* por el virus de la cabeza amarilla genotipo 1 designa una *infección* causada por el agente patógeno *Okavirus flavicapitis* ~~del genotipo 1 del virus de la cabeza amarilla,~~ ~~del género *Okavirus*~~, ~~y~~ de la familia Rodiviridae ~~y del orden de los Nidovirales~~.

La información sobre los métodos de *diagnóstico* figura en el *Manual Acuático*.

[…]

SECCIÓN 10

ENFERMEDADES DE LOS PECES

CAPÍTULO 10.4.

INFECCIÓN POR EL VIRUS DE LA ANEMIA INFECCIOSA DEL SALMÓN

Artículo 10.4.1.

A efectos del *Código Acuático*, la *infección* por el virus de la anemia infecciosa del salmón designa una *infección* causada por el *agente patógeno* *Isavirus salaris* de la familia *Orthomyxoviridae*. La *infección* por el virus de la anemia infecciosa incluye dos genotipos; con supresión (HPR) o sin supresión en la región altamente polifórmica (HPR0) ~~del género Isavirus y de la familia de los Orthomyxoviridae~~. Ambos genotipos deberán notificarse de conformidad con el Capítulo 2.1.

Las formas no patógenas del virus (HPR0) y el virus patógeno con supresión en la HPR están interrelacionadas, y ciertos *brotes* se producen como resultado de la aparición de supresión en la HPR a partir de HPR0.

Las disposiciones del presente capítulo reconocen tres niveles posibles de *estatus zoosanitario* con respecto al virus de la anemia infecciosa del salmón:

1. libre de virus de la anemia infecciosa del salmón HPR0 y de virus de la anemia infecciosa del salmón con supresión en la HPR;

2. libre de virus de la anemia infecciosa del salmón HPR0 endémico (pero libre de virus de la anemia infecciosa del salmón con supresión en la HPR);

3. libre de virus de la anemia infecciosa del salmón HPR0 y virus de la anemia infecciosa del salmón con supresión en la HPR endémicos.

La información sobre los métodos de *diagnóstico* figura en el *Manual Acuático*.

[…]

CAPÍTULO 10.5.

INFECCIÓN POR EL ALFAVIRUS DE LOS SALMÓNIDOS

Artículo 10.5.1.

A efectos del *Código Acuático*, la *infección* por el alfavirus de los salmónidos designa una *infección* causada por cualquier genotipo del ~~alfavirus de los salmónidos, un~~ *agente patógeno* *Alphavirus salmon* ~~del género *Alphavirus* y~~ de la familia Togaviridae.

La información sobre los métodos de *diagnóstico* figura en el *Manual Acuático*.

[…]

CAPÍTULO 10.6.

INFECCIÓN POR EL VIRUS DE LA NECROSIS HEMATOPOYÉTICA INFECCIOSA

Artículo 10.6.1.

A efectos del *Código Acuático*, la *infección* por el virus de la necrosis hematopoyética infecciosa designa una *infección* causada por el ~~virus de la necrosis hematopoyética infecciosa, un~~ *agente patógeno Novirhabdovirus salmonid* ~~del género Novirhabdovirus del salmónido (también conocido como el virus de la necrosis hematopoyética infecciosa) y~~ de la familia Rhabdoviridae.

La información sobre los métodos de *diagnóstico* figura en el *Manual Acuático*.

[…]

CAPÍTULO 10.7.

INFECCIÓN POR EL HERPESVIRUS DE LA CARPA KOI

Artículo 10.7.1.

A efectos del *Código Acuático*, la *infección* por el herpesvirus de la carpa koi designa una *infección* causada por el ~~herpesvirus de la carpa koi, un~~ *agente patógeno* *Cyvirus cyprinidallo 3* ~~del género Cyprinivirus y~~ de la familia Alloherpesviridae.

La información sobre los métodos de *diagnóstico* figura en el *Manual Acuático*.

[…]

CAPÍTULO 10.9.

INFECCIÓN POR EL VIRUS DE LA VIREMIA PRIMAVERAL DE LA CARPA

Artículo 10.9.1.

A efectos del *Código Acuático*, la *infección* por el virus de la viremia primaveral de la carpa designa una *infección* causada por el ~~virus de la viremia primaveral de la carpa, un~~ *agente patógeno* ~~del género~~ *Sprivivirus cyprinus* ~~Sprivivirus y~~ de la familia Rhabdoviridae.

La información sobre los métodos de *diagnóstico* figura en el *Manual Acuático*.

[…]

CAPÍTULO 10.10.

INFECCIÓN POR EL VIRUS DE LA SEPTICEMIA HEMORRÁGICA VIRAL

Artículo 10.10.1.

A efectos del presente *Código Acuático*, la *infección* por el virus de la septicemia hemorrágica viral designa una *infección* causada por el ~~*virus de la septicemia hemorrágica viral, un*~~ *agente patógeno Novirhabdovirus piscine* ~~del género Novirhabdovirus y~~ de la familia Rhabdoviridae.

La información sobre los métodos de *diagnóstico* figura en el *Manual Acuático.*

[…]

CAPÍTULO 10.11.

INFECCIÓN POR EL VIRUS DE LA TILAPIA DEL LAGO

Artículo 10.11.1.

A efectos del *Código Acuático*, la *infección* por el virus de la tilapia del lago (TiLV) designa una *infección* causada por el *agente patógeno* *Tilapinevirus tilapiae* ~~*Tilapia tilapinevirus*, del género Tilapinevirus y~~ de la familia Amnoonviridae.

La información sobre los métodos de *diagnóstico* figura en el *Manual Acuático.*

[…]

SECCIÓN 11

ENFERMEDADES DE LOS MOLUSCOS

CAPÍTULO 11.1.

INFECCIÓN POR EL HERPESVIRUS DEL ABALÓN

Artículo 11.1.1.

A efectos del *Código Acuático*, la *infección* por el herpesvirus del abalón designa una *infección* causada por el *agente patógeno* *Aurivirus haliotidmalaco 1* ~~Haliotid herpesvirus 1 (HaHV-1)~~, ~~herpesvirus 1 de la familia de los haliótidos (HaHV-1), del género Aurivirus y~~ de la familia Malacoherpesviridae.

La información sobre los métodos de *diagnóstico* figura en el *Manual Acuático*.

[…]

Anexo 18. – Secciones 2.2.1. y 2.2.2. del Capítulo 2.2.2. ‘Infección por *Aphanomyces astaci* (plaga del cangrejo del río)’

CHAPTER 2.2.9.

INFECTION WITH *APHANOMYCES ASTACI* (CRAYFISH PLAGUE)

[…]

2.2. Host factors

2.2.1. Susceptible host species

Species that fulfil the criteria for listing as susceptible to infection with *A. astaci* according to Chapter 1.5. of the *Aquatic Animal Health Code* (*Aquatic Code*) are:

Family	Scientific name	Common name
Astacidae	*Astacus astacus*	noble crayfish
	Austropotamobius spp. (all species) ~~*pallipes*~~	N/A ~~no common name~~
	Pacifastacus leniusculus	signal crayfish
	Pontastacus leptodactylus	Danube crayfish
Cambaridae	*Faxonius* spp. (all species)	N/A
	Procambarus spp. (all species)	N/A
Cambaroididae	*Cambaroides japonicus*	no common name
Parastacidae	*Cherax quadricarinatus*	red claw crayfish
Potamidae	*Potamon potamios*	no common name
Varunidae	*Eriocheir sinensis*	Chinese mitten crab

~~The recommendations in this chapter apply to all species of crayfish in all three crayfish families (Cambaridae, Astacidae and Parastacidae).~~

~~[Note: an assessment of species that meet the criteria for listing as susceptible to infection with *A. astaci* in accordance with Chapter 1.5. has not yet been completed]~~

2.2.2. Species with incomplete evidence for susceptibility

Species for which there is incomplete evidence to fulfil the criteria for listing as susceptible to infection with *A. astaci* according to Chapter 1.5. of the *Aquatic Code* are:

Family	Scientific name	Common name
Astacidae	*Austropotamobius torrentium*	Stone crayfish ~~no common name~~
Cambaridae	*Cambarellus* (*Cambarellus*) *montezumae*	no common name
	Cambarellus patzcuarensis	no common name
	Cambarellus shufeldtii	Cajun dwarf crayfish
	Cambarus bartonii	Appalachian brook crayfish

In addition, pathogen-specific positive polymerase chain reaction (PCR) results have been reported in the following species, but no active infection has been demonstrated:

Family	Scientific name	Common name
Cambaridae	*Cambarus latimanus*	no common name
	Cambarus striatus	Hay crayfish
	Creaserinus fodiens	no common name
	Creaserinus oryktes	no common name
Palaemonidae	*Palaemon kadiakensis*	no common name

~~[Under study]~~

[…]

_____

Anexo 19. – Capítulo 2.4.5.
'Infección por *Perkinsus marinus*'

CHAPTER 2.4.5.

INFECTION WITH *PERKINSUS MARINUS*

1. Scope

Infection with *Perkinsus marinus* means infection with the pathogenic agent *Perkinsus marinus,* of the Family Perkinsidae.

2. Disease information

2.1. Agent factors

2.1.1. Aetiological agent

Perkinsus marinus is a Perkinsid parasite initially described infecting eastern oysters, *Crassostrea virginica* and causing disease and mortality (Mackin *et al.,* 1950).

Current phylogenomic studies place *P. marinus* within the class Perkinsea of the phylum Perkinsozoa, under the Kingdom Alveolata (Reece *et al.,* 1997).

The parasite occurs as motile zoospores, trophozoites, and schizonts. Following infection, trophozoites proliferate within oyster tissues, particularly in connective tissue and haemolymph. As trophozoites grow, they undergo schizogony: cleavage furrows form, leading to the production of multiple merozoites within a morula-like stage. Fully developed schizonts rupture to release large numbers of merozoites, which disseminate through host tissues or into the environment (Andrews, 1988; Villalba *et al.,* 2004).

As in *P. olseni*, the ribosomal RNA transcription units in *P. marinus* are highly duplicated and variable, complicating the use of rDNA as a reliable quantitative marker for infection intensity (Bogema *et al.,* 2021). Alternative genomic regions, including internal transcribed spacer (ITS) sequences and non-ribosomal single-copy genes, have been explored for molecular diagnostics (Audemard *et al.,* 2004; De Faveri *et al.,* 2009; Gauthier *et al.,* 2006; Reece *et al.,* 1997; Robledo *et al.,* 1999).

Cytogenetic and molecular studies suggest that *P. marinus* may also display variable ploidy. Early work using microsatellite loci and karyotyping suggested diploidy (Reece *et al.,* 1997; Robledo *et al.,* 1999), but subsequent genomic sequencing has raised the possibility of polyploidy or aneuploidy, with variation across life stages and isolates (Bogema *et al.,* 2021; Thompson *et al.,* 2011). Further investigation is needed to resolve the extent and biological implications of ploidy variation in this species.

2.1.2. Survival and stability in processed or stored samples

The survival and stability of *P. marinus* varies according to the conditions when processing and storing samples. Refrigeration (2–6°C) and low salinity (7 ppt) significantly decreases the viability and metabolic activity of *P. marinus* but could not eliminate *P. marinus* from oysters (La Peyre *et al.,* 2008b; 2010). It is likely that *P. marinus* can survive in dead or frozen oysters for an unknown, potentially long period (Andrews & Hewatt, 1957).

2.1.3. Survival and stability outside the host

Perkinsus marinus meront cells can survive for at least 14 days in the water column, with their infectivity potentially persisting beyond 7days (Chu & Lund, 2006).

For inactivation methods, see Section 2.4.5.

2.2. Host factors

2.2.1. Susceptible host species

Species that fulfil the criteria for listing as susceptible to infection with *P. marinus* according to Chapter 1.5. of the *Aquatic Animal Health Code* (*Aquatic Code*) are:

Family	Scientific name	Common name
Ostreidae	*Crassostrea corteziensis*	Cortez oyster
	Crassostrea virginica	American cupped oyster
	Magallana [syn. *Crassostrea*] *ariakensis*	Ariake cupped oyster
	Saccostrea palmula	palmate oyster

2.2.2. Species with incomplete evidence for susceptibility

Species for which there is incomplete evidence to fulfil the criteria for listing as susceptible to infection with *P. marinus* according to Chapter 1.5. of the *Aquatic Code* are:

Family	Scientific name	Common name
Ostreidae	*Crassostrea gasar* ~~*tulipa*~~	Gasar cupped oyster
	Crassostrea rhizophorae	mangrove cupped oyster
	Magallana [syn. *Crassostrea*] *gigas*	Pacific cupped oyster

In addition, pathogen-specific positive polymerase chain reaction (PCR) results have been reported in the following species, but no active infection has been demonstrated:

Family	Scientific name	Common name
Myidae	*Mya arenaria*	soft shell clam
Ostreidae	*Crassostrea columbiensis*	Columbia black oyster
	Striostrea prismatica	stone oyster

2.2.3. Likelihood of infection by species, host life stage, population or sub-populations

Perkinsus marinus usually infects and kills oysters between 1 and 3 years of age. Differences in clinical expression to the infection with *Perkinsus marinus* has been shown between *Crassostrea virginica* populations (e.g. Bushek & Allen, 1996; Green-Beach *et al.,* 2011).

Compared with *C. virginica, Crassostrea corteziensis, Saccostrea palmula* and triploid *M. ariakensis* were found to exhibit partial resistance to *P. marinus* infection and associated disease (Cáceres-Martínez *et al.,* 2012; 2016; Calvo *et al.* 2000; 2001).

2.2.4. Distribution of the pathogen in the host

While *P. marinus* can be found in various tissues and organs, it mostly infects intestine, vesicular connective tissue, haemocytes and digestive gland (Remacha-Triviño *et al.,* 2008). The proliferation of the parasite causes systemic disruption of connective tissue and epithelial cells.

2.2.5. Aquatic animal reservoirs of infection

None known

2.2.6. Vectors

None known

2.3. Disease pattern

2.3.1. Mortality, morbidity and prevalence

Infection is often lethal for *C. virginica*. Death usually occurs 1 or 2 years after infection, during or shortly after the warmest annual water temperatures (Burreson & Ragone Calvo, 1996). Prevalence is expected to be higher in individuals with more than 1 year of exposure to the pathogen (Andrews, 1996; Burreson & Ragone Calvo, 1996).

Prevalence is highly variable depending on host populations, pathogen strain and environmental factors (Kim *et al.,* 2025; Proestou *et al.,* 2023). Along the US east coast, *C. virginica* populations consistently exhibit high *P. marinus* infection rates, typically exceeding 80% and sometimes reaching 100% prevalence (Smolowitz*,* 2013). While populations from the Mexico Gulf typically exhibit low to moderate prevalence, with infection intensities generally remaining light (Ammons *et al.,* 2023; Cáceres-Martínez *et al.,* 2016; Gullian-Klanian *et al.,* 2008).

Infection prevalence and intensity are consistently low in *M. ariakensis*, *C. corteziensis* and *S. palmula* populations (Cáceres-Martínez *et al.,* 2012; 2016; Calvo *et al.,* 2000; 2001). Notably, no significant *P. marinus*-associated mortality has been observed in natural populations of *C. corteziensis* and *S. palmula* (Cáceres-Martínez *et al.,* 2012; 2016). Furthermore, *M. ariakensis* demonstrates superior survival, faster growth, and reduced disease susceptibility compared to *C. virginica* across varying salinity regimes (low, medium, and high) (Calvo *et al.,* 2001).

2.3.2. Clinical signs, including behavioural changes

Perkinsus marinus causes a chronic wasting disease.

Infected oysters may have a pale appearance to the digestive gland, reductions in condition index, severe emaciation, gaping, shrinkage of the mantle away from the outer edge of the shell, reduced gonadal development and/or retardation of growth (Ford & Tripp, 1996). Occasionally, pus-like pockets may occur in the soft tissues. However, these signs are not pathognomonic of perkinsosis.

2.3.3 Gross pathology

Gross signs are thin, watery tissue, eventually presence of pus-like pockets, but these gross signs are not specific to infection with *P. marinus*.

2.3.4. Modes of transmission and life cycle

The life cycle is direct from host to host (Andrews, 1996; Villalba *et al.,* 2004). *Perkinsus marinus* is transmitted directly from host to host through the water column following release of parasites in faeces or the decay or scavenging of moribund tissues (Bushek *et al.,* 2002). The parasite enters the paleal cavity of the oyster during filter-feeding. Labial palps and particularly the pseudo-faeces discharge area of the mantle are important sites of infection (Allam *et al.,* 2013; Winnicki *et al.,* 2008). Parasites infect and multiply within haemocytes. The infected circulating haemocytes migrate throughout the host tissues.

Experimental infection in controlled conditions is possible by exposing animals to *in vitro* culture of *P. marinus* (Dungan *et al.,* 2007).

2.3.5. Environmental factors

The prevalence and intensity of *P. marinus* are primarily driven by salinity and temperature. On the east coast of US, infections are acquired during the warm months of the year, when the water temperatures reach above 18°C (Smolowitz, 2013). Proliferation of the parasite and associated lesions are correlated with temperature. Temperature controls the annual cycle of *P. marinus*, with maximum prevalence and intensity lagging 1–2 months behind maximum summer water temperatures and minimum prevalence and intensity lagging 1–2 months behind minimum winter temperatures. Thus, *P. marinus* infections are most intense in autumn and least intense in early spring (Burreson & Ragone Calvo, 1996). Parasite infection intensity peaked at about 35°C (Malek *et al*., 2018). In tropical regions such as the Gulf of Mexico, however, *P. marinus* can proliferate~~s~~ even at significantly higher temperatures, suggesting an adaptation of host-parasite dynamics to these conditions (Cáceres-Martínez *et al.,* 2016).

Perkinsus marinus cells ~~were~~ are released on a diurnal cycle, with most cells released during the hottest and brightest period of the day (12:00–18:00). Temperature ~~had~~ has a strong and immediate effect on the number of cells released, but salinity ~~did~~ does not, only influencing the intensity of infection over the course of several months (Gignoux-Wolfsohn *et al*., 2020). Prevalence and intensity of *P. marinus* infections are greatest at salinities greater than 12 practical salinity units (psu). Transmission can occur between 9 and 12 psu, but infections remain low in intensity. *Perkinsus marinus* can persist for long periods in hosts at salinities less than 9 psu, but replication is low and no host mortality occurs.

2.3.6. Geographical distribution

Infection with *P. marinus* has been reported from the Atlantic coast of North and South America (da Silva *et al.,* 2013). It was introduced to the Pacific coast of North America (Cáceres-Martínez *et al.,* 2008). It has also been reported in Asia (Kim *et al*., 2024).

See WAHIS (https://wahis.woah.org/#/home) for recent information on distribution at the country level.

2.4. Biosecurity and disease control strategies

2.4.1. Vaccination

None.

2.4.2. Chemotherapy including blocking agents

N-Halamine disinfectant compounds ~~killed~~ can kill cultured *P. marinus* cells without affecting oyster larvae (Delaney *et al.,* 2003). Bacitracin, cycloheximide and freshwater have been shown to reduce, but not eliminate *P. marinus* in infected oyster hosts (Calvo & Burreson, 1994; Faisal *et al.,* 1999; La Peyre *et al.,* 2003). Moreover, the antimicrobial drug triclosan (5-chloro-2-[2,4 dichlorophenoxy]) phenol, may be effective in treating *P. marinus*-infected oysters (Chu *et al.,* 2008; Lund *et al.,* 2005). Finally copper (as $CuCl_2$) ~~was shown to~~ can greatly reduce infection levels of *P. marinus* in haemocytes *in vivo* (Foster *et al.,* 2011). These treatments may be relevant for aquaculture but are not practical in the natural environment.

2.4.3. Immunostimulation

None.

2.4.4. Breeding resistant strains

Selective breeding of surviving oysters from epizootics has demonstrated effectiveness for reducing mortality caused by *P. marinus* (Ragone Calvo *et al.,* 2003).

It has been reported that survival in genomically selected individuals is 20% higher than survival in individuals selected through phenotypic selection (Wang *et al.,* 2025).

2.4.5. Inactivation methods

Desiccation, chlorination (>0.3 mg ml^{-1} = 300 ppm [parts per million]), UV light (>28,000 µWs cm^{-2}) and freshwater have all been shown to inactivate *P. marinus* cells (Bushek *et al.,* 1997; Bushek & Howell 2000). UV irradiation from 4000 to 14,000 µWs cm^{-2} will inhibit proliferation of *P. marinus* (Bushek & Howell, 2000).

2.4.6. Disinfection of eggs and larvae

Perkinsus marinus is not known to infect eggs or larvae, but cells could occur extracellulary. Disinfection of eggs or larvae may be possible using N-halamine disinfectant compounds (Delaney *et al.,* 2003).

2.4.7. General husbandry

Farming in areas where salinity is less than 12 psu and use of fast-growing, disease-tolerant strains has shown some benefit (Andrews, 1996; Burreson & Ragone Calvo, 1996).

Water filtration to 1 µm followed by UV exposure should allow protecting hatchery produced seed against infection (Ford *et al.,* 2001). Inversely, treating water effluents should minimise the transmission of *P. marinus* to local oyster populations from infected oysters maintained in facilities such as processing plants, depuration facilities or hatcheries (Bushek & Howell, 2000).

Cultured off-bottom and air daily exposed oysters from infected area showed increased survival and higher condition indexes (La Peyre *et al.,* 2008).

3. Specimen selection, sample collection, transportation and handling

This section draws on information in Sections 2.2, 2.3 and 2.4 to identify populations, individuals and samples that are most likely to be infected.

3.1. Selection of populations and individual specimens

Gaping or freshly dead individuals (2 or more years old) should be sampled by priority, to increase the chances of finding infected animals. For histology, only live (including moribund) animals should be sampled.

Sampling of bivalves should be organised once a year when prevalence is known to be at a maximum. When such data is not available in a particular ecosystem, sampling should preferably be carried out during periods of oyster spawning which also corresponded with warmer temperatures (Burrell *et al.,* 1984).

3.2. Selection of organs or tissues

Connective tissue of all organs, and haemocytes. For Ray's fluid thioglycollate medium (RFTM), the whole animal should be ideally sampled if its size allows. If not, pieces of gill followed by mantle ~~and rectum~~ or foot for abalone are typically used. For culture purposes, tissue slices that can include gill, mantle, or foot (for abalone) can be cultured. For histology, a 5-mm thick section through the visceral mass that includes digestive gland, gill and mantle is used. For PCR, a pool of organs including digestive gland, gill or mantle tissue is ~~best~~ recommended.

3.3. Samples or tissues not suitable for pathogen detection

Tissues other than digestive gland, mantle and gills are less suitable.

3.4. Non-lethal sampling

At this time, there are no non-lethal tissue samples that can support detection of *P.marinus*.

A real-time PCR assay targeting the multicopy internal transcribed spacer region of the genome ~~was~~ has been developed to detect ~~*P. marinus*~~ DNA in ~~environmental~~ water~~s allowing detecting~~, with a reported detection limit equivalent ~~of~~ to 3.3 × 10^{-2} cells per 10~~-microl~~ µl reaction mixture (Audemard *et al.,* 2004). *Perkinsus* cells can also be detected in haemolymph, faeces, or water samples using RFTM assays (Bushek *et al.,* 2002; Ellin & Bushek, 2006; Gauthier & Fisher, 1990; Gauthier & Vasta, 1995). Detection limits (diagnostic sensitivity and specificity) have not been established for these analyses.

3.5. Preservation of samples for submission

For guidance on sample preservation methods for the intended test methods, see Chapter 2.4.0 General information (diseases of molluscs).

3.5.1. Samples for pathogen isolation

The results of bioassay depend strongly on the quality of samples (time since collection and time in storage). Fresh specimens should be kept on ice and preferably sent to the laboratory within 24 hours of collection. To avoid degradation of samples, use alternate storage methods only after consultation with the receiving laboratory.

Standard sample collection, preservation and processing methods for molecular techniques can be found in Section B.5.5 of Chapter 2.4.0 *General information* (diseases of molluscs).

For diagnosis using RFTM, samples must be fresh.

3.5.2. Preservation of samples for molecular detection

For polymerase chain reaction (PCR) assays, samples must be preserved in ~~70–100%~~ analytical grade ethanol (80%) at room temperature ~~and not denatured alcohol~~.

Standard sample collection, preservation and processing methods for molecular techniques can be found in Section B.5.5 of Chapter 2.4.0 *General information* (diseases of molluscs).

3.5.3. Samples for histopathology, immunohistochemistry or *in-situ* hybridisation (ISH)

For histology, the best preservative is Davidson's AFA, but 10% buffered formalin or other standard histology fixatives are also acceptable.

Standard sample collection, preservation and processing methods for histological techniques can be found in Section B.5.3 of Chapter 2.4.0 *General information* (diseases of molluscs).

3.5.4. Samples for other tests

None.

3.6. Pooling of samples

Pooling of samples from more than one individual animal for a given purpose is only recommended where robust supporting data on diagnostic sensitivity and diagnostic specificity have been evaluated and found to be suitable. If the effect of pooling on diagnostic sensitivity has not been thoroughly evaluated, larger specimens should be processed and tested individually. Small life stages such as spat can be pooled to obtain the minimum amount of

material for molecular detection. ~~Pooling of very small spat (5–10 depending on size) is acceptable for PCR analyses.~~

4. Diagnostic methods

The methods currently available for pathogen detection that can be used in i) surveillance of apparently healthy animals, ii) presumptive diagnosis in clinically affected animals and iii) confirmatory diagnostic purposes are listed in Table 4.1. by animal life stage.

Ratings for purposes of use. For each recommended assay a qualitative rating for the purpose of use is provided. The ratings are determined based on multiple performance and operational factors relevant to application of an assay for a defined purpose. These factors include appropriate diagnostic performance characteristics, level of assay validation, availability cost, timeliness, and sample throughput and operability. For a specific purpose of use, assays are rated as:

+++ =	Methods are most suitable with desirable performance and operational characteristics.
++ =	Methods are suitable with acceptable performance and operational characteristics under most circumstances.
+ =	Methods are suitable, but performance or operational characteristics may limit application under some circumstances.
Shaded boxes =	Not appropriate for this purpose.

Validation stage. The validation stage corresponds to the assay development and validation pathway in chapter 1.1.2. The validation stage is specific to each purpose of use. Where available, information on the diagnostic performance of recommended assays is provided in Section 6.3.

WOAH Reference Laboratories welcome feedback on diagnostic performance of recommended assays, in particular PCR methods. Of particular interest are any factors affecting expected assay sensitivity (e.g. tissue components inhibiting amplification) or expected specificity (e.g. failure to detect particular genotypes, detection of homologous sequences within the host genome). These issues should be communicated to the WOAH Reference Laboratories so that advice can be provided to diagnostic laboratories and the standards amended if necessary.

Table 4.1. WOAH recommended diagnostic methods and their level of validation for surveillance of apparently healthy animals and investigation of clinically affected animals

Method	A. Surveillance of apparently healthy animals				B. Presumptive diagnosis of clinically affected animals				C. Confirmatory diagnosis[1] of a suspect result from surveillance or presumptive diagnosis			
	Early life stages[2]	Juveniles[2]	Adults	LV	Early life stages[2]	Juveniles[2]	Adults	LV	Early life stages[2]	Juveniles[2]	Adults	LV
Wet mounts												
Histopathology		++	++	1		+++	+++	NA				
Cell culture												
Transmission electron microscopy									++	++	++	NA
Real-time PCR	+ ~~++~~	+++	+++	~~2~~ 1	+ ~~++~~	+++	+++	~~2~~ **1**	+ ~~++~~	+++	+++	~~2~~ **1**
Conventional PCR	+ ~~++~~	++	++	1	+ ~~++~~	++	++	1				
Conventional PCR followed by amplicon sequencing									+++	+++	+++	1
In-situ hybridisation										+	+	1
Bioassay												
LAMP												
Ab-ELISA												
Ag-ELISA												
RFTM		++	+++	~~2~~ 1		++	+++	NA				

LV = level of validation, refers to the stage of validation in the WOAH Pathway (chapter 1.1.2); PCR = polymerase chain reaction; LAMP = loop-mediated isothermal amplification; Ab- or Ag-ELISA = antibody or antigen enzyme-linked immunosorbent assay, respectively. RFTM = Ray's fluid thioglycollate culture method

[1]For confirmatory diagnoses, methods need to be carried out in combination (see Section 6). [2]Susceptibility of early and juvenile life stages is described in Section 2.2.3.

[3]Specify the test used. Shading indicates the test is inappropriate or should not be used for this purpose.

4.1. Smears

Not recommended as a diagnostic method.

4.2. Histopathology

Samples to be taken consist of fresh, gaping or freshly dead bivalves.

Sections of tissue that include gills, digestive gland, mantle, and gonad should be fixed for 24 hours minimum in a recommended fixative followed by standard processing for histology as described in section 5.3 of Chapter 2.4.0 *General information* (diseases of molluscs). Observations are made at increasing magnifications up to ×1000.

Histology is generally less sensitive than PCR methods (see Sections 6.1. and 6.2).

A positive result is the occurrence of spherical, uninucleated cells, trophozoïtes, ranging from about 2 to 5 µm (sometimes to 10 µm) in diameter with a large vacuole and an eccentrically displaced nucleus. These are typically associated with host gut or sometimes epithelium in lighter infections, with colonisation of connective tissues characteristic of more advanced cases. Binucleated *P. marinus* schizonts (dividing forms) may be observed, though larger schizonts of greater nuclear count are not reliably presented. Many of the parasites may occur within oyster haemocytes. *Perkinsus marinus* cells stain basophilic. A change in *Perkinsus marinus* phenotype (large trophozoïtes at low infection intensity were replaced by abundant tinny cells) was reported since 1986 in oysters from Chesapeake Bay by Carnegie *et al.,* 2021).

4.3. Electron microscopy

Fixed sections reveal large multifocal lesions in gut epithelium or connective tissue of any organ containing *P. marinus* cells (Mackin, 1951). Haemocyte infiltration and phagocytosis of *P. marinus* cells occurs in most infections. In high intensity infections, the gut epithelium may be almost completely destroyed.

Perkinsus marinus zoospores, like zoospores of all species of *Perkinsus,* show an ornamented anterior flagellum with hair-like and spurs-like structures and a glabrous posterior flagellum. The zoospore contains an apical complex consisting of a conoid, subpellicular microtubules, rhoptries, rectilinear micronemes and conoid-associated micronemes. Large vacuoles also occur at the anterior end of the zoospore (Perkins 1976; 1996).

4.4. Nucleic acid amplification

PCR assays should always be run with the controls specified in Section B.5.5 *Molecular methods* Chapter 2.4.0 *General information* (diseases of molluscs). Molluscs are known to potentially contain substances that can inhibit PCR reactions. It is recommended to check for the presence of PCR inhibitors in DNA extracts to avoid false negative results. In case PCR inhibitors are present, DNA samples can be diluted prior to PCR analyses (a 1/10 dilution usually resolves most cases of PCR inhibition). Each sample should be tested in duplicate.

Several PCR assays have been developed for the detection of *Perkinsus* sp. and *P. marinus*, most of them target portions of the rRNA gene complex. Primers that target the NTS region have demonstrated good species specificity for *P. marinus*, however, little is known of the variation within species for the NTS region and there is a risk of false negatives. The sequence variation in the ITS region is more broadly characterised (see the GenBank database) and primers targeting the ITS region are more thoroughly tested for specificity. For these reasons, primers that target the ITS region are recommended

Depending on the context of analysis, *Perkinsus* genus PCR assays be conducted first, and then samples with positive results should be tested with a *P. marinus* specific assay.

Extraction of nucleic acids

Samples to be taken consist of live or freshly dead molluscs. 2–3 mm^2 tissue pieces are excised aseptically from gill, mantle and digestive gland placed into 1.5 ml microcentrifuge. Dissecting utensils should be flamed between samples to prevent cross-contamination.

Different kits and procedures can be used for nucleic acid extraction. DNA is usually extracted by proteinase K digestion at 56°C (generally overnight), and the spin-column methodology using commercially available kits. Faster extraction protocols using Chelex resin has also been described (De Faveri *et al.,* 2009; Piesz *et al.,* 2022). The quality and concentration of the extracted nucleic acid is important and can be checked using a suitable method as appropriate to the circumstances.

4.4.1. Real-time PCR

40. Primers and probes (sequences)

Pathogen/ target gene	Primer/probe (5'–3')	Concentration	Cycling parameters[(a)]
Method 1: TaqMan PCR: Gauthier *et al.,* 2006, amplicon size: 86 bp; GenBank Accession No.: AF149876.1			
Perkinsus sp./ITS (detects at least *P. marinus, P. olseni* and *P. chesapeaki*)	PERK-F: TCC-GTG-AAC-CAG-TAG-AAA-TCT-CAA-C PERK-R: GGA-AGA-AGA-GCG-ACA-CTG-ATA-TGT-A PERK Probe: (FAM) CCC-TTT-GTG-CAG-TAT-GC-MGB	0.9 µM 0.9 µM 0.25 µM	40 cycles of: 95°C/15 sec and 60°C/1 min
Method 2: TaqMan PCR: Gauthier *et al.,* 2006; GenBank Accession No.: AF149876.1; amplicon size: 72 bp			
Perkinsus marinus/ITS	PMAR F: TTG-TTA-ACG-CAA-CTC-AAT-GCT-TTG-T PMAR R: AAG-CGC-ACA-TAA-CGA-ACC-ACC PMAR-MGB Probe: (FAM)-GCT-TGA-ACT-AAC-TCT-MGB-	0.9 µM 0.9 µM 0.25 µM	40 cycles of: 95°C/15 sec and 60°C/1 min
Method 3: Real-time PCR: De Faveri *et al.,* 2009; GenBank Accession No.: No data; amplicon size: 90 bp			
Perkinsus marinus/ITS	F: CGC-CTG-TGA-GTA-TCT-CTC-GA R: GTT-GAA-GAG-AAG-AAT-CGC-GTG-AT Probe: [FAM]CGC-AAA-CTC-GAC-TGT-GTT-GTG-GTG	0.2 µM 0.2 µM 0.04 µM	35 cycles at 95°C for 30 sec, and 60°C for 45 sec
Method 4: SYBR Green PCR Audemard *et al.,* 2004; 509 bp, GenBank Accession No.: AF149876.1			
Perkinsus marinus/ITS	PmarITS-70F: CTT-TTG-YTW-GAG-WGT-TGC-GAG-ATG PmarITS-600R: CGA-GTT-TGC-GAG-TAC-CTC-KAG-AG	0.5 µM 0.5 µM	50 cycles of: 95°C/10 sec and 69°C/5 sec and 72°C/25 sec Final elongation 72°C/10 min Meting curve analysis: from 60°C to 95°C, increment 1°C for 30 sec

[(a)]A denaturation step prior to cycling has not been included.

Interpretation of results:

A sample is considered positive when a PCR amplification curve is observed (with the expected Ct value when a cut-off Ct value has been established) with the appropriate melting temperature peak (Tm) in case of SYBR Green PCR assays, with all negative controls being negative and all positive controls being positive.

Depending on the context/objective of the analysis, a cut-off Ct value can be used to define if samples are positives or negatives. The cut-off Ct value may vary depending on the equipment, reagents and consumables used for the test.

If PCR inhibitors are detected in a sample producing a negative result, this sample should be considered as uninterpretable.

4.4.2. Conventional PCR

Pathogen/ target gene	Primer/probe (5'–3')	Concentration	Cycling parameters[(a)]
Method 1: Audemard *et al.*, 2004 (primers from Casas *et al.*, 2002); GenBank Accession No.: AF149876.1, amplicon size: ~~703~~ 666bp			
Perkinsus sp./ITS (detects all known *Perkinsus* species *except P. qugwadi*)	PerkITS-85: CCG-CTT-TGT-TTG-GAT-CCC PerkITS-750: ACA-TCA-GGC-CTT-CTA-ATG-ATG	0.5 µM 0.5 µM	40 cycles of: 95°C/1 min and 55°C/1min and 72°C/1min Final elongation 72°C/10 min
Method 2: Audemard *et al.*, 2004; GenBank Accession No.: AF149876.1, amplicon size: 509 bp			
Perkinsus marinus/ITS	PmarITS-70F: CTT-TTG-YTW-GAG-WGT-TGC-GAG-ATG PmarITS-600R: CGA-GTT-TGC-GAG-TAC-CTC-KAG-AG	0.5 µM 0.5 µM	40 cycles of: 95°C/1 min and 57°C/1min and 72°C/3min Final elongation 72°C/10 min

[(a)]A denaturation step prior to cycling has not been included.

4.4.3. Other nucleic acid amplification methods

None.

4.5. Amplicon sequencing

The size of the PCR amplicon should be verified, for example by agarose gel electrophoresis. ~~Obtained sequences are analysed in comparison with reference sequences.~~ Both DNA strands of the PCR product must be sequenced and analysed in comparison with reference sequences.

4.6. *In-situ* hybridisation

Samples to be taken consist of live or freshly dead molluscs.

A specific DNA probe that targets the small subunit rRNA gene has been developed for the genus *Perkinsus*: Perksp700DIG (Elston *et al.,* 2004) and a DNA probe that targets the LSU of the rRNA gene has been developed for the specific detection of *P. marinus:* PmarLSU-181 (Moss *et al.,* 2006). Additionally, two other *P. marinus* probes also targeting the LSU region has been developed: Pmar LSU-420 and LSU 560 (Reece *et al.,* 2008) and can be used in cocktails with the first one. The probe should be 5' end-labelled with digoxigenin. Alternatively, probes can be labelled with a fluorochrome (ex. Alexa Fluor 488®)

Pathogen/target	**ISH probe type**	**ISH probe (5'-3')**	**Reference**
Perkinsus sp./SSU (detects all known *Perkinsus* species *except P. qugwadi)*	Labelled oligonucleotide	PerkspSSU-700 CGC-ACA-GTT-AAG-TRC-GTG-RGC-ACG	Elston *et al.* (2004)
Perkinsus marinus/LSU	Labelled oligonucleotide(s) (PmarLSU-181 alone or cocktail of the 3)	PmarLSU-181 GAC-AAA-CGG-CGA-ACG-ACT-C PmarLSU-420 GAA-GAC-AGG-AGC-GAG-CAG-C PmarLSU-560 AAC-CAA-TTC-ACA-GAT-AGC-G	Moss *et al.* (2006); Reece *et al.* (2008)

4.7. Immunohistochemistry

Not available.

4.8. Bioassay

Not available.

4.9. Antibody- or antigen-based detection methods (ELISA, etc.)

Polyclonal antibody-based analyte-capture ELISAs are reported to detect *P. marinus* extracellular secretions (ECP) in tissue homogenates from oysters with infection intensities of one parasite cell/g oyster tissue (Ottinger *et al.,* 2001). The specificity of polyclonal antibodies against *P. marinus* ECP was not tested against other *Perkinsus* spp. or marine mollusc protistan associates.

4.10. Other methods

4.10.1. Ray's fluid thioglycollate culture method (RFTM)

Incubation in thioglycollate is routinely used for surveillance of *P. marinus.* The technique is simple, inexpensive and very sensitive, but not species specific. Trophozoites of *P. marinus* in oyster tissue will enlarge when cultured for at least 5 days in fluid thioglycollate medium containing dextrose that is fortified with antibiotics (penicillin, streptomycin) and an antifungal compound (nystatin) to reduce bacterial and fungal growth. When the tissue is macerated after culture to allow penetration of aqueous iodine solution (Lugol's), the enlarged trophozoites (hypnospores or prezoosporangia in the old terminology) readily take up Lugol's and become easily visible at low power because of their bluish–black colouration and spherical shape.

Samples to be taken consist of live or freshly dead molluscs.

Tissue assay (*Ray, 1966*): tissue samples measuring approximately 5–10 mm are excised, giving preference to rectal, gill and mantle tissue from oysters, and placed in test tubes containing thioglycollate medium (thioglycollate medium containing dextrose 14.6 g; NaCl, 10.0 g; sterile distilled water (dH_2O), 485 ml). A total of 9.5 ml is dispensed into disposable test tubes, which are autoclaved for 15 minutes at 1.2 kg cm^{-2} pressure. The autoclaved solution can be stored in tubes for up to 3 weeks. Dissecting utensils should be rinsed in 95% ethanol and flamed between hosts to prevent carry-over. The recommended antifungal/antibiotics are: 500 units ml^{-1} penicillin G and 500 units ml^{-1} dihydro-streptomycin in media (penicillin, 3.13 g; streptomycin, 6.55 g; 500 ml dH_2O; freeze in 50 ml aliquots; add 0.5 ml to each tube), and 50 µl of mycostatin (nystatin) per tube. Chloromycetin can be used in place of penicillin/streptomycin. The tube is plugged with a foam rubber or cotton stopper. Incubation is at 22–25°C for between 5 and 7 days, in the dark. After incubation, the fragments of tissue are collected and chopped with a scalpel blade on a glass slide, a drop of Lugol's iodine solution is added (stock Lugol's iodine solution: potassium iodide, 6.0 g; iodine, 4.0 g; dH_2O, 100 ml. Lugol's iodine working solution: dH_2O, 30.0 ml; Lugol's stock solution, 15.0 ml) and the preparation is covered with a cover-slip and allowed to sit for 10 minutes. The preparations are examined in the fresh state.

Whole body burden assay (*Fisher & Oliver, 1996*): the entire host, cut into 2–5 mm pieces, is placed in fluid thioglycollate culture medium and incubated as in the tissue assay above. The solution is centrifuged at 1500 ***g*** for 10 minutes and the supernatant is discarded. 2 M NaOH (20 ml g^{-1} tissue) is added and the solution is incubated at 60°C for 2–6 hours until tissue is digested. The solution is centrifuged at 1500 ***g*** for 10 minutes and the supernatant is discarded. The solution is washed three times in deionised water, the pellet is resuspended in 1 ml Lugol's iodine working solution, and the cells are counted. Serial dilutions may have to be made to reduce the total cell number to a manageable number.

Interpretation of results:

Cultured parasites enlarge from 2–10 to 20–70 µm during incubation. *Perkinsus* spp. cells are spherical and the walls stain blue or bluish-black with Lugol's iodine solution (Bushek *et al.,* 1994; Ray 1966).

In susceptible host species, within the known range of *P. marinus*, a positive result is presumptive evidence of *P. marinus* infection, but should be confirmed in accordance with Section 6.

4.10.2. Cell culture/artificial media

Perkinsus marinus cells are easily cultured in a variety of media (e.g. La Peyre *et al.,* 1993). Culture medium is usually inoculated with heart, haemolymph, or gill tissue. Comparisons of commercially available media have been made (Dungan & Hamilton, 1995) and growth was supported in all media but was at a maximum in 1/1 DME (Dulbecco's Modified Eagle's)/Ham's F-12 medium.

5. Test(s) recommended for surveillance to demonstrate freedom in apparently healthy populations

Real-time PCR and ~~eventually~~ RFTM ~~tissue or~~ whole-body burden assays should be used for targeted surveillance to declare freedom from infection with *P. marinus*.

6. Corroborative diagnostic criteria

This section only addresses the diagnostic test results for detection of infection in the absence (Section 6.1.) or in the presence (Section 6.2.) of clinical signs ~~(Section 6.2.)~~ but does not evaluate whether the infectious agent is the cause of the clinical event.

The case definitions for a suspect and confirmed case have been developed to support decision making related to trade and confirmation of disease status at the country, zone or compartment level. Case definitions for disease confirmation in endemically affected areas may be less stringent. If a Competent Authority does not have the capability to undertake the necessary diagnostic tests it should seek advice from the appropriate WOAH Reference Laboratory, and if necessary, refer samples to that laboratory for confirmatory testing of samples from the index case in a country, zone or compartment considered free. There ~~are~~ is currently no WOAH Reference Laboratory for *Perkinsus marinus*.

When two molecular methods are used for confirmation of a case, it is preferable to use two species-specific methods targeting non-overlapping regions of the pathogen genome.

6.1. Apparently healthy animals or animals of unknown health status[1]

Apparently healthy populations may fall under suspicion, and therefore be sampled, if there is an epidemiological link(s) to an infected population. Hydrographical proximity to, or movement of animals or animal products or equipment, etc., from a known infected population equate to an epidemiological link. Alternatively, healthy populations are sampled in surveys to demonstrate disease freedom.

6.1.1. Definition of suspect case in apparently healthy animals

The presence of infection with *P. marinus* shall be suspected if at least one of the following criteria is met:

i) Histopathological changes consistent with the presence of the pathogen or the disease

ii) Positive result by conventional PCR

iii) Positive result by real-time PCR

iv) Positive result by RFTM

6.1.2. Definition of confirmed case in apparently healthy animals

The presence of infection with *P. marinus* is considered to be confirmed if at least one of the following criteria is met:

i) Positive result by real-time PCR and positive result by conventional PCR followed by amplicon sequencing

ii) Positive result by histology and positive result by conventional PCR followed by amplicon sequencing

iii) Positive result by *in-situ* hybridisation and positive result by conventional PCR followed by amplicon sequencing

iv) Positive result by histology and positive result by species-specific real-time PCR.

v) Positive result by *in-situ* hybridisation and positive result by species-specific real-time PCR.

6.2 Clinically affected animals

Clinical signs are not pathognomonic for a single disease; however, they may narrow the range of possible diagnoses.

[1] For example transboundary commodities.

6.2.1. Definition of suspect case in clinically affected animals

The presence of infection with *P. marinus* shall be suspected if at least one of the following criteria is met:

i) Gross pathology or clinical signs associated with the disease as described in this chapter~~, with or without elevated mortality~~

ii) Histopathological changes consistent with the presence of the pathogen or the disease

iii) Positive result by conventional PCR

iv) Positive result by real-time PCR

v) Positive result by RFTM

6.2.2. Definition of confirmed case in clinically affected animals

The presence of infection with *P. marinus* is considered to be confirmed if at least one of the following criteria is met:

i) Positive result by real-time PCR and positive result by conventional PCR followed by ~~sequence analysis~~ amplicon sequencing

ii) Positive result by ~~ISH~~ *in-situ* hybridisation and positive result by conventional PCR followed by ~~sequence analysis~~ amplicon sequencing

ii) Positive result by i*n-situ* hybridisation and positive result by species-specific real-time PCR ~~real-time PCR and ISH~~

6.3. Diagnostic sensitivity and specificity for diagnostic tests

The diagnostic performance of tests recommended for surveillance or diagnosis of infection with *P. marinus* are provided in Tables 6.3.1. and 6.3.2 (no data are currently available for either). Data are only presented where tests are validated to at least level 2 of the validation pathway described in Chapter 1.1.2. and the information is available within published diagnostic accuracy studies.

~~Some data on analytical performances (stage 1 validation) are available for most diagnostic tests described in this chapter but are not always complete (for example for inclusivity and exclusivity).~~ RFTM ~~assays~~ and histopathology ~~allow a diagnostic~~ enable diagnosis only at the genus level and ~~do not allow~~ cannot differentiate between species ~~discrimination~~. RFTM ~~assays are~~ is considered ~~to be~~ specific for all members of the genus *Perkinsus* except *P. qugwadi* (incertae sedis). However, ~~the apparent~~ its exclusivity ~~of RFTM assays~~ for ~~detection of~~ detecting only *Perkinsus* species ~~lacks exhaustive confirmation~~ has not been exhaustively confirmed through testing of all other protists (Dungan & Reece, 2020). Molecular tools have been evaluated for ~~their~~ analytical sensitivity and specificity to varying ~~extents.~~ degrees but estimates of diagnostic sensitivity (DSe) and specificity (DSp) (stage 2 validation) ~~have~~ are generally lacking. ~~not been estimated, however~~ Comparative data ~~comparing sensitivity performances of the different diagnostic tests are available.~~ indicate that histopathology is ~~considered as~~ less sensitive than both PCR and RFTM assays. The whole-body burden (WBB) RFTM assay is considered ~~very~~ highly sensitive, with a theoretical detection limit ~~sensitivity~~ of one *Perkinsus* sp. cell per host. In contrast, tissue and haemolymph RFTM assays have ~~a~~ limited sensitivity when infection intensity ~~is~~ falls below 1000 *perkinsus* cells per gram wet tissue (Bushek *et al.,* 1994).

6.3.1. For presumptive diagnosis of clinically affected animals (no data are currently available)

Test type	Test purpose	Source populations	Tissue or sample types	Species	DSe (*n*)	DSp (*n*)	Reference test	Citation

41. DSe = diagnostic sensitivity, DSp = diagnostic specificity, *n* = number of animals used in the validation study,
PCR: = polymerase chain reaction.

6.3.2. For surveillance of apparently healthy animals (no data are currently available)

Test type	Test purpose	Source populations	Tissue or sample types	Species	DSe (*n*)	DSp (*n*)	Reference test	Citation

42. DSe = diagnostic sensitivity, DSp = diagnostic specificity, *n* = number of animals used in the validation study,
PCR: = polymerase chain reaction.

7. References

ALLAM B., CARDEN W.E., WARD J.E., RALPH G., WINNICKI S. & PALES ESPINOSA E. (2013). Early host-pathogen interactions in marine bivalves: evidence that the alveolate parasite *Perkinsus marinus* infects through the oyster mantle during rejection of pseudofeces. *J. Invertebr. Pathol.*, **113**, 26–34. doi: 10.1016/j.jip.2012.12.011.

AMMONS D., GONZALEZ D., LU M.T. & RAMPERSAD J. (2023). Assessment of Dermo *Perkinsus marinus* in the "Southern Oyster" from a High-Salinity Environment. *N. Am. J. Aquac.,* **85**, 235–240.

ANDREWS J.D. (1988). Epizootiology of the disease caused by the oyster pathogen *Perkinsus marinus* and its effects on the Oyster industry. *Am. Fisheries Soc. Special Publications* **18**, 47–63.

ANDREWS J.D. (1996). History of *Perkinsus marinus*, a pathogen of oysters in Chesapeake Bay 1950–1984. *J. Shellfish Res.,* **15**, 13–16.

ANDREWS J.D. & HEWATT W.G. (1957). Oyster Mortality Studies in Virginia. II. The Fungus Disease Caused by *Dermocystidium marinum* in Oysters of Chesapeake Bay. *Ecological Monographs*, **27**, 1–25. Published by: Ecological Society of America. http://www.jstor.org/stable/1948568.

AUDEMARD C., REECE K.S. & BURRESON E.M. (2004). Real-time PCR for the detection and quantification of the protistan parasite *Perkinsus marinus* in environmental waters. *Appl. Environ. Microbiol.,* **70**, 6611–6618.

BOGEMA D.R., YAM J., MICALLEF M.L., GHOLIPOURKANANI H., GO J., JENKINS C. & DANG C. (2021). Draft genomes of *Perkinsus olseni* and *Perkinsus chesapeaki* reveal polyploidy and regional differences in heterozygosity. *Genomics,* **113**, 677–688.

BURRELL V.G., BOBO M.Y. & MANZI J.J. (1984). A comparison of seasonal incidence and intensity of *Perkinsus marinus* between subtidal and intertidal oyster populations in South Carolina. *J. World Mariculture Soc.,* **15**, 301–309.

BURRESON E.M. & RAGONE CALVO L.M. (1996). Epizootiology of *Perkinsus marinus* disease of oysters in Chesapeake Bay, with emphasis on data since 1985. *J. Shellfish Res*., **15**, 17–34.

BUSHEK D. & ALLEN S.K. (1996). Host-parasite interactions among broadly distributed populations of the eastern oyster *Crassostrea virginica* and the protozoan *Perkinsus marinus*. *Mar. Ecol. Progr. Series*, **139**, 127–141.

BUSHEK D., FORD S.E. & ALLEN S.K. (1994). Evaluation of methods using Ray's fluid thioglycollate medium for diagnosis of *Perkinsus marinus* infection in the eastern oyster, *Crassostrea virginica*. *Ann. Rev. Fish Dis.,* **4**, 201–217.

BUSHEK D., FORD S.E. & CHINTALA M.M. (2002). Comparison of *in vitro*-cultured and wild type *Perkinsus marinus*. III. Fecal elimination and its role in transmission. *Dis. Aquat. Organ*.,**51**, 217–225.

BUSHEK D., HOLLEY R. & KELLY M. (1997). Treatment of *Perkinsus marinus*-contaminated materials. *J. Shellfish Res.,* **16**, 330.

BUSHEK D. & HOWELL T.L. (2000). The effect of UV irradiation on *Perkinsus marinus* and its potential use to reduce transmission via shellfish effluents. Northeastern Regional Aquaculture Center (NRAC) Publication No. 00-008. North Dartmouth, Massachusetts, USA, 4p.

CÁCERES-MARTÍNEZ J., MADERO-LÓPEZ L.H., PADILLA-LARDIZÁBAL G. & VÁSQUEZ-YEOMANS R. (2016). Epizootiology of *Perkinsus marinus*, parasite of the pleasure oyster *Crassostrea corteziensis*, in the Pacific coast of Mexico. *J. Invertebr. Pathol.*, **139**, 12–18.

CÁCERES-MARTÍNEZ J., ORTEGA M.G., VASQUEZ-YEOMANS R., GARCIA T. DE J., STOKES N.A. & CARNEGIE R.B. (2012). Natural and cultured populations of the mangrove oyster *Saccostrea palmula* from Sinaloa, Mexico, infected by *Perkinsus marinus*. *J. Invertebr. Pathol.,* **110**, 321–325.

CÁCERES-MARTÍNEZ J., VÁSQUEZ-YEOMANS R., PADILLA-LARDIZÁBAL G. & DEL RÍO PORTILLA M.A. (2008). *Perkinsus marinus* in pleasure oyster *Crassostrea corteziensis* from Nayarit, Pacific coast of México. *J. Invert. Pathol.,* **99**, 66–73.

CALVO G.W. & BURRESON E.M. (1994). *In vitro* and *in vivo* effects of eight chemotherapeutants on the oyster parasite *Perkinsus marinus* (Mackin, Owen, and Collier). *J. Shellfish Res.,* **13**, 101–107.

CALVO G.W., LUCKENBACH M.W. & BURRESON E.M. (2000). High Performance of *Crassostrea ariakensis* in Chesapeake Bay. *J. Shellfish Res*., **19**, 643. (Abstract).

CALVO G.W., M.W. LUCKENBACH, ALLEN S.K. JR & BURRESON E.M. (2001). A comparative field study of *Crassostrea ariakensis* (Fujita 1913) and *Crassostrea virginica* (Gmelin 1791) in relation to salinity in Virginia. *J. Shellfish Res*., **20**, 221–229.

CARNEGIE R.B., FORD S.E., CROCKETT R.K., KINGSLEY-SMITH P.R., BIENLIEN L.M., SAFI L.S.L., WHITEFLEET-SMITH L.A. & BURRESON E.M. (2021). A rapid phenotype change in the pathogen *Perkinsus marinus* was associated with a historically significant marine disease emergence in the eastern oyster. *Sci. Rep*., **11**, 12872.

CASAS S.M., VILLALBA A. & REECE K.S. (2002). Study of the perkinsosis of the carpet shell clam *Tapes decussatus* in Galicia (NW Spain). I. Identification of the etiological agent and *in vitro* modulation of zoosporulation by temperature and salinity. *Dis. Aquat. Organ.*, **50**, 51–65.

CHU F.L. & LUND E. (2006). Viability, infectivity and fatty acid synthetic activity of *Perkinsus marinus* meront cells incubated in estuarine and artificial seawater. *Dis. Aquat. Organ.,* **71**, 131–139.

CHU F.-L.E., LUND E.D. & PODBESEK J.A. (2008). Effects of triclosan on the oyster parasite, *Perkinsus marinus* and its host, the eastern oyster, *Crassostrea virginica J. Shellfish Res*., **27**, 769–773.

DA SILVA P.M., VIANNA R.T., GUERTLER C., FERREIRA L.P., SANTANA L.N., FERNÁNDEZ-BOO S., RAMILO A., CAO A. & VILLALBA A. (2013). First report of the protozoan parasite Perkinsus marinus in South America, infecting mangrove oysters *Crassostrea rhizophorae* from the Paraíba River (NE, Brazil). *J. Invertebr. Pathol.,* **113**, 96–103.

DE FAVERI J., SMOLOWITZ R.M. & ROBERTS S.B. (2009). Development and validation of a real-time quantitative PCR assay for the detection and quantification of *Perkinsus marinus* in the eastern oyster, *Crassostrea virginica*. *J. Shellfish Res.,* **28**, 459-464.

DELANEY M.A., BRADY Y.J. WORLEY S.D. & HUELS K.L. (2003). The effectiveness of N-Halamine disinfectant compounds on *Perkinsus marinus*, a parasite of the eastern oyster *Crassostrea virginica*. *J. Shellfish Res.,* **22,** 91–94.

DUNGAN C.F. & HAMILTON R.M. (1995). Use of a tetrazolium-based cell proliferation assay to measure effects of *in vitro* conditions on *Perkinsus marinus* (Apicomplexan) proliferation. *J. Euk. Microbiol.,* **42**, 379–388.

DUNGAN C.F. & REECE K.S. (2020). Chapter 5.2. 1 *Perkinsus* spp. Infections of Marine Molluscs. *In:* FHS Blue Book: Suggested procedures for the detection and identification of certain finfish and shellfish pathogens. AFS-FHS (American Fisheries Society-Fish Health Section), Bethesda, Maryland, USA, 1–24.

DUNGAN C.F., REECE K.S., HAMILTON R.M., STOKES N.A. & BURRESON E.M. (2007). Experimental cross-infection by *Perkinsus marinus* and *P. chesapeaki* in three sympatric species of Chesapeake Bay oysters and clams. *Dis. Aquat. Organ*., **76**, 67–75.

ELLIN R. & BUSHEK D. (2006). Adaptation of ray's fluid thioglycollate medium assay to detect and quantify planktonic stages of *Perkinsus* spp. parasites. *J. Shellfish Res*., **25**, 1037–1042.

ELSTON R.A., DUNGAN C.F., MEYERS T.R. & REECE K.S. (2004). *Perkinsus* sp. infection risk for manila clams, *Venerupis philippinarum* (A. Adams and Reeve, 1850) on the Pacific coast of North and Central America. *J. Shellfish Res.,* **23,** 101–105.

FAISAL M., LA PEYRE J.F. & ELSAYED E.E. (1999). Bacitracin inhibits the oyster pathogen *Perkinsus marinus in vitro* and *in vivo*. *J. Aquat. Anim. Health*, **11**, 130–138.

FISHER W.S. & OLIVER L.M. (1996). A whole-oyster procedure for diagnosis of *Perkinsus marinus* disease using Ray's fluid thioglycollate culture medium. *J. Shellfish Res.,* **15**, 109–117.

FORD S.E. & TRIPP M.R. (1996). Diseases and Defense Mechanisms. *In:* The Eastern Oyster *Crassostrea virginica*, Kennedy V.S., Newell R.I.E.& Eble A.F., eds. Maryland Sea Grant College, College Park, Maryland, USA, 581–660.

FORD S.E., XU Z. & DEBROSSE G. (2001). Use of particle filtration and UV irradiation to prevent infection by *Haplosporidium nelsoni* (MSX) and *Perkinsus marinus* (Dermo) in hatchery-reared larval and juvenile oysters. *Aquaculture*, **194**, 37–49.

FOSTER B., GREWAL S., GRAVES O., HUGHES F.M. JR & SOKOLOVA I.M. (2011). Copper exposure affects hemocyte apoptosis and *Perkinsus marinus* infection in eastern oysters *Crassostrea virginica* (Gmelin). *Fish Shellfish Immunol*., **31**, 341–349.

GAUTHIER J.D. & FISHER W.S. (1990). Hemolymph assay for diagnosis of *Perkinsus marinus* in oysters *Crassostrea virginica* (Gmelin, 1791). *J. Shellfish Res*., **9**, 367–371.

GAUTHIER J.D., MILLER C.R., & WILBUR A.E. (2006). TaqMan® MGB real-time PCR approach to quantification of *Perkinsus marinus* and *Perkinsus* spp. in oysters. *J. Shellfish Res.,* **25,** 619-624.

GAUTHIER J.D. & VASTA G.R. (1995). *In Vitro* Culture of the Eastern Oyster Parasite *Perkinsus marinus:* Optimization of the Methodology. *J. Invertebr. Pathol*., **66**, 156–168.

GIGNOUX-WOLFSOHN S.A., NEWCOMB M.S.R., RUIZ G. & PAGENKOPP LOHAN K. (2020). Environmental factors drive release of Perkinsus marinus from infected oysters. *Parasitol*., **148**, 1–31.

GREEN-BEACH E., GUO X., SMOUSE P. & BUSHEK D. (2011). Population structure of oysters on Martha's Vineyard, MA in response to selection by *Perkinsus marinus*, using microsatellite markers. *J. Shellfish Res*., **30**, 512. (Abstract).

GULLIAN-KLANIAN M., HERRERA-SILVEIRA J.A., RODRÍGUEZ-CANUL R. & AGUIRRE-MACEDO L. (2008). Factors associated with the prevalence of *Perkinsus marinus* in *Crassostrea virginica* from the southern Gulf of Mexico. *Dis. Aquat. Organ.,* **79**, 237–247.

KIM S.H., BATHIGE S.D.N.K., JEON H.B., LEE D., CHOI K.S., KIM H.J. & PARK K.I. (2024). First report of *Perkinsus marinus* occurrence associated with wild Pacific oysters *Crassostrea gigas* from the west coast of Korea. *J. Invertebr. Pathol*., **204**, 108119. https://doi.org/10.1016/j.jip.2024.108119.

KIM S.-H., KIM H.J., BATHIGE S.D.N.K., KIM S. & PARK K.-I. (2025). Strain-specific virulence of *Perkinsus marinus* and related species in Eastern oysters: A comprehensive analysis of immune responses and mortality. *Fish Shellfish Immunol.,* **157**, 110112

LA PEYRE J.F., FAISAL M. & BURRESON E.M. (1993). *In vitro* propagation of the protozoan *Perkinsus marinus*, a pathogen of the eastern oyster, *Crassostrea virginica. J. Euk. Microbiol.,* **40**, 304–310.

LA PEYRE J.F, CASAS S., LI Y. & SUPAN J. (2008a). Evaluation of an adjustable long line system to increase oyster survival in Dermo endemic areas and refrigerated shelf-life after harvest. *J. Shellfish Res.,* **27**, 1041.

LA PEYRE M.K., CASAS S.M., GALYE W. & LA PEYRE J.F. (2010). The combined influence of sub-optimal temperature and salinity on the *in vitro* viability of *Perkinsus marinus*, a protistan parasite of the eastern oyster *Crassostrea virginica*. *J. Invertrebr. Pathol.,* **105**, 176–181. doi: 10.1016/j.jip.2010.06.010.

LA PEYRE M.K., CASAS S.M., VILLALBA A. & LA PEYRE J.F. (2008b). Determination of the effects of temperature on viability, metabolic activity and proliferation of two *Perkinsus* species, and its significance to understanding seasonal cycles of perkinsosis. *Parasitology* **135**, 505–519. doi:10.1017/S0031182008004150

LA PEYRE M.K., NICKENS A.D., VOLETY A. K., TOLLEY G.S., LA PEYRE J.F. (2003). Environmental significance of freshets in reducing *Perkinsus marinus* infection in eastern oysters *Crassostrea virginica*: potential management applications. *Mar. Ecol. Prog. Ser*., **248**, 165–176.

LUND E.D., SOUDANT P., CHU F.-L.E., HARVEY E., BOLTON S. & FLOWERS A. (2005). Effects of triclosan on growth, viability and fatty acid synthesis of the oyster protozoan parasite *Perkinsus marinus. Dis. Aquat. Organ*., **67**, 217–224.

MACKIN J.G., H.M. OWEN & A. COLLIER A. (1950). Preliminary note on the occurrence of a new protistan parasite, *Dermocystidium marinum* n.sp. in *Crassostrea virginica* (Gmelin). *Science* (Washington DC), **111**, 328–329.

MACKIN J.G. (1951). Histopathology of infection of *Crassostrea virginica* Gmelin by *Dermocystidium marinum* Mackin, Owen and Collier. *Bull. Marine Sci. Gulf Caribb.,* **1**, 72–87.

MALEK J.C. & BYERS J.E. (2018). Responses of an oyster host (*Crassostrea virginica*) and its protozoan parasite (Perkinsus marinus) to increasing air temperature. *PeerJ.,* 6:e5046. doi: 10.7717/peerj.5046.

MOSS J.A., BURRESON E.M. & REECE K.S. (2006). Advanced *Perkinsus marinus* infections in *Crassostrea ariakensis* maintained under laboratory conditions. *J. Shellfish Res.,* **25,** 65–72.

OTTINGER C.A., LEWIS T.D., SHAPIRO D.A., FAISAL M. & KAATTARI S.L. (2001). Detection of *Perkinsus marinus* extracellular proteins in tissues of the eastern oyster *Crassostrea virginica:* potential use in diagnostic assays. *J. Aquat. Animal Health*, **13**, 133–141.

PERKINS F.O. (1976). Zoospores of the oyster pathogen, *Dermocystidium marinum*. I. Fine structure of the conoid and other sporozoan-like organelles. *J. Parasitol*., **62**, 959–974.

PERKINS F.O. (1996). The structure of *Perkinsus marinus* (Mackin, Owen and Collier, 1950) Levine, 1978 with comments on taxonomy and phylogeny of *Perkinsus* spp. *J. Shellfish Res.*, **15**, 67–87.

PIESZ J.L., SCRO A.K., CORBETT R., LUNDGREN K.M., SMOLOWITZ R.& GOMEZ-CHIARRI M. (2022). Development of a multiplex qPCR for the quantification of three protozoan parasites of the eastern oyster *Crassostrea virginica. Dis. Aquat. Organ.,* **151**, 111–121.

PROESTOU D.A., SULLIVAN M.E., LUNDGREN K.M., BEN-HORIN T., WITKOP E.M. & HART K.M. (2023). Understanding Crassostrea virginica tolerance of *Perkinsus marinus* through global gene expression analysis. *Front. Genet*., **14**, 1054558.

RAGONE CALVO L.M., DUNGAN C.F., ROBERSON B.S. & BURRESON E.M. (2003). Systematic evaluation of factors controlling *Perkinsus marinus* transmission dynamics in lower Chesapeake Bay. *Dis. Aquat. Organ*., **56**, 75–86. doi: 10.3354/dao056075.

RAY S.M. (1966). A review of the culture method of detecting *Dermocystidium marinum* with suggested modifications and precautions. *Proc. Natl Shellfish Assoc.,* **54**, 55–69.

REECE K.S., SIDDALL M.E., BURRESON E.M. & GRAVES J.E. (1997). Phylogenetic analysis of *Perkinsus* based on actin gene sequences. *J. Parasitol.,* **83**, 417–423.

REECE K.S., DUNGAN C.F. & BURRESON E.M. (2008). Molecular epizootiology of *Perkinsus marinus* and *P. chesapeaki* infections among wild oysters and clams in Chesapeake Bay, USA. *Dis. Aquat Organ.,* **82**, 237–248.

REMACHA-TRIVIÑO A., BORSAY-HOROWITZ D., DUNGAN C., GUAL-ARNAU X., GÓMEZ-LEON J., VILLAMIL L. & GÓMEZ-CHIARRI M. (2008). Numerical quantification of *Perkinsus marinus* in the American oyster *Crassostrea virginica* (Gmelin, 1791) (Mollusca: Bivalvia) by modern stereology. *J. Parasitol.,* **94**, 125–136.

ROBLEDO J.A.F., WRIGHT A.C., MARSH A.G. & VASTA G.R. (1999). Nucleotide sequence variability in the nontranscribed spacer of the rRNA locus in the oyster parasite *Perkinsus marinus*. *J. Parasitol.,* **85**, 650–656.

SMOLOWITZ R. (2013). A Review of Current State of Knowledge Concerning *Perkinsus marinus* Effects on *Crassostrea virginica* (Gmelin) (the Eastern Oyster). *Vet. Pathol.,* **50**, 404–411.

THOMPSON P.C., ROSENTHAL B.M. & HARE M.P. (2011). An evolutionary legacy of sex and clonal reproduction in the protistan oyster parasite *Perkinsus marinus. Infect. Genet. Evol*., **11**, 598–609 doi: 10.1016/j.meegid.2011.01.008.

VILLALBA A., REECE K.S., ORDAS M.C., CASAS S.M. & FIGUERAS A. (2004). Perkinsosis in molluscs: a review. *Aquat. Living Resour.,* **17**, 411–432.

WANG Z., CASAS S., LA PEYRE J., RIKARD S., WILLIAMS M.L., TARNECKI A., BUSHEK D. & GUO X. (2025). Genomic Selection for Dermo Resistance in the Eastern Oyster *Crassostrea virginica:* Production and Laboratory Testing of F1 Generation. *J. Shellfish Res*., **44**, 75–87. https://doi.org/10.2983/035.044.0108.

WINNICKI S.M., CARDEN W., HOLOHAN B., RALPH G., WARD E. & B. ALLAM (2008). Establishment of *Perkinsus marinus* infection in *Crassostrea virginica:* insights into the potrtal of entry and the potential role of marine aggregates. *J. Shellfish Res*., **27**, 1064. (Abstract).

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* *

NB: Currently (2026) there is no WOAH Reference Laboratory for infection with *Perkinsus marinus* (please consult the WOAH web site: https://www.woah.org/en/what-we-offer/expertise-network/reference-laboratories/#ui-id-3).

NB: FIRST ADOPTED IN 1995 AS PERKINSOSIS. MOST RECENT UPDATES ADOPTED IN 2024 (SECTIONS 2.2.1 AND 2.2.2).

Anexo 20. – Capítulo 2.4.6. 'Infección por *Perkinsus olseni*'

CHAPTER 2.4.6.

INFECTION WITH *PERKINSUS OLSENI*

1. Scope

Infection with *Perkinsus olseni* is considered to be infection with the pathogenic agent *Perkinsus olseni* of the ~~(~~Family Perkinsidae~~)~~.

2. Disease information

2.1. Agent factors

2.1.1. Aetiological agent

The aetiological agent is the protozoan parasite *Perkinsus olseni*.

Current evidence using genomic sequencing data have placed *Perkinsus olseni* in the class Perkinsea of the phylum Perkinsozoa, and Kingdom Alveolata (Reece *et al.,* 1997). *Perkinsus olseni* was formerly known as *Perkinsus atlanticus* in Europe (Azevedo 1989). As trophozoites grow *in vitro*, cleavage furrows begin to form on the cell surface, ~~signaling~~ marking the early stages of ~~their~~ cellular subdivision associated with~~, or~~ the onset of schizogony (Gajamange *et al.,* 2020). As cleavage advances, the number of daughter cells increases, progressing to the morula stage, where approximately 100 irregularly shaped merozoites are observed. Once schizonts are fully developed, they release hundreds of merozoites upon the rupture or abrasion of the schizont cellular membrane.

A study investigating genome-wide comparison of five *P. olseni* isolates from Australia ~~(00978-12)~~, New Zealand ~~(PRA-205 and 207)~~, Japan ~~(PRA-179)~~, and Spain ~~(PRA-31)~~ observed differences between different geographical locations. Indeed, *P. olseni* from Oceania (Australia and New Zealand) displayed a high heterozygosity of 5.47–7.71% compared with Eurasia (Spain and Japan) with 0.1 to 0.2% of heterozygosity (Bogema *et al.,* 2021). The gene numbers differed substantially, with the Oceanian isolates sharing a high proportion of unique orthogroups in comparison to the Eurasian isolates (Bogema *et al.,* 2021). The Oceanian isolates had a slightly higher proportion of repetitive content such as tandem gene duplication. The characterised gene contents appear to be highly conserved across *Perkinsus* species such as in *P. olseni*, *P. marinus*, and *P. chesapeaki* (Bogema *et al.,* 2021).

The ribosomal RNA transcription unit is highly duplicated with variations between the isolates from different parts of the world, which does not make it a suitable region for designing real-time PCR tests to assess the intensity of infection (Bogema *et al.,* 2021).

More studies are needed to confirm the ploidy of *P. olseni*. Indeed, some authors using genome-wide data suggested that *Perkinsus* is polyploid with a ploidy variation between individual cells and populations (Bogema *et al.,* 2021) whereas other using microsatellite loci or restriction fragment length polymorphism proposed a diploidy (Pardo *et al.,* 2011; Reece *et al.,* 1997; 2001; Robledo *et al.,* 1999; Thompson *et al.,* 2011).

2.1.2. Survival and stability in processed or stored samples

Perkinsus olseni can be propagated *in vitro* in various media formulations such as Dulbecco's Modified Eagle medium and Ham's F-12 nutrient mixture (Burreson *et al.,* 2005; Dungan & Reece, 2006) or JL-ODR-2A (La Peyre *et al.,* 2006). Both media are supplemented with various salts, FBS, lipid mixtures, and yeast ultrafiltrates. Isolates can be cryopreserved and stored indefinitely (Dungan et al., 2007).

2.1.3. Survival and stability outside the host

Long-term survival of *P. olseni* outside its bivalve host is not known. but it is at least 4 months for prezoosporangia (Casas *et al.,* 2002b).

2.2. Host factors

2.2.1. Susceptible host species

Species that fulfil the criteria for listing as susceptible to infection with *Perkinsus olseni* according to Chapter 1.5. of the *Aquatic Animal Health Code* (*Aquatic Code*) are:

Family	Scientific name	Common name
Arcidae	*Anadara kagoshimensis*	half-crenated ark
	Anadara trapezia	no common name
Cardiidae	*Tridacna crocea*	crocus giant clam
Haliotidae	*Haliotis laevigata*	greenlip abalone
	Haliotis rubra	blacklip abalone
	Haliotis iris	black paua
Margaritidae	*Pinctada fucata*	Japanese pearl oyster
Mytilidae	*Mytilus galloprovincialis*	Mediterranean mussel
	Perna canaliculus	New Zealand mussel
Veneridae	*Austrovenus stutchburyi*	Stutchbury's venus
	Leukoma jedoensis	Jedo venus
	Paratapes undulatus	undulate venus
	Protapes gallus	rooster venus
	Proteopitar patagonicus	no common name
	Ruditapes decussatus	grooved carpet shell
	Ruditapes philippinarum	Japanese carpet shell

2.2.2. Species with incomplete evidence for susceptibility

Species for which there is incomplete evidence to fulfil the criteria for listing as susceptible to infection with *P. olseni* according to Chapter 1.5. of the *Aquatic Code* are:

Family	Scientific name	Common name
Cardiidae	*Cerastoderma edule*	common edible cockle
Mytilidae	*Mytilus chilensis*	Chilean mussel
Ostreidae	*Crassostrea gasar*	gasar cupped oyster
	Ostrea angasi	Australian mud oyster
Pectinidae	*Pecten novaezelandiae*	New Zealand scallop
Psammobiidae	*Hiatula acuta*	no common name
Veneridae	*Venerupis corrugata*	corrugated venus clam

In addition, pathogen-specific positive polymerase chain reaction (PCR) results have been reported in the following species, but no active infection has been demonstrated:

Family	Scientific name	Common name
Cardiidae	*Cerastoderma glaucum*	olive green cockle
Chamidae	*Chama pacifica*	reflexed jewel box
Haliotidae	*Haliotis diversicolor*	small abalone
Isognomonidae	*Isognomon alatus*	flat tree oyster
	Isognomon sp.	N/A
Margaritidae	*Pinctada imbricata*	Atlantic pearl oyster
Ostreidae	*Crassostrea rhizophorae*	mangrove cupped oyster
	Dendostrea frons	Frons oyster
	Magallana [syn. *Crassostrea*] *gigas*	Pacific oyster
	Magallana [syn. *Crassostrea*] *hongkongensis*	no common name
	Saccostrea sp.	N/A
Pectinidae	*Mimachlamys crassicostata*	noble scallop
Pharidae	*Sinonovacula constricta*	constricted tagelus clam
Veneridae	*Meretrix lyrata*	lyrate hard clam
	Polititapes aureus	golden carpet shell
	Venus verrucosa	warty venus

2.2.3. Likelihood of infection by species, host life stage, population or sub-populations

Perkinsus olseni is known to infect and cause clinical signs in many bivalve species. All stages after settlement are susceptible. *P. olseni* infection intensity increases with host age (Villalba *et al.,* 2005).

2.2.4. Distribution of the pathogen in the host

Perkinsus olseni trophozoites and schizonts can be found in various tissues and organs, including connective tissues, siphons, mantle, gills, digestive system, foot, adductor muscle, and haemolymph (Carella *et al.,* 2023; Park & Choi, 2001; Leethochavalit *et al.,* 2004). In some clams, the infection was heaviest in the digestive gland and gills (Leethochavalit *et al.,* 2004; Park & Choi, 2001). However, infection in the gills of bivalves is representative of the infection throughout the entire body and is therefore considered the organ of choice for diagnosing *P. olseni* infection (Choi *et al.,* 2002; Cui *et al.,* 2018; Dang *et al.,* 2013; Park *et al.,* 1999). *Perkinsus olseni* has also been detected in faeces of infected animals (Park *et al.,* 2010).

2.2.5. Aquatic animal reservoirs of infection

None known.

2.2.6. Vectors

None known.

2.3. Disease pattern

2.3.1. Mortality, morbidity and prevalence

Infections in ~~clam hosts~~ shellfish can be lethal depending on environmental conditions, and death may occur 1 or 2 years after infection. Studies have suggested that the impact on the host depends on the intensity of infection but also on host species and locations; for example, in clam hosts it can start being deleterious and lethal at densities of 10^6 parasite cells/g wet tissue (Dang *et al.,* 2013; Waki *et al.,* 2018).

Prevalence is highly variable depending on host and environmental conditions, up to 100% in susceptible host species as determined by histology or polymerase chain reaction (PCR). Prevalence and intensity of infection may be higher in individuals with more than 1 year of exposure to the pathogen because they have been exposed to *P. olseni* for longer, and larger animal have a higher filtration rate (Choi *et al.,* 2002; Villalba *et al.,* 2005). Prevalence can vary according to the season and is often higher in spring (Cui *et al.,* 2018; Villalba *et al.,* 2005).

2.3.2. Clinical signs, including behavioural changes

Perkinsus olseni infection can lead to reduced growth, reproduction issues, and mass mortalities in shellfish populations (Cho & Park, 2010). Clinical signs include mantle retraction, byssal tissue sloughing, gaping or dead molluscs but these clinical signs are not specific to infection with *P. olseni.* Individual bivalves with late-stage infections may exhibit slow responses to stimuli (Sheppard & Phillips, 2008).

2.3.3 Gross pathology

Gross signs are thin, watery tissue, pale digestive gland and nodules in several tissues such as mantle, gills, and foot of some hosts, but these signs are not specific to infection with *P. olseni*.

At high infection intensities the infected molluscs can present several milky-white cysts, abscesses or brown nodules (Azevedo, 1989; Gudkovs *et al.,* 2016; Ruano *et al.,* 2015). The cysts or nodules contain individual and grouped encapsulated trophozoites at different stages of maturation, and result from a natural defensive reaction, the infiltration of hemocytes (Abdel-Baki *et al.,* 2014).

2.3.4. Modes of transmission and life cycle

The life cycle is horizontal, direct from host to host (Villalba *et al.,* 2004). Faecal discharge and decomposition of infected tissues after the host death have been suggested as two transmission pathways for *P. olseni* (Cui *et al.,* 2018; Park *et al.,* 2010).

The life cycle consists of three main life stages: trophozoite, prezoosporangium, and zoospore (Villalba *et al.,* 2004). The trophozoite is a stage occurring in the tissues of the live host where vegetative proliferation occurs. The trophozoite undergoes successive bipartitioning to yield up to 32 daughter cells that stay together in a rosette-like arrangement inside a wall. After rupture of the wall, immature trophozoites enlarge and form a vacuole, becoming mature trophozoites. At the host death, trophozoites evolve into a prezoosporangia, which evolve into zoosporangia when they are released in the environment. Zoosporangia produce zoospores, which are the infective stage.

2.3.5. Environmental factors

Environmental factors such as temperature, salinity, oxygen levels, pH, and nutrient availability influence *Perkinsus* infection dynamics and hypnospore formation (~~2013;~~ Delisle *et al.,* 2025; Villalba *et al.,* 2004).

Temperature and salinity appear to be the most important environmental factors controlling the transmission of *P. olseni* infection with zoosporulation occurring between 19 and 28°C and increasing with higher temperature and salinity (Kyoung & Ki, 2001; Umeda *et al.,* 2020). Delisle *et al.* (2025), showed the development of *P. olseni* increased significantly above 20°C, reached an optimal level at 22°C, and was considerably reduced at 35°C.

Temperature and salinity also appear to be the two major environmental factors influencing the prevalence and seasonality of *P. olseni* infection, with several studies reporting higher prevalence and intensity of infection in spring (Park & Choi 2001; Soudant *et al.,* 2013; Villalba *et al.,* 2005; Waki & Yoshinaga, 2013; 2015).

2.3.6. Geographical distribution

Infections are widespread throughout the tropical Pacific Ocean, Oceania, Asia, Europe and South America (Cremonte *et al.,* 2005; Goggin & Lester, 1995; Villalba *et al.,* 2004). *Perkinsus olseni* is not known from North America.

See WAHIS (https://wahis.woah.org/#/home) for recent information on distribution at the country level.

2.4. Biosecurity and disease control strategies

2.4.1. Vaccination

None.

2.4.2. Chemotherapy including blocking agents

Cyclohexamide, pyrimethamine, deferoxamine (DFO) and 2, 2-bipyridyl inhibit *P. olseni* development/replication *in vitro*, and DFO inhibits *P. olseni* development/infection *in vivo*. (Elandalloussi *et al.,* 2005).

2.4.3. Immunostimulation

None.

2.4.4. Breeding resistant strains

None.

2.4.5. Inactivation methods

Isolated *P. olseni* cells were killed when immersed in freshwater within 10 minutes at room temperature. Another study showed that free zoospores, free prezoosporangia and prezoosporangia in gill tissues were killed after 1 hour incubation with 50, 200, and 3000 ppm of chlorine, respectively (Casas *et al.,* 2002b). *Perkinsus olseni* cells in host tissue were much more resistant to these treatments. UV-C irradiation has been shown to be effective in inactivating *Perkinsus* parasites, with a minimum dose of 94 mJ/cm^2 required to inhibit proliferation and 450 mJ/cm^2 to completely kill all parasites (Fernandez-Boo *et al.,* 2021).

2.4.6. Disinfection of eggs and larvae

Perkinsus olseni is not known to infect eggs or larvae of its hosts, but parasite cells may occur intercellularly.

2.4.7. General husbandry

Management strategies to mitigate perkinsosis impacts include modifying culture procedures such as reducing stocking density, ~~selective breeding for resistant strains,~~ and using triploid or allochthonous oyster species (Villalba *et al.,* 2004).

3. Specimen selection, sample collection, transportation and handling

This section draws on information in Sections 2.2, 2.3 and 2.4 to identify populations, individuals and samples that are most likely to be infected.

3.1. Selection of populations and individual specimens

Bivalves that are gaping, ~~clams~~ that are found at the sediment surface ~~instead of being~~ when they would normally be buried, or freshly dead animals should be targeted to increase the chances of ~~finding~~ detecting infected animals. Abalone with foot blisters should be sampled. For histology, only live animals should be taken.

3.2. Selection of organs or tissues

Connective tissue of all organs, and haemocytes. For Ray's fluid thioglycollate medium (RFTM), the whole animal should be ideally sampled if its size allows. If not, pieces of gill followed by mantle or foot for abalone are typically used. For culture purposes, tissue slices that can include gill, mantle, or foot (for abalone) can be cultured. For histology, a 5-mm thick section through the visceral mass that includes digestive gland, gill and mantle is used. For PCR, a pool of organs including gill or mantle tissue is recommended.

3.3. Samples or tissues not suitable for pathogen detection

Rectal tissue is not reliable for PCR assays because of the presence of inhibitors.

3.4. Non-lethal sampling

For *P. marinus*, Gauthier & Vasta (1995) proposed haemolymph incubation in RFTM as an alternative to the traditional tissue RFTM, enabling non-lethal sampling of individuals. This method was further refined by Nickens *et al.* (2002). Rodriguez & Navas (1995) reviewed and compared various RFTM assays for *Perkinsus olseni*, highlighting that whole host body incubation in RFTM is the most sensitive method. Detection limits (diagnostic sensitivity and specificity) have not been established for these analyses.

3.5. Preservation of samples for submission

For guidance on sample preservation methods for the intended test methods, see Chapter 2.4.0 General information (diseases of molluscs).

3.5.1. Samples for pathogen isolation

The results of bioassay depend strongly on the quality of samples (time since collection, time in storage, preservative used). Fresh specimens should be kept on ice and preferably sent to the laboratory within 24 hours of collection. To avoid degradation of samples, use alternative storage methods only after consultation with the receiving laboratory.

3.5.2. Preservation of samples for molecular detection

Tissue samples for PCR testing should be preserved in 80% (v/v) analytical-grade ethanol. The recommended ratio of ethanol to tissue is 10:1 based on studies in terrestrial animal and human health. The use of lower grade (laboratory or industrial grade) ethanol is not recommended. If material cannot be fixed it may be frozen.

Standard sample collection, preservation and processing methods for molecular techniques can be found in Section B.5.5 of Chapter 2.4.0 *General information* (diseases of molluscs).

3.5.3. Samples for histopathology, immunohistochemistry or *in-situ* hybridisation (ISH)

Standard sample collection, preservation and processing methods for histological techniques can be found in Section B.5.3 of Chapter 2.4.0 *General information* (diseases of molluscs).

3.5.4. Samples for other tests

None.

3.6. Pooling of samples

Pooling of samples from more than one individual animal for a given purpose is only recommended where robust supporting data on diagnostic sensitivity and diagnostic specificity have been evaluated and found to be suitable. If the effect of pooling on diagnostic sensitivity has not been thoroughly evaluated, larger specimens should be processed and tested individually. Small life stages such as spat can be pooled to obtain the minimum amount of material for molecular detection. ~~Pooling of very small spat (5–10 depending on size) is acceptable for PCR analyses.~~

4. Diagnostic methods

The methods currently available for pathogen detection that can be used in i) surveillance of apparently healthy animals, ii) presumptive diagnosis in clinically affected animals and iii) confirmatory diagnostic purposes are listed in Table 4.1. by animal life stage.

Ratings for purposes of use. For each recommended assay a qualitative rating for the purpose of use is provided. The ratings are determined based on multiple performance and operational factors relevant to application of an assay for a defined purpose. These factors include appropriate diagnostic performance characteristics, level of assay validation, availability cost, timeliness, and sample throughput and operability. For a specific purpose of use, assays are rated as:

+++ =	Methods are most suitable with desirable performance and operational characteristics.
++ =	Methods are suitable with acceptable performance and operational characteristics under most circumstances.
+ =	Methods are suitable, but performance or operational characteristics may limit application under some circumstances.

Shaded boxes = Not appropriate for this purpose.

Validation stage. The validation stage corresponds to the assay development and validation pathway in chapter 1.1.2. The validation stage is specific to each purpose of use. Where available, information on the diagnostic performance of recommended assays is provided in Section 6.3.

WOAH Reference Laboratories welcome feedback on diagnostic performance of recommended assays, in particular PCR methods. Of particular interest are any factors affecting expected assay sensitivity (e.g. tissue components inhibiting amplification) or expected specificity (e.g. failure to detect particular genotypes, detection of homologous sequences within the host genome). These issues should be communicated to the WOAH Reference Laboratories so that advice can be provided to diagnostic laboratories and the standards amended if necessary.

Table 4.1. WOAH recommended diagnostic methods and their level of validation for surveillance of apparently healthy animals and investigation of clinically affected animals

Method	D. Surveillance of apparently healthy animals				E. Presumptive diagnosis of clinically affected animals				F. Confirmatory diagnosis[1] of a suspect result from surveillance or presumptive diagnosis			
	Early life stages[2]	Juveniles[2]	Adults	LV	Early life stages[2]	Juveniles[2]	Adults	LV	Early life stages[2]	Juveniles[2]	Adults	LV
Wet mounts												
Histopathology		++	++	~~2~~ 1		+++	+++	NA				
Cell culture												
Transmission electron microscopy												
Real-time PCR	~~+~~ ~~++~~	+++	+++	3	~~+~~ ~~++~~	+++	+++	NA	~~+~~ ~~++~~	+++	+++	3
Conventional PCR	~~+~~ ~~++~~	~~++~~ ~~+~~	~~++~~ ~~+~~	~~3~~ 1	~~+~~ ~~++~~	+++	+++	NA				
Conventional PCR followed by amplicon sequencing									~~+~~ ~~++~~	+++	+++	~~3~~ 1
In-situ hybridisation										~~++~~ ~~+~~	~~++~~ ~~+~~	~~3~~ 1
Bioassay												
LAMP												
Ab-ELISA												
Ag-ELISA												
RFTM		+++	+++	3		+++	+++	~~3~~ NA				

LV = level of validation, refers to the stage of validation in the WOAH Pathway (chapter 1.1.2); PCR = polymerase chain reaction; LAMP = loop-mediated isothermal amplification; Ab- or Ag-ELISA = antibody or antigen enzyme-linked immunosorbent assay, respectively; RRTM = Ray's fluid thioglycollate medium

[1]For confirmatory diagnoses, methods need to be carried out in combination (see Section 6). [2]Susceptibility of early and juvenile life stages is described in Section 2.2.3.

Shading indicates the test is inappropriate or should not be used for this purpose.

4.1. Smears

Not recommended as a diagnostic method.

4.2. Histopathology

Samples to be taken consist of live or moribund bivalves.

*S*ections of tissues that include gills, digestive gland and mantle should be fixed for a minimum of 24 hours in a recommended fixative followed by standard processing for histology as described in Section 5.3 of Chapter 2.4.0 *General information* (diseases of molluscs). Observations are made at increasing magnifications up to ×400.

Fixed sections reveal large multifocal lesions in connective tissue containing *P. olseni* cells. Haemocyte infiltration (haemocytosis) occurs in most infections. In clam hosts, *P. olseni* cells are often encapsulated by a thick layer of eosinophilic material derived from haemocyte degranulation (Villalba *et al.,* 2004).

The occurrence of spherical, uninucleate cells ranging from approximately 5 to 15 µm in diameter with a large vacuole and an eccentrically displaced nucleus with a prominent nucleolus, may indicate infection with *Perkinsus olseni*. Multinucleate schizonts (dividing forms) often accompany the uninucleate trophozoites. Cells may be phagocytosed by host haemocytes Cells may be phagocytosed by host haemocytes. *Perkinsus olseni* cells stain lightly basophilic.

4.3. Electron microscopy

Ultrastructural data show that the lysis of haemocytes and coalescence of metachromatic granules result in the nodule that encapsulates trophozoites (Sagrista *et al.,* 1995).

4.4. Nucleic acid amplification

Samples to be taken consist of live or freshly dead molluscs. 2–3 mm^2 tissue pieces are excised aseptically from gill and mantle and placed into 1.5 ml microcentrifuge tubes containing 80% ethanol. Dissecting utensils should be flamed between samples to prevent cross-contamination.

PCR assays should always be run with the controls specified in Section B.5.5 *Molecular methods* Chapter 2.4.0 *General information* (diseases of molluscs). Each sample should be tested in duplicate.

Extraction of nucleic acids

DNA is extracted by proteinase K digestion overnight at 56°C and the spin-column methodology using commercially available kits. Different kits and procedures can be used for nucleic acid extraction. The quality and concentration of the extracted nucleic acid is important and can be checked using a suitable method as appropriate to the circumstances.

4.4.1. Real-time PCR

A real-time *Perkinsus* genus PCR assay targeting the ITS region has been developed for use with host tissue (Gauthier *et al.,* 2006). It has been tested only with *P. marinus*, *P. olseni* and *P. chesapeaki*, and was shown to be more sensitive in a limited validation against the RFTM assay. This assay needs to be tested more thoroughly for specificity but may be useful for laboratories that possess the necessary equipment.

Several real-time PCR assays all targeting the ITS region have been developed to detect and quantify *P. olseni* (Cui *et al.,* 2018; Gajamange *et al.,* 2011; Itoïz *et al.,* 2021; Ríos *et al.,* 2020; Umeda & Yoshinaga, 2012). One needs to be careful when attempting to quantify *P. olseni* as genome sequencing revealed that this region is highly duplicated, which does not make it the ideal region for quantifying parasite infection. Of all those assays, only the assay developed by Ríos *et al.* (2020) presented some level of validation such as sensitivity and reproducibility. ~~More validation~~ Further method optimisation is required before a real-time PCR assay can be recommended (see chapter 1.1.2 *Principles and methods of validation of diagnostic assays for infectious diseases*). In addition, Ríos *et al.* (2020) detected non-specific hybridisation when using the Umeda & Yoshinaga (2012) and Gajamange *et al.* (2011) assays.

43. Primers and probes (sequences)

Pathogen/ target gene	Primer/probe (5'–3')	Concentration	Cycling parameters[a]
Method 1: TaqMan PCR Gauthier *et al.,* 2006; GenBank Accession No.: AF149876.1			
Perkinsus sp./ITS (detects at least *P. marinus, P. olseni* and *P. chesapeaki*).	~~PERK-F: CGT-GAA-CCA-GTA-GAA-ATC-TCA-A~~ ~~PERK-R: ACA-TAT-CAG-TGT-CGC-TCT-TCT-TCC~~ ~~Probe: GCA-TAC-TGC-ACA-AAG-GG~~ PERK-F: TCC-GTG-AAC-CAG-TAG-AAA-TCT-CAA-C PERK-R: GGA-AGA-AGA-GCG-ACA-CTG-ATA-TGT-A Probe: (FAM)-CCC-TTT-GTG-CAG-TAT-GC-MGB	0.9 µM 0.9 µM 0.25 µM	40 cycles of: 95°C/15 sec and 60°C/60 sec
Method 2: TaqMan PCR Itoiz *et al.,* 2021; GenBank Accession No.: MW187111; amplicon size: 76 bp			
P. olseni/ITS 2	PolsITS2-F: CAC-CAC-AAC-ACA-GTC-GGA-C PolsITS2-R: CGT-ATT-GTA-GCC-CCT-CCG-A PolsITS2-probe: GAC-ACT-CAC-AGG-CGC-GGT-CC	0.2 µM 0.2 µM 0.5 µM	45 cycles of: 95°C/10 sec and 55°C/20 sec
Method 3: SYBR Green PCR Rios *et al.,* 2020; GenBank Accession No.[b]: ~~???~~; amplicon size 346 bp			
P. olseni/ITS	Perk-ITS-qF1: CTG-ACC-GCC-TTA-ACG-GGC Perk-ITS-qR2: CTA-TCT-CCG-AAG-AGT-TAG-TCC-	10 µM 10 µM	40 cycles of: 95°C/30 sec and 60°C/60 sec
Method 4: Cui *et al*., 2018; GenBank Accession No.: ~~???~~ AF369975; amplicon size 217 bp			
P. olseni/ITS	PO-F: GAG-TGT-CTC-TGG-TTG-CTC-GCA PO-R: ACA-TCA-GGC-CTT-CTA-ATG-ATG	10 µM 10 µM	40 cycles of: 95°C/15 sec and 60°C/60 sec
Method ~~6~~ 5: Umeda &Yoshinaga, 2012; GenBank Accession No. ~~???~~[b]			
P. olseni/ITS	OF: CTT-AAC-GGG-CCG-TGT-TA OR: CAT-AAC-GAA-CTA-TCT-CCG-AAG	0.5 µM 0.5 µM	40 cycles of: 98°C/2 sec and 60°C/5 sec
Method ~~5~~ 6: Gajamange *et al.,* 2011; GenBank Accession No.: ~~???~~ AF473840, amplicon size: 97 bp			
P. olseni/**5.8S & ITS2**	PK-ITS-F: CAG-AAT-TCC-GTG-AAC-CAG-TAG-A PK-ITS-R: TGT-CGC-TCT-TCT-TCC-CGA-TA Probe: TCA-ACG-CAT-ACT-GCA-CAA-AGG-GGA	10 pM 10 pM 10 pM	40 cycles of: 95°C/10 sec and 56°C/30 sec

[a]A denaturation step prior to cycling has not been included.
[b]For GenBank Accession numbers see original publications

4.4.2. Conventional PCR

Pathogen/ target gene	Primer/probe (5'–3')	Concentration	Cycling parameters[a]
Method 1: Moss *et al.,* 2006; GenBank Accession No.: ~~???~~ AF369975; amplicon size: ~~???~~ 457 bp			
P. olseni	PolsITS-140F: GAC-CGC-CTT-AAC-GGG-CCG-TGT-T PolsITS-600R: GGR-CTT-GCG-AGC-ATC-CAA-AG ~~450 bp~~	0.1 µM 0.1 µM	40 cycles of: 94°C/60 sec and 62°C/60 sec and 65°C/180 sec
Method 2: ~~Casas *et al.,* 2002a; GenBank Accession No.: ??? AF369975; amplicon size: 655 bp~~ Audemard *et al.*, 2004 (primers from Casas *et al.*, 2002); GenBank Accession No.: AF149876.1, amplicon size: 666 bp			
Perkinsus spp. (except *P. qugwadi*)	PerkITS-85: CCG-CTT-TGT-TTG-GAT-CCC PerkITS-750: ACA-TCA-GGC-CTT-CTA-ATG-ATG ~~675bp product~~	~~0.1~~ 0.5 µM ~~0.1~~ 0.5 µM	40 cycles of: 95°C/1 min and 55°C/1 min and 72°C/1 min

[a]A denaturation step prior to cycling has not been included.

4.4.3. Other nucleic acid amplification methods

A PCR-restriction fragment length polymorphism (RFLP) assay has been developed that may be useful for specific diagnoses of *P. olseni* (Abollo *et al.,* 2006), although it has not been tested for specificity against all known *Perkinsus* species.

A LAMP assay was developed by (Feng *et al.,* 2013) targeting ITS-2 and appeared to be rapid, sensitive (detection limit of 10 copies of plasmid DNA), and specific for *Perkinsus* spp. detection.

4.5. Amplicon sequencing

The size of the PCR amplicon should be verified, for example by agarose gel electrophoresis. Both DNA strands of the PCR product must be sequenced and analysed in comparison with reference sequences.

4.6. *In-situ* hybridisation

~~Samples to be taken consist of~~ Live or freshly dead molluscs should be targeted for sampling.

A specific DNA probe that targets the small subunit rRNA gene has been developed for the genus *Perkinsus*: Perksp700DIG (Elston *et al.,* 2004) and a DNA probe that targets the LSU of the rRNA gene has been developed for the specific detection of *P. olseni:* PolsLSU-464DIG (Moss *et al.,* 2006). Muznebin *et al.* (2023) also used a DIG labelled probe using primers targeting the ITS gene region.

Pathogen/target	**ISH probe type**	**ISH probe (5'-3')**	**Reference**
Perkinsus sp./SSU (detects all known *Perkinsus* species *except P. qugwadi)*	Labelled oligonucleotide	PerkspSSU-700-CGC-ACA-GTT-AAG-TRC-GTG-RGC-ACG	Elston *et al.* (2004)
Perkinsus olseni/LSU	Labelled oligonucleotide	PolsLSU-464DIG-CTC-ACA-AGT-GCC-AAA-CAA-CTG	Moss *et al.* (2006);
Perkinsus olseni/ITS	Labelled primers	Pols140F-GAC-CGC-CTT-AAC-GGG-CCG-TGT-T and PolsITS-600R-GGR-CTT-GCG-AGC-ATC-CAA-AG-3	Muznebin *et al.*, (2023)

~~***Perkinsus* genus assays (PCR and *in-situ* hybridisation)~~

~~For *in-situ* hybridisation (ISH), probes have been developed that target the small subunit (SSU) of the rRNA gene complex (Elston *et al.,* 2004).~~

~~***Perkinsus genus-specific in-situ hybridisation***~~

~~Samples to be taken: follow the procedure for 'fixed sections' above, except that tissue sections must be placed on positively charged glass slides or slides coated with 3-aminopropyl triethoxylane, without staining. Deparaffinise sections in xylene for 10 minutes and then rehydrate in an alcohol series. Wash sections twice for 5 minutes in phosphate-buffered saline (PBS).~~

~~A specific DNA probe that targets the small subunit rRNA gene has been developed for the genus *Perkinsus* (Elston *et al.,* 2004): Perksp700DIG (5'-CGC-ACA-GTT-AAG-TRC-GTG-RGC-ACG-3'). The probe should be 5' end-labelled with digoxigenin.~~

~~The tissue sections are treated with 125 µg ml−1 pronase in PBS, at 37°C for 30 minutes. The reaction is then stopped by washing the sections in PBS with 0.2% glycine for 5 minutes. The sections are then placed in 2× SSC (standard saline citrate; 20× SSC = 3 M NaCl; 0.3 M Na citrate; pH 7.0) for 10 minutes.~~

~~The sections are prehybridised for 1 hour at 42°C in prehybridisation solution (4× SSC, 50% formamide, 5× Denhardt's solution, 0.5 mg ml−1 yeast tRNA, and 0.5 mg ml−1 heat-denatured salmon sperm DNA) in a humid chamber.~~

~~The prehybridisation solution is then replaced with prehybridisation buffer containing 7 ng µl−1 of the digoxigenin-labelled *Perkinsus* genus probe. The sections are covered with in situ hybridisation plastic cover slips and placed on a heating block at 90°C for 12 minutes. The slides are then cooled on ice for 1 minute before hybridisation overnight at 42°C in a humid chamber.~~

~~The sections are washed twice for 5 minutes each in 2× SSC at room temperature, twice for 5 minutes each in 1× SSC at room temperature, and twice for 10 minutes each in 0.5× SSC at 42°C. The sections are then placed in Buffer 1 (100 mM Tris, pH 7.5, 150 mM NaCl) for 1–2 minutes.~~

~~The sections are placed in Buffer 1 (see above) supplemented with 0.3% Triton X-100 and 2% sheep serum for 30 minutes. Anti-digoxigenin-alkaline phosphatase antibody conjugate is diluted 1/500 (or according to the manufacturer's recommendations) in Buffer 1 supplemented with 0.3% Triton X-100 and 1% sheep serum and applied to the tissue sections. The sections are covered with in-situ hybridisation cover-slips and incubated for 3 hours at room temperature in a humid chamber.~~

~~The slides are washed twice in Buffer 1 for 5 minutes each and twice in Buffer 2 (100 mM Tris, pH 9.5, 100 mM NaCl, 50 mM $MgCl_2$) for 5 minutes each. The slides are then placed in colour development solution (337.5 µg ml−1 nitroblue tetrazolium, 175 µg ml−1 5-bromo-4-chloro-3-indolylphosphate p-toluidine salt, 240 µg ml^{-1} levamisole in Buffer 2) for 2 hours in the dark. The colour reaction is stopped by washing in TE buffer (10 mM Tris, pH 8.0, 1 mM EDTA [ethylene diamine tetra-acetic acid]).~~

~~The slides are then rinsed in sterile distilled water (dH_2O). The sections are counterstained with Bismarck Brown Y, rinsed in dH_2O, and cover slips are applied using an aqueous mounting medium.~~

~~Positive/negative controls: these are compulsory. Positive controls are tissue sections from any Perkinsus sp.-infected mollusc. Negative controls are either no-probe assays or assays with uninfected oysters.~~

~~***Perkinsus olseni-specific in-situ hybridisation***~~

~~The probe should be end-labelled with digoxygenin. The ISH procedures are the same as for the *Perkinsus* genus probe presented above.~~

~~*Positive/negative controls:* these are compulsory. Positive controls are tissue sections from any susceptible host infected with *P. olseni*. Negative controls are either no-probe assays or assays with uninfected oysters.~~

~~*A* DNA probe that targets the LSU of the rRNA gene of *P. olseni* has been developed (Moss *et al.*, 2006) (PolsLSU-464DIG 5'-CTC-ACA-AGT-GCC-AAA-CAA-CTG-3').~~

~~Muznebin *et al.*, (2023) also used ISH using the DIG PCR probe synthesis kit (Roche). A DIG-labelled probe was generated using the PCR primers Pols140F (5' GAC CGC CTT AAC GGG CCG TGT T 3') and PolsITS 600R (5' GGR CTT GCG AGC ATC CAA AG 3') (Moss *et al.*, 2006). The DIG-labelled probe was used at a concentration of 5 ng/µl.~~

4.7. Immunohistochemistry

~~Not available.~~ The successful production and characterisation of monoclonal antibodies against *P. olseni* by Kaewsalabnil *et al.* (2024) enabled specific immunoreactivity with trophozoite stages in infected clam tissues using immunohistochemistry. These findings suggest that immunohistochemistry has potential as a tool for the detection and monitoring of *P. olseni* infections in mollusc tissues, particularly in commercially cultured clams.

4.8. Bioassay

Not available.

4.9. Antibody- or antigen-based detection methods (ELISA, etc.)

Not currently available for diagnostic purposes but monoclonal antibodies have been developed (Hanrio et al., 2021; 2022; Park et al., 2010;).

4.10. Other methods

4.10.1. Ray's fluid thioglycollate culture method (RFTM)

Incubation in thioglycollate is routinely used for surveillance of *P. olseni.* The technique is simple, inexpensive and very sensitive, but not species-specific. Trophozoites of *P. olseni* in host tissue will enlarge when cultured for at least 5 days in fluid thioglycollate medium containing dextrose that is supplemented with antibiotics (penicillin, streptomycin) and an antifungal compound (nystatin) to reduce bacterial and fungal growth. When the tissue is macerated after culture to allow penetration of aqueous iodine solution (Lugol's), the enlarged trophozoites (hypnospores or prezoosporangia in the old terminology) readily take

up Lugol's and they easily become visible at low power because of their generally bluish–black coloration and their spherical shape.

Samples to be taken consist of live or freshly dead molluscs.

Tissue assay (Ray, 1966): tissue samples measuring approximately 5–10 mm are excised giving preference to rectal, gill and mantle tissue from oysters and clams, and adductor or foot muscles or mantle for abalone, and placed in test tubes containing thioglycollate medium (thioglycollate medium containing dextrose 14.6 g; NaCl, 10.0 g; sterile distilled water (dH_2O), 485 ml). A total of 9.5 ml is dispensed into disposable test tubes, which are autoclaved for 15 minutes at 1.2 kg cm^{-2} pressure. The autoclaved solution can be stored in tubes for up to 3 weeks. Dissecting utensils should be rinsed in 95% ethanol and flamed between hosts to prevent carry-over. The recommended antifungal/antibiotics are: 500 units ml^{-1} penicillin G and 500 units ml^{-1} dihydro-streptomycin in media (penicillin, 3.13 g; streptomycin, 6.55 g; 500 ml dH_2O; freeze in 50 ml aliquots; add 0.5 ml to each tube), and 50 µl of mycostatin (nystatin) per tube. Chloromycetin can be used in place of penicillin/streptomycin. The tube is plugged with a foam rubber or cotton stopper. Incubation is at 22–25°C for between 5 and 7 days, in the dark. After incubation, the fragments of tissue are collected and chopped with a scalpel blade on a glass slide, a drop of Lugol's iodine solution is added (stock Lugol's iodine solution: potassium iodide, 6.0 g; iodine, 4.0 g; dH_2O, 100 ml. Lugol's iodine working solution: dH_2O, 30.0 ml; Lugol's stock solution, 15.0 ml) and the preparation is covered with a cover-slip and allowed to sit for 10 minutes. The preparations are examined in the fresh state.

Whole body burden assay (Fisher & Oliver, 1996): the entire host, cut into 2–5 mm pieces, is placed in fluid thioglycollate culture medium and incubated as in the tissue assay above. If host organisms are too large to use the entire host, then selected target tissue can be used. The solution is centrifuged at 1500 ***g*** for 10 minutes and the supernatant is discarded. 2 M NaOH (20 ml g^{-1} tissue) is added and the solution is incubated at 60°C for 2–6 hours until tissue is digested. The solution is centrifuged at 1500 ***g*** for 10 minutes and the supernatant is discarded. The solution is washed three times in deionised water, the pellet is resuspended in 1 ml Lugol's iodine working solution, and the cells are counted. Serial dilutions may have to be made to reduce the total cell number to a manageable number.

Interpretation of results:

Cultured parasites enlarge from 2–10 to 20–70 µm during incubation. *Perkinsus* spp. cells are spherical and the walls stain blue or bluish-black with Lugol's iodine solution (Bushek *et al.*, 1994; Ray 1966).

In susceptible host species, within the known range of *P. olseni*, a positive result is presumptive evidence of *P. olseni* infection but should be confirmed in accordance with Section 6.

5. Test(s) recommended for surveillance to demonstrate freedom in apparently healthy populations

Real-time PCR and RFTM ~~tissue or~~ whole-body burden assays are recommended for targeted surveillance to declare freedom from infection with *P. olseni*.

6. Corroborative diagnostic criteria

This section only addresses the diagnostic test results for detection of infection in the absence (Section 6.1.) or in the presence (Section 6.2.) of clinical signs ~~(Section 6.2.)~~ but does not evaluate whether the infectious agent is the cause of the clinical event.

The case definitions for a suspect and confirmed case have been developed to support decision making related to trade and confirmation of disease status at the country, zone or compartment level. Case definitions for disease confirmation in endemically affected areas may be less stringent. If a Competent Authority does not have the capability to undertake the necessary diagnostic tests it should seek advice from the appropriate WOAH Reference Laboratory, and if necessary, refer samples to that laboratory for confirmatory testing of samples from the index case in a country, zone or compartment considered free. There are currently no WOAH Reference Laboratory for *Perkinsus olseni*.

When two molecular methods are used for confirmation of a case, it is preferable to use two species-specific methods targeting non-overlapping regions of the pathogen genome.

6.1. Apparently healthy animals or animals of unknown health status[2]

Apparently healthy populations may fall under suspicion, and therefore be sampled, if there is an epidemiological link(s) to an infected population. Hydrographical proximity to, or movement of animals or animal products or

[2] For example transboundary commodities.

equipment, etc., from a known infected population equate to an epidemiological link. Alternatively, healthy populations are sampled in surveys to demonstrate disease freedom.

6.1.1. Definition of suspect case in apparently healthy animals

The presence of infection with *P. olseni* shall be suspected if at least one of the following criteria is met:

i) Histopathological changes consistent with the presence of the pathogen or the disease

ii) Positive result by conventional PCR

iii) Positive result by real-time PCR

iv) Positive result by RFTM

6.1.2. Definition of confirmed case in apparently healthy animals

The presence of infection with *P. olseni* is considered to be confirmed if at least one of the following ~~criterion~~ criteria is met:

i) Positive result by real-time PCR and positive result by conventional PCR followed by amplicon sequencing

ii) Positive result by histology and positive result by conventional PCR followed by amplicon sequencing

iii) Positive result by *in-situ* hybridisation and positive result by conventional PCR followed by amplicon sequencing

iv) Positive result by RFTM and positive result by conventional PCR followed by amplicon sequencing

v) Positive result by histology and positive result by species-specific real-time PCR

vi) Positive result by *in-situ* hybridisation and positive result by species-specific real-time PCR

vii) Positive result by or RFTM and positive result by species-specific real-time PCR

6.2 Clinically affected animals

Clinical signs are not pathognomonic for a single disease; however, they may narrow the range of possible diagnoses.

6.2.1. Definition of suspect case in clinically affected animals

The presence of infection with *P. olseni* shall be suspected if at least one of the following criteria is met:

i) Gross pathology or clinical signs associated with the disease as described in this chapter, with or without elevated mortality

ii) Histopathological changes consistent with the presence of the pathogen or the disease

iii) Positive result by conventional PCR

iv) Positive result by real-time PCR

v) Positive result by RFTM

6.2.2. Definition of confirmed case in clinically affected animals

The presence of infection with *P. olseni* is considered to be confirmed if at least one of the following criteria is met:

i) Positive result by real-time PCR and and positive result by conventional PCR followed by ~~sequence analysis~~ amplicon sequencing

ii) Positive result ~~ISH~~ by *in-situ* hybridisation and positive result by and conventional PCR followed by ~~sequence analysis~~ amplicon sequencing

ii) Positive result ~~of real-time PCR and ISH~~ by i*n-situ* hybridisation and positive result by species-specific real-time PCR

6.3. Diagnostic sensitivity and specificity for diagnostic tests

The diagnostic performance of tests recommended for surveillance or diagnosis of infection with *P. olseni* are provided in Tables 6.3.1. (no data are currently available) and 6.3.2 ~~(no data are currently available for either)~~. Data are only presented where tests are validated to at least level 2 of the validation pathway described in Chapter 1.1.2. and the information is available within published diagnostic accuracy studies.

RFTM and histopathology enable diagnosis only at the genus level and cannot differentiate between species. RFTM is considered specific for all members of the genus *Perkinsus* except *P. qugwadi* (incertae sedis). However, its exclusivity for detecting only *Perkinsus* species has not been exhaustively confirmed through testing against all other protists (Dungan & Reece, 2020). Molecular tools have been evaluated for analytical sensitivity and specificity to varying degrees but estimates of diagnostic sensitivity (DSe) and specificity (DSp) (stage 2 validation) are generally lacking. Comparative data indicate that histopathology is less sensitive than both PCR and RFTM assays. The whole-body burden (WBB) RFTM assay is considered highly sensitive, with a theoretical detection limit of one *Perkinsus* sp. cell per host. In contrast, tissue and haemolymph RFTM assays have limited sensitivity when infection intensity falls below 1000 *Perkinsus* cells per gram of wet tissue (Bushek *et al*., 1994).

6.3.1. For presumptive diagnosis of clinically affected animals (no data are currently available)

Test type	Test purpose	Source populations	Tissue or sample types	Species	DSe (*n*)	DSp (*n*)	Reference test	Citation

44. DSe = diagnostic sensitivity, DSp = diagnostic specificity, *n* = number of animals used in the validation study,
PCR: = polymerase chain reaction.

6.3.2. For surveillance of apparently healthy animals ~~(no data are currently available)~~

Test type	Test purpose	Source populations	Tissue or sample types	Species	DSe (*n*)	DSp (*n*)	Reference test	Citation
Real-time PCR	Targeted surveillance	Wild populations	Gill tissue Haemolymph	*Ruditapes philippinarum*	100 (79)	50 (125)	RFTM	Rios *et al.*, 2020

45. DSe = diagnostic sensitivity, DSp = diagnostic specificity, *n* = number of animals used in the validation study,
PCR: = polymerase chain reaction; RFTM = Ray's fluid thioglycollate medium.

7. References

Abdel-Baki A.A.S., Al-Quraishy S., Dkhil M.A., Oliveira E., Casal G. & 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.

Abollo E., Casas S.M., Ceschia G. & Villalba A. (2006). Differential diagnosis of *Perkinsus* species by polymerase chain reaction-restriction fragment length polymorphism assay. *Mol. Cell. Probes,* **20**, 323–329.

Azevedo C. (1989). Fine structure of *Perkinsus atlanticus* n. sp. (Apicomplexa, Perkinsea) parasite of the clam *Ruditapes decussatus* from Portugal. *J. Parasitol.,* **75**, 627–635.

Bushek D., Ford S.E. & Allen S.K. (1994). Evaluation of methods using Ray's fluid thioglycollate medium for diagnosis of *Perkinsus marinus* infection in the eastern oyster, *Crassostrea virginica*. *Ann. Rev. Fish Dis.*, **4**, 201–217.

Bogema D.R., Yam J., Micallef M.L., Gholipourkanani H., Go J., Jenkins C. & Dang C. (2021). Draft genomes of *Perkinsus olseni* and *Perkinsus chesapeaki* reveal polyploidy and regional differences in heterozygosity. *Genomics*, **113**, 677–688.

Burreson E.M., Reece K.S. & Dungan C.F. (2005). Molecular, morphological, and experimental evidence support the synonymy of *Perkinsus chesapeaki* and *Perkinsus andrewsi*. *J. Eukaryot. Microbiol*., **52**, 258–270.

Casas S.M., La Peyre J.F., Reece K.S., Azevedo C. & Villalba A. (2002a). Continuous in vitro culture of the carpet shell clam *Tapes decussatus* protozoan parasite *Perkinsus atlanticus*. *Dis. Aquat. Organ.*, **52**, 217–231.

Casas S.M., Villalba A. & Reece K.S. (2002b). Study of the perkinsosis of the carpet shell clam *Tapes decussatus* in Galicia (NW Spain). I. Identification of the etiological agent and *in vitro* modulation of zoosporulation by temperature and salinity. *Dis. Aquat. Organ.*, **50**, 51–65.

Carella F., Fernandez Tejedor M., Villari G., Andree K.B. & De Vico G. (2023). The endoparasite *Perkinsus olseni* affecting the Mediterranean mussels (*Mytilus galloprovincialis*) in the Italian and Spanish waters: A new possible threat for mussel aquaculture and wild animal population. *Front. Mar. Sci.*, **10**.

Cho K.-S. & Park K. (2010). Review on the Protozoan Parasite *Perkinsus olseni* (Lester and Davis 1981) Infection in Asian Waters. Coastal Environmental and Ecosystem Issues of the East China Sea. https://api.semanticscholar.org/CorpusID:40174231

CREMONTE F., BALSEIRO P. & FIGUERAS A. (2005). Occurrence of *Perkinsus olseni* (Protozoa: Apicomplexa) and other parasites in the venerid commercial clam *Pitar rostrata* from Uruguay, southwestern Atlantic coast. *Dis. Aquat. Organ.,* **64**, 85–90.

CUI Y.Y., YE L.T., WU L. & WANG J.Y. (2018). Seasonal occurrence of *Perkinsus* spp. and tissue distribution of *P. olseni* in clam (*Soletellina acuta*) from coastal waters of Wuchuan County, southern China. *Aquaculture*, **492**, 300–305.

DANG C., DE MONTAUDOUIN X., BINIAS C., SALVO F., CAILL-MILLY N., BALD J. & SOUDANT P. (2013). Correlation between perkinsosis and growth in clams ruditapes spp. *Dis. Aquat. Organ*., **106**, 255–265.

DELISLE L., BUI T., COPEDO J., LAROCHE O., VON AMMON U., LANE H.S. & HUTSON K.S. (2025). Isolation, culture, and optimal growth conditions for the shellfish protozoan parasite, *Perkinsus olseni*. *Int. J. Pathol*., **55**, 339–350. https://doi.org/10.1016/j.ijpara.2025.04.011

DUNGAN C.F. & REECE K.S. (2006). *In vitro* propagation of two *Perkinsus* spp. parasites from Japanese Manila clams *Venerupis philippinarum* and description of *Perkinsus honshuensis* n. sp. *J. Eukaryot. Microbiol*., **53**, 316–326.

DUNGAN C.F. & REECE K.S. (2020). Chapter 5.2. 1 *Perkinsus* spp. Infections of Marine Molluscs. *In:* FHS Blue Book: Suggested procedures for the detection and identification of certain finfish and shellfish pathogens. AFS-FHS (American Fisheries Society-Fish Health Section), Bethesda, Maryland, USA, 1–24.

DUNGAN C.F., REECE K.S., MOSS J.A., HAMILTON R.M. & DIGGLES B.K. (2007). *Perkinsus olseni in vitro* isolates from the New Zealand clam *Austrovenus stutchburyi. J. Eukaryot. Microbiol.*, **54**, 263–270.

ELANDALLOUSSI L.M., LEITE R.B., RODRIGUES P.M., AFONSO R., NUNES P.A. & CANCELA M.L. (2005). Effect of antiprotozoal drugs on the proliferation of the bivalve parasite *Perkinsus olseni*. *Aquaculture,* **243**, 9–17.

ELSTON R.A., DUNGAN C.F., MEYERS T.R. & REECE K.S. (2004). *Perkinsus* sp. infection risk for manila clams, *Venerupis philippinarum* (A. Adams and Reeve, 1850) on the Pacific coast of North and Central America. *J. Shellfish Res*., **23**, 101–105.

FENG C., WANG C., LIN X., ZHANG Y., LV J., DENG J., YUAN X., MEI L. & WU S. (2013). Development of a loop-mediated isothermal amplification method for detection of *Perkinsus* spp. in mollusks. *Dis. Aquat. Organ.*, **104**, 141–148.

FERNANDEZ-BOO S., PROVOT C., LECADET C., STAVRAKAKIS C., PAPIN M., CHOLLET B., AUVRAY J. & ARZUL I. (2021). Inactivation of marine bivalve parasites using UV-C irradiation: Examples of *Perkinus olseni* and *Bonamia ostreae*. *Aquaculture Rep.*, **21**, 100859.

FISHER W.S. & OLIVER L.M. (1996). A whole-oyster procedure for diagnosis of *Perkinsus marinus* disease using Ray's fluid thioglycollate culture medium. *J. Shellfish Res.,* **15**, 109–117.

GAJAMANGE D., JONG-MAN Y. & PARK J.K. (2011). Development of a real-time PCR method for detection and quantification of the parasitic protozoan *Perkinsus olseni. Korean J. Malacol.*, **27**, 1225–3480.

GAJAMANGE D., KIM S.H., CHOI K.S., AZEVEDO C. & PARK K.I. (2020). Scanning electron microscopic observation of the in vitro cultured protozoan, *Perkinsus olseni,* isolated from the Manila clam, R*uditapes philippinarum. BMC Microbiol.*, **20**.

GAUTHIER J.D., MILLER C.R. & WILBUR A.E. (2006). TaqMan® MGB real-time PCR approach to quantification of *Perkinsus marinus* and *Perkinsus* spp. in oysters. *J. Shellfish Res.,* **25**, 619–624.

GAUTHIER J.D. & VASTA G.R. (1995). *In Vitro* Culture of the Eastern Oyster Parasite *Perkinsus marinus:* Optimization of the Methodology. *J. Invertebr. Pathol.*, **66**, 156–168.

GOGGIN C.L. & LESTER R.J.G. (1995). *Perkinsus*, a protistan parasite of abalone in Australia: a review. *Aust. J. Mar. Freshwater Res*., **46**, 639–646.

GUDKOVS N., CUMMINS D., JONES B., COLLING A., SINGANALLUR B.S. & CRANE M. (2016). Aquatic Animal Health Subprogram: Development of improved molecular diagnostic tests for *Perkinsus olseni* in Australian molluscs. *In:* Fisheries Research and Development Corporation (FRDC) Project No. 2011/004, CSIRO Publishing, p. 128.

HANRIO E., BATLEY J., DAVERN K. & DANG C. (2022). Development of monoclonal antibodies against *Perkinsus olseni* using whole cells. *Aquaculture Rep.*, **24**.

HANRIO E., BATLEY J., DUNGAN C.F. & DANG C. (2021). Immunoassays and diagnostic antibodies for *Perkinsus* spp. pathogens of marine molluscs. *Dis. Aquat. Organ*., **147**, 13–23.

ITOÏZ S., PERENNOU M., MOURONVALLE C., DERELLE E., LE GOÏC N., BIDAULT A., DE MONTAUDOUIN X., ARZUL I., SOUDANT P. & CHAMBOUVET A. (2021). Development of duplex TaqMan-based real-time PCR assay for the simultaneous detection of *Perkinsus olseni* and *P. chesapeaki* in host Manila clam tissue samples. *J. Invertebr. Pathol.,* **184**.

KAEWSALABNIL S., BUEWKEAW A., WATANACHOTE J., LEETHOCHAVALIT S. & KHONGCHAREONPORN N. (2024). Anti *Perkinsus olseni* Monoclonal Antibody Generation Using Hypnospores as Antigens. *Curr. Appl. Sci. Tech*., e0257223-e0257223. https://doi.org/10.55003/cast.2023.257223

KYOUNG J.A. & KI H.K. (2001). Effect of temperature and salinity on in vitro zoosporulation of *Perkinsus* sp. in Manila clams *Ruditapes philippinarum. Dis. Aquat. Organ.*, **48**, 43–46.

LA PEYRE M., CASAS S. LA PEYRE J. (2006). Salinity effects on viability, metabolic activity and proliferation of three *Perkinsus* species. *Dis. Aquat. Organ.,* **71,** 59–74.

LEETHOCHAVALIT S., CHALERMWAT K., UPATHAM E.S., CHOI K.S., SAWANGWONG P. & KRUATRACHUE M. (2004). Occurrence of Perkinsus sp. in undulated surf clams *Paphia undulata* from the Gulf of Thailand. *Dis. Aquat. Organ*., **60**, 165–171.

MOSS J.A., BURRESON E.M. & REECE K.S. (2006). Advanced *Perkinus marinus* infections in *Crassostrea ariakensis* maintained under laboratory conditions. *J. Shellfish Res.,* **25**, 65–72.

MUZNEBIN F., ALFARO A.C.& WEBB S.C. (2023). Occurrence of *Perkinsus olseni* and other parasites in New Zealand black-footed abalone (*Haliotis iris*). *NZ J. Marine Freshw. Res.*, **57**, 261–281.

NICKENS A.D., LA PEYRE J.F., WAGNER E.S. & TIERSCH T.R. (2002). An improved procedure to count *Perkinsus marinus* in eastern oyster hemolymph. *J. Shellfish Res*., **21**, 725–732.

PARDO B.G., CAO A., VILAS R., ABOLLO E., VILLALBA A. & MARTÍNEZ P. (2011). Microsatellite marker development in the protozoan parasite *Perkinsus olseni. Dis. Aquat. Organ*., **94**, 161–165.

PARK K.I. & CHOI K.S. (2001). Spatial distribution of the protozoan parasite *Perkinsus* sp. found in the Manila clams, *Ruditapes philippinarum*, in Korea. *Aquaculture*, **203**, 9–22.

PARK K.I., CHOI K.S. & CHOI J.W. (1999). Epizootiology of *Perkinsus* sp. found in the manila clam *Ruditapes philippinarum* in Komsoe Bay, Korea. *J. Korean Fish. Soc*., **32**, 303–309.

PARK K.I., YANG H.S., KANG H.S., CHO M, PARK KJ. & CHOI K.S. (2010). Isolation and identification of *Perkinsus olseni* from feces and marine sediment using immunological and molecular techniques. *J. Invertebr. Pathol.*, **105**, 261–269.

RAY S.M. (1966). A review of the culture method of detecting *Dermocystidium marinum* with suggested modifications and precautions. *Proc. Natl. Shellfish Assoc.,* **54**, 55–69.

REECE K., BUSHEK D., HUDSON K. & GRAVES J. (2001). Geographic distribution of *Perkinsus marinus* genetic strains along the Atlantic and Gulf coasts of the USA. *Marine Biol.*, **139**, 1047–1055.

REECE K.S., SIDDAL M.E., BURRESON E.M. & GRAVES J.E. (1997). Phylogenetic analysis of *Perkinsus* based on actin gene sequences. *J. Parasitol.*, **83**, 417–423.

RÍOS R., ARANGUREN R., GASTALDELLI M., ARCANGELI G., NOVOA B. & FIGUERAS A. (2020). Development and validation of a specific real-time PCR assay for the detection of the parasite *Perkinsus olseni. J. Invertebr. Pathology*, **169**, 107301.

ROBLEDO J.A.F., WRIGHT A.C., MARSH A.G. & VASTA G.R. (1999). Nucleotide sequence variability in the nontranscribed spacer of the rRNA locus in the oyster parasite *Perkinsus marinus. J. Parasitol.*, **85**, 650–656.

RODRIGUEZ F. & NAVAS J.I. (1995). A comparison of gill and hemolymph assays for the thioglycollate diagnosis of *Perkinsus atlanticus* (Apicomplexa, Perkinsea) in clams, *Ruditapes decussatus* (L.) and *Ruditapes philippinarum* (Adams et Reeve). *Aquaculture*, **132**, 145–152.

RUANO F., BATISTA F.M. & ARCANGELI G. (2015). Perkinsosis in the clams *Ruditapes decussatus* and *R. philippinarum* in the Northeastern Atlantic and Mediterranean Sea: A review. *J. Invertebr. Pathol.,* **131**, 58-67.

SAGRISTA E., DURFORT M. & AZEVEDO C. (1995). *Perkinsus* sp. (Phylum Apicomplexea) in Mediterranean clam *Ruditapes semidecussatus*: ultrastructural observations of the cellular response of the host. *Aquaculture,* **132**, 153–160.

SHEPPARD B.J. & PHILLIPS A.C. (2008). *Perkinsus olseni* detected in Vietnamese aquacultured reef clams Tridacna crocea imported to the USA, following a mortality event. *Dis. Aquat. Organ*., **79**, 229–235.

SOUDANT P.E., CHU F.L. & VOLETY A. (2013). Host-parasite interactions: Thompson PC, Rosenthal BM, Hare MP. 2011. An evolutionary legacy of sex and clonal reproduction in the protistan oyster parasite Perkinsus marinus. Infection, Genetics and Evolution 11:598-609.

UMEDA K., YANG X., WAKI T., YOSHINAGA T. & ITOH N. (2020). The effects of environmental and nutritional conditions on the development of *Perkinsus olseni* prezoosporangia. *Exp. Parasitol.*, **209**.

UMEDA K. & YOSHINAGA T. (2012). Development of real-time PCR assays for discrimination and quantification of two *Perkinsus* spp. in the Manila clam *Ruditapes philippinarum*. *Dis. Aquat. Organ.*, **99**, 215–225.

VILLALBA A., CASAS S.M., LÓPEZ C., & CARBALLAL M.J. (2005). Study of perkinsosis in the carpet shell clam *Tapes decussatus* in Galicia (NW Spain). II. Temporal pattern of disease dynamics and association with clam mortality. *Dis. Aquat. Organ.,* **65**, 257–267.

VILLALBA A., REECE K.S., ORDAS M.C., CASA S.M. & FIGUERAS A. (2004). Perkinsosis in molluscs: a review. *Aquat. Living Resour.,* **17**, 411–432.

WAKI T., TAKAHASHI M., EKI T., HIASA M., UMEDA K., KARAKAWA N. & YOSHINAGA T. (2018). Impact of *Perkinsus olseni* infection on a wild population of Manila clam *Ruditapes philippinarum* in Ariake Bay, Japan. *J. Inverteb. Pathol.*, **153**, 134–144.

WAKI T. & YOSHINAGA T. (2013). Experimental challenges of juvenile and adult Manila clams with the protozoan *Perkinsus olseni* at different temperatures. *Fisheries Sci.*, **79**, 779–786.

WAKI T. & YOSHINAGA T. (2015). Suppressive effects of low salinity and low temperature on *in-vivo* propagation of the protozoan *Perkinsus olseni* in Manila clam. *Fish Pathol.*, **50**, 16–22.

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* *

NB: Currently (2026) there is no WOAH Reference Laboratory for infection with *Perkinsus olseni* (please consult the WOAH web site: https://www.woah.org/en/what-we-offer/expertise-network/reference-laboratories/#ui-id-3).

NB: FIRST ADOPTED IN 1995 AS PERKINSOSIS. MOST RECENT UPDATES ADOPTED IN 2015.

Anexo 21. – Capítulo 2.4.7. 'Infeción por *Xenohaliotis californiensis*'

CHAPTER 2.4.7

INFECTION WITH *XENOHALIOTIS CALIFORNIENSIS*

1. Scope

Infection with Xenohaliotis californiensis means infection with the pathogenic agent *Candidatus* Xenohaliotis californiensis of the Family Anaplasmataceae. For the purposes of this chapter, the pathogenic agent will be referred to as *Xenohaliotis californiensis.*

2. Disease information

2.1. Agent factors

2.1.1. Aetiological agent

Xenohaliotis californiensis is an intracellular bacterium in the family Anaplasmataceae (Dumler *et al.,* 2001) and is closely related to members of the genera *Ehrlichia, Anaplasma* and *Cowdria* (Friedman *et al.,* 2000). The disease caused by this bacterium is known as withering syndrome (Friedman *et al.,* 2002; Haaker *et al.,* 1992) and may be more appropriately termed abalone rickettsiosis. Some *X. californiensis* may be infected with a phage (Friedman & Crosson, 2012). The dimorphic rod-to-spherical-shaped bacterium measures an average of 332 × 1550 nm in the bacillus form and an average of 1405 nm in the spherical morphotype. The bacterium reproduces within intracytoplasmic vacuoles 14–56 µm in diameter within gastrointestinal epithelia (Friedman *et al.,* 2000).

2.1.2. Survival and stability in processed or stored samples

As the pathogen has not been cultured, its survival and stability in stored samples are unknown.

2.1.3. Survival and stability outside the host

Although *X. californiensis* is thought to be an obligate intracellular organism, the bacterium ~~may~~ can survive outside the host ~~for an undetermined period of time~~ as evidenced by water-borne transmission studies (Balseiro *et al*., 2006; Braid *et al*., 2005; Friedman *et al*., 2002; 2007; Rosenblum *et al*., 2008).

~~For inactivation methods, see Section 2.4.5.~~

2.2. Host factors

2.2.1. Susceptible host species

Species that fulfil the criteria for listing as susceptible to infection with *Xenohaliotis californiensis* according to Chapter 1.5. of the *Aquatic Animal Health Code* (*Aquatic Code*) are:

Family	Scientific name	Common name
Haliotidae	*Haliotis corrugata*	pink abalone
	Haliotis cracherodii	black abalone
	Haliotis discus discus	Japanese abalone
	Haliotis diversicolor	small abalone
	Haliotis fulgens	green abalone
	Haliotis kamtschatkana	pinto abalone

Family	Scientific name	Common name
	Haliotis rufescens	red abalone
	Haliotis rufescens X *Haliotis discus hannai* hybrid	hybrid red and Japanese abalone
	Haliotis sorenseni	white abalone
	Haliotis tuberculata	tuberculate abalone

2.2.2. Species with incomplete evidence for susceptibility

Species for which there is incomplete evidence to fulfil the criteria for listing as susceptible to infection with *X. californiensis* according to Chapter 1.5. of the *Aquatic Code* is:

Family	Scientific name	Common name
Haliotidae	*Haliotis gigantea*	giant abalone

In addition, pathogen-specific positive polymerase chain reaction (PCR) results have been reported in the following species, but no active infection has been demonstrated:

Family	Scientific name	Common name
Haliotidae	*Haliotis discus hannai*	Japanese disc abalone

2.2.3. Likelihood of infection by species, host life stage, population or sub-populations

The bacterium divides by binary fission (Friedman *et al.,* 2000) and has direct, horizontal transmission (Braid *et al*., 2005; Friedman *et al*., 2002; Moore *et al*., 2001). Although not typically observed in farmed abalones until they are in grow-out conditions (>2.5 cm maximum size), polymerase chain reaction (PCR) examination of exposed 6-week-old abalones suggested that 1–2 mm abalones may become infected (Moore *et al*., unpublished observations). Probability of detection increases with increasing abalone size. Animals less than 10 mm in size have a reduced probability of detection using histology but equal probability of detection using PCR (Friedman *et al*., 2007; Moore *et al*., 2011).

While all post-larval life stages have been demonstrated susceptible to infection with *X. californiensis*, clinical disease is typically observed in animals >1 years of age in farmed abalones (Friedman, unpublished observations) and all abalone size classes observed in wild populations surveyed to date (e.g. Balseiro *et al*., 2006; Braid *et al*., 2005; Friedman *et al*., 1997; Haaker *et al*., 1992; Steinbeck *et al*., 1992; Van Blaricom *et al*., 1993).

2.2.4. Distribution of the pathogen in the host

Xenohaliotis californiensis infects the gastrointestinal epithelial cells of the posterior oesophagus, digestive gland and, to a lesser extent, intestine (Friedman *et al*., 2000).

2.2.5. Aquatic animal reservoirs of infection

None.

2.2.6. Vectors

None.

2.3. Disease pattern

2.3.1. Mortality, morbidity and prevalence

Susceptibility varies with species as the bacterium is known to cause disease in *H. cracherodii* (up to 99% mortality; Moore *et al*., 2009), *H. sorenseni* (up to 100% mortality; Friedman & McCormick, unpublished observations), *H. rufescens* (up to 35% mortality; Moore *et al*., 2000; 2001), *H. corrugata* and *H. fulgens* (Tinajero *et al*., 2002). Unlike the other abalone species studied to date, the magnitude of abalone mortality is not well documented in *H. corrugata* and *H. fulgens*. However, in Baja California, Mexico, up to 100% of *H. fulgens* and 63% of *H. corrugata* may be infected, with up to 43% of *H. fulgens* and 71% of *H. corrugata* having microscopic signs of disease (degenerated or metaplastic digestive gland; Tinajero *et al*., 2002).

The incubation period varies with temperature but typically involves a prolonged 3- to 7-month prepatent period. Mortality typically occurs 1–2.5 months after the onset of visible clinical signs (Friedman *et al*., 1997). Prevalence has not been well documented but up to 61% of *H. diversicolor supertexta* were infected at a farm in Thailand, however, like the European abalone, *H. tuberculata*, no abalones exhibited clinical signs of withering syndrome (Balseiro *et al*., 2001).

Infections may persist for long periods without the development of clinical disease when the host is maintained at cool water temperatures (e.g. 15°C for *H. rufescens*), and exposure to elevated seawater temperatures (e.g. >17°C for *H. rufescens*, *H. cracherodii* and *H. sorenseni*) typically results in clinical disease (Friedman & Finley, 2003; Moore *et al*., 2000; Steinbeck *et al*., 1992). Varying seawater temperatures with a lower mean temperature (e.g. 16.5°C for *H. rufescens*) may exacerbate losses (Moore *et al*., 2011). There is some suggestion that species, especially those inhabiting warmer waters may harbour the bacterium without the development of clinical disease (Wetchateng, 2008; Wetchateng *et al*., 2010).

2.3.2. Clinical signs, including behavioural changes

This intracellular pathogen infects the gastrointestinal epithelial cells, leading to clinical signs of starvation, including pedal and digestive gland atrophy. Abalones with *X. californiensis* infections may be sub-clinically infected during the prepatent period or at water temperatures ≤15°C. Infected individuals may be slightly to severely emaciated (atrophied) under permissive water temperatures.

During an epidemic, affected abalones will often cling to horizontal (as opposed to vertical or inverted) substrates and appear weak (easily removed from the substrate by hand) and emaciated (withered) (Haaker *et al*., 1992). Farmed abalones will also be anorexic. In addition, the presence of an abnormally high number of fresh shells may also indicate disease.

2.3.3 Gross pathology

Clinical disease is characterised by morphological changes in the digestive gland, which vary between species and may include degeneration (atrophy of tubules, increase in connective tissues and inflammation) and/or metaplasia of the digestive tubules. Metaplasia involves the replacement of terminal secretory/absorptive acini with absorptive/transport ducts similar in appearance to the post-oesophagus. These morphological changes are accompanied by anorexia, depletion of glycogen reserves, followed by use of the foot muscle as an energy source and subsequent death (Balseiro *et al*., 2006; Braid *et al*., 2005; Friedman *et al*., 1997; 2007; Kismohandaka *et al*., 1995; Moore *et al*., 2000; 2001). The foot of affected abalones contains fewer and less organised muscle bundles, abundant connective tissue and may contain more cerous cells than unaffected individuals (Friedman *et al*., 2007; Moore *et al*., 2000; Van Blaricom *et al*., 1993). Surviving abalones appear to remain infected, even in low water temperature environments, such as in northern California (Friedman & Finley, 2003).

2.3.4. Modes of transmission and life cycle

Transmission of *X. californiensis* is horizontal and is postulated to be via a faecal–oral route. Exposure of abalones to seawater containing infectious material is sufficient for transmission of the bacterium, and no direct animal contact is required (Balseiro *et al*., 2006; Braid *et al*., 2005; Friedman *et al*., 2002; 2007; Moore *et al*., 2000; 2001; Rosenblum *et al.,* 2008). Temperatures below 13°C have been demonstrated to limit transmission of the bacterium (i.e. less than 1% transmission) relative to those held at ~18°C (72–94% transmission) (Braid *et al*., 2005).

2.3.5. Environmental factors

Disease (withering syndrome) occurs at elevated water temperatures (~18–25°C in abalones with moderate to severe infections (Braid *et al*., 2005; Friedman *et al*., 2000; 2002; Moore *et al*., 2000; 2011; Rosenblum *et al*., 2008). Parasite transmission is enhanced in fed (94%) as opposed to starved. (72%) abalones (Balseiro *et al*., 2006; Braid *et al*., 2005). Subclinical infections have been observed in *H. diversicolor supertexta* raised at 27–29°C. As abalones are obligate marine species, salinity tolerances of the Rickettsia-like organism (RLO) have not been investigated.

2.3.6. Geographical distribution

Xenohaliotis californiensis occurs along the south-west coast of North America. However, as infected abalones have been transported to South America, Asia-Pacific, and Europe and possibly other regions, the geographical range of the aetiological agent is suspected to be broad where California red abalones, *Haliotis rufescens*, are cultured or areas where native species have been exposed to red abalones (e.g. Wetchateng, 2008)

See WAHIS (https://wahis.woah.org/#/home) for recent information on distribution at the country level.

2.4. Biosecurity and disease control strategies

2.4.1. Vaccination

No vaccines are available.

2.4.2. Chemotherapy including blocking agents

Reducing densities and application of an oxytetracycline-medicated diet may reduce losses (Friedman *et al*., 2003; 2007; Rosenblum *et al*., 2008). Oral administration of 12–19% TM-100 (90–100 mg kg^{-1}) in a medicated diet for 10 or 20 days provides protection against bacterial re-infection for several months. A single day oral administration of 12% TM-100 may reduce bacterial infections from 80% to 10% prevalence and mean infection intensity from 1.4 to 0.1 on a scale of 0–3 (Friedman *et al*., 2000; 2007).

2.4.3. Immunostimulation

No immunostimulants are currently known

2.4.4. Breeding resistant strains

Interest in selecting for resistant abalones, particularly for restoration purposes, is increasing. Wild black abalones have continued to recruit along the California Channel Islands since 2002 and some recruits survive, suggesting that these individuals may be more resistant to this rickettsial disease (Tinajero *et al*., 2002).

2.4.5. Inactivation methods

Based on successful decontamination in the laboratory, this bacterium is readily inactivated by immersion in <10% bleach. In addition, exposure of seawater containing the bacterium to >10 mg $litre^{-1}$ [ppm] calcium hypochlorite and disinfection of equipment in a bath of 1% tamed iodine in freshwater for 1 hour are effective disinfectants based on the use of these disinfection methods at a marine laboratory with flow-through seawater and a lack of detection of this pathogen in adjacent abalone populations (Friedman & Finley, 2003).

2.4.6. Disinfection of eggs and larvae

No attempts to disinfect eggs and larvae have been undertaken.

2.4.7. General husbandry

Husbandry practices to control *X. californiensis* are typical of those for any bacterial disease and include the purchase of inspected seed (devoid of evidence of infection), maintaining separate families or groups (i.e. avoid high grading and mixing of disparate groups), rinsing hands and equipment in freshwater or iodinated water and drying them ~~in~~ thoroughly between uses. Isolation of infected groups is recommended if possible.

3. Specimen selection, sample collection, transportation and handling

This section draws on information in Sections 2.2, 2.3 and 2.4 to identify populations, individuals and samples that are most likely to be infected.

3.1. Selection of populations and individual specimens

To optimise detection (targeted sampling), selection of abalones exhibiting the clinical sign of reduced weight (atrophied pedal muscle) is recommended. If possible, animals should be sampled after exposure to a period (e.g. 30 days) of warm water (e.g. >18°C).

3.2. Selection of organs or tissues

The best target tissue is the posterior oesophagus, and the second-best tissue is the digestive gland/intestine complex (Friedman, 2014; Friedman *et al.,* 2007).

3.3. Samples or tissues not suitable for pathogen detection

Non-digestive tissues do not contain rickettsial DNA and should be avoided.

3.4. Non-lethal sampling

None

3.5. Preservation of samples for submission

3.5.1. Samples for pathogen isolation

Not applicable.

3.5.2. Preservation of samples for molecular detection

~~Tissue samples for PCR testing should be preserved in 80% (v/v) analytical grade ethanol. The recommended ratio of ethanol to tissue is 10:1 based on studies in terrestrial animal and human health. The use of lower grade (laboratory or industrial grade) ethanol is not recommended. If material cannot be fixed it may be frozen.~~

Standard sample collection, preservation and processing methods for molecular techniques can be found in Section B.5.5 of Chapter 2.4.0 *General information* (diseases of molluscs).

3.5.3. Samples for histopathology, immunohistochemistry or *in-situ* hybridisation (ISH)

Standard sample collection, preservation and processing methods for histological techniques can be found in Section B.5.3 of Chapter 2.4.0 *General information* (diseases of molluscs).

3.5.4. Samples for other tests

None.

3.6. Pooling of samples

Pooling of samples from more than one individual animal for a given purpose is only recommended where robust supporting data on diagnostic sensitivity and diagnostic specificity have been evaluated and found to be suitable. If the effect of pooling on diagnostic sensitivity has not been thoroughly evaluated, specimens should be processed and tested individually. Small life stages such as spat can be pooled to obtain the minimum amount of material for virus isolation or molecular detection.

4. Diagnostic methods

The methods currently available for pathogen detection that can be used in i) surveillance of apparently healthy animals, ii) presumptive diagnosis in clinically affected animals and iii) confirmatory diagnostic purposes are listed in Table 4.1. by animal life stage.

Ratings for purposes of use. For each recommended assay a qualitative rating for the purpose of use is provided. The ratings are determined based on multiple performance and operational factors relevant to application of an assay for a defined purpose. These factors include appropriate diagnostic performance characteristics, level of assay validation, availability cost, timeliness, and sample throughput and operability. For a specific purpose of use, assays are rated as:

+++ = Methods are most suitable with desirable performance and operational characteristics.
++ = Methods are suitable with acceptable performance and operational characteristics under most circumstances.
+ = Methods are suitable, but performance or operational characteristics may limit application under some circumstances.
Shaded boxes = Not appropriate for this purpose.

Validation stage. The validation stage corresponds to the assay development and validation pathway in chapter 1.1.2. The validation stage is specific to each purpose of use. Where available, information on the diagnostic performance of recommended assays is provided in Section 6.3.

WOAH Reference Laboratories welcome feedback on diagnostic performance of recommended assays, in particular PCR methods. Of particular interest are any factors affecting expected assay sensitivity (e.g. tissue components inhibiting amplification) or expected specificity (e.g. failure to detect particular genotypes, detection of homologous sequences within the host genome). These issues should be communicated to the WOAH Reference Laboratories so that advice can be provided to diagnostic laboratories and the standards amended if necessary.

Table 4.1. WOAH recommended diagnostic methods and their level of validation for surveillance of apparently healthy animals and investigation of clinically affected animals

Method	G. Surveillance of apparently healthy animals				H. Presumptive diagnosis of clinically affected animals				I. Confirmatory diagnosis[1] of a suspect result from surveillance or presumptive diagnosis			
	Early life stages[2]	Juveniles[2]	Adults	LV	Early life stages[2]	Juveniles[2]	Adults	LV	Early life stages[2]	Juveniles[2]	Adults	LV
Wet mounts												
Histopathology	+	++	++	1	+	+++	+++	1				
Cell cultures												
Real-time PCR	+++	+++	+++	3	+++	+++	+++	NA ~~3~~	+++	+++	+++	3
Conventional PCR	+++	+++	+++	1	+++	+++	+++	1				
Conventional PCR followed by amplicon sequencing									+++	+++	+++	1
In-situ hybridisation					+	+	+	1	+	+	+	1
Bioassay												
LAMP												
Ab-ELISA												
Ag-ELISA												
Other antigen detection methods[3]												
Other methods[3]												

LV = level of validation, refers to the stage of validation in the WOAH Pathway (chapter 1.1.2); PCR = polymerase chain reaction; LAMP = loop-mediated isothermal amplification; Ab- or Ag-ELISA = antibody or antigen enzyme-linked immunosorbent assay, respectively; IFAT = indirect fluorescent antibody test.
[1]For confirmatory diagnoses, methods need to be carried out in combination (see Section 6). [2]Susceptibility of early and juvenile life stages is described in Section 2.2.3.
[3]Specify the test used. Shading indicates the test is inappropriate or should not be used for this purpose.

4.1. Wet mounts

Not applicable.

4.2. Histopathology and cytopathology

The presence of basophilic, ovoid intracytoplasmic bacterial inclusions in digestive epithelia (posterior oesophagus, transport ducts and metaplastic epithelia of the digestive gland, and/or intestine). Metaplastic changes in the digestive gland that include the transformation of the terminal digestive tubules into absorptive/transport epithelia occurs in abalones infected with *X. californiensis* (Balseiro *et al*., 2006; Braid *et al*., 2005; Friedman *et al*., 2002; Moore *et al*., 2000; 2001). Although metaplasia has been observed in all affected species examined to date, the response to infection may vary between hosts. Red abalones and white abalones, for example, typically respond with a metaplastic change (Balseiro *et al*., 2006; Braid *et al*., 2005; Moore *et al*., 2000), while black abalones generally respond with a combination of metaplasia, digestive tubule degeneration and inflammation (Friedman *et al*., 1997; 2002). Affected individuals contain less pedal glycogen and fewer muscle bundles than do unaffected individuals (Balseiro *et al*., 2006; Braid *et al*., 2005; Gardner *et al*., 1995). In some abalones, an increase in serous cells may be observed in the foot muscle (Van Blaricom *et al*., 1993), but these signs are not pathognomonic for this disease. Juvenile white abalone may contain an apparently metaplastic digestive without presence of *X. californiensis* (Friedman *et al*., 2007). Thus, the presence of *X. californiensis* in digestive epithelia in conjunction with the morphological changes noted above indicate the presence of clinical withering syndrome.

4.3. Cell culture for isolation

Not applicable.

4.4. Nucleic acid amplification

PCR assays should always be run with the controls specified in Section B.5.5 *Molecular methods* Chapter 2.4.0 *General information* (diseases of molluscs). Each sample should be tested in duplicate.

Extraction of nucleic acids

Different kits and procedures can be used for nucleic acid extraction. The quality and concentration of the extracted nucleic acid is important and can be checked using a suitable method as appropriate to the circumstances.

4.4.1. Real-time PCR

Pathogen/ target gene	Primer/probe (5'–3')	Concentration	Cycling parameters
Method 1: Friedman *et al.,* 2014; GenBank Accession No.: AF133090, amplicon size: 147 bp			
16S rDNA	Fwd WSN1 F: AGT-TTA-CTG-AAG-GCA-AGT-AGC-AGA Rev WSN1 R: TCT-AAC-TTG-GAC-TCA-TTC-AAA-AGC Probe WS-RLO_P: TGC-TTG-GAA-ATC-TAC-TCA-GAA-GAC-ATG-A	320 nM 320 nM 200 nM	45 cycles of: 95°C/15 sec and 60°C/30 sec

4.4.2. Conventional PCR

Pathogen/ target gene	Primer (5'–3')	Concentration	Cycling parameters[(a)]
Method 1: Andree *et al.,* 2000; GenBank Accession No.: AF133090, amplicon size: 160 bp			
16S rDNA	Fwd RA 5-1: GTT-GAA-CGT-GCC-TTC-AGT-TTA-C Rev RA 3-6**:** ACT-TGG-ACT-CAT-TCA-AAA-GCG-GA	500 nM 500 nM	40 cycles of: 95°C/60 sec, 50°C/30 sec and 72°C/30 sec
Method 2: Cicala *et al.,* 2017; GenBank Accession No.: KU645900, amplicon size: 426 bp			
16S rDNA	Fwd ss16S-F: GCC-TCA-GTT-TGG-CTG-GGT-TCT-TCA Rev ss16S-R: GAA-TTG-CCA-CTT-TAA-AGT-ATG-GAC-GG	300 nM 300 nM	40 cycles of: 94°C/60 sec, 66°C/30 sec and 72°C/30 sec

4.4.3. Other nucleic acid amplification methods

Not applicable.

4.5. Amplicon sequencing

The size of the PCR amplicon should be verified, for example by agarose gel electrophoresis. Both DNA strands of the PCR product must be sequenced and analysed in comparison with reference sequences.

4.6. *In-situ* hybridisation

Use of Rickettsiales-like prokaryotes specific DNA probes with histological sections is useful to demonstrate the presence of *X. californiensis* nucleic acid in infected cells (Antonio *et al*., 2000). See Chapter 2.4.0 Section 5.5.4 for general comments on *in-situ* hybridisation.

Antonio *et al.* (2000) developed an ISH method targeting the 16S rDNA gene. This method allows the detection of Rickettsiales-like prokaryotes in tissue sections. Although this method has not been formally validated, tests for specificity using several bivalve and fish rickettsial organisms suggested that the test was specific for *X. californiensis*.

Reference	Pathogen/target gene	ISH probe	Probe ~~size~~ type
Antonio *et al.* (2000)	16S rDNA	RA 5-1: GTT-GAA-CGT-GCC-TTC-AGT-TTA-C RA 3-6: ACT-TGG-ACT-CAT-TCA-AAA-GCG-GA; RA 3-8: CCA-CTG-TGA-GTG-GTT-ATC-TCC-TG; RA 5-6: GAA-GCA-ATA-TTG-TGA-GAT-AAA-GCA.	oligo nucleotide probes

4.7. Immunohistochemistry

Not applicable.

4.8. Bioassay

Not applicable.

4.9. Antibody- or antigen-based detection methods (ELISA, etc.)

Not applicable.

4.10. Other methods

Not applicable.

5. Test(s) recommended for surveillance to demonstrate freedom in apparently healthy populations

The recommended method for surveillance is real-time PCR using the assay by Friedman *et al.* (2014).

6. Corroborative diagnostic criteria

This section only addresses the diagnostic test results for detection of infection in the absence (Section 6.1.) or in the presence (Section 6.2.) of clinical signs ~~(Section 6.2.)~~ but does not evaluate whether the infectious agent is the cause of the clinical event.

The case definitions for a suspect and confirmed case have been developed to support decision making related to trade and confirmation of disease status at the country, zone or compartment level. Case definitions for disease confirmation in endemically affected areas may be less stringent. If a Competent Authority does not have the capability to undertake the necessary diagnostic tests it should seek advice from the appropriate WOAH Reference Laboratory, and if necessary, refer samples to that laboratory for confirmatory testing of samples from the index case in a country, zone or compartment considered free. There are currently no WOAH Reference Laboratories for *Xenohaliotis californiensis*.

When two molecular methods are used for confirmation of a case, it is preferable to use two species-specific methods targeting non-overlapping regions of the pathogen genome.

6.1. Apparently healthy animals or animals of unknown health status[3]

Apparently healthy populations may fall under suspicion, and therefore be sampled, if there is an epidemiological link(s) to an infected population. Hydrographical proximity to, or movement of animals or animal products or

[3] For example transboundary commodities.

equipment, etc., from a known infected population equate to an epidemiological link. Alternatively, healthy populations are sampled in surveys to demonstrate disease freedom.

6.1.1. Definition of suspect case in apparently healthy animals

The presence of infection with *X. californiensis* shall be suspected if at least one of the following criteria is met:

i) Histopathological changes consistent with the presence of the pathogen or the disease

ii) Positive result by conventional PCR

iii) Positive result by real-time PCR

6.1.2. Definition of confirmed case in apparently healthy animals

The presence of infection with *X. californiensis* is considered to be confirmed if at least one of the following criteria is met:

i) Positive result by real-time PCR and positive result by conventional PCR followed by amplicon sequencing

ii) Positive result by *in-situ* hybridisation and positive result by conventional PCR followed by amplicon sequencing

iii) Positive result by species-specific real-time PCR and positive result by *in-situ* hybridisation ~~and positive result by real-time PCR~~

6.2 Clinically affected animals

Clinical signs are not pathognomonic for a single disease; however they may narrow the range of possible diagnoses.

6.2.1. Definition of suspect case in clinically affected animals

The presence of infection with *X. californiensis* shall be suspected if at least one of the following criteria is met:

i) Histopathological changes consistent with the presence of the pathogen or the disease

ii) Positive result by *in-situ* hybridisation

iii) Positive result by conventional PCR

iv) Positive result by real-time PCR

6.2.2. Definition of confirmed case in clinically affected animals

The presence of infection with *X. californiensis* is considered to be confirmed if at least at least one of the following criteria is met:

i) Positive result by real-time PCR and positive result by conventional PCR followed by amplicon sequencing.

ii) Positive result by *in-situ* hybridisation and positive result by conventional PCR followed by amplicon sequencing.

iii) Positive result by species-specific real-time PCR and positive result by *in-situ* hybridisation ~~and positive result by real-time PCR~~

6.3. Diagnostic sensitivity and specificity for diagnostic tests

The diagnostic performance of tests recommended for surveillance or diagnosis of infection with *Xenohaliotis californiensis* are provided in Tables 6.3.1. [no data currently available] and 6.3.2). Data are only presented where tests are validated to at least level 2 of the validation pathway described in Chapter 1.1.2. and the information is available within published diagnostic accuracy studies.

6.3.1. For presumptive diagnosis of clinically affected animals (no data are currently available)

Test type	Test purpose	Source populations	Tissue or sample types	Species	DSe (*n*)	DSp (*n*)	Reference test	Citation

~~Real-time PCR~~	~~Diagnosis~~	~~Clinically diseased abalone from farms~~	~~Posterior esophagus and digestive gland tissue~~	~~*Haliotis rufescens, H. sorensoni, H. fulgens, H. discushannai, H. cracherodii, H. kamtschatkana*~~	~~100 (518)~~	~~99.7 (518)~~	~~Histopathology~~	~~Friedman *et al.* (2014)~~

46. DSe = diagnostic sensitivity, DSp = diagnostic specificity, *n* = number of animals used in the validation study,
PCR: = polymerase chain reaction.

6.3.2. For surveillance of apparently healthy animals ~~(no data are currently available)~~

Test type	Test purpose	Source populations	Tissue or sample types	Species	DSe (*n*)	DSp (*n*)	Reference test	Citation
Real-time PCR	Diagnosis	Farmed and wild abalone with known historical health status ~~Clinically diseased abalone from farms~~	Posterior esophagus and digestive gland tissue	*Haliotis rufescens, H. sorensoni, H. fulgens, H. discushannai, H. cracherodii, H. kamtschatkana*	100 (518)	99.7 (518)	Histopathology	Friedman *et al.* (2014)

47. DSe = diagnostic sensitivity, DSp = diagnostic specificity, *n* = number of animals used in the validation study,
PCR: = polymerase chain reaction.

7. References

ANDREE K.B., FRIEDMAN C.S., MOORE J.D. & HEDRICK R.P. (2000). A polymerase chain reaction for detection of genomic DNA of a Rickettsiales-like prokaryote associated with Withering Syndrome in Black Abalone (*Haliotis cracherodii*). *J. Shellfish Res*., **19**, 213–218.

ANTONIO D.B., ANDREE K.B., MOORE J.D., FRIEDMAN C.S. & HEDRICK R.P. (2000). Detection of Rickettsiales-like prokaryotes (RLPs) by *in situ* hybridization in black abalone *Haliotis cracherodii* with withering syndrome. *J. Invertebr. Pathol.*, **75**, 180–182.

BALSEIRO P., ARANGUREN R., GESTAL C., NOVOA B. & FIGUERAS A. (2006). *Candidatus* Xenohaliotis californiensis and *Haplosporidium montforti* associated with mortalities of abalone *Haliotis tuberculata* cultured in Europe. *Aquaculture*, **258**, 63–72.

BRAID B.A., MOORE J.D., ROBBINS T.T., HEDRICK R.P., TJEERDEMA R.S. & FRIEDMAN C.S. (2005). Health and survival of red abalone, *Haliotis rufescens*, under varying temperature, food supply and exposure to the agent of withering syndrome. *J. Invertebr. Pathol.,* **89**, 219–231.

CICALA F., MOORE J.D., CACERES-MARTINEZ J., DEL RIO-PORTILLA M.A., HERNANDEX-RODRIGUES M., VASQUEZ_YEOMANS R. & ROCHA-OLIVARES A. (2017). Multigenic characterisation of "*Çandidatus* Xenohaliotis californiensis". *Int. J. Syst. Evol. Microbiol.*, **67**, 42–49.

DUMLER J.S., BARBET A.F., BEKKER C.P.J., DASCH G.A., PALMER G.H., RAY S.C., RIKIHISA Y. & RURANGIRWA F.R. (2001). Reorganization of general in the families Rickettsiaceae and Anaplasmataceae in the order Rickettsiales: unification of some species of *Ehrlichia* with *Anaplasma*, *Cowdria* with *Ehrlichia* and *Ehrlichia* with *Neorickettsia*, descriptions of six new species combinations and designation of *Ehrlichia equi* and 'HGE agent' as subjective synonyms of E*hrlichia phagocytophila. Int. J. System. Evol. Microbiol*., **51**, 2145–2165.

FRIEDMAN C.S., ANDREE K.B., BEAUCHAMP K.A., MOORE J.D., ROBBINS T.T., SHIELDS J.D. & HEDRICK R.P. (2000). "*Candidatus* Xenohaliotis californiensis" a newly described pathogen of abalone, *Haliotis* spp., along the west coast of North America. *Int. J. Syst. Evol. Microbiol.,* **50**, 847–855.

FRIEDMAN C.S., BIGGS W., SHIELDS J.D. & HEDRICK R.P. (2002). Transmission of withering syndrome in black abalone, *Haliotis cracherodii* Leach. *J. Shellfish Res*., **21**, 817–824.

FRIEDMAN C.S. & CROSSON L.M. (2012). Putative phage hyperparasite in the Rickettsial Pathogen of Abalone, "*Candidatus* Xenohaliotis californiensis". *Invertebr. Microbiol*., **64**, 1064–1072.

FRIEDMAN C.S. & FINLEY C.A. (2003). Evidence for an anthropogenic introduction of "*Candidatus* Xenohaliotis californiensis", the etiological agent of withering syndrome, into northern California abalone populations via conservation efforts. *Can. J. Fish Aquat. Sci.,* **60**, 1424–1431.

FRIEDMAN C.S., THOMSON M., CHUN C., HAAKER P.L. & HEDRICK, R.P. (1997). Withering syndrome of the black abalone, *Haliotis cracherodii* (Leach): Water temperature, food availability, and parasites as possible causes. *J. Shellfish Res.*, **16**, 403–411.

FRIEDMAN C.S., TREVELYAN G., MULDER E.P. & FIELDS R. (2003). Development of an oral administration of oxytetracycline to control losses due to withering syndrome in cultured red abalone *Haliotis rufescens. Aquaculture*, **224**, 1–23.

FRIEDMAN C.S., SCOTT B.B., STRENGE R.E. & MCCORMICK T.B. (2007). Oxytetracycline as a tool to manage and prevent losses of the endangered white abalone, *Haliotis sorenseni,* due to withering syndrome. *J. Shellfish Res*., **26**, 877–885.

FRIEDMAN C.S., WIGHT N., CROSSON L.M., WHITE S.J. & STRENGE R.M. (2014). Validation of a qualitative PCR assay for detection and quantification of '*Candidatus* Xenohaliotis californiensis'. *Dis. Aquat. Organ*.,**108**, 251–259.

GARDNER G.R., HARSHBARGER J.C., LAKE J., SAWYER T.K., PRICE K.L., STEPHENSON M.D., HAAKER P.L. & TOGSTAD H.A. (1995). Association of prokaryotes with symptomatic appearance of withering syndrome in black abalone *Haliotis craherodii. J. Invertebr. Pathol.*, **66**, 111–120.

HAAKER P.L., PARKER D.O., TOGSTAD H., RICHARDS D.V., DAVIS G.E. & FRIEDMAN C.S. (1992). Mass mortality and withering syndrome in black abalone *Haliotis cracherodii*, in California. *In:* Abalone of the World, Shepard S.A., Tegner M.J. & Guzman del Proo S.A., eds, Blackwell Scientific, Oxford, UK, 214–224.

KISMOHANDAKA G., ROBERTS W., HEDRICK R.P. & FRIEDMAN C.S. (1995). Physiological alterations of the black abalone, *Haliotis cracherodii* Leach, with withering syndrome. *J. Shellfish Res*., **14**, 269–270.

MOORE J.D., JUHASZ C.I., ROBBINS T.T. & VILCHIS I. (2009). Green abalone, *Haliotis fulgens*, infected with the agent of withering syndrome do not express disease signs under a temperature regime permissive for red abalone, *Haliotis rufescens*. *Marine Biol.,* **156**, 2325–2330.

MOORE J.D., MARSHMAN B.C. & CHUN C.C. (2011). Health and survival of red abalone *Haliotis rufescens* from San Miguel Island, California, USA, in a laboratory simulation of La Niña and El Niño conditions. *J. Aquat. Anim. Health*, **23**, 78–84.

MOORE J.D., ROBBINS T.T. & FRIEDMAN C.S. (2000). Withering syndrome in farmed red abalone *Haliotis rufescens:* Thermal induction and association with a gastrointestinal rickettsia-like prokaryote. *J. Aquat. Anim. Health,* **12**, 26–34.

MOORE J.D., ROBBINS T.T., HEDRICK R.P. & FRIEDMAN C.S. (2001) Transmission of the Rickettsiales-like procaryote "*Candidatus* Xenohaliotis californiensis" and its role in withering syndrome of California abalone *Haliotis* spp. *J. Shellfish Res.*, **20**, 867–874.

ROSENBLUM E.S., JUHASZ C., FRIEDMAN C.S., ROBBINS T.T., CRAIGMILL A., TJEERDEMA R.S. & MOORE J.D. (2008). Oxytetracycline as a treatment for abalone withering syndrome Part II: Efficacy, pharmacokinetics, and long term resistance to re-infection at elevated sea water temperatures. *Aquaculture*, **277**, 138–148.

STEINBECK J.R., GROFF J.M., FRIEDMAN C.S., MCDOWELL T. & HEDRICK R.P. (1992). Investigations into mortality among populations of the California black abalone, *Haliotis cracherodii,* on the central coast of California, USA. *In:* Abalone of the World, Shepard S.A., Tegner M.J. & Guzman del Proo S.A., eds. Blackwell Scientific, Oxford, UK, 203–213.

TINAJERO M.D.C.A., CACERES-MARTINEZ J., & AVILES J.G.G. (2002) Histopathological evaluation of the yellow abalone *Haliotis corrugata* and the blue abalone *Haliotis fulgen*s from Baja California, Mexico. *J. Shellfish Res*., **21**, 825–830.

VAN BLARICOM G.R., RUEDIGER J.L., FRIEDMAN C.S., WOODARD D.D. & HEDRICK R.P. (1993). Discovery withering syndrome among black abalone populations at San Nicolas Island, California *J. Shellfish Res.*, **12**, 185–188.

WETCHATENG T. (2008). *Rickettsia*-like organism (RLO) infection in the abalone *Haliotis diversicolor supertexta*: Histopathology, diagnosis and treatment. PhD. Dissertation, Mahidol University, Bangkok, Thailand, 49 pp.

WETCHATENG T., FRIEDMAN C.S., WIGHT N.A., LEE P.-Y., TENG P.H., SRIURAIRATTANA S., WONGPRASERT K. & WITHYACHUMNARNKUL B. (2010). Withering syndrome in abalone *Halioits diversicolor supertexta*. *Dis. Aquat. Organ.,* **90**, 69–76.

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NB: Currently (2026) there is no WOAH Reference Laboratory for infection with *Xenohaliotis californiensis* (please consult the WOAH web site: https://www.woah.org/en/what-we-offer/expertise-network/reference-laboratories/#ui-id-3).
NB: FIRST ADOPTED IN 2006. MOST RECENT UPDATES ADOPTED IN 2012.

Anexo 22. – Actualización de la taxonomía del ámbito de aplicación de los capítulos 2.2.3., 2.2.5., 2.2.6., 2.2.7., 2.2.8., 2.2.10., 2.3.2., 2.3.4., 2.3.5., 2.3.6., 2.3.8., 2.3.9., 2.3.10., et 2.4.1.

CHAPTER 2.2.3.

INFECTION WITH INFECTIOUS DECAPOD IRIDESCENT VIRUS 1

1. Scope

Infection with decapod iridescent virus 1 (DIV1) means infection with the pathogenic agent *Decapodiridovirus litopenaeus1* ~~decapod iridescent virus 1 (DIV1), Genus *Decapodiridovirus*,~~ of the Subfamily *Betairidovirinae*, Family *Iridoviridae*.

[…]

CHAPTER 2.2.5.

INFECTION WITH INFECTIOUS HYPODERMAL AND HAEMATOPOIETIC NECROSIS VIRUS

1. Scope

Infection with infectious hypodermal and haematopoietic necrosis virus (IHHNV) means infection with the pathogenic agent *Penstylhamaparvovirus decapod 1* ~~Decapod penstylhamaparvovirus 1~~, of the ~~Genus *Penstylhamaparvovirus* and~~ Family *Parvoviridae*.

[…]

CHAPTER 2.2.6.

INFECTION WITH INFECTIOUS MYONECROSIS VIRUS

1. Scope

Infection with infectious myonecrosis virus (IMNV) means infection with the pathogenic agent *Artirivirus ichi* ~~infectious myonecrosis virus (IMNV) that is tentatively assigned to the Family *Totiviridae*~~ of the ~~genus Artivirus and~~ Family *Artiviridae*.

2. Disease information

2.1. Agent factors

2.1.1. Aetiological agent

48. IMNV is ~~tentatively~~ assigned to the family Artiviridae (ICTV, 2025) ~~*Totiviridae* (Lightner, 2011; Nibert, 2007; Poulos *et al.*, 2006; Wickner *et al.*, 2011)~~. The IMNV isolate of Poulos *et al.* (2006) (Genbank accession number AY570982) has been assigned as *Artivirus ichi* within the family *Artiviridae* (ICTV, 2025).

49. IMNV particles are icosahedral in shape and 40 nm in diameter, with a buoyant density of 1.366 g ml^{-1} in caesium chloride. The genome consists of a single, double-stranded (ds) RNA molecule of 8226–8230 bp (Loy *et al*., 2015; Naim *et al.,* 2015). Sequencing of the viral genome reveals two non-overlapping open reading frames (ORFs). The first ORF (ORF1, 470–5596 nt) encodes a putative RNA-binding protein and a capsid protein. The coding region of the RNA-binding protein is located in the first half of ORF1 and contains a dsRNA-binding motif in the first 60 amino acids. The second half of ORF1 encodes a capsid protein, as determined by amino acid sequencing, with a molecular mass of 106 kDa. The second ORF (ORF2, 5884–8133 nt) encodes a putative RdRp (Poulos *et al.,* 2006). The most variable region of IMNV genome is located in the first half of ORF1, coinciding with a region which probably encodes the capsid protrusions (Dantas *et al*., 2015).

50. The complete genomes of IMNV types originating from Brazil and Indonesia have been sequenced and found to be 99.6% identical at the nucleotide level (Poulos *et al.,* 2006; Senapin *et al.,* 2007). The 99.6% full genome sequence identity (and anecdotal information on the introduction of *Penaeus vannamei* stocks from Brazil) indicate that the disease was introduced from Brazil to Indonesia in 2006. A new genotype was analysed in infected samples in 2018 in Indonesia, including an isolate that contains a deletion of 622 amino acids (Mai *et al.,* 2019).

[…]

CHAPTER 2.2.7.

INFECTION WITH MACROBRACHIUM ROSENBERGII NODAVIRUS (WHITE TAIL DISEASE)

1. Scope

Infection with Macrobrachium rosenbergii nodavirus means infection with the pathogenic agent Macrobrachium rosenbergii nodavirus (MrNV) in the Family *Nodaviridae*. The disease is commonly known as white tail disease (WTD).

Extra small virus (XSV) is associated with disease, but its role has not been determined.

2. Disease information

2.1. Agent factors

2.1.1. Aetiological agent

51. Two viruses are associated with WTD, namely MrNV (primary) and extra small virus (XSV) (associate) (Qian *et al.,* 2003; Romestand & Bonami, 2003). MrNV is a necessary cause of WTD in prawns, however, the role of XSV in pathogenicity remains unclear.

52. MrNV belongs in the family *Nodaviridae* (Bonami *et al.,* 2005). While the physico-chemical properties of MrNV are consistent with those of other members of the *Nodaviridae*, it differs structurally and genetically from other nodaviruses within the two recognised genera, *Alphanodavirus* and *Betanodavirus* (Ho *et al*., 2017, 2018; Naveenkumar *et al*., 2013). Consequently, a third genus, *Gammanodavirus*, has been proposed for nodaviruses that infect crustaceans, including MrNV and *Penaeus vannamei* nodavirus (*Pv*NV) (Naveenkumar *et al*., 2013).

53. XSV is the first sequenced satellite virus in aquatic animals and it is also the first record of a satellite-nodavirus association (Bonami *et al.,* 2005). XSV has been classified by the ICTV as *Macronovirus macrobrachii* ~~*Macrobrachium satellite virus 1*~~ of the family *Sarthroviridae*.

[…]

CHAPTER 2.2.8.

INFECTION WITH TAURA SYNDROME VIRUS

1. Scope

Infection with Taura syndrome virus (TSV) means infection with the pathogenic agent *Aparavirus tauraense* ~~Taura syndrome virus (TSV)~~, ~~Genus *Aparavirus*,~~ of the Family *Dicistroviridae*, Order *Picornavirales*.

[…]

CHAPTER 2.3.4.

INFECTION WITH ~~HRP~~ HPR-DELETED OR HPR0 INFECTIOUS SALMON ANAEMIA VIRUS

1. Scope

Infection with infectious salmon anaemia virus (ISAV) means infection with the pathogenic agent *Isavirus salaris* of the ~~Genus *Isavirus* and~~ Family *Orthomyxoviridae*. Infection with ISAV includes two genotypes: highly polymorphic region ~~(HPR)~~ deleted ISAV (HPR-deleted ISAV), or ~~the non-pathogenic HPR0~~ full length highly polymorphic region-0 ISAV (HPRO ISAV) ~~(non-deleted HPR) ISAV of the Genus Isavirus and Family Orthomyxoviridae~~.

HPR-deleted ISAV may cause disease in Atlantic salmon (Salmo salar), which may progress to a generalised and lethal condition characterised by severe anaemia, and variable haemorrhages and necrosis in several organs.

Detection of HPR0 ISAV has not been associated with clinical signs of disease in Atlantic salmon (Christiansen et al., 2011). A link between non-pathogenic HPR0 ISAV and pathogenic HPR-deleted ISAV has been suggested, with some disease outbreaks potentially occurring as a result of the emergence of HPR-deleted ISAV from HPR0 ISAV (Cardenas et al., 2014; Christiansen et al., 2017; Cunningham et al., 2002; Gagne & Leblanc, 2017; Mjaaland, et al., 2002).

[…]

CHAPTER 2.3.5.

INFECTION WITH INFECTIOUS HAEMATOPOIETIC NECROSIS VIRUS

1. Scope

Infection with infectious haematopoietic necrosis virus (IHNV) means infection with the pathogenic agent *Novirhabdovirus salmonid* (previously known as Salmonid novirhabdovirus) ~~Salmonid novirhabdovirus (commonly known as infectious haematopoietic necrosis virus [IHNV]) Genus *Novirhabdovirus* and~~ of the Family *Rhabdoviridae*.

[…]

CHAPTER 2.3.6.

INFECTION WITH KOI HERPESVIRUS

1. Scope

Infection with koi herpesvirus (KHV) means infection with the pathogenic agent *Cyvirus cyprinidallo3* (previously known as cyprinid herpesvirus-3 [CyHV-3]), of ~~the Genus *Cyprinivirus* in~~ the Family *Alloherpesviridae*.

2. Disease information

2.1. Agent factors

2.1.1. Aetiological agent

KHV, also known as carp interstitial nephritis and gill necrosis virus (CNGV) (Ilouze *et al.,* 2010), has been classified as *Cyvirus cyprinidallo 3* ~~cyprinid herpesvirus-3 (CyHV-3) following the nomenclature of other cyprinid herpesviruses: CyHV-1 (carp pox virus, fish papilloma virus) and CyHV-2 (goldfish haematopoietic necrosis virus)~~. Analysis of the complete genome has shown that CyHV-3 is closely related to CyHV-1 (carp pox virus), CyHV-2 (goldfish haematopoietic necrosis virus), anguillid herpesvirus-1 (AngHV-1) and distantly related to channel catfish virus (Ictalurid herpesvirus: IcHV-1) and Ranid (frog) herpesvirus (RaHV-1) (Waltzek *et al.,* 2005). CyHV-3 was designated the type species of the new *Cyprinivirus* genus within the *Alloherpesviridae* family, that also contains CyHV-1 and CyHV-2. However, the designation KHV has been retained in the *Aquatic Code* and *Aquatic Manual* for reasons of continuity and is used here synonymously with CyHV-3.

The size of the KHV genome is now confirmed as 295 kbp. Virus nucleocapsids have been measured at 100–110 nm in diameter and are surrounded by an envelope (Ilouze *et al.,* 2010). The enveloped virions range in size from 170 to 230 nm in the different infected cell types (Hedrick *et al.,* 2000; Miwa *et al.,* 2007; Miyazaki *et al,* 2008a). Aoki *et al.* (2007) initially described the complete genome sequence of three isolates of KHV and the genome includes 164 open reading frames (ORFs) of which 156 are unique protein-coding genes. They suggested that the finding that 15 KHV genes are homologous with genes in IcHV-1 confirms the proposed place of KHV in the family Herpesviridae. Forty viral proteins and 18 cellular proteins are incorporated into mature virions.

Engelsma *et al.* (2013) detected novel strains of cyprinid herpesvirus closely related to KHV. These strains may represent low or non-pathogenic variants of CyHV-3, but further investigation is required to establish the true genetic relationship between these strains, and KHV.

[…]

CHAPTER 2.3.8.

INFECTION WITH ~~INFECTIOUS~~ SALMONID ALPHAVIRUS

1. Scope

Infection with salmonid alphavirus (SAV) means infection with any genotype of the pathogenic agent *Alphavirus salmon* ~~SAV~~, ~~Genus *Alphavirus* and~~ of the Family *Togaviridae*.

[…]

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CHAPTER 2.3.9.

INFECTION WITH SPRING VIRAEMIA OF CARP VIRUS

1. Scope

Infection with spring viraemia of carp virus (SVCV) means infection with the pathogenic agent *Sprivivirus cyprinus* ~~(commonly known as spring viraemia of carp virus [SVCV])~~, of ~~the Genus *Sprivivirus* and~~ the Family *Rhabdoviridae*.

[…]

CHAPTER 2.3.10.

INFECTION WITH VIRAL HAEMORRHAGIC SEPTICAEMIA VIRUS

1. Scope

Infection with viral haemorrhagic septicaemia virus (VHSV) means infection with the pathogenic agent *Novirhabdovirus piscine* ~~viral haemorrhagic septicaemia virus~~ of the ~~Genus *Novirhabdovirus* and~~ Family *Rhabdoviridae.*

[…]

_____

CHAPTER 2.4.1.

INFECTION WITH ABALONE HERPESVIRUS

1. Scope

Infection with abalone herpesvirus (AbHV) means infection with the pathogenic agent *Aurivirus haliotidmalaco1* (previously known as *Haliotid herpesvirus 1*, ~~and *abalone herpesvirus* [AbHV]~~) of ~~the genus *Aurivirus* and~~ the Family *Malacoherpesviridae*.

[…]