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Activités des Commissions spécialisées

COMMISSION DES NORMES SANITAIRES POUR LES ANIMAUX AQUATIQUES

Propositions de modifications du *Code aquatique* et du *Manuel aquatique*

[Document de travail technique]

Organisation mondiale de la santé animale
Fondée en tant qu'OIE

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I. Introduction

1. Depuis la 90e Session générale en mai 2023, la Commission des normes sanitaires pour les animaux aquatiques (la Commission des animaux aquatiques) s'est réunie à deux reprises, du 13 au 20 septembre 2023 ainsi que du 14 au 21 février 2024. Dans le cadre de ses activités, la Commission a fait avancer les travaux ayant trait à l'élaboration de textes nouveaux et révisés du *Code sanitaire pour les animaux aquatiques* (le *Code aquatique*) et du *Manuel des tests de diagnostic des animaux aquatiques* (le *Manuel aquatique),* conformément à son plan de travail. Les détails relatifs aux réunions de la Commission des animaux aquatiques sont disponibles sur le site web destiné aux Délégués et sur le site web de l'OMSA.

2. Le présent document apporte des informations générales portant sur chacun des textes nouveaux et révisés du *Code aquatique* et du *Manuel aquatique* qui seront proposés pour adoption lors de la 91e Session générale en mai 2024. Lors de la révision de ces textes, la Commission a pris en considération les commentaires transmis par les Membres, les recommandations formulées dans les rapports de plusieurs Groupes *ad hoc*, ainsi que par des experts des Laboratoires de référence. La Commission des animaux aquatiques a également travaillé en collaboration avec la Commission des normes sanitaires pour les animaux terrestres en ce qui concerne toutes les activités pertinentes.

3. Les informations détaillées ayant trait à l'examen par la Commission des commentaires transmis portant sur les projets de textes diffusés afin de recueillir les commentaires ont été présentées dans les rapports de septembre 2023 et de février 2024 de la Commission. **La Commission invite les Membres à consulter ces rapports pour obtenir des informations plus détaillées ayant trait aux textes amendés qui sont proposés à l'adoption**.

4. Les modifications du *Code aquatique* et du *Manuel aquatique* présentées en annexes 4 à 6, 8 à 39 et 51 à 59 seront proposées pour adoption lors de la 91e Session générale. La numérotation des annexes utilisée dans le présent document correspond à celle du rapport de février 2024 de la Commission.

1. Textes du *Code aquatique* qui seront proposés à l'adoption

1.1 Utilisation des définitions du Glossaire : « Services chargés de la santé des animaux aquatiques », « Autorité compétente » et « Autorité vétérinaire » (annexe 4)

5. Les propositions de modifications visent à assurer une utilisation cohérente des définitions révisées du Glossaire de termes employés dans l'ensemble des parties pertinentes du *Code aquatique*. Les définitions révisées du Glossaire qui ont été adoptées en 2022 concernent les termes « Services chargés de la santé des animaux aquatiques », « Autorité compétente » et « Autorité vétérinaire ».

6. Ces modifications ont été élaborées en coordination avec la Commission des normes sanitaires pour les animaux terrestres, qui a proposé des amendements concernant l'utilisation des termes « Services vétérinaires », « Autorité compétente » et « Autorité vétérinaire » dans l'ensemble du *Code terrestre.*

7. Le texte révisé a été diffusé à trois reprises, la première fois dans le rapport de février 2023 de la Commission.

8. Le texte révisé portant sur les termes « Services chargés de la santé des animaux aquatiques », « Autorité compétente » et « Autorité vétérinaire » est présenté en annexe 4 et sera proposé pour adoption lors de la 91e Session générale en mai 2024.

1.2 Utilisation de la définition du Glossaire : « Produits issus d'animaux aquatiques » (annexe 5)

9. Les modifications proposées visent à remplacer dans la version anglaise le terme « products of aquatic animal origin » par le terme défini du Glossaire « aquatic animal products », afin de veiller à ce que les définitions du Glossaire soient utilisées de manière cohérente dans les parties pertinentes du *Code aquatique.*

10. Le texte révisé a été diffusé à deux reprises, la première fois dans le rapport de septembre 2023 de la Commission.

11. Le texte révisé portant sur le terme « Produits issus d'animaux aquatiques » est présenté en annexe 5 et sera proposé à l'adoption lors de la 91e Session générale en mai 2024.

1.3 Article 1.1.5. du chapitre 1.1. « Notification des maladies et communication des informations épidémiologiques » (annexe 6)

12. La proposition de modification vise à supprimer l'article 1.1.5., car les dispositions qui y figurent sont maintenant traitées dans le chapitre 1.4. révisé « Surveillance des maladies des animaux aquatiques ». Cette modification permet également d'assurer l'harmonisation avec le chapitre 1.1. du *Code terrestre*.

13. Le texte révisé a été diffusé à trois reprises, la première fois dans le rapport de février 2023 de la Commission de février 2023.

14. L'article 1.1.5. révisé du chapitre 1.1. « Notification des maladies et communication des informations épidémiologiques » est présenté en annexe 6 et sera proposé pour adoption lors de la 91e Session générale en mai 2024.

1.4 Article 1.3.1. du chapitre 1.3. « Maladies listées par l'OMSA » (annexe 8)

15. Il est proposé de remplacer la dénomination de la maladie « Infection par l'iridovirus de la daurade japonaise » par « Infection par les génogroupes de l'espèce virale de la nécrose infectieuse rénale et splénique ».

16. Les virus du genre *Megalocytivirus* peuvent provoquer des maladies graves chez les poissons, et ce genre comprend l'espèce virale de la nécrose infectieuse rénale et splénique (ISKNV). Un des génogroupes de l'ISKNV appelé iridovirus de la daurade japonaise (RSIV) est responsable de la maladie listée par l'OMSA, « Infection par l'iridovirus de la daurade japonaise ». Outre le RSIV, l'ISKNV comprend également le génogroupe de l'ISKNV et le génogroupe de l'iridovirus du corps rougeâtre du turbot (TRBIV).

17. L'espèce virale ISKNV a été évaluée pour ses trois génogroupes au regard des critères figurant dans l'article 1.2.2. du chapitre 1.2. « Critères d'inclusion des maladies des animaux aquatiques ». La Commission est convenue que les trois génogroupes de l'ISKNV satisfont aux critères d'inclusion 1, 2, 3 et 4b. Il a été proposé de remplacer le nom de la maladie par « Infection par les génogroupes de l'espèce virale de la nécrose infectieuse rénale et splénique » et qu'elle serait définie de manière à couvrir les trois génogroupes ISKNV, RSIV et TRBIV.

18. Le texte révisé a été diffusé à quatre reprises, la première fois dans le rapport de septembre 2022 de la Commission.

19. L'article 1.3.1. révisé du chapitre 1.3. « Maladies listées par l'OMSA » est présenté en annexe 8 et sera proposé pour adoption lors de la 91e Session générale en mai 2024.

1.5 Article 8.1.3. du chapitre 8.1. « Infection à Batrachochytrium dendrobatidis *» (annexe 9)*

20. Les propositions de modifications de l'article 8.1.3. visent à ce qu'il soit en ligne avec l'approche révisée des couples temps / température concernant les traitements thermiques pour les marchandises dénuées de risques.

21. Le texte révisé a été diffusé à quatre reprises, la première fois dans le rapport de février 2022 de la Commission.

22. L'article 8.1.3. révisé du chapitre 8.1. « Infection à *Batrachochytrium dendrobatidis* » est présenté en annexe 9 et sera proposé pour adoption lors de la 91e Session générale en mai 2024.

1.6 Article 8.2.3. du chapitre 8.2. « Infection à Batrachochytrium salmandrivorans *» (annexe 10)*

23. Les propositions de modifications de l'article 8.2.3. visent à ce qu'il soit en ligne avec l'approche révisée des couples temps / température concernant les traitements thermiques pour les marchandises dénuées de risques.

24. Le texte révisé a été diffusé à quatre reprises, la première fois dans le rapport de février 2022 de la Commission.

25. L'article 8.2.3. révisé du chapitre 8.2. « Infection à *Batrachochytrium salmandrivorans* » est présenté en annexe 10 et sera proposé pour adoption lors de la 91e Session générale en mai 2024.

1.7 Article 8.3.3. du chapitre 8.3. « Infection par les espèces du genre Ranavirus *» (annexe 11)*

26. Les propositions de modifications de l'article 8.3.3. visent à ce qu'il soit en ligne avec l'approche révisée des couples temps / température concernant les traitements thermiques pour les marchandises dénuées de risques.

27. Le texte révisé a été diffusé à quatre reprises, la première fois dans le rapport de février 2022 de la Commission.

28. L'article 8.3.3. révisé du chapitre 8.3. « Infection par les espèces du genre *Ranavirus* » est présenté en annexe 11 et sera proposé pour adoption lors de la 91e Session générale en mai 2024.

1.8 Article 9.3.3. du chapitre 9.3. « Infection par le virus 1 iridescent des décapodes » (annexe 12)

29. Les propositions de modifications de l'article 9.3.3. visent à ce qu'il soit en ligne avec l'approche révisée des couples temps / température concernant les traitements thermiques pour les marchandises dénuées de risques.

30. Le texte révisé a été diffusé à trois reprises, la première fois dans le rapport de février 2023 de la Commission.

31. L'article 9.3.3. révisé du chapitre 9.3. « Infection par le virus 1 iridescent des décapodes » est présenté en annexe 12 et sera proposé pour adoption lors de la 91e Session générale en mai 2024.

1.9 Article 9.4.3. du chapitre 9.4. « Infection à Hepatobacter penaei *(hépatopancréatite nécrosante) » (annexe 13)*

32. Les propositions de modifications de l'article 9.4.3. visent à ce qu'il soit en ligne avec l'approche révisée des couples temps / température concernant les traitements thermiques pour les marchandises dénuées de risques.

33. Le texte révisé a été diffusé à trois reprises, la première fois dans le rapport de février 2023 de la Commission.

34. L'article 9.4.3. révisé du chapitre 9.4. « Infection à *Hepatobacter penaei* (hépatopancréatite nécrosante) » est présenté en annexe 13 et sera proposé pour adoption lors de la 91e Session générale en mai 2024.

1.10 Article 9.6.3. du chapitre 9.6. « Infection par le virus de la myonécrose infectieuse » (annexe 14)

35. Les propositions de modifications de l'article 9.6.3. visent à ce qu'il soit en ligne avec l'approche révisée des couples temps / température concernant les traitements thermiques pour les marchandises dénuées de risques.

36. Le texte révisé a été diffusé à trois reprises, la première fois dans le rapport de février 2023 de la Commission.

37. L'article 9.6.3. révisé du chapitre 9.6. « Infection par le virus de la myonécrose infectieuse » est présenté en annexe 14 et sera proposé pour adoption lors de la 91e Session générale en mai 2024.

1.11 Article 9.7.3. du chapitre 9.7. « Infection par le nodavirus de Macrobrachium rosenbergii » (annexe 15)

38. Les propositions de modifications de l'article 9.7.3. visent à ce qu'il soit en ligne avec l'approche révisée des couples temps / température concernant les traitements thermiques pour les marchandises dénuées de risques.

39. Le texte révisé a été diffusé à trois reprises, la première fois dans le rapport de février 2023 de la Commission.

40. L'article 9.7.3. révisé du chapitre 9.7. « Infection par le nodavirus de Macrobrachium rosenbergii » est présenté en annexe 15 et sera proposé pour adoption lors de la 91e Session générale en mai 2024.

1.12 Article 9.8.3. du chapitre 9.8. « Infection par le virus du syndrome de Taura » (annexe 16)

41. Les propositions de modifications de l'article 9.8.3. visent à ce qu'il soit en ligne avec l'approche révisée des couples temps / température concernant les traitements thermiques pour les marchandises dénuées de risques.

42. Le texte révisé a été diffusé à trois reprises, la première fois dans le rapport de février 2023 de la Commission.

43. L'article 9.8.3. révisé du chapitre 9.8. « Infection par le virus du syndrome de Taura » est présenté en annexe 16 et sera proposé pour adoption lors de la 91e Session générale en mai 2024.

1.13 Article 10.1.3. du chapitre 10.1. « Infection par le virus de la nécrose hématopoïétique épizootique » (annexe 17)

44. Les propositions de modifications de l'article 10.1.3. visent à ce qu'il soit en ligne avec l'approche révisée des couples temps / température concernant les traitements thermiques pour les marchandises dénuées de risques.

45. Le texte révisé a été diffusé à trois reprises, la première fois dans le rapport de février 2023 de la Commission.

46. L'article 10.1.3. révisé du chapitre 10.1. « Infection par le virus de la nécrose hématopoïétique épizootique » est présenté en annexe 17 et sera proposé pour adoption lors de la 91e Session générale en mai 2024.

1.14 Article 10.2.3. du chapitre 10.2. « Infection à Aphanomyces invadans *(syndrome ulcératif épizootique) » (annexe 18)*

47. Les propositions de modifications de l'article 10.2.3. visent à ce qu'il soit en ligne avec l'approche révisée des couples temps / température concernant les traitements thermiques pour les marchandises dénuées de risques.

48. Le texte révisé a été diffusé à trois reprises, la première fois dans le rapport de février 2023 de la Commission.

49. L'article 10.2.3. révisé du chapitre 10.2. « Infection à *Aphanomyces invadans* (syndrome ulcératif épizootique) » est présenté en annexe 18 et sera proposé pour adoption lors de la 91e Session générale en mai 2024.

1.15 Article 10.3.3. du chapitre 10.3. « Infection à Gyrodactylus salaris *» (annexe 19)*

50. Les propositions de modifications de l'article 10.3.3. visent à ce qu'il soit en ligne avec l'approche révisée des couples temps / température concernant les traitements thermiques pour les marchandises dénuées de risques.

51. Le texte révisé a été diffusé à trois reprises, la première fois dans le rapport de février 2023 de la Commission.

52. L'article 10.3.3. révisé du chapitre 10.3. « Infection à *Gyrodactylus salaris* » est présenté en annexe 19 et sera proposé pour adoption lors de la 91e Session générale en mai 2024.

1.16 Article 10.4.3. du chapitre 10.4. « Infection par le virus de l'anémie infectieuse du saumon » (annexe 20)

53. Les propositions de modifications de l'article 10.4.3. visent à ce qu'il soit en ligne avec l'approche révisée des couples temps / température concernant les traitements thermiques pour les marchandises dénuées de risques.

54. Le texte révisé a été diffusé à trois reprises, la première fois dans le rapport de février 2023 de la Commission.

55. L'article 10.4.3. révisé du chapitre 10.4. « Infection par le virus de l'anémie infectieuse du saumon » est présenté en annexe 20 et sera proposé pour adoption lors de la 91e Session générale en mai 2024.

1.17 Article 10.5.3. du chapitre 10.5. « Infection par l'alphavirus des salmonidés » (annexe 21)

56. Les propositions de modifications de l'article 10.5.3. visent à ce qu'il soit en ligne avec l'approche révisée des couples temps / température concernant les traitements thermiques pour les marchandises dénuées de risques.

57. Le texte révisé a été diffusé à trois reprises, la première fois dans le rapport de février 2023 de la Commission.

58. L'article 10.5.3. révisé du chapitre 10.5. « Infection par l'alphavirus des salmonidés » est présenté en annexe 21 et sera proposé pour adoption lors de la 91e Session générale en mai 2024.

1.18 Article 10.6.3. du chapitre 10.6. « Infection par le virus de la nécrose hématopoïétique infectieuse » (annexe 22)

59. Les propositions de modifications de l'article 10.6.3. visent à ce qu'il soit en ligne avec l'approche révisée des couples temps / température concernant les traitements thermiques pour les marchandises dénuées de risques.

60. Le texte révisé a été diffusé à trois reprises, la première fois dans le rapport de février 2023 de la Commission.

61. L'article 10.6.3. révisé du chapitre 10.6. « Infection par le virus de la nécrose hématopoïétique infectieuse » est présenté en annexe 22 et sera proposé pour adoption lors de la 91e Session générale en mai 2024.

1.19 Article 10.7.3. du chapitre 10.7. « Infection par l'herpèsvirus de la carpe koï » (annexe 23)

62. Les propositions de modifications de l'article 10.7.3. visent à ce qu'il soit en ligne avec l'approche révisée des couples temps / température concernant les traitements thermiques pour les marchandises dénuées de risques.

63. Le texte révisé a été diffusé à trois reprises, la première fois dans le rapport de février 2023 de la Commission.

64. L'article 10.7.3. révisé du chapitre 10.7. « Infection par l'herpèsvirus de la carpe » est présenté en annexe 23 et sera proposé pour adoption lors de la 91e Session générale en mai 2024.

1.20 Article 10.8.3. du chapitre 10.8. « Infection par l'iridovirus de la daurade japonaise » (annexe 24)

65. Les propositions de modifications de l'article 10.8.3. visent à ce qu'il soit en ligne avec l'approche révisée des couples temps / température concernant les traitements thermiques pour les marchandises dénuées de risques.

66. Le texte révisé a été diffusé à trois reprises, la première fois dans le rapport de février 2023 de la Commission.

67. L'article 10.8.3. révisé du chapitre 10.8. « Infection par l'iridovirus de la daurade japonaise » est présenté en annexe 24 et sera proposé pour adoption lors de la 91e Session générale en mai 2024.

1.21 Article 10.9.3. du chapitre 10.9. « Infection par le virus de la virémie printanière de la carpe » (annexe 25)

68. Les propositions de modifications de l'article 10.9.3. visent à ce qu'il soit en ligne avec l'approche révisée des couples temps / température concernant les traitements thermiques pour les marchandises dénuées de risques.

69. Le texte révisé a été diffusé à trois reprises, la première fois dans le rapport de février 2023 de la Commission.

70. L'article 10.9.3. du chapitre 10.9. « Infection par le virus de la virémie printanière de la carpe » est présenté en annexe 25 et sera proposé pour adoption lors de la 91e Session générale en mai 2024.

1.22 Article 10.10.3. du chapitre 10.10. « Infection par le virus de la septicémie hémorragique virale » (annexe 26)

71. Les propositions de modifications de l'article 10.10.3. visent à ce qu'il soit en ligne avec l'approche révisée des couples temps / température concernant les traitements thermiques pour les marchandises dénuées de risques.

72. Le texte révisé a été diffusé à trois reprises, la première fois dans le rapport de février 2023 de la Commission.

73. L'article 10.10.3. révisé du chapitre 10.10. « Infection par le virus de la septicémie hémorragique virale » est présenté en annexe 26 et sera proposé pour adoption lors de la 91e Session générale en mai 2024.

1.23 Article 10.11.3. du chapitre 10.11. « Infection par le virus du tilapia lacustre » (annexe 27)

74. Les propositions de modifications de l'article 10.11.3. visent à ce qu'il soit en ligne avec l'approche révisée des couples temps / température concernant les traitements thermiques pour les marchandises dénuées de risques.

75. Le texte révisé a été diffusé à deux reprises, la première fois dans le rapport de septembre 2023 de la Commission.

76. L'article 10.11.3. révisé du chapitre 10.11. « Infection par le virus du tilapia lacustre » est présenté en annexe 27 et sera proposé pour adoption lors de la 91e Session générale en mai 2024.

1.24 Article 11.1.3. du chapitre 11.1. « Infection par l'herpèsvirus de l'ormeau » (annexe 28)

77. Les propositions de modifications de l'article 11.1.3. visent à ce qu'il soit en ligne avec l'approche révisée des couples temps / température concernant les traitements thermiques pour les marchandises dénuées de risques.

78. Le texte révisé a été diffusé à quatre reprises, la première fois dans le rapport de février 2022 de la Commission.

79. L'article 11.1.3. révisé du chapitre 11.1. « Infection par l'herpèsvirus de l'ormeau » est présenté en annexe 28 et sera proposé pour adoption lors de la 91e Session générale en mai 2024.

1.25 Article 11.2.3. du chapitre 11.2. « Infection à Bonamia exitiosa *» (annexe 29)*

80. Les propositions de modifications de l'article 11.2.3. visent à ce qu'il soit en ligne avec l'approche révisée des couples temps / température concernant les traitements thermiques pour les marchandises dénuées de risques.

81. Le texte révisé a été diffusé à quatre reprises, la première fois dans le rapport de février 2022 de la Commission.

82. L'article 11.2.3. révisé du chapitre 11.2. « Infection à *Bonamia exitiosa* » est présenté en annexe 29 et sera proposé pour adoption lors de la 91e Session générale en mai 2024.

1.26 Article 11.3.3. du chapitre 11.3. « Infection à Bonamia ostreae *» (annexe 30)*

83. Les propositions de modifications de l'article 11.3.3. visent à ce qu'il soit en ligne avec l'approche révisée des couples temps / température concernant les traitements thermiques pour les marchandises dénuées de risques.

84. Le texte révisé a été diffusé à quatre reprises, la première fois dans le rapport de février 2022 de la Commission.

85. L'article 11.3.3. révisé du chapitre 11.3. « Infection à *Bonamia ostreae* » est présenté en annexe 30 et sera proposé pour adoption lors de la 91e Session générale en mai 2024.

1.27 Article 11.4.3. du chapitre 11.4. « Infection à Marteilia refringens *» (annexe 31)*

86. Les propositions de modifications de l'article 11.4.3. visent à ce qu'il soit en ligne avec l'approche révisée des couples temps / température concernant les traitements thermiques pour les marchandises dénuées de risques.

87. Le texte révisé a été diffusé à quatre reprises, la première fois dans le rapport de février 2022 de la Commission.

88. L'article 11.4.3. révisé du chapitre 11.4. « Infection à *Marteilia refringens* » est présenté en annexe 31 et sera proposé pour adoption lors de la 91e Session générale en mai 2024.

1.28 Article 11.5.3. du chapitre 11.5. « Infection à Perkinsus marinus *» (annexe 32)*

89. Les propositions de modifications de l'article 11.5.3. visent à ce qu'il soit en ligne avec l'approche révisée des couples temps / température concernant les traitements thermiques pour les marchandises dénuées de risques.

90. Le texte révisé a été diffusé à quatre reprises, la première fois dans le rapport de février 2022 de la Commission.

91. L'article 11.5.3. révisé du chapitre 11.5. « Infection à *Perkinsus marinus* » est présenté en annexe 32 et sera proposé pour adoption lors de la 91e Session générale en mai 2024.

1.29 Article 11.6.3. du chapitre 11.6. « Infection à Perkinsus olseni *» (annexe 33)*

92. Les propositions de modifications de l'article 11.6.3. visent à ce qu'il soit en ligne avec l'approche révisée des couples temps / température concernant les traitements thermiques pour les marchandises dénuées de risques.

93. Le texte révisé a été diffusé à quatre reprises, la première fois dans le rapport de février 2022 de la Commission.

94. L'article 11.6.3. révisé du chapitre 11.6. « Infection à *Perkinsus olseni* » est présenté en annexe 33 et sera proposé pour adoption lors de la 91e Session générale en mai 2024.

1.30 Article 11.7.3. du chapitre 11.7. « Infection à Xenohaliotis californiensis *» (annexe 34)*

95. Les propositions de modifications de l'article 11.7.3. visent à ce qu'il soit en ligne avec l'approche révisée des couples temps / température concernant les traitements thermiques pour les marchandises dénuées de risques.

96. Le texte révisé a été diffusé à quatre reprises, la première fois dans le rapport de février 2022 de la Commission.

97. L'article 11.7.3. révisé du chapitre 11.7. « Infection à *Xenohaliotis californiensis* » est présenté en annexe 34 et sera proposé pour adoption lors de la 91e Session générale en mai 2024.

1.31 Modèles d'articles X.X.5. et X.X.6. destinés aux chapitres spécifiques à des maladies (annexe 35)

98. Les propositions de modifications des modèles d'articles X.X.5. « Pays indemne d'infection par / à [agent pathogène X] » et X.X.6. « Zone indemne d'infection par / à [agent pathogène X] » visent à assurer l'harmonisation des articles pertinents pour le statut indemne des pays et des zones, qui figurent dans l'ensemble des chapitres spécifiques à des maladies du *Code aquatique*.

99. Le texte révisé a été diffusé à deux reprises, la première fois dans le rapport de septembre 2023 de la Commission.

100. Les modèles d'articles X.X.5. et X.X.6. révisés destinés aux chapitres spécifiques à des maladies sont présentés en annexe 35 et seront proposés pour adoption lors de la 91e Session générale en mai 2024.

1.32 Article 9.3.2. du chapitre 9.3. « Infection par le virus 1 iridescent des décapodes » (annexe 36)

101. Une liste modifiée des espèces sensibles, destinée à l'article 9.3.2., est proposée.

102. Le rapport de mars 2023 du Groupe *ad hoc* sur la sensibilité des espèces de crustacés à l'infection par une maladie listée par l'OMSA présente des informations détaillées sur les évaluations entreprises pour établir une proposition de liste des espèces sensibles. Lors de sa réunion de février 2024, la Commission des animaux aquatiques a demandé au Groupe *ad hoc* d'examiner les données probantes relatives à deux espèces, sur la base des commentaires d'un Membre. La réévaluation de ces deux espèces figure dans le rapport de février 2024 de la Commission.

103. Le texte révisé a été diffusé à deux reprises, la première fois dans le rapport de septembre 2023 de la Commission.

104. L'article 9.3.2. révisé du chapitre 9.3. « Infection par le virus 1 iridescent des décapodes » est présentée en annexe 36 et sera proposé pour adoption lors de la 91e Session générale en mai 2024.

1.33 Article 10.6.2. du chapitre 10.6. « Infection par le virus de la nécrose hématopoïétique infectieuse » (annexe 37)

105. Un reformatage de la liste des espèces sensibles destinée à l'article 10.6.2. est proposé afin de l'aligner sur la convention utilisée dans l'article X.X.2. du *Code aquatique* ; cette modification vise à présenter la liste des espèces sensibles sous la forme d'un tableau.

106. Le texte révisé a été diffusé à deux reprises, la première fois dans le rapport de septembre 2023 de la Commission.

107. L'article 10.6.2. révisé du chapitre 10.6. « Infection par le virus de la nécrose hématopoïétique infectieuse » est présenté en annexe 37 et sera proposé pour adoption lors de la 91e Session générale en mai 2024.

1.34 Article 10.11.2. du chapitre 10.11. « Infection par le virus du tilapia lacustre » (annexe 38)

108. Une liste modifiée des espèces sensibles, destinée à l'article 10.11.2., est proposée.

109. Le rapport d'avril 2023 du Groupe *ad hoc* sur la sensibilité des espèces de poissons à l'infection par une maladie listée par l'OMSA présente des informations détaillées sur les évaluations entreprises pour établir une proposition de liste des espèces sensibles.

110. Le texte révisé a été diffusé à deux reprises, la première fois dans le rapport de septembre 2023 de la Commission.

111. L'article 10.11.2. révisé du chapitre 10.11. « Infection par le virus du tilapia lacustre » est présenté en annexe 38 et sera proposé pour adoption lors de la 91e Session générale en mai 2024.

1.35 Articles 11.5.1. et 11.5.2. du chapitre 11.5. « Infection à Perkinsus marinus *» (annexe 39)*

112. Les propositions de modifications dans le cadre de la révision partielle de l'article 11.5.1. visent à assurer la cohérence avec d'autres chapitres spécifiques aux mollusques. Le libellé « aux espèces ci-après, satisfaisant aux critères permettant de les lister comme étant sensibles conformément au chapitre 1.5. » a été ajouté après « Les recommandations du présent chapitre s'appliquent » pour des raisons d'harmonisation avec d'autres chapitres spécifiques à des maladies des mollusques.

113. Une liste modifiée des espèces sensibles destinée à l'article 11.5.2. est proposée.

114. Le rapport de novembre / décembre 2022 du Groupe *ad hoc* sur la sensibilité des espèces de mollusques à une infection par une maladie listée de l'OMSA présente des informations détaillées sur les évaluations entreprises pour établir une proposition de liste des espèces sensibles.

115. Le texte révisé a été diffusé à trois reprises, la première fois dans le rapport de février 2023 de la Commission.

116. Les articles 11.5.1. et 11.5.2. révisés du chapitre 11.5. « Infection à *Perkinsus marinus* » sont présentés en annexe 39 et seront proposés pour adoption lors de la 91e Session générale en mai 2024.

2. Textes du *Manuel aquatique* qui seront proposés pour adoption

2.1 Chapitre 2.2.0. « Informations générales : maladies des crustacés » (annexe 51)

117. Une révision approfondie du chapitre 2.2.0. « Informations générales : maladies des crustacés » est proposée afin de fournir des informations actualisées.

118. Le texte modifié a été diffusé à quatre reprises, la première fois dans le rapport de septembre 2022 de la Commission.

119. Le chapitre 2.2.0. révisé « Informations générales : maladies des crustacés » est présenté en annexe 51 et sera proposé pour adoption lors de la 91e Session générale en mai 2024.

2.2 Chapitre 2.2.2. « Infection à Aphanomyces astaci *(peste de l'écrevisse) » (annexe 52)*

120. Une révision approfondie du chapitre 2.2.2. « Infection à *Aphanomyces astaci* (peste de l'écrevisse) », comprenant une proposition de reformatage en vue de son alignement avec le nouveau modèle de chapitres spécifiques à des maladies, a été proposée.

121. Le chapitre révisé a été diffusé à quatre reprises, la première fois dans le rapport de septembre 2022 de la Commission.

122. Le chapitre 2.2.2. révisé « Infection à *Aphanomyces astaci* (peste de l'écrevisse) » est présenté en annexe 52 et sera proposé pour adoption lors de la 91e Session générale en mai 2024.

2.3 Chapitre 2.2.6. « Infection par le nodavirus de Macrobrachium rosenbergii (maladie des queues blanches) » (annexe 53)

123. Une révision approfondie du chapitre 2.2.6. « Infection par le nodavirus de Macrobrachium rosenbergii (maladie des queues blanches) », comprenant une proposition de reformatage en vue de son alignement avec le nouveau modèle de chapitres spécifiques à des maladies, a été proposée.

124. Le chapitre révisé a été diffusé à trois reprises, la première fois dans le rapport de février 2023 de la Commission.

125. Le chapitre 2.2.6. révisé « Infection par le nodavirus de Macrobrachium rosenbergii (maladie des queues blanches) » est présenté en annexe 53 et sera proposé pour adoption lors de la 91e Session générale en mai 2024.

2.4 Chapitre 2.2.9. « Infection par le génotype 1 du virus de la tête jaune » (annexe 54)

126. Une révision approfondie du chapitre 2.2.9. « Infection par le génotype 1 du virus de la tête jaune », comprenant une proposition de reformatage en vue de son alignement avec le nouveau modèle de chapitres spécifiques à des maladies, est prévue.

127. Le chapitre révisé a été diffusé à trois reprises, la première fois dans le rapport de février 2023 de la Commission.

128. Le chapitre révisé 2.2.9. « Infection par le génotype 1 du virus de la tête jaune » est présenté en annexe 54 et sera proposé pour adoption lors de la 91e Session générale en mai 2024.

2.5 Chapitre 2.2.X. « Infection par le virus 1 iridescent des décapodes » (annexe 55)

129. Un nouveau chapitre 2.2.X. « Infection par le virus 1 iridescent des décapodes » qui est aligné sur le nouveau modèle de chapitres spécifiques à des maladies, est proposé.

130. Le nouveau chapitre a été diffusé à deux reprises, la première fois dans le rapport de septembre 2023 de la Commission.

131. Le nouveau chapitre 2.2.X. « Infection par le virus 1 iridescent des décapodes » est présenté en annexe 55 et sera proposé pour adoption lors de la 91e Session générale en mai 2024.

2.6 Chapitre 2.4.0. « Informations générales : maladies des mollusques » (annexe 56)

132. Une révision approfondie du chapitre 2.4.0. « Informations générales : maladies des mollusques » comprenant des propositions de modifications visant à présenter des informations actualisées, est prévue.

133. Le chapitre révisé a été diffusé à deux reprises, la première fois dans le rapport de septembre 2023 de la Commission.

134. Le chapitre révisé 2.4.0. « Informations générales : maladies des mollusques » est présenté en annexe 56 et sera proposé pour adoption lors de la 91e Session générale en mai 2024.

2.7 Chapitre 2.4.1. « Infection par l'herpèsvirus de l'ormeau » (annexe 57)

135. Une révision approfondie du chapitre 2.4.1. « Infection par l'herpèsvirus de l'ormeau », comprenant une proposition de reformatage en vue de son alignement avec le nouveau modèle de chapitres spécifiques à des maladies, est prévue.

136. Le chapitre révisé a été diffusé à deux reprises, la première fois dans le rapport de septembre 2023 de la Commission.

137. Le chapitre révisé 2.4.1. « Infection par l'herpèsvirus de l'ormeau » est présenté en annexe 57 et sera proposé pour adoption lors de la 91e Session générale en mai 2024.

2.8 Chapitre 2.4.4. « Infection à Marteilia refringens *» (annexe 58)*

138. Une révision approfondie du chapitre 2.4.4. « Infection à *Marteilia refringens* », comprenant une proposition de reformatage en vue de son alignement avec le nouveau modèle de chapitres spécifiques à des maladies, est prévue.

139. Le chapitre révisé a été diffusé à deux reprises, la première fois dans le rapport de septembre 2023 de la Commission.

140. Le chapitre révisé 2.4.4. « Infection à *Marteilia refringens* » est présenté en annexe 58 et sera proposé pour adoption lors de la 91e Session générale en mai 2024.

2.9 Sections 2.2.1. et 2.2.2. du chapitre 2.4.5. « Infection à Perkinsus marinus *» (annexe 59)*

141. Des modifications de la liste des espèces sensibles, destinée aux sections 2.2.1. et 2.2.2., sont proposées.

142. Le rapport de décembre 2022 du Groupe *ad hoc* sur la sensibilité des espèces de mollusques à une infection par une maladie listée par l'OMSA présente des informations détaillées sur les évaluations entreprises pour établir une proposition de liste des espèces sensibles.

143. Les sections révisées ont été diffusés à trois reprises, la première fois dans le rapport de février 2023 de la Commission.

144. Les sections révisées 2.2.1. et 2.2.2. du chapitre 2.4.5. « Infection à *Perkinsus marinus* » sont présentées en annexe 59 et seront proposées pour adoption lors de la 91e Session générale en mai 2024.

3. Annexes

Annexe 4. Point 6.1. – Définitions du Glossaire : « Services chargés de la santé des animaux aquatiques », « Autorité compétente » et « Autorité vétérinaire »

Article	Usage
Guide de l'utilisateur : B.5.	Les normes figurant dans les chapitres du titre 3 ont pour objet la mise en place, le maintien et l'évaluation des Services chargés de la santé des animaux aquatiques, y compris les questions afférentes à la communication. Ces normes visent à aider les Services chargés de la santé des animaux aquatiques et les Autorités compétentes des États membres à atteindre leurs objectifs d'amélioration de la santé des animaux aquatiques et du bien-être des poissons d'élevage, ainsi qu'à instaurer et préserver la confiance dans leurs certificats sanitaires internationaux relatifs aux animaux aquatiques.
Guide de l'utilisateur : C.8.	Certificats sanitaires internationaux pour les animaux aquatiques Un certificat sanitaire international applicable aux animaux aquatiques est un document officiel que l'Autorité compétente du pays exportateur délivre conformément aux chapitres 5.1. et 5.2. Il énonce les exigences auxquelles répondent les marchandises exportées en matière de santé des animaux aquatiques. C'est de la qualité des Services chargés de la santé des animaux aquatiques du pays exportateur, notamment des principes éthiques de l'Autorité compétente pertinente régissant l'établissement des certificats sanitaires et de l'expérience ~~des Services chargés de la santé des animaux aquatiques~~ de l'Autorité vétérinaire dans la satisfaction des obligations en matière de notification, que dépend l'assurance qu'auront les partenaires commerciaux de la sécurité sanitaire des marchandises issues d'animaux aquatiques.
Glossaire	***NOTIFICATION*** désigne la procédure par laquelle : a) ~~l'*Autorité compétente*~~ l'*Autorité vétérinaire* porte à la connaissance du *Siège*, b) le Siège porte à la connaissance ~~des Autorités compétentes~~ de l'*Autorité vétérinaire* des États membres l'apparition d'une *maladie*, conformément aux dispositions prévues au chapitre 1.1.
Article 1.1.1.	Aux fins du *Code aquatique* et conformément aux dispositions prévues aux articles 5, 9 et 10 des Statuts organiques de l'OMSA, les États membres reconnaissent au *Siège* le droit de communiquer directement avec ~~l'*Autorité compétente*~~ l'*Autorité vétérinaire* de son ou de ses *territoires*. Toute *notification* ou toute information adressée par l'OMSA à ~~l'*Autorité compétente*~~ l'*Autorité vétérinaire* est considérée comme ayant été adressée à l'État dont elle relève et toute *notification* ou toute information adressée à l'OMSA par ~~l'*Autorité compétente*~~ *l'Autorité vétérinaire* est considérée comme ayant été envoyée par l'État dont elle relève.
Article 1.1.3. paragraphe 1	Sous la responsabilité du Délégué, ~~l'*Autorité compétente*~~ l'*Autorité vétérinaire* adressera au *Siège* :
Article 1.1.4. paragraphe 1	Sous la responsabilité du Délégué, ~~l'*Autorité compétente*~~ l'*Autorité vétérinaire* adressera au *Siège* :
Article 1.1.5. point 1	~~L'*Autorité compétente*~~ L'*Autorité vétérinaire* d'un pays comptant une *zone* ou un *compartiment* infecté avisera le *Siège* dès que ce pays, cette *zone* ou ce *compartiment* aura recouvré le statut indemne au regard de la *maladie* considérée.
Article 1.1.5. point 3	~~L'*Autorité compétente*~~ L'*Autorité vétérinaire* d'un État membre qui a établi une ou plusieurs *zones indemnes* ou un ou plusieurs *compartiments indemnes*, doit en informer le *Siège* en donnant les détails nécessaires, notamment les critères sur lesquels repose le statut de territoire indemne et les conditions applicables de maintien de ce statut, et en indiquant clairement l'emplacement de ces *zones* et de ces *compartiments* sur une carte du territoire de l'État membre.

Article	**Usage**
Article 3.1.2. point 7 paragraphe 3	~~Les *Services chargés de la santé des animaux aquatiques*~~ Les *Autorités compétentes* doivent définir et consigner par écrit les responsabilités et l'organisation (notamment de la chaîne de commandement) de la structure chargée de la délivrance des *certificats sanitaires internationaux applicables aux animaux aquatiques*.
Article 3.1.2. point 10	Demandes d'information, réclamations et recours ~~Les *Services chargés de la santé des animaux aquatiques*~~ L'*Autorité compétente* pertinente doi~~vent~~ s'engager à répondre aux sollicitations ~~des *Services chargés de la santé des animaux aquatiques*~~ de l'*Autorité compétente* des autres États membres ~~ou de toute autre autorité~~, en veillant notamment à ce que les demandes d'information, les réclamations et les recours soient traités dans un délai raisonnable. Un relevé de toutes ces réclamations et de tous ces recours, ainsi que des suites que ~~les *Services chargés de la santé des animaux aquatiques*~~ l'*Autorité compétente* leur ~~auront~~a réservées, doit être tenu.
Article 3.1.5. paragraphe 4	L'(les) expert(s) réalise(nt) l'évaluation des *Services chargés de la santé des animaux aquatiques* de l'État membre en prenant pour guide l'ouvrage « *Outil* de l'OMSA *pour l'évaluation des performances des ~~Services vétérinaires ou des~~ Services chargés de la santé des animaux aquatiques* (*Outil PVS* de l'OMSA : *animaux aquatiques*) ». La mise en pratique de l'outil doit être adaptée au contexte de l'évaluation. L'(les) expert(s) rédige(nt) un rapport après consultation des *Services chargés de la santé des animaux aquatiques* de l'État membre.
Article 3.2.1. paragraphe 2	Il est primordial de reconnaître la communication en tant que discipline au sein des *Services chargés de la santé des animaux aquatiques* et de l'y intégrer afin de permettre le bon fonctionnement de ces *Services*. L'intégration de compétences en santé des *animaux aquatiques* et en communication est essentielle pour une communication efficace. ~~La communication entre les *Services chargés de la santé des animaux aquatiques* et les Services vétérinaires (en particulier lorsque les *Services chargés de la santé des animaux aquatiques* sont distincts et indépendants des Services vétérinaires) est capitale.~~
Article 4.2.3. point 1	L'étendue d'une *zone* doit être fixée par ~~le *Service chargé de la santé des animaux aquatiques*~~ l'*Autorité compétente*, en s'appuyant sur la définition du terme *zone*, et être rendue publique par des canaux officiels.
Article 4.2.3. point 3	Les facteurs définissant un *compartiment* doivent être établis par ~~le Service chargé de la santé des animaux aquatiques~~ l'*Autorité compétente*, en s'appuyant sur des critères pertinents tels que les pratiques de gestion et d'élevage reposant sur la sécurité biologique. Ils doivent être rendus publics par des canaux officiels.
Article 4.2.3. Point 6	Le *plan de sécurité biologique* fourni pour un *compartiment* doit consigner par écrit le partenariat ~~entre~~ auprès de l'entreprise ou ~~le~~ du secteur industriel concerné, ~~et le *Service chargé de la santé des animaux aquatiques*~~ l'*Autorité compétente* et les *Services chargés de la santé des animaux aquatiques*, ainsi que leurs responsabilités respectives (procédures de supervision de l'opération relative au *compartiment* par ~~le *Service chargé de la santé des animaux aquatiques*~~ l'*Autorité compétente* y compris).
Article 5.3.4. point 2) a)	infrastructure : comprend le support réglementaire (par exemple, les lois relatives à la santé des animaux aquatiques) et les systèmes administratifs (par exemple, organisation ~~des Services vétérinaires ou des Services chargés de la santé des animaux aquatiques~~ de l'*Autorité compétente*) ;
Article 5.3.7. point 1) d) i)	l'évaluation des ~~*Services* vétérinaires ou des~~ *Services chargés de la santé des animaux aquatiques* du *pays exportateur* ;
Article 5.3.7. point 2) e) i)	l'évaluation des ~~*Services* vétérinaires ou des~~ *Services chargés de la santé des animaux aquatiques* du *pays exportateur* ;

Annexe 5. Point 6.2. – Définitions du Glossaire : « Produits issus d'animaux aquatiques »

Article	**Usage**
Article 4.3.1. paragraphe 1	Les recommandations du présent chapitre fournissent un cadre structuré pour l'application et la reconnaissance des *compartiments* au sein de pays ou de *zones*, en vertu des dispositions prévues au chapitre 4.2., en vue de faciliter le commerce d'*animaux aquatiques* et de ~~produits d'origine *animale aquatique*~~*produits issus d'animaux aquatiques* et de disposer d'un outil pour la gestion des *maladies*.
Article 5.11.1. titre	Notes explicatives sur les certificats sanitaires relatifs au commerce international des animaux aquatiques vivants et des ~~produits qui en sont issus~~produits issus d'animaux aquatiques

Annexe 6. Point 6.3. – Article 1.1.5. du chapitre 1.1. « Notification des maladies et communication des informations épidémiologique »

CHAPITRE 1.1.

NOTIFICATION DES MALADIES ET COMMUNICATION DES INFORMATIONS ÉPIDÉMIOLOGIQUES

[…]

Article 1.1.5.

~~1) L'*Autorité compétente* d'un pays comptant une *zone* ou un *compartiment* infecté avisera le *Siège* dès que ce pays, cette *zone* ou ce *compartiment* aura recouvré le statut indemne au regard de la *maladie* considérée.~~

~~2) Un pays, une *zone* ou un *compartiment* peut être considéré comme ayant recouvré le statut indemne d'une *maladie* déterminée s'il remplit toutes les conditions énoncées dans le *Code aquatique*.~~

~~3) L'*Autorité compétente* d'un État membre qui a établi une ou plusieurs *zones indemnes* ou un ou plusieurs *compartiments indemnes*, doit en informer le *Siège* en donnant les détails nécessaires, notamment les critères sur lesquels repose le statut de territoire indemne et les conditions applicables de maintien de ce statut, et en indiquant clairement l'emplacement de ces *zones* et de ces *compartiments* sur une carte du territoire de l'État membre.~~

Article 1.1.~~6~~5.

1) Bien qu'ils soient tenus de notifier seulement les *maladies listées* et les *maladies émergentes*, les États membres sont encouragés à fournir à l'OMSA toute autre information importante relative à la santé des *animaux aquatiques*.

2) Le *Siège* transmettra aux *Autorités compétentes* par courrier électronique ou par le biais de l'application WAHIS toutes les *notifications* reçues conformément aux articles 1.1.2. à 1.1.~~5~~4., ainsi que toute autre information jugée pertinente.

[…]

Annexe 8. Point 6.4. – Article 1.3.1. du chapitre 1.3. « Maladies listées par l'OMSA »

CHAPITRE 1.3.

MALADIES LISTÉES PAR L'OMSA

[…]

Article 1.3.1.

Les *maladies* suivantes de poissons, sont des *maladies listées* :

- Infection à *Aphanomyces invadans* (syndrome ulcératif épizootique)
- Infection à *Gyrodactylus salaris*
- Infection par des variants délétés dans la RHP du virus de l'anémie infectieuse du saumon ou par des variants RHP0 de ce virus
- Infection par l'alphavirus des salmonidés
- Infection par l'herpèsvirus de la carpe koï
- ~~Infection par l'iridovirus de la daurade japonaise~~
- Infection par le virus de la nécrose hématopoïétique épizootique
- Infection par le virus de la nécrose hématopoïétique infectieuse
- Infection par ~~le~~tous les génogroupes de l'espèce virus de la nécrose infectieuse rénale et splénique
- Infection par le virus de la septicémie hémorragique virale
- Infection par le virus de la virémie printanière de la carpe
- Infection par le virus du tilapia lacustre.

[…]

Annexe 9. Point 6.5.1. – Article 8.1.3. du chapitre 8.1. « Infection à *Batrachochytrium dendrobatidis* »

(VERSION AVEC LES MARQUES DE RÉVISION)

CHAPITRE 8.1.

INFECTION À *BATRACHOCHYTRIUM DENDROBATIDIS*

[…]

Article 8.1.3.

Mesures pour l'importation ou le transit par le territoire de produits issus d'animaux aquatiques indépendamment de l'usage auquel ils sont destinés et du statut sanitaire du pays, de la zone ou du compartiment d'exportation au regard de l'infection à *B. dendrobatidis*

~~1) Il a été démontré que~~ Les *produits issus d'animaux aquatiques* ~~suivants~~ énumérés ci-dessous ont été évalués comme satisfaisai~~e~~nt aux critères d'évaluation de la sécurité des *produits issus d'animaux aquatiques* conformément à l'article 5.4.1. Lorsqu'elles autorisent~~, pour quelque usage que ce soit,~~ l'importation ou le transit par leur *territoire* ~~des~~ de ces *produits issus d'animaux aquatiques* ~~énumérés ci-dessous quand elles autorisent, pour quelque usage que ce soit, l'importation, ou le transit par leur *territoire*, des *produits issus d'animaux aquatiques* énumérés ci-dessous lorsqu'il s'agit de l'une des espèces visées à l'article 8.1.2. et que ces produits satisfont aux dispositions prévues à l'article 5.1.4.~~, les *Autorités compétentes* ne doivent imposer aucune ~~condition~~ *mesure sanitaire* ayant trait à *B. dendrobatidis*, quel que soit le statut sanitaire du pays, de la *zone* ou du *compartiment* d'exportation au regard de l'infection à *B. dendrobatidis* :

1) *produits issus d'animaux aquatiques* ayant été soumis à un traitement thermique suffisant pour atteindre une température à cœur d'au moins 60°C pendant au moins cinq minutes, ou à un couple temps/température équivalent qui inactive *B. dendrobatidis* ;

   ~~a) produits à base d'amphibiens stérilisés par la chaleur (c'est-à-dire exposés à une température de 121 °C pendant au moins 3,6 minutes ou à toute combinaison de température et de temps dont l'équivalence a été démontrée en termes d'inactivation de *B. dendrobatidis*) et présentés en conditionnement hermétique ;~~

   ~~b) produits à base d'amphibiens cuits ayant subi un traitement thermique à 100 °C pendant au moins une minute (ou à toute combinaison de température et de temps dont l'équivalence a été démontrée en termes d'inactivation de *B. dendrobatidis*) ;~~

   ~~c) produits à base d'amphibiens pasteurisés ayant subi un traitement thermique à 90 °C pendant au moins dix minutes (ou à toute combinaison de température et de temps dont l'équivalence a été démontrée en termes d'inactivation de *B. dendrobatidis*) ;~~

   ~~d)~~2) ~~produits à base d'amphibiens séchés par un procédé mécanique (ayant subi~~ été soumis à un traitement thermique ~~suffisant pour atteindre une température à cœur d'au moins~~ 100 °C 60°C pendant au moins 30 cinq minutes, ~~ou à~~ un couple temps/température équivalent qui inactive *B. dendrobatidis* ~~toute combinaison de température et de temps dont l'équivalence a été démontrée en termes d'inactivation de *B. dendrobatidis*) ;~~

~~e)~~3)2) cuir élaboré à partir de peau d'amphibien.

~~2) Lorsqu'elles autorisent l'importation ou le transit par leur *territoire* de *produits issus d'animaux aquatiques* appartenant à l'une des espèces visées à l'article 8.1.2., autres que ceux visés au point 1 de l'article 8.1.3., les *Autorités compétentes* doivent imposer le respect des conditions prescrites aux articles 8.1.9. à 8.1.14. en fonction du statut sanitaire du pays, de la *zone* ou du *compartiment* d'exportation au regard de l'infection à *B. dendrobatidis.*~~

~~3) Lorsqu'elle envisage l'importation ou le transit par son *territoire* de *produits issus d'animaux aquatiques* appartenant à une espèce non visée à l'article 8.1.2. mais dont on peut raisonnablement s'attendre à ce qu'ils posent un *risque* en termes de transmission de *B. dendrobatidis*, l'*Autorité compétente* doit procéder à une *analyse des risques* conformément aux recommandations contenues dans le chapitre 2.1. L'*Autorité compétente* du *pays exportateur* doit être tenue informée du résultat de cette analyse.~~

[…]

(VERSION SANS LES MARQUES DE RÉVISION)

CHAPITRE 8.1.

INFECTION À *BATRACHOCHYTRIUM DENDROBATIDIS*

[…]

Article 8.1.3.

Mesures pour l'importation ou le transit par le territoire de produits issus d'animaux aquatiques indépendamment de l'usage auquel ils sont destinés et du statut sanitaire du pays, de la zone ou du compartiment d'exportation au regard de l'infection à *B. dendrobatidis*

Les *produits issus d'animaux aquatiques* énumérés ci-dessous ont été évalués comme satisfaisant aux critères d'évaluation de la sécurité des *produits issus d'animaux aquatiques* conformément à l'article 5.4.1. Lorsqu'elles autorisent l'importation ou le transit par leur *territoire* de ces *produits issus d'animaux aquatiques*, les *Autorités compétentes* ne doivent imposer aucune *mesure sanitaire* ayant trait à *B. dendrobatidis*, quel que soit le statut sanitaire du pays, de la *zone* ou du *compartiment* d'exportation au regard de l'infection à *B. dendrobatidis* :

1) *produits issus d'animaux aquatiques* ayant été soumis à un traitement thermique suffisant pour atteindre une température à cœur d'au moins 60°C pendant au moins cinq minutes, ou à un couple temps/température équivalent qui inactive *B. dendrobatidis* ;

2) cuir élaboré à partir de peau d'amphibien.

[…]

Annexe 10. Point 6.5.1. – Article 8.2.3. du chapitre 8.2. « Infection à *Batrachochytrium salamandrivorans* »

(VERSION AVEC LES MARQUES DE RÉVISION)

CHAPITRE 8.2

INFECTION À *BATRACHOCHYTRIUM SALAMANDRIVORANS*

[…]

Article 8.2.3.

Mesures pour l'~~i~~importation ou le transit par le territoire de produits issus d'animaux aquatiques indépendamment de l'usage auquel ils sont destinés et du statut sanitaire du pays, de la zone ou du compartiment d'exportation au regard de l'infection à *B. salamandrivorans*

~~Il a été démontré que~~ ~~l~~Les *produits issus d'animaux aquatiques* ~~suivants~~ énumérés ci-dessous ont été évalués comme satisfaisa~~ie~~nt aux critères d'évaluation de la sécurité des *produits issus d'animaux aquatiques* conformément à l'article 5.4.1. Lorsqu'elles autorisent~~, pour quelque usage que ce soit,~~ l'importation ou le transit par leur *territoire* ~~des~~ de ces *produits issus d'animaux aquatiques* ~~énumérés ci-dessous quand elles autorisent, pour quelque usage que ce soit, l'importation, ou le transit par leur *territoire*, des *produits issus d'animaux aquatiques* énumérés ci-dessous lorsqu'il s'agit de l'une des espèces visées à l'article 8.2.2. et que ces produits satisfont aux dispositions prévues à l'article 5.1.4.~~, les *Autorités compétentes* ne doivent imposer aucune ~~condition~~ *mesure sanitaire* ayant trait à *B. salamandrivorans*, quel que soit le statut sanitaire du pays, de la *zone* ou du *compartiment* d'exportation au regard de l'infection à *B. salamandrivorans* :

1) *produits issus d'animaux aquatiques* ayant été soumis à un traitement thermique suffisant pour atteindre une température à cœur d'au moins 60°C pendant au moins cinq minutes, ou à un couple temps/température équivalent qui inactive *B. salamandrivoran ;*

   ~~a)~~ ~~produits à base d'amphibiens stérilisés par la chaleur (c'est-à-dire exposés à une température de 121 °C pendant au moins 3,6 minutes ou à toute combinaison de température et de temps dont l'équivalence a été démontrée en termes d'inactivation de *B. salamandrivorans)* et présentés en conditionnement hermétique ;~~

   ~~b)~~ ~~produits à base d'amphibiens cuits ayant subi un traitement thermique à 100 °C pendant au moins une minute (ou à toute combinaison de température et de temps dont l'équivalence a été démontrée en termes d'inactivation de *B. salamandrivorans*) ;~~

   ~~c)~~ ~~produits à base d'amphibiens pasteurisés ayant subi un traitement thermique à 90 °C pendant au moins dix minutes (ou à toute combinaison de température et de temps dont l'équivalence a été démontrée en termes d'inactivation de *B. salamandrivorans*) ;~~

   d~~2~~) ~~produits à base d'amphibiens séchés par un procédé mécanique (ayant subi été soumis à un traitement thermique suffisant pour atteindre une température à cœur d'au moins 100°C 60°C pendant au moins cinq minutes, ou à un couple temps/température équivalent qui inactive *B. salamandrivorans* toute combinaison de température et de temps dont l'équivalence a été démontrée en termes d'inactivation de *B. salamandrivorans*) ;~~

   e~~3~~2) cuir élaboré à partir de peau d'amphibien.

~~2) Lorsqu'elles autorisent l'importation ou le transit par leur *territoire* de *produits issus d'animaux aquatiques* appartenant à l'une des espèces visées à l'article 8.2.2., autres que ceux visés au point 1 de l'article 8.2.3., les *Autorités compétentes* doivent imposer le respect des conditions prescrites aux articles 8.2.9. à 8.2.14. en fonction du statut sanitaire du *pays exportateur* ou de la *zone* ou du *compartiment* d'exportation au regard de l'infection à *B. salamandrivorans*.~~

~~3) Lorsqu'elle envisage l'importation ou le transit par son *territoire* de *produits issus d'animaux aquatiques* appartenant à une espèce non visée à l'article 8.2.2. mais dont on peut raisonnablement s'attendre à ce qu'ils posent un *risque* en termes de transmission de *B. salamandrivorans*, l'*Autorité compétente* du *pays exportateur* doit être tenue informée~~

~~du résultat de cette analyse. L'*Autorité compétente* doit procéder à une *analyse des risques* conformément aux recommandations contenues dans le chapitre 2.1.~~

[…]

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(VERSION SANS LES MARQUES DE RÉVISION)

CHAPITRE 8.2.

INFECTION À *BATRACHOCHYTRIUM SALAMANDRIVORANS*

[…]

Article 8.2.3.

Mesures pour l'importation ou le transit par le territoire de produits issus d'animaux aquatiques indépendamment de l'usage auquel ils sont destinés et du statut sanitaire du pays, de la zone ou du compartiment d'exportation au regard de l'infection à *B. salamandrivorans*

Les *produits issus d'animaux aquatiques* énumérés ci-dessous ont été évalués comme satisfaisant aux critères d'évaluation de la sécurité des *produits issus d'animaux aquatiques* conformément à l'article 5.4.1. Lorsqu'elles autorisent l'importation ou le transit par leur *territoire* de ces *produits issus d'animaux aquatiques*, les *Autorités compétentes* ne doivent imposer aucune *mesure sanitaire* ayant trait à *B. salamandrivorans*, quel que soit le statut sanitaire du pays, de la *zone* ou du *compartiment* d'exportation au regard de l'infection à *B. salamandrivorans* :

1) *produits issus d'animaux aquatiques* ayant été soumis à un traitement thermique suffisant pour atteindre une température à cœur d'au moins 60°C pendant au moins cinq minutes, ou à un couple temps/température équivalent qui inactive *B. salamandrivorans* ;

2) cuir élaboré à partir de peau d'amphibien.

[…]

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Annexe 11. Point 6.5.1. – Article 8.3.3. du chapitre 8.3. « Infection par les espèces du genre *Ranavirus* »

(VERSION AVEC LES MARQUES DE RÉVISION)

CHAPITRE 8.3.

INFECTION PAR LES ESPÈCES DU GENRE *RANAVIRUS*

[…]

Article 8.3.3.

Mesures pour l'~~i~~importation ou le transit par le territoire de produits issus d'animaux aquatiques indépendamment de l'usage auquel ils sont destinés et du statut sanitaire du pays, de la zone ou du compartiment d'exportation au regard de l'infection par les espèces du genre *Ranavirus*

~~Il a été démontré que~~ ~~I~~Les *produits issus d'animaux aquatiques* ~~suivants~~ énumérés ci-dessous ont été évalués comme satisfaisa~~ie~~nt aux critères d'évaluation de la sécurité des *produits issus d'animaux aquatiques* conformément à l'article 5.4.1. Lorsqu'elles autorisent~~, pour quelque usage que ce soit,~~ l'importation ou le transit par leur *territoire* ~~des~~ de ces *produits issus d'animaux aquatiques* ~~énumérés ci-dessous quand elles autorisent, pour quelque usage que ce soit, l'importation, ou le transit par leur *territoire*, des *produits issus d'animaux aquatiques* énumérés ci-dessous lorsqu'il s'agit de l'une des espèces visées à l'article 8.3.2. et que ces produits satisfont aux dispositions prévues à l'article 5.1.4.~~, les *Autorités compétentes* ne doivent imposer aucune ~~condition~~ *mesure sanitaire* ayant trait aux espèces du genre *Ranavirus*, quel que soit le statut sanitaire du pays, de la *zone* ou du *compartiment* d'exportation au regard de l'infection par les espèces du genre *Ranavirus* :

1) *produits issus d'animaux aquatiques* ayant été soumis à un traitement thermique suffisant pour atteindre une température à cœur d'au moins ~~65~~60°C pendant au moins 30 minutes, ou à un couple temps/température équivalent qui inactive les espèces du genre *Ranavirus*.~~;~~

   ~~a) produits à base d'amphibiens stérilisés par la chaleur (c'est-à-dire exposés à une température de 121 °C pendant au moins 3,6 minutes ou à toute combinaison de température et de temps dont l'équivalence a été démontrée en termes d'inactivation des espèces du genre *Ranavirus*) et présentés en conditionnement hermétique ;~~

   ~~b) produits à base d'amphibiens cuits ayant subi un traitement thermique à 65 °C pendant au moins 30 minutes (ou à toute combinaison de température et de temps dont l'équivalence a été démontrée en termes d'inactivation des espèces du genre *Ranavirus*) ;~~

   ~~c) produits à base d'amphibiens pasteurisés ayant subi un traitement thermique à 90 °C pendant au moins dix minutes (ou à toute combinaison de température et de temps dont l'équivalence a été démontrée en termes d'inactivation des espèces du genre *Ranavirus*) ;~~

   ~~d2))produits à base d'amphibiens séchés par un procédé mécanique (ayant subi été soumis à un traitement thermique suffisant pour atteindre une température à cœur d'au moins 65°C à 100 °C pendant au moins 30 minutes, ou à toute combinaison de température et de temps dont l'équivalence a été démontrée en termes d'inactivation à un couple temps/température équivalent qui inactive les espèces du genre *Ranavirus*).~~

~~2) Lorsqu'elles autorisent l'importation ou le transit par leur *territoire* de *produits issus d'animaux aquatiques* appartenant à l'une des espèces visées à l'article 8.3.2., autres que ceux visés au point 1 de l'article 8.3.3., les *Autorités compétentes* doivent imposer le respect des conditions prescrites aux articles 8.3.9. à 8.3.14. en fonction du statut sanitaire du *pays exportateur* ou de la *zone* ou du *compartiment* d'exportation au regard de l'infection par les espèces du genre *Ranavirus*.~~

~~2) Lorsqu'elle envisage l'importation ou le transit par son *territoire* de *produits issus d'animaux aquatiques* appartenant à une espèce non visée à l'article 8.3.2. mais dont on peut raisonnablement s'attendre à ce qu'ils posent un *risque* en termes de transmission des espèces du genre *Ranavirus*, l'*Autorité compétente* doit procéder à une *analyse des risques* conformément aux recommandations contenues dans le chapitre 2.1. L'*Autorité compétente* du *pays exportateur* doit être tenue informée du résultat de cette analyse.~~

[…]

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(VERSION SANS LES MARQUES DE RÉVISION)

CHAPITRE 8.3.

INFECTION PAR LES ESPÈCES DU GENRE *RANAVIRUS*

[…]

Article 8.3.3.

Mesures pour l'importation ou le transit par le territoire de produits issus d'animaux aquatiques indépendamment de l'usage auquel ils sont destinés et du statut sanitaire du pays, de la zone ou du compartiment d'exportation au regard de l'infection par les espèces du genre *Ranavirus*

Les *produits issus d'animaux aquatiques* énumérés ci-dessous ont été évalués comme satisfaisant aux critères d'évaluation de la sécurité des *produits issus d'animaux aquatiques* conformément à l'article 5.4.1. Lorsqu'elles autorisent l'importation ou le transit par leur *territoire* de ces *produits issus d'animaux aquatiques,* les *Autorités compétentes* ne doivent imposer aucune *mesure sanitaire* ayant trait aux espèces du genre *Ranavirus*, quel que soit le statut sanitaire du pays, de la *zone* ou du *compartiment* d'exportation au regard de l'infection par les espèces du genre *Ranavirus* :

1) *produits issus d'animaux aquatiques* ayant été soumis à un traitement thermique suffisant pour atteindre une température à cœur d'au moins 60°C pendant au moins 30 minutes, ou à un couple temps/température équivalent qui inactive les espèces du genre *Ranavirus*.

[…]

Annexe 12. Point 6.5.2. – Article 9.3.3. du chapitre 9.3. « Infection par le virus 1 iridescent des décapodes »

CHAPITRE 9.3.

INFECTION PAR LE VIRUS 1 IRIDESCENT DES DÉCAPODES

[…]

Article 9.3.3.

Mesures pour l'importation ou le transit par le territoire de produits issus d'animaux aquatiques indépendamment de l'usage auquel ils sont destinés et du statut sanitaire du pays, de la zone ou du compartiment d'exportation au regard de l'infection par le virus 1 iridescent des décapodes

Les *produits issus d'animaux aquatiques* énumérés ci-dessous ont été évalués comme satisfaisant aux critères d'évaluation de la sécurité des *produits issus d'animaux aquatiques* conformément à l'article 5.4.1. Lorsqu'elles autorisent l'importation ou le transit par leur *territoire* de ces *produits issus d'animaux aquatiques*, les *Autorités compétentes* ne doivent imposer aucune *mesure sanitaire* ayant trait au virus 1 iridescent des décapodes, quel que soit le statut sanitaire du pays, de la *zone* ou du *compartiment* d'exportation au regard de l'infection par le virus 1 iridescent des décapodes :

1) ~~[~~*produits issus d'animaux aquatiques* ayant été soumis à un traitement thermique suffisant pour atteindre une température à cœur d'au moins ~~56~~80°C pendant au moins 30 minutes, ou à un couple temps/température équivalent qui inactive le virus 1 iridescent des décapodes ;

2) *farine* de crustacés ayant été soumise à un traitement thermique suffisant pour atteindre une température à cœur d'au moins ~~56~~80°C pendant au moins 30 minutes, ou à un couple temps/température équivalent qui inactive le virus 1 iridescent des décapodes ;

3) huile de crustacés ;

4) chitine extraite par un procédé chimique.~~] (à l'étude)~~

[…]

Annexe 13. Point 6.5.2. – Article 9.4.3. du chapitre 9.4. « Infection à *Hepatobacter penaei* »

CHAPITRE 9.4.

INFECTION À *HEPATOBACTER PENAEI* (HEPATOPANCREATITE NECROSANTE)

[…]

Article 9.4.3.

Mesures pour l'importation ou le transit par le territoire de produits issus d'animaux aquatiques indépendamment de l'usage auquel ils sont destinés et du statut sanitaire du pays, de la zone ou du compartiment d'exportation au regard de l'infection à *H. penaei*

Les *produits issus d'animaux aquatiques* énumérés ci-dessous ont été évalués comme satisfaisant aux critères d'évaluation de la sécurité des *produits issus d'animaux aquatiques* conformément à l'article 5.4.1. Lorsqu'elles autorisent l'importation ou le transit par leur *territoire* de ces *produits issus d'animaux aquatiques*, les *Autorités compétentes* ne doivent imposer aucune *mesure sanitaire* ayant trait à *H. penaei*, quel que soit le statut sanitaire du pays, de la *zone* ou du *compartiment* d'exportation au regard de l'infection à *H. penaei* :

1) *produits issus d'animaux aquatiques* ayant été soumis à un traitement thermique suffisant pour atteindre une température à cœur d'au moins ~~63~~95°C pendant au moins ~~30~~cinq minutes, ou à un couple temps/température équivalent qui inactive *H. penaei* ;

2) *farine* de crustacés ayant été soumise à un traitement thermique suffisant pour atteindre une température à cœur d'au moins ~~63~~95°C pendant au moins ~~30~~cinq minutes, ou à un couple temps/température équivalent qui inactive *H. penaei* ;

3) huile de crustacés ;

4) chitine extraite par un procédé chimique.

[…]

Annexe 14. Point 6.5.2. – Article 9.6.3. du chapitre 9.6. « Infection par le virus de la myonécrose infectieuse »

CHAPITRE 9.6.

INFECTION PAR LE VIRUS DE LA MYONÉCROSE INFECTIEUSE

[…]

Article 9.6.3.

Mesures pour l'importation ou le transit par le territoire de produits issus d'animaux aquatiques indépendamment de l'usage auquel ils sont destinés et du statut sanitaire du pays, de la zone ou du compartiment d'exportation au regard de l'infection par le virus de la myonécrose infectieuse

Les *produits issus d'animaux aquatiques* énumérés ci-dessous ont été évalués comme satisfaisant aux critères d'évaluation de la sécurité des *produits issus d'animaux aquatiques* conformément à l'article 5.4.1. Lorsqu'elles autorisent l'importation ou le transit par leur *territoire* de ces *produits issus d'animaux aquatiques*, les *Autorités compétentes* ne doivent imposer aucune *mesure sanitaire* ayant trait au virus de la myonécrose infectieuse, quel que soit le statut sanitaire du pays, de la *zone* ou du *compartiment* d'exportation au regard de l'infection par le virus de la myonécrose infectieuse :

1) *produits issus d'animaux aquatiques* ayant été soumis à un traitement thermique suffisant pour atteindre une température à cœur d'au moins ~~60~~75°C pendant au moins ~~60~~cinq minutes, ou à un couple temps/température équivalent qui inactive le virus de la myonécrose infectieuse ;

2) *farine* de crustacés ayant été soumise à un traitement thermique suffisant pour atteindre une température à cœur d'au moins ~~60~~75°C pendant au moins ~~60~~cinq minutes, ou à un couple temps/température équivalent qui inactive le virus de la myonécrose infectieuse ;

3) huile de crustacés ;

4) chitine extraite par un procédé chimique.

[…]

Annexe 15. Point 6.5.2. – Article 9.7.3. du chapitre 9.7. « Infection par le nodavirus de Macrobrachium rosenbergii »

CHAPITRE 9.7.

INFECTION PAR LE NODAVIRUS DE MACROBRACHIUM ROSENBERGII
(MALADIE DES QUEUES BLANCHES)

[…]

Article 9.7.3.

Mesures pour l'importation ou le transit par le territoire de produits issus d'animaux aquatiques indépendamment de l'usage auquel ils sont destinés et du statut sanitaire du pays, de la zone ou du compartiment d'exportation au regard de l'infection par le nodavirus de Macrobrachium rosenbergii

Les *produits issus d'animaux aquatiques* énumérés ci-dessous ont été évalués comme satisfaisant aux critères d'évaluation de la sécurité des *produits issus d'animaux aquatiques* conformément à l'article 5.4.1. Lorsqu'elles autorisent l'importation ou le transit par leur *territoire* de ces *produits issus d'animaux aquatiques*, les *Autorités compétentes* ne doivent imposer aucune *mesure sanitaire* ayant trait au nodavirus de Macrobrachium rosenbergii, quel que soit le statut sanitaire du pays, de la *zone* ou du *compartiment* d'exportation au regard de l'infection par le nodavirus de Macrobrachium rosenbergii :

1) *produits issus d'animaux aquatiques* ayant été soumis à un traitement thermique suffisant pour atteindre une température à cœur d'au moins ~~60~~50°C pendant au moins ~~60~~cinq minutes, ou à un couple temps/température équivalent qui inactive le nodavirus de Macrobrachium rosenbergii ;

2) *farine* de crustacés ayant été soumise à un traitement thermique suffisant pour atteindre une température à cœur d'au moins ~~60~~50°C pendant au moins ~~60~~cinq minutes, ou à un couple temps/température équivalent qui inactive le nodavirus de Macrobrachium rosenbergii ;

3) huile de crustacés ;

4) chitine extraite par un procédé chimique.

[…]

Annexe 16. Point 6.5.2. – Article 9.8.3. du chapitre 9.8. « Infection par le virus du syndrome de taura »

CHAPITRE 9.8.

INFECTION PAR LE VIRUS DU SYNDROME DE TAURA

[…]

Article 9.8.3.

Mesures pour l'importation ou le transit par le territoire de produits issus d'animaux aquatiques indépendamment de l'usage auquel ils sont destinés et du statut sanitaire du pays, de la zone ou du compartiment d'exportation au regard de l'infection par le virus du syndrome de Taura

Les *produits issus d'animaux aquatiques* énumérés ci-dessous ont été évalués comme satisfaisant aux critères d'évaluation de la sécurité des *produits issus d'animaux aquatiques* conformément à l'article 5.4.1. Lorsqu'elles autorisent l'importation ou le transit par leur *territoire* de ces *produits issus d'animaux aquatiques*, les *Autorités compétentes* ne doivent imposer aucune *mesure sanitaire* ayant trait au virus du syndrome de Taura, quel que soit le statut sanitaire du pays, de la *zone* ou du *compartiment* d'exportation au regard de l'infection par le virus du syndrome de Taura :

1) *produits issus d'animaux aquatiques* ayant été soumis à un traitement thermique suffisant pour atteindre une température à cœur d'au moins 70°C pendant au moins ~~30~~108 minutes, ou à un couple temps/température équivalent qui inactive le virus du syndrome de Taura ;

2) *farine* de crustacés ayant été soumise à un traitement thermique suffisant pour atteindre une température à cœur d'au moins 70°C pendant au moins ~~30~~108 minutes, ou à un couple temps/température équivalent qui inactive le virus du syndrome de Taura ;

3) huile de crustacés ;

4) chitine extraite par un procédé chimique.

[…]

Annexe 17. Point 6.5.3. – Article 10.1.3. du chapitre 10.1. « Infection par le virus de la nécrose hématopoïétique épizootique »

CHAPITRE 10.1.

INFECTION PAR LE VIRUS DE LA NÉCROSE HÉMATOPOÏÉTIQUE ÉPIZOOTIQUE

[…]

Article 10.1.3.

Mesures pour l'importation ou le transit par le territoire de produits issus d'animaux aquatiques indépendamment de l'usage auquel ils sont destinés et du statut sanitaire du pays, de la zone ou du compartiment d'exportation au regard de l'infection par le virus de la nécrose hématopoïétique épizootique

Les *produits issus d'animaux aquatiques* énumérés ci-dessous ont été évalués comme satisfaisant aux critères d'évaluation de la sécurité des *produits issus d'animaux aquatiques* conformément à l'article 5.4.1. Lorsqu'elles autorisent l'importation ou le transit par leur *territoire* de ces *produits issus d'animaux aquatiques*, les *Autorités compétentes* ne doivent imposer aucune *mesure sanitaire* ayant trait au virus de la nécrose hématopoïétique épizootique, quel que soit le statut sanitaire du pays, de la *zone* ou du *compartiment* d'exportation au regard de l'infection par le virus de la nécrose hématopoïétique épizootique :

1) *produits issus d'animaux aquatiques* ayant été soumis à un traitement thermique suffisant pour atteindre une température à cœur d'au moins 60°C pendant au moins 15 minutes, ou à un couple temps/température équivalent qui inactive le virus de la nécrose hématopoïétique épizootique ;

~~2)~~ ~~poissons éviscérés et séchés par un procédé mécanique ayant été soumis à un traitement thermique suffisant pour atteindre une température à cœur d'au moins 60°C pendant au moins 15 minutes, ou à un couple temps/température équivalent qui inactive le virus de la nécrose hématopoïétique épizootique ;~~

~~3~~2) *farine* de poisson ayant été soumise à un traitement thermique suffisant pour atteindre une température à cœur d'au moins 60°C pendant au moins 15 minutes, ou à un couple temps/température équivalent qui inactive le virus de la nécrose hématopoïétique épizootique ;

~~4~~3) huile de poisson ;

~~5~~4) cuir élaboré à partir de peau de poisson.

[…]

Annexe 18. Point 6.5.3. – Article 10.2.3. du chapitre 10.2. « Infection à *Aphanomyces invadans* »

CHAPITRE 10.2.

INFECTION À *APHANOMYCES INVADANS* (syndrome ulcératif épizootique)

[…]

Article 10.2.3.

Mesures pour l'importation ou le transit par le territoire de produits issus d'animaux aquatiques indépendamment de l'usage auquel ils sont destinés et du statut sanitaire du pays, de la zone ou du compartiment d'exportation au regard de l'infection à *A. invadans*

Les *produits issus d'animaux aquatiques* énumérés ci-dessous ont été évalués comme satisfaisant aux critères d'évaluation de la sécurité des *produits issus d'animaux aquatiques* conformément à l'article 5.4.1. Lorsqu'elles autorisent l'importation ou le transit par leur *territoire* de ces *produits issus d'animaux aquatiques*, les *Autorités compétentes* ne doivent imposer aucune *mesure sanitaire* ayant trait à *A. invadans*, quel que soit le statut sanitaire du pays, de la *zone* ou du *compartiment* d'exportation au regard de l'infection à *A. invadans* :

1) *produits issus d'animaux aquatiques* ayant été soumis à un traitement thermique suffisant pour atteindre une température à cœur d'au moins ~~60~~100°C pendant au moins ~~cinq~~une minute~~s~~, ou à un couple temps/température équivalent qui inactive *A. invadans* ;

~~2) poissons éviscérés et séchés par un procédé mécanique ayant été soumis à un traitement thermique suffisant pour atteindre une température à cœur d'au moins 60°C pendant au moins cinq minutes, ou à un couple temps/température équivalent qui inactive *A. invadans* ;~~

~~3~~2) *farine* de poisson ayant été soumise à un traitement thermique suffisant pour atteindre une température à cœur d'au moins ~~60~~100°C pendant au moins ~~cinq~~une minute~~s~~, ou à un couple temps/température équivalent qui inactive *A. invadans* ;

~~4~~3) huile de poisson ;

~~5~~4) poissons éviscérés congelés ;

~~6~~5) filets ou darnes / pavés de poisson congelés.

[…]

Annexe 19. Point 6.5.3. – Article 10.3.3. du chapitre 10.3. « Infection à *Gyrodactylus salaris* »

CHAPITRE 10.3.

INFECTION À *GYRODACTYLUS SALARIS*

[…]

Article 10.3.3.

Mesures pour l'importation ou le transit par le territoire de produits issus d'animaux aquatiques indépendamment de l'usage auquel ils sont destinés et du statut sanitaire du pays, de la zone ou du compartiment d'exportation au regard de l'infection à *G. salaris*

Les *produits issus d'animaux aquatiques* énumérés ci-dessous ont été évalués comme satisfaisant aux critères d'évaluation de la sécurité des *produits issus d'animaux aquatiques* conformément à l'article 5.4.1. Lorsqu'elles autorisent l'importation ou le transit par leur *territoire* de ces *produits issus d'animaux aquatiques*, les *Autorités compétentes* ne doivent imposer aucune *mesure sanitaire* ayant trait à *G. salaris*, quel que soit le statut sanitaire du pays, de la *zone* ou du *compartiment* d'exportation au regard de l'infection à *G. salaris* :

1) *produits issus d'animaux aquatiques* ~~ayant subi un traitement thermique et qui sont présentés en conditionnement hermétique~~ ayant été soumis à un traitement thermique suffisant pour atteindre une température à cœur d'au moins 40°C pendant au moins une minute, ou à un couple temps/température équivalent qui inactive *G. salaris* ;

~~2)~~ ~~poissons éviscérés et séchés par un procédé mécanique ;~~

~~3~~2) poissons éviscérés et séchés dans des conditions naturelles (c'est-à-dire à l'air ou au soleil) ;

~~4~~3) poissons éviscérés et congelés ayant été soumis à des températures inférieures ou égales à moins 18 °C ;

~~5~~4) filets ou darnes / pavés de poisson congelés ayant été soumis à des températures inférieures ou égales à moins 18 °C ;

~~6~~5) poissons éviscérés réfrigérés ayant été pêchés dans une eau de mer de salinité supérieure ou égale à 25 ppt pendant au moins 14 jours consécutifs ;

~~7~~6) filets ou darnes / pavés réfrigérés de poissons ayant été pêchés dans une eau de mer de salinité supérieure ou égale à 25 ppt pendant au moins 14 jours consécutifs ;

~~8~~7) produits réfrigérés à base de poisson dont la peau, les arêtes et les nageoires ont été retirés ;

~~9~~8) œufs de poisson non viables ;

~~10~~9) huile de poisson ;

~~11~~10) *farine* de poisson ;

~~12~~11) cuir élaboré à partir de peau de poisson.

[…]

Annexe 20. Point 6.5.3. – Article 10.4.3. du chapitre 10.4. « Infection par le virus de l'anémie infectieuse du saumon »

CHAPITRE 10.4.

INFECTION PAR LE VIRUS DE L'ANÉMIE INFECTIEUSE DU SAUMON

[…]

Article 10.4.3.

Mesures pour l'importation ou le transit par le territoire de produits issus d'animaux aquatiques indépendamment de l'usage auquel ils sont destinés et du statut sanitaire du pays, de la zone ou du compartiment d'exportation au regard de l'infection par le virus de l'anémie infectieuse du saumon

Les dispositions figurant au présent article s'appliquent à l'ensemble des formes du virus de l'anémie infectieuse du saumon, y compris les variants RHP0.

Les *produits issus d'animaux aquatiques* énumérés ci-dessous ont été évalués comme satisfaisant aux critères d'évaluation de la sécurité des *produits issus d'animaux aquatiques* conformément à l'article 5.4.1. Lorsqu'elles autorisent l'importation ou le transit par leur *territoire* de ces *produits issus d'animaux aquatiques,* les *Autorités compétentes* ne doivent imposer aucune *mesure sanitaire* ayant trait au virus de l'anémie infectieuse du saumon, quel que soit le statut sanitaire du pays, de la *zone* ou du *compartiment* d'exportation au regard de l'infection par le virus de l'anémie infectieuse du saumon :

1) *produits issus d'animaux aquatiques* ayant été soumis à un traitement thermique suffisant pour atteindre une température à cœur d'au moins 56°C pendant au moins cinq minutes, ou à un couple temps/température équivalent qui inactive le virus de l'anémie infectieuse du saumon ;

~~2)~~ ~~poissons éviscérés et séchés par un procédé mécanique ayant été soumis à un traitement thermique suffisant pour atteindre une température à cœur d'au moins 56°C pendant au moins cinq minutes, ou à un couple temps/température équivalent qui inactive le virus de l'anémie infectieuse du saumon ;~~

~~3~~2) *farine* de poisson ayant été soumise à un traitement thermique suffisant pour atteindre une température à cœur d'au moins 56°C pendant au moins cinq minutes, ou à un couple temps/température équivalent qui inactive le virus de l'anémie infectieuse du saumon ;

~~4~~3) huile de poisson ;

~~5~~4) cuir élaboré à partir de peau de poisson.

[…]

Annexe 21. Point 6.5.3. – Article 10.5.3. du chapitre 10.5. « Infection par le virus de l'alphavirus des salmonidés »

CHAPITRE 10.5.

INFECTION PAR L'ALPHAVIRUS DES SALMONIDÉS

[…]

Article 10.5.3.

Mesures pour l'importation ou le transit par le territoire de produits issus d'animaux aquatiques indépendamment de l'usage auquel ils sont destinés et du statut sanitaire du pays, de la zone ou du compartiment d'exportation au regard de l'infection par l'alphavirus des salmonidés

Les *produits issus d'animaux aquatiques* énumérés ci-dessous ont été évalués comme satisfaisant aux critères d'évaluation de la sécurité des *produits issus d'animaux aquatiques* conformément à l'article 5.4.1. Lorsqu'elles autorisent l'importation ou le transit par leur *territoire* de ces *produits issus d'animaux aquatiques*, les *Autorités compétentes* ne doivent imposer aucune *mesure sanitaire* ayant trait à l'alphavirus des salmonidés, quel que soit le statut sanitaire du pays, de la *zone* ou du *compartiment* d'exportation au regard de l'infection par l'alphavirus des salmonidés :

1) *produits issus d'animaux aquatiques* ayant été soumis à un traitement thermique suffisant pour atteindre une température à cœur d'au moins 60°C pendant au moins 60 minutes, ou à un couple temps/température équivalent qui inactive l'alphavirus des salmonidés ;

~~2)~~ ~~poissons éviscérés et séchés par un procédé mécanique ayant été soumis à un traitement thermique suffisant pour atteindre une température à cœur d'au moins 60°C pendant au moins 60 minutes, ou à un couple temps/température équivalent qui inactive l'alphavirus des salmonidés ;~~

~~3~~2) *farine* de poisson ayant été soumise à un traitement thermique suffisant pour atteindre une température à cœur d'au moins 60°C pendant au moins 60 minutes, ou à un couple temps/température équivalent qui inactive l'alphavirus des salmonidés ;

~~4~~3) huile de poisson ;

~~5~~4) cuir élaboré à partir de peau de poisson.

[…]

Annexe 22. Point 6.5.3. – Article 10.6.3. du chapitre 10.6. « Infection par le virus de la nécrose hématopoïétique infectieuse »

CHAPITRE 10.6.

INFECTION PAR LE VIRUS DE LA NÉCROSE HÉMATOPOÏÉTIQUE INFECTIEUSE

[…]

Article 10.6.3.

Mesures pour l'importation ou le transit par le territoire de produits issus d'animaux aquatiques indépendamment de l'usage auquel ils sont destinés et du statut sanitaire du pays, de la zone ou du compartiment d'exportation au regard de l'infection par le virus de la nécrose hématopoïétique infectieuse

Les *produits issus d'animaux aquatiques* énumérés ci-dessous ont été évalués comme satisfaisant aux critères d'évaluation de la sécurité des *produits issus d'animaux aquatiques* conformément à l'article 5.4.1. Lorsqu'elles autorisent l'importation ou le transit par leur *territoire* de ces *produits issus d'animaux aquatiques*, les *Autorités compétentes* ne doivent imposer aucune *mesure sanitaire* ayant trait au virus de la nécrose hématopoïétique infectieuse, quel que soit le statut sanitaire du pays, de la *zone* ou du *compartiment* d'exportation au regard de l'infection par le virus de la nécrose hématopoïétique infectieuse :

1) *produits issus d'animaux aquatiques* ayant été soumis à un traitement thermique suffisant pour atteindre une température à cœur d'au moins 90°C pendant au moins 30 secondes, ou à un couple temps/température équivalent qui inactive le virus de la nécrose hématopoïétique infectieuse ;

~~2)~~ ~~poissons éviscérés et séchés par un procédé mécanique ayant été soumis à un traitement thermique suffisant pour atteindre une température à cœur d'au moins 90°C pendant au moins 30 secondes, ou à un couple temps/température équivalent qui inactive le virus de la nécrose hématopoïétique infectieuse ;~~

~~3~~2) *farine* de poisson ayant été soumise à un traitement thermique suffisant pour atteindre une température à cœur d'au moins 90°C pendant au moins 30 secondes, ou à un couple temps/température équivalent qui inactive le virus de la nécrose hématopoïétique infectieuse ;

~~4~~3) huile de poisson ;

~~5~~4) cuir élaboré à partir de peau de poisson.

[…]

Annexe 23. Point 6.5.3. – Article 10.7.3. du chapitre 10.7. « Infection par l'herpèsvirus de la carpe koï »

CHAPITRE 10.7.

INFECTION PAR L'HERPESVIRUS DE LA CARPE KOÏ

[…]

Article 10.7.3.

Mesures pour l'importation ou le transit par le territoire de produits issus d'animaux aquatiques indépendamment de l'usage auquel ils sont destinés et du statut sanitaire du pays, de la zone ou du compartiment d'exportation au regard de l'infection par l'herpèsvirus de la carpe koï

Les *produits issus d'animaux aquatiques* énumérés ci-dessous ont été évalués comme satisfaisant aux critères d'évaluation de la sécurité des *produits issus d'animaux aquatiques* conformément à l'article 5.4.1. Lorsqu'elles autorisent l'importation ou le transit par leur *territoire* de ces *produits issus d'animaux aquatiques,* les *Autorités compétentes* ne doivent imposer aucune *mesure sanitaire* ayant trait à l'herpèsvirus de la carpe koï, quel que soit le statut sanitaire du pays, de la *zone* ou du *compartiment* d'exportation au regard de l'infection par l'herpèsvirus de la carpe koï :

1) *produits issus d'animaux aquatiques* ayant été soumis à un traitement thermique suffisant pour atteindre une température à cœur d'au moins 50°C pendant au moins ~~trois~~une minute~~s~~, ou à un couple temps/température équivalent qui inactive l'herpèsvirus de la carpe koï ;

~~2)~~ ~~poissons éviscérés et séchés par un procédé mécanique ayant été soumis à un traitement thermique suffisant pour atteindre une température à cœur d'au moins 50°C pendant au moins trois minutes, ou à un couple temps/température équivalent qui inactive l'herpèsvirus de la carpe koï ;~~

~~3~~2) *farine* de poisson ayant été soumise à un traitement thermique suffisant pour atteindre une température à cœur d'au moins 50°C pendant au moins ~~trois~~une minute~~s~~, ou à un couple temps/température équivalent qui inactive l'herpèsvirus de la carpe koï ;

~~4~~3) huile de poisson.

[…]

Annexe 24. Point 6.5.3. – Article 10.8.3. du chapitre 10.8. « Infection par l'iridovirus de la daurade japonaise »

CHAPITRE 10.8.

INFECTION PAR L'IRIDOVIRUS DE LA DAURADE JAPONAISE

[…]

Article 10.8.3.

Mesures pour l'importation ou le transit par le territoire de produits issus d'animaux aquatiques indépendamment de l'usage auquel ils sont destinés et du statut sanitaire du pays, de la zone ou du compartiment d'exportation au regard de l'infection par l'iridovirus de la daurade japonaise

Les *produits issus d'animaux aquatiques* énumérés ci-dessous ont été évalués comme satisfaisant aux critères d'évaluation de la sécurité des *produits issus d'animaux aquatiques* conformément à l'article 5.4.1. Lorsqu'elles autorisent l'importation ou le transit par leur *territoire* de ces *produits issus d'animaux aquatiques*, les *Autorités compétentes* ne doivent imposer aucune *mesure sanitaire* ayant trait à l'iridovirus de la daurade japonaise, quel que soit le statut sanitaire du pays, de la *zone* ou du *compartiment* d'exportation au regard de l'infection par l'iridovirus de la daurade japonaise :

1) *produits issus d'animaux aquatiques* ayant été soumis à un traitement thermique suffisant pour atteindre une température à cœur d'au moins 56°C pendant au moins 30 minutes, ou à un couple temps/température équivalent qui inactive l'iridovirus de la daurade japonaise ;

~~2)~~ ~~poissons éviscérés et séchés par un procédé mécanique ayant été soumis à un traitement thermique suffisant pour atteindre une température à cœur d'au moins 56°C pendant au moins 30 minutes, ou à un couple temps/température équivalent qui inactive l'iridovirus de la daurade japonaise ;~~

~~3~~2) *farine* de poisson ayant été soumise à un traitement thermique suffisant pour atteindre une température à cœur d'au moins 56°C pendant au moins 30 minutes, ou à un couple temps/température équivalent qui inactive l'iridovirus de la daurade japonaise ;

~~4~~3) huile de poisson ;

~~5~~4) cuir élaboré à partir de peau de poisson.

[…]

Annexe 25. Point 6.5.3. – Article 10.9.3. du chapitre 10.9. « Infection par le virus de la virémie printanière de la carpe »

CHAPITRE 10.9.

INFECTION PAR LE VIRUS DE LA VIRÉMIE PRINTANIÈRE DE LA CARPE

[…]

Article 10.9.3.

Mesures pour l'importation ou le transit par le territoire de produits issus d'animaux aquatiques indépendamment de l'usage auquel ils sont destinés et du statut sanitaire du pays, de la zone ou du compartiment d'exportation au regard de l'infection par le virus de la virémie printanière de la carpe

Les *produits issus d'animaux aquatiques* énumérés ci-dessous ont été évalués comme satisfaisant aux critères d'évaluation de la sécurité des *produits issus d'animaux aquatiques* conformément à l'article 5.4.1. Lorsqu'elles autorisent l'importation ou le transit par leur *territoire* de ces *produits issus d'animaux aquatiques*, les *Autorités compétentes* ne doivent imposer aucune *mesure sanitaire* ayant trait au virus de la virémie printanière de la carpe, quel que soit le statut sanitaire du pays, de la *zone* ou du *compartiment* d'exportation au regard de l'infection par le virus de la virémie printanière de la carpe :

1) *produits issus d'animaux aquatiques* ayant été soumis à un traitement thermique suffisant pour atteindre une température à cœur d'au moins ~~90~~60°C pendant au moins 60 ~~secondes~~minutes, ou à un couple temps/température équivalent qui inactive le virus de la virémie printanière de la carpe ;

~~2)~~ ~~poissons éviscérés et séchés par un procédé mécanique ayant été soumis à un traitement thermique suffisant pour atteindre une température à cœur d'au moins 90°C pendant au moins 60 secondes, ou à un couple temps/température équivalent qui inactive le virus de la virémie printanière de la carpe ;~~

~~3~~2) *farine* de poisson ayant été soumise à un traitement thermique suffisant pour atteindre une température à cœur d'au moins ~~90~~60°C pendant au moins 60 ~~secondes~~minutes, ou à un couple temps/température équivalent qui inactive le virus de la virémie printanière de la carpe ;

~~4~~3) huile de poisson.

[…]

Annexe 26. Point 6.5.3. – Article 10.10.3. du chapitre 10.10. « Infection par le virus de la septicémie hémorragique virale »

CHAPITRE 10.10.

INFECTION PAR LE VIRUS DE LA SEPTICÉMIE HÉMORRAGIQUE VIRALE

[…]

Article 10.10.3.

Mesures pour l'importation ou le transit par le territoire de produits issus d'animaux aquatiques indépendamment de l'usage auquel ils sont destinés et du statut sanitaire du pays, de la zone ou du compartiment d'exportation au regard de l'infection par le virus de la septicémie hémorragique virale

Les *produits issus d'animaux aquatiques* énumérés ci-dessous ont été évalués comme satisfaisant aux critères d'évaluation de la sécurité des *produits issus d'animaux aquatiques* conformément à l'article 5.4.1. Lorsqu'elles autorisent l'importation ou le transit par leur *territoire* de ces *produits issus d'animaux aquatiques*, les *Autorités compétentes* ne doivent imposer aucune *mesure sanitaire* ayant trait au virus de la septicémie hémorragique virale, quel que soit le statut sanitaire du pays, de la *zone* ou du *compartiment* d'exportation au regard de l'infection par le virus de la septicémie hémorragique virale :

1) *produits issus d'animaux aquatiques* ayant été soumis à un traitement thermique suffisant pour atteindre une température à cœur d'au moins ~~90~~60°C pendant au moins 60 ~~secondes~~minutes, ou à un couple temps/température équivalent qui inactive le virus de la septicémie hémorragique virale ;

~~2)~~ ~~poissons éviscérés et séchés par un procédé mécanique ayant été soumis à un traitement thermique suffisant pour atteindre une température à cœur d'au moins 90°C pendant au moins 60 secondes, ou à un couple temps/température équivalent qui inactive le virus de la septicémie hémorragique virale ;~~

~~3~~2) *farine* de poisson ayant été soumise à un traitement thermique suffisant pour atteindre une température à cœur d'au moins ~~90~~60°C pendant au moins 60 ~~secondes~~minutes, ou à un couple temps/température équivalent qui inactive le virus de la septicémie hémorragique virale ;

~~4~~3) poissons éviscérés et séchés dans des conditions naturelles (c'est-à-dire à l'air ou au soleil) ;

~~5~~4) huile de poisson ;

~~6~~5) cuir élaboré à partir de peau de poisson.

[…]

Annexe 27. Point 6.5.3. – Article 10.11.3. du chapitre 10.11. « Infection par le virus du tilapia lacustre »

CHAPITRE 10.11.

INFECTION PAR LE VIRUS DU TILAPIA LACUSTRE

[…]

Article 10.10.3.

Mesures pour l'importation ou le transit par le territoire de produits issus d'animaux aquatiques indépendamment de l'usage auquel ils sont destinés et du statut sanitaire du pays, de la zone ou du compartiment d'exportation au regard de l'infection par le virus du tilapia lacustre

Les *produits issus d'animaux aquatiques* énumérés ci-dessous ont été évalués comme satisfaisant aux critères d'évaluation de la sécurité des *produits issus d'animaux aquatiques* conformément à l'article 5.4.1. Lorsqu'elles autorisent l'importation ou le transit par leur *territoire* de ces *produits issus d'animaux aquatiques*, les *Autorités compétentes* ne doivent imposer aucune *mesure sanitaire* ayant trait au virus du tilapia lacustre, quel que soit le statut sanitaire du pays, de la *zone* ou du *compartiment* d'exportation au regard de l'infection par le virus du tilapia lacustre :

1) ~~[~~*produits issus d'animaux aquatiques* ayant été soumis à un traitement thermique suffisant pour atteindre une température à cœur d'au moins ~~56~~60°C pendant au moins ~~cinq~~120 minutes, ou à un couple temps/température équivalent qui inactive le virus du tilapia lacustre ;

2) *farine* de poisson ayant été soumise à un traitement thermique suffisant pour atteindre une température à cœur d'au moins ~~56~~60°C pendant au moins ~~cinq~~120 minutes, ou à un couple temps/température équivalent qui inactive le virus du tilapia lacustre~~] (à l'étude)~~ ;

3) poissons éviscérés et séchés dans des conditions naturelles (c'est-à-dire à l'air ou au soleil) ;

4) huile de poisson ;

5) cuir élaboré à partir de peau de poisson.

[…]

Annexe 28. Point 6.5.4. – Article 11.1.3. du chapitre 11.1. « Infection par l'herpèsvirus de l'ormeau »

(VERSION AVEC LES MARQUES DE RÉVISION)

CHAPITRE 11.1.

INFECTION PAR L'HERPÈSVIRUS DE L'ORMEAU

[…]

Article 11.1.3.

Mesures pour l'~~i~~importation ou le transit par le territoire de produits issus d'animaux aquatiques indépendamment de l'usage auquel ils sont destinés et du statut sanitaire du pays, de la zone ou du compartiment d'exportation au regard de l'infection par l'herpèsvirus de l'ormeau

~~1.~~Les *produits issus d'animaux aquatiques* énumérés ci-dessous ont été évalués comme satisfaisant aux critères de l'évaluation de la sécurité des *produits issus d'animaux aquatiques* conformément à l'article 5.4.1. Lorsqu'elles autorisent~~, pour quelque usage que ce soit,~~ l'importation ou le transit par leur *territoire* ~~des~~ de ces *produits issus d'animaux aquatiques* ~~énumérés ci-dessous quand elles autorisent, pour quelque usage que ce soit, l'importation, ou le transit par leur *territoire*, des *produits issus d'animaux aquatiques* énumérés ci-dessous lorsqu'il s'agit de l'une des espèces visées à l'article 11.1.2. et que ces produits satisfont aux dispositions prévues à l'article 5.4.1.,~~ les *Autorités compétentes* ne doivent imposer aucune ~~condition~~ *mesure sanitaire* ayant trait à l'herpèsvirus de l'ormeau, quel que soit le statut sanitaire du pays, de la *zone* ou du *compartiment* d'exportation au regard de l'infection par l'herpèsvirus de l'ormeau :

1) *produits issus d'animaux aquatiques* ayant été soumis à un traitement thermique suffisant pour atteindre une température à cœur d'au moins ~~121~~50°C pendant au moins ~~3~~cinq minutes ~~et 36 secondes~~, ou à un couple temps/température équivalent qui inactive l'herpèsvirus de l'ormeau. ~~;~~

   ~~a) produits à base d'ormeaux stérilisés par la chaleur (c'est-à-dire exposés à une température de 121 °C pendant au moins 3,6 minutes ou à toute combinaison de température et de temps équivalente) et présentés dans un conditionnement hermétique ;~~

   ~~b2) produits à base d'ormeaux séchés par un procédé mécanique (c'est-à-dire ayant subi un traitement thermique à 100 °C pendant au moins 30 minutes ou à toute combinaison de température et de temps dont l'équivalence a été démontrée en termes d'inactivation de l'herpèsvirus de l'ormeau) ayant été soumis à un traitement thermique suffisant pour atteindre une température à cœur d'au moins 121°C pendant au moins 3 minutes et 36 secondes, ou à un couple temps/température équivalent qui inactive l'herpèsvirus de l'ormeau.~~

~~2) Lorsqu'elles autorisent l'importation ou le transit par leur *territoire* d'*animaux aquatiques* ou de *produits issus d'animaux aquatiques* appartenant à une espèce visée à l'article 11.1.2. autres que ceux énumérés au point 1 de l'article 11.1.3., les *Autorités compétentes* doivent exiger le respect des conditions prescrites par les articles 11.1.7. à 11.1.11. en fonction du statut sanitaire du pays, de la *zone* ou du *compartiment* d'exportation au regard de l'infection par l'herpèsvirus de l'ormeau.~~

~~3) L'*Autorité compétente* doit procéder à une *analyse des risques* conformément aux recommandations contenues dans le chapitre 2.1. lorsqu'elle envisage l'importation ou le transit par son *territoire* d'*animaux aquatiques* ou de *produits issus d'animaux aquatiques* appartenant à une espèce non visée à l'article 11.1.2., mais dont on peut raisonnablement s'attendre à ce qu'ils posent un *risque* en termes de propagation de l'infection par l'herpèsvirus de l'ormeau. L'*Autorité compétente* du *pays exportateur* doit être tenue informée du résultat de cette analyse.~~

[…]

(VERSION SANS LES MARQUES DE RÉVISION)

CHAPITRE 11.1.

INFECTION PAR L'HERPÈSVIRUS DE L'ORMEAU

[…]

Article 11.1.3.

Mesures pour l'importation ou le transit par le territoire de produits issus d'animaux aquatiques indépendamment de l'usage auquel ils sont destinés et du statut sanitaire du pays, de la zone ou du compartiment d'exportation au regard de l'infection par l'herpèsvirus de l'ormeau

Les *produits issus d'animaux aquatiques* énumérés ci-dessous ont été évalués comme satisfaisant aux critères d'évaluation de la sécurité des *produits issus d'animaux aquatiques* conformément à l'article 5.4.1. Lorsqu'elles autorisent l'importation ou le transit par leur *territoire* de ces *produits issus d'animaux aquatiques*, les *Autorités compétentes* ne doivent imposer aucune *mesure sanitaire* ayant trait à l'herpèsvirus de l'ormeau, quel que soit le statut sanitaire du pays, de la *zone* ou du *compartiment* d'exportation au regard de l'infection par l'herpèsvirus de l'ormeau *:*

1) *produits issus d'animaux aquatiques* ayant été soumis à un traitement thermique suffisant pour atteindre une température à cœur d'au moins 50°C pendant au moins cinq minutes, ou à un couple temps/température équivalent qui inactive l'herpèsvirus de l'ormeau.

[…]

Annexe 29. Point 6.5.4. – Article 11.2.3. du chapitre 11.2. « Infection à *Bonamia exitiosa* »

(VERSION AVEC LES MARQUES DE RÉVISION)

CHAPITRE 11.2.

INFECTION À *BONAMIA EXITIOSA*

[…]

Article 11.2.3.

Mesures pour l'~~i~~importation ou le transit par le territoire de produits issus d'animaux aquatiques indépendamment de l'usage auquel ils sont destinés et du statut sanitaire du pays, de la zone ou du compartiment d'exportation au regard de l'infection à *B. exitiosa*

~~1.~~Les *produits issus d'animaux aquatiques* énumérés ci-dessous ont été évalués comme satisfaisant aux critères de l'évaluation de la sécurité des *produits issus d'animaux aquatiques* conformément à l'article 5.4.1. Lorsqu'elles autorisent~~, pour quelque usage que ce soit,~~ l'importation ou le transit par leur *territoire* ~~des~~ de ces *produits issus d'animaux aquatiques* ~~énumérés ci-dessous quand elles autorisent, pour quelque usage que ce soit, l'importation, ou le transit par leur *territoire*, des *produits issus d'animaux aquatiques* énumérés ci-dessous lorsqu'il s'agit de l'une des espèces visées à l'article 11.2.2. et que ces produits satisfont aux dispositions prévues à l'article 5.4.1.~~, les *Autorités compétentes* ne doivent imposer aucune ~~condition~~ *mesure sanitaire* ayant trait à *B. exitiosa*, quel que soit le statut sanitaire du pays*,* de la *zone* ou du *compartiment* d'exportation au regard de l'infection à *B. exitiosa* :

1) *produits issus d'animaux aquatiques* ayant été soumis à un traitement thermique suffisant pour atteindre une température à cœur d'au moins 100°C pendant au moins 15 minutes, ou à un couple temps/température équivalent qui inactive *B. exitiosa ;*

~~a)~~2) chair d'huître à l'état congelé ; ~~, et~~

~~b)~~3) huîtres congelées en demi-coquille.

~~2) Lorsqu'elles autorisent l'importation ou le transit par leur *territoire* d'*animaux aquatiques* ou de *produits issus d'animaux aquatiques* appartenant à une espèce visée à l'article 11.2.2. autres que ceux énumérés au point 1 de l'article 11.2.3., les *Autorités compétentes* doivent exiger le respect des conditions prescrites par les articles 11.2.7. à 11.2.11. en fonction du statut sanitaire du pays, de la *zone* ou du *compartiment* d'exportation au regard de l'infection à *B. exitiosa.*~~

~~3) L'*Autorité compétente* doit procéder à une *analyse des risques* conformément aux recommandations contenues dans le chapitre 2.1. lorsqu'elle envisage l'importation ou le transit par son *territoire* d'*animaux aquatiques* ou de *produits issus d'animaux aquatiques* appartenant à une espèce non visée à l'article 11.2.2., mais dont on peut raisonnablement s'attendre à ce qu'ils posent un *risque* en termes de propagation de l'infection à *B. exitiosa.* L'*Autorité compétente* du *pays exportateur* doit être tenue informée du résultat de cette analySE.~~

[…]

(VERSION SANS LES MARQUES DE RÉVISION)

CHAPITRE 11.2.

INFECTION À *BONAMIA EXITIOSA*

[…]

Article 11.2.3.

Mesures pour l'importation ou le transit par le territoire de produits issus d'animaux aquatiques indépendamment de l'usage auquel ils sont destinés et du statut sanitaire du pays, de la zone ou du compartiment d'exportation au regard de l'infection à *B. exitiosa*

Les *produits issus d'animaux aquatiques* énumérés ci-dessous ont été évalués comme satisfaisant aux critères d'évaluation de la sécurité des *produits issus d'animaux aquatiques* conformément à l'article 5.4.1. Lorsqu'elles autorisent l'importation ou le transit par leur *territoire* de ces *produits issus d'animaux aquatiques*, les *Autorités compétentes* ne doivent imposer aucune *mesure sanitaire* ayant trait à *B. exitiosa*, quel que soit le statut sanitaire du pays, de la *zone* ou du *compartiment* d'exportation au regard de l'infection à *B. exitiosa* :

1) *produits issus d'animaux aquatiques* ayant été soumis à un traitement thermique suffisant pour atteindre une température à cœur d'au moins 100°C pendant au moins 15 minutes, ou à un couple temps/température équivalent qui inactive *B. exitiosa* ;

2) chair d'huître à l'état congelé ;

3) huîtres congelées en demi-coquille.

[…]

Annexe 30. Point 6.5.4. – Article 11.3.3. du chapitre 11.3. « Infection à *Bonamia ostreae* »

(VERSION AVEC LES MARQUES DE RÉVISION)

CHAPITRE 11.3.

INFECTION À *BONAMIA OSTREAE*

[…]

Article 11.3.3.

Mesures pour l'~~i~~importation ou le transit par le territoire de produits issus d'animaux aquatiques indépendamment de l'usage auquel ils sont destinés et du statut sanitaire du pays, de la zone ou du compartiment d'exportation au regard de l'infection à *B. ostreae*

~~1.~~Les *produits issus d'animaux aquatiques* énumérés ci-dessous ont été évalués comme satisfaisant aux critères de l'évaluation de la sécurité des *produits issus d'animaux aquatiques* conformément à l'article 5.4.1. Lorsqu'elles autorisent~~, pour quelque usage que ce soit,~~ l'importation ou le transit par leur *territoire* ~~des~~ de ces *produits issus d'animaux aquatiques* ~~énumérés ci-dessous quand elles autorisent, pour quelque usage que ce soit, l'importation, ou le transit par leur *territoire*, des *produits issus d'animaux aquatiques* énumérés ci-dessous lorsqu'il s'agit de l'une des espèces visées à l'article 11.3.2. et que ces produits satisfont aux dispositions prévues à l'article 5.4.1.~~, les *Autorités compétentes* ne doivent imposer aucune ~~condition~~ *mesure sanitaire* ayant trait à *B. ostreae*, quel que soit le statut sanitaire du pays*,* de la *zone* ou du *compartiment* d'exportation au regard de l'infection à *B. ostreae* :

1) *produits issus d'animaux aquatiques* ayant été soumis à un traitement thermique suffisant pour atteindre une température à cœur d'au moins 100°C pendant au moins 15 minutes, ou à un couple temps/température équivalent qui inactive *B. ostreae ;*

~~a)~~12) chair d'huître à l'état congelé ; ~~, et~~

~~b)~~23) huîtres congelées en demi-coquille.

~~2) Lorsqu'elles autorisent l'importation ou le transit par leur *territoire* d'*animaux aquatiques* ou de *produits issus d'animaux aquatiques* appartenant à une espèce visée à l'article 11.3.2. autres que ceux énumérés au point 1 de l'article 11.3.3., les *Autorités compétentes* doivent exiger le respect des conditions prescrites dans les articles 11.3.7. à 11.3.11. en fonction du statut sanitaire du pays, de la *zone* ou du *compartiment* d'exportation au regard de l'infection à *B. ostreae*.~~

~~3) L'*Autorité compétente* doit procéder à une *analyse des risques* conformément aux recommandations contenues dans le chapitre 2.1. lorsqu'elle envisage l'importation ou le transit par son *territoire* d'*animaux aquatiques* et de *produits issus d'animaux aquatiques* appartenant à une espèce non visée à l'article 11.3.2., mais dont on peut raisonnablement s'attendre à ce qu'ils posent un *risque* en termes de propagation de l'infection à *B. ostreae*. L'*Autorité compétente* du *pays exportateur* doit être tenue informée du résultat de cette analyse.~~

[…]

(VERSION SANS LES MARQUES DE RÉVISION)

CHAPITRE 11.3.

INFECTION À *BONAMIA OSTREAE*

[…]

Article 11.3.3.

Mesures pour l'importation ou le transit par le territoire de produits issus d'animaux aquatiques indépendamment de l'usage auquel ils sont destinés et du statut sanitaire du pays, de la zone ou du compartiment d'exportation au regard de l'infection à *B. ostreae*

Les *produits issus d'animaux aquatiques* énumérés ci-dessous ont été évalués comme satisfaisant aux critères d'évaluation de la sécurité des *produits issus d'animaux aquatiques* conformément à l'article 5.4.1. Lorsqu'elles autorisent l'importation ou le transit par leur *territoire* de ces *produits issus d'animaux aquatiques*, les *Autorités compétentes* ne doivent imposer aucune *mesure sanitaire* ayant trait à *B. ostreae*, quel que soit le statut sanitaire du pays, de la *zone* ou du *compartiment* d'exportation au regard de l'infection à *B. ostreae* :

1) *produits issus d'animaux aquatiques* ayant été soumis à un traitement thermique suffisant pour atteindre une température à cœur d'au moins 100°C pendant au moins 15 minutes, ou à un couple temps/température équivalent qui inactive *B. ostreae* ;

2) chair d'huître à l'état congelé ;

3) huîtres congelées en demi-coquille.

[…]

Annexe 31. Point 6.5.4. – Article 11.4.3. du chapitre 11.4. « Infection à *Marteilia refringens* »

(VERSION AVEC LES MARQUES DE RÉVISION)

CHAPITRE 11.4.

INFECTION À *MARTEILIA REFRINGENS*

[…]

Article 11.4.3.

Mesures pour l'~~i~~importation ou le transit par le territoire de produits issus d'animaux aquatiques indépendamment de l'usage auquel ils sont destinés et du statut sanitaire du pays, de la zone ou du compartiment d'exportation au regard de l'infection à *M. refringens*

~~1.~~Les *produits issus d'animaux aquatiques* énumérés ci-dessous ont été évalués comme satisfaisant aux critères de l'évaluation de la sécurité des *produits issus d'animaux aquatiques* conformément à l'article 5.4.1. Lorsqu'elles autorisent~~, pour quelque usage que ce soit,~~ l'importation ou le transit par leur *territoire* ~~des~~ de ces *produits issus d'animaux aquatiques* ~~énumérés ci-dessous quand elles autorisent, pour quelque usage que ce soit, l'importation, ou le transit par leur *territoire*, des *produits issus d'animaux aquatiques* énumérés ci-dessous lorsqu'il s'agit de l'une des espèces visées à l'article 11.4.2. et que ces produits satisfont aux dispositions prévues à l'article 5.4.1.~~, les *Autorités compétentes* ne doivent imposer aucune ~~condition~~ *mesure sanitaire* ayant trait à *M. refringens*, quel que soit le statut sanitaire du pays, de la *zone* ou du *compartiment* d'exportation au regard de l'infection à *M. refringens* :

1) *produits issus d'animaux aquatiques* ayant été soumis à un traitement thermique suffisant pour atteindre une température à cœur d'au moins 121°C pendant au moins trois~~3~~ minutes et 36 secondes, ou à un couple temps/température équivalent qui inactive *M. refringens.*

~~2) Lorsqu'elles autorisent l'importation ou le transit par leur *territoire* d'*animaux aquatiques* ou de *produits issus d'animaux aquatiques* appartenant à une espèce visée à l'article 11.4.2. autres que ceux mentionnés au point 1 de l'article 11.4.3., les *Autorités compétentes* doivent exiger le respect des conditions prescrites par les articles 11.4.7. à 11.4.11. en fonction du statut sanitaire du pays, de la *zone* ou du *compartiment* d'exportation au regard de l'infection à *M. refringens*.~~

~~3) L'*Autorité compétente* doit procéder à une *analyse des risques* conformément aux recommandations contenues dans le chapitre 2.1. lorsqu'elle envisage l'importation ou le transit par son *territoire* d'*animaux aquatiques* ou de *produits issus d'animaux aquatiques* appartenant à une espèce non visée à l'article 11.4.2., mais dont on peut raisonnablement s'attendre à ce qu'ils posent un *risque* en termes de propagation de l'infection à *M. refringens*. L'*Autorité compétente* du *pays exportateur* doit être tenue informée du résultat de cette analyse.~~

[…]

(VERSION SANS LES MARQUES DE RÉVISION)

CHAPITRE 11.4.

INFECTION À *MARTEILIA REFRINGENS*

[…]

Article 11.4.3.

Mesures pour l'importation ou le transit par le territoire de produits issus d'animaux aquatiques indépendamment de l'usage auquel ils sont destinés et du statut sanitaire du pays, de la zone ou du compartiment d'exportation au regard de l'infection à *M. refringens*

Les *produits issus d'animaux aquatiques* énumérés ci-dessous ont été évalués comme satisfaisant aux critères d'évaluation de la sécurité des *produits issus d'animaux aquatiques* conformément à l'article 5.4.1. Lorsqu'elles autorisent l'importation ou le transit par leur *territoire* de ces *produits issus d'animaux aquatiques*, les *Autorités compétentes* ne doivent imposer aucune *mesure sanitaire* ayant trait à *M. refringens*, quel que soit le statut sanitaire du pays, de la *zone* ou du *compartiment* d'exportation au regard de l'infection à *M. refringens*:

1) *produits issus d'animaux aquatiques* ayant été soumis à un traitement thermique suffisant pour atteindre une température à cœur d'au moins 121°C pendant au moins trois minutes et 36 secondes, ou à un couple temps/température équivalent qui inactive *M. refringens.*

[…]

Annexe 32. Point 6.5.4. – Article 11.5.3. du chapitre 11.5. « Infection à *Perkinsus marinus* »

(VERSION AVEC LES MARQUES DE RÉVISION)

CHAPITRE 11.5.

INFECTION À *PERKINSUS MARINUS*

[…]

Article 11.5.3.

Mesures pour l'~~i~~importation ou le transit par le territoire de produits issus d'animaux aquatiques indépendamment de l'usage auquel ils sont destinés et du statut sanitaire du pays, de la zone ou du compartiment d'exportation au regard de l'infection à *P. marinus*

~~1.~~Les *produits issus d'animaux aquatiques* énumérés ci-dessous ont été évalués comme satisfaisant aux critères de l'évaluation de la sécurité des *produits issus d'animaux aquatiques* conformément à l'article 5.4.1. Lorsqu'elles autorisent~~, pour quelque usage que ce soit,~~ l'importation ou le transit par leur *territoire* ~~des~~ de ces *produits issus d'animaux aquatiques* ~~énumérés ci-dessous quand elles autorisent, pour quelque usage que ce soit, l'importation, ou le transit par leur *territoire*, des *produits issus d'animaux aquatiques* énumérés ci-dessous lorsqu'il s'agit de l'une des espèces visées à l'article 11.5.2. et que ces produits satisfont aux dispositions prévues à l'article 5.4.1.~~, les *Autorités compétentes* ne doivent imposer aucune ~~condition~~ *mesure sanitaire* ayant trait à *P. marinus,* quel que soit le statut sanitaire du pays*,* de la *zone* ou du *compartiment* d'exportation au regard de l'infection à *P. marinus* :

1) *produits issus d'animaux aquatiques* ayant été soumis à un traitement thermique suffisant pour atteindre une température à cœur d'au moins ~~121~~60°C pendant au moins ~~3~~60 minutes ~~et 36 secondes~~, ou à un couple temps/température équivalent qui inactive *P. marinus.*

~~2) Lorsqu'elles autorisent l'importation ou le transit par leur *territoire* d'*animaux aquatiques* ou de *produits issus d'animaux aquatiques* appartenant à une espèce visée à l'article 11.5.2. autres que ceux énumérés au point 1 de l'article 11.5.3., *les Autorités compétentes* doivent exiger le respect des conditions prescrites par les articles 11.5.7. à 11.5.11. en fonction du statut sanitaire du pays, de la *zone* ou du *compartiment* d'exportation au regard de l'infection à *P. marinus*.~~

~~3) L'*Autorité compétente* doit procéder à une *analyse des risques* conformément aux recommandations contenues dans le chapitre 2.1. lorsqu'elle envisage l'importation ou le transit par son *territoire* d'*animaux aquatiques* ou de *produits issus d'animaux aquatiques* appartenant à une espèce non visée à l'article 11.5.2., mais dont on peut raisonnablement s'attendre à ce qu'ils posent un *risque* en termes de propagation de l'infection à *P. marinus*. L'*Autorité compétente* du *pays exportateur* doit être tenue informée du résultat de cette analyse.~~

[…]

(VERSION SANS LES MARQUES DE RÉVISION)

CHAPITRE 11.5.

INFECTION À *PERKINSUS MARINUS*

[…]

Article 11.5.3.

Mesures pour l'importation ou le transit par le territoire de produits issus d'animaux aquatiques indépendamment de l'usage auquel ils sont destinés et du statut sanitaire du pays, de la zone ou du compartiment d'exportation au regard de l'infection à *P. marinus*

Les *produits issus d'animaux aquatiques* énumérés ci-dessous ont été évalués comme satisfaisant aux critères d'évaluation de la sécurité des *produits issus d'animaux aquatiques* conformément à l'article 5.4.1. Lorsqu'elles autorisent l'importation ou le transit par leur *territoire* de ces *produits issus d'animaux aquatiques*, les *Autorités compétentes* ne doivent imposer aucune *mesure sanitaire* ayant trait à *P. marinus*, quel que soit le statut sanitaire du pays, de la *zone* ou du *compartiment* d'exportation au regard de l'infection à *P. marinus* :

1) *produits issus d'animaux aquatiques* ayant été soumis à un traitement thermique suffisant pour atteindre une température à cœur d'au moins 60°C pendant au moins 60 minutes, ou à un couple temps/température équivalent qui inactive *P. marinus.*

[…]

Annexe 33. Point 6.5.4. – Article 11.6.3. du chapitre 11.6. « Infection à *Perkinsus olseni* »

(VERSION AVEC LES MARQUES DE RÉVISION)

CHAPITRE 11.6.

INFECTION À *PERKINSUS OLSENI*

[…]

Article 11.6.3.

Mesures pour l'~~i~~importation ou le transit par le territoire de produits issus d'animaux aquatiques indépendamment de l'usage auquel ils sont destinés et du statut sanitaire du pays, de la zone ou du compartiment d'exportation au regard de l'infection à *P. olseni*

~~1.~~Les *produits issus d'animaux aquatiques* énumérés ci-dessous ont été évalués comme satisfaisant aux critères de l'évaluation de la sécurité des *produits issus d'animaux aquatiques* conformément à l'article 5.4.1. Lorsqu'elles autorisent~~, pour quelque usage que ce soit,~~ l'importation ou le transit par leur *territoire* ~~des~~ de ces *produits issus d'animaux aquatiques* ~~énumérés ci-dessous quand elles autorisent, pour quelque usage que ce soit, l'importation, ou le transit par leur *territoire*, des *produits issus d'animaux aquatiques* énumérés ci-dessous lorsqu'il s'agit de l'une des espèces visées à l'article 11.6.2. et que ces produits satisfont aux dispositions prévues à l'article 5.4.1.~~, les *Autorités compétentes* ne doivent imposer aucune ~~condition~~ *mesure sanitaire* ayant trait à *P. olseni,* quel que soit le statut sanitaire du pays*,* de la *zone* ou du *compartiment* d'exportation au regard de l'infection à *P. olseni* :

1) *produits issus d'animaux aquatiques* ayant été soumis à un traitement thermique suffisant pour atteindre une température à cœur d'au moins ~~121~~60°C pendant au moins ~~3~~60 minutes ~~et 36 secondes~~, ou à un couple temps/température équivalent qui inactive P*. olseni.*

~~2) Lorsqu'elles autorisent l'importation ou le transit par leur *territoire* d'*animaux aquatiques* ou de *produits issus d'animaux aquatiques* appartenant à une espèce visée à l'article 11.6.2. autres que ceux énumérés au point 1 de l'article 11.6.3., les *Autorités compétentes* doivent exiger le respect des conditions prescrites par les articles 11.6.7. à 11.6.11. en fonction du statut sanitaire du pays, de la *zone* ou du *compartiment* d'exportation au regard de l'infection à *P. olseni*.~~

~~3) L'*Autorité compétente* doit procéder à une *analyse des risques* conformément aux recommandations contenues dans le chapitre 2.1. lorsqu'elle envisage l'importation ou le transit par son *territoire* d'*animaux aquatiques* ou de *produits issus d'animaux aquatiques* appartenant à une espèce non visée à l'article 11.6.2., mais dont on peut raisonnablement s'attendre à ce qu'ils posent un *risque* en termes de propagation de l'infection à *P. olseni*. L'*Autorité compétente* du *pays exportateur* doit être tenue informée du résultat de cette analyse.~~

[…]

(VERSION SANS LES MARQUES DE RÉVISION)

CHAPITRE 11.6.

INFECTION À *PERKINSUS OLSENI*

[…]

Article 11.6.3.

Mesures pour l'importation ou le transit par le territoire de produits issus d'animaux aquatiques indépendamment de l'usage auquel ils sont destinés et du statut sanitaire du pays, de la zone ou du compartiment d'exportation au regard de l'infection à *P. olseni*

Les *produits issus d'animaux aquatiques* énumérés ci-dessous ont été évalués comme satisfaisant aux critères d'évaluation de la sécurité des *produits issus d'animaux aquatiques* conformément à l'article 5.4.1. Lorsqu'elles autorisent l'importation ou le transit par leur *territoire* de ces *produits issus d'animaux aquatiques*, les *Autorités compétentes* ne doivent imposer aucune *mesure sanitaire* ayant trait à *P. olseni*, quel que soit le statut sanitaire du pays, de la *zone* ou du *compartiment* d'exportation au regard de l'infection à *P. olseni* :

1) *produits issus d'animaux aquatiques* ayant été soumis à un traitement thermique suffisant pour atteindre une température à cœur d'au moins 60°C pendant au moins 60 minutes, ou à un couple temps/température équivalent qui inactive *P. olseni.*

[…]

Annexe 34. Point 6.5.4. – Article 11.7.3. du chapitre 11.7. « Infection à *Xenohaliotis californiensis* »

(VERSION AVEC LES MARQUES DE RÉVISION)

CHAPITRE 11.7.

INFECTION À *XENOHALIOTIS CALIFORNIENSIS*

[…]

Article 11.7.3.

Mesures pour l'~~i~~importation ou le transit par le territoire de produits issus d'animaux aquatiques indépendamment de l'usage auquel ils sont destinés et du statut sanitaire du pays, de la zone ou du compartiment d'exportation au regard de l'infection à *X. californiensis*

~~1.~~Les *produits issus d'animaux aquatiques* énumérés ci-dessous ont été évalués comme satisfaisant aux critères de l'évaluation de la sécurité des *produits issus d'animaux aquatiques* conformément à l'article 5.4.1. Lorsqu'elles autorisent~~, pour quelque usage que ce soit,~~ l'importation ou le transit par leur *territoire* ~~des~~ de ces *produits issus d'animaux aquatiques* ~~énumérés ci-dessous quand elles autorisent, pour quelque usage que ce soit, l'importation, ou le transit par leur *territoire*, des *produits issus d'animaux aquatiques* énumérés ci-dessous lorsqu'il s'agit de l'une des espèces visées à l'article 11.7.2. et que ces produits satisfont aux dispositions prévues à l'article 5.4.1.~~, les *Autorités compétentes* ne doivent imposer aucune ~~condition~~ *mesure sanitaire* ayant trait à *X. californiensis,* quel que soit le statut sanitaire du pays*,* de la *zone* ou du *compartiment* d'exportation au regard de l'infection à *X. californiensis* :

1) *produits issus d'animaux aquatiques* ayant été soumis à un traitement thermique suffisant pour atteindre une température à cœur d'au moins ~~121~~95°C pendant au moins ~~3~~cinq minutes ~~et 36 secondes~~, ou à un couple temps/température équivalent qui inactive *X. californiensis.*

~~2) Lorsqu'elles autorisent l'importation ou le transit par leur *territoire* d'*animaux aquatiques* ou de *produits issus d'animaux aquatiques* appartenant à une espèce visée à l'article 11.7.2. autres que ceux énumérés au point 1 de l'article 11.7.3., les *Autorités compétentes* doivent exiger le respect des conditions prescrites par les articles 11.7.7. à 11.7.11. en fonction du statut sanitaire du pays, de la *zone* ou du *compartiment* d'exportation au regard de l'infection à *X. californiensis.*~~

~~3) L'*Autorité compétente* doit procéder à une *analyse des risques* conformément aux recommandations contenues dans le chapitre 2.1. lorsqu'elle envisage l'importation ou le transit par son *territoire* d'*animaux aquatiques* ou de *produits issus d'animaux aquatiques* appartenant à une espèce non visée à l'article 11.7.2., mais dont on peut raisonnablement s'attendre à ce qu'ils posent un *risque* en termes de propagation de l'infection à *X. californiensis*. L'*Autorité compétente* du *pays exportateur* doit être tenue informée du résultat de cette analyse.~~

[…]

(VERSION SANS LES MARQUES DE RÉVISION)

CHAPITRE 11.7.

INFECTION À *XENOHALIOTIS CALIFORNIENSIS*

[…]

Article 11.7.3.

Mesures pour l'importation ou le transit par le territoire de produits issus d'animaux aquatiques indépendamment de l'usage auquel ils sont destinés et du statut sanitaire du pays, de la zone ou du compartiment d'exportation au regard de l'infection à *X. californiensis*

Les *produits issus d'animaux aquatiques* énumérés ci-dessous ont été évalués comme satisfaisant aux critères d'évaluation de la sécurité des *produits issus d'animaux aquatiques* conformément à l'article 5.4.1. Lorsqu'elles autorisent l'importation ou le transit par leur *territoire* de ces *produits issus d'animaux aquatiques*, les *Autorités compétentes* ne doivent imposer aucune *mesure sanitaire* ayant trait à *X. californiensis*, quel que soit le statut sanitaire du pays, de la *zone* ou du *compartiment* d'exportation au regard de l'infection à *X. californiensis* :

1) *produits issus d'animaux aquatiques* ayant été soumis à un traitement thermique suffisant pour atteindre une température à cœur d'au moins 95°C pendant au moins cinq minutes, ou à un couple temps/température équivalent qui inactive *X. californiensis*.

[…]

Annexe 35. Point 6.6. – Modèles d'articles X.X.5. et X.X.6. destinés aux chapitres spécifiques aux maladies

Modèles d'articles X.X.5. et X.X.6. destinés aux chapitres spécifiques aux maladies

CHAPITRE X.X.

INFECTION PAR [L'AGENT PATHOGÈNE X]

[…]

Article X.X.5.

Pays indemne d'infection par [l'agent pathogène X]

En cas de partage des étendues d'eau avec d'autres pays, un pays ne peut déposer une auto-déclaration d'absence d'infection par [l'agent pathogène X] que si toutes les étendues d'eau partagées sont situées dans des pays ou des *zones* déclarés indemnes de cette *infection* (voir l'article X.X.6.).

Comme indiqué dans l'article 1.4.4., un État membre peut déposer une auto-déclaration d'absence d'infection par [l'agent pathogène X] pour l'ensemble de son *territoire* s'il peut démontrer :

1) qu'aucune des *espèces sensibles* visées à l'article X.X.2. n'est présente dans le pays et que les *conditions élémentaires de sécurité biologique* sont réunies sans discontinuer depuis au moins [six mois] ;

OU

2) qu'aucune infection par [l'agent pathogène X] n'est apparue depuis au moins [10] ans, et :

   a) que l'État membre peut démontrer que les conditions propices à l'expression clinique de l'infection par [l'agent pathogène X] sont réunies, comme indiqué au chapitre correspondant du *Manuel aquatique*, et

   b) que les *conditions élémentaires de sécurité biologique* telles que décrites dans le chapitre 1.4. sont réunies sans discontinuer depuis au moins [10] ans ;

OU

3) qu'une *surveillance ciblée*, comme décrit au chapitre 1.4., est mise en œuvre depuis au moins [deux] ans sans que la présence de [l'agent pathogène X] ait été décelée, et que les *conditions élémentaires de sécurité biologique* ont été réunies sans discontinuer et mises en œuvre au moins [un] an avant le commencement de la *surveillance ciblée* ;

OU

4) que le pays, après avoir déposé une auto-déclaration d'absence d'infection par [l'agent pathogène X], a perdu son statut indemne par suite de la détection du virus du tilapia lacustre, mais que les conditions suivantes sont remplies :

   a) dès la détection de [l'agent pathogène X], le secteur touché a été déclaré *zone infectée* et une *zone de protection* a été établie, et

   b) les populations touchées par l'*infection* de la *zone infectée* ont été abattues et éliminées par des moyens réduisant autant que possible la probabilité de nouvelle transmission de [l'agent pathogène X], et les opérations appropriées de *désinfection* (décrites au chapitre 4.4.) ont été menées à bien et suivies d'une période de *vide sanitaire* comme indiqué au chapitre 4.7., et

c) les *conditions élémentaires de sécurité biologique* existant antérieurement ont été réexaminées, et sont en place sans discontinuer, avec les modifications éventuellement nécessaires, depuis l'éradication de l'infection par [l'agent pathogène X], et

d) une *surveillance ciblée*, comme décrit au chapitre 1.4., est exercée :

i) depuis au moins [deux] ans sur les *espèces sensibles* d'élevage et sauvages sans que la présence de [l'agent pathogène X] ait été décelée, ou

ii) depuis au moins [un] an sans que la présence de [l'agent pathogène X] ait été décelée dans le cas où les *établissements d'aquaculture* touchés ne présentent aucun lien épidémiologique avec des populations sauvages d'*espèces sensibles*.

Entre-temps, ~~tout ou partie du pays, à l'exclusion des *zones infectées* et des *zones de protection*,~~ la partie du pays, à l'exclusion de la *zone infectée* et de la *zone de protection*, peut être déclarée *zone indemne* ~~sous réserve que les conditions énoncées au point 2 de l'article X.X.6. soient remplies~~ comme indiqué dans l'article 1.4.4.

Article X.X.6.

Zone indemne d'infection par [l'agent pathogène X]

En cas d'extension au-delà du *territoire* de plus d'un pays, une *zone* ne peut être déclarée indemne d'infection par [l'agent pathogène X] que si l'ensemble des *Autorités compétentes* concernées confirment que toutes les conditions voulues sont remplies.

Comme indiqué dans l'article 1.4.4., un État membre peut déposer une auto-déclaration d'absence d'infection par [l'agent pathogène X] pour une *zone* établie sur son *territoire* s'il peut démontrer :

1) qu'aucune des *espèces sensibles* visées à l'article X.X.2. n'est présente et que *les conditions élémentaires de sécurité biologique* sont réunies sans discontinuer depuis au moins [six mois] ;

OU

2) qu'aucune infection par [l'agent pathogène X] n'est apparue depuis au moins [dix] ans, et :

a) que l'État membre peut démontrer que les conditions propices à l'expression clinique de l'infection par [l'agent pathogène X] sont réunies, comme décrit à l'article 1.4.8. du chapitre 1.4., et

b) que les *conditions élémentaires de sécurité biologique* telles que décrites dans le chapitre 1.4. sont réunies sans discontinuer dans la zone depuis au moins [dix] ans ;

OU

3) qu'une *surveillance ciblée*, comme décrit au chapitre 1.4., est mise en œuvre dans la zone depuis au moins [deux] ans sans que la présence de [l'agent pathogène X] ait été décelée, et que les *conditions élémentaires de sécurité biologique* ont été réunies sans discontinuer et mises en œuvre au moins [un] an avant le commencement de la *surveillance ciblée* ;

OU

4) que le pays, après avoir déposé une auto-déclaration d'absence d'infection par [l'agent pathogène X] pour une *zone*, a perdu son statut indemne par suite de la détection de [l'agent pathogène X] dans cette *zone*, mais que les conditions suivantes sont remplies :

a) dès la détection de [l'agent pathogène X], le secteur touché a été déclaré *zone infectée* et une *zone de protection* a été établie, et

b) les populations touchées par l'*infection* de la *zone infectée* ont été abattues et éliminées par des moyens réduisant autant que possible la probabilité de nouvelle transmission de [l'agent pathogène X], et les opérations appropriées de *désinfection* (décrites au chapitre 4.4.) ont été menées à bien et suivies d'une période de *vide sanitaire* comme indiqué au chapitre 4.7., et

c) les *conditions élémentaires de sécurité biologique* existant antérieurement ont été réexaminées, et sont en place sans discontinuer, avec les modifications éventuellement nécessaires, depuis l'éradication de l'infection par [l'agent pathogène X], et

d) une *surveillance ciblée*, comme décrit au chapitre 1.4., est mise en œuvre depuis au moins [deux] ans sans que la présence de [l'agent pathogène X] ait été décelée.

Entre-temps, une partie de la *zone*, à l'exclusion de la *zone infectée* et de la *zone de protection*, peut être déclarée comme une nouvelle *zone indemne* comme indiqué dans l'article 1.4.4.

[…]

Annexe 36. Point 6.7. – Article 9.3.2. du chapitre 9.3. « Infection par le virus 1 iridescent des décapodes »

CHAPITRE 9.3.

INFECTION PAR LE VIRUS 1 IRIDESCENT DES DÉCAPODES

[…]

Article 9.3.2.

Champ d'application

Les recommandations du présent chapitre s'appliquent aux espèces ci-après, satisfaisant aux critères permettant de les lister comme étant sensibles conformément au chapitre 1.5. : ~~[la crevette charnue (*Penaeus chinensis*), le crabe gazami (*Portunus trituberculatus*), le bouquet géant (*Macrobrachium rosenbergii*), la crevette kuruma (*Penaeus japonicus*), le bouquet nippon (*Macrobrachium nipponense*), *Cherax quadricarinatus*, l'écrevisse rouge de marais (*Procambarus clarkii*), le bouquet quille (*Exopalaemon carinicauda*) et la crevette pattes blanches (*Penaeus vannamei*), la crevette géante tigrée (*Penaeus monodon*), *Cherax quadricarinatus*, le bouquet géant (*Macrobrachium rosenbergii*), l'écrevisse rouge des marais (*Procambarus clarkii*), le bouquet nippon (*Macrobrachium nipponense*) et le bouquet quille (*Exopalaemon carinicauda*)] (à l'étude)~~.

Famille	Nom scientifique	Nom vernaculaire
Cambaridae	*Procambarus clarkii*	écrevisse rouge de marais
Palaemonidae	*Macrobrachium nipponense*	bouquet nippon
	Macrobrachium rosenbergii	bouquet géant
	Palaemon carinicauda	bouquet quille
Parastacidae	*Cherax quadricarinatus*	[red claw crayfish]
Penaeidae	*Penaeus japonicus*	crevette kuruma
	Penaeus vannamei	crevette pattes blanches
Portunidae	*Portunus trituberculatus*	crabe gazami

[…]

_____

Annexe 37. Point 6.8. – Article 10.6.2. du Chapitre 10.6. « Infection par le virus de la nécrose hématopoïétique infectieuse »

CHAPTER 10.6.

INFECTION PAR LE VIRUS DE LA NÉCROSE HÉMATOPOÏÉTIQUE INFECTIEUSE

[…]

Article 10.6.2.

Champ d'application

Les recommandations du présent chapitre s'appliquent aux espèces ci-après, satisfaisant aux critères permettant de les lister comme étant sensibles conformément au chapitre 1.5. : ~~l'omble chevalier (*Salvelinus alpinus*), le saumon de l'Atlantique (*Salmo salar*), le saumon de fontaine (Salvelinus fontinalis), la truite de mer (*Salmo trutta*), le saumon royal (*Oncorhynchus tshawytscha*), le saumon chien (*Oncorhynchus keta*), le saumon coho (*Oncorhynchus kisutch*), la truite cutthroat (*Onchorynchus clarkii*), l'omble du Canada (*Salvelinus namaycush*), le saumon du Japon (*Oncorhynchus masou*), la truite marbrée (*Salmo marmoratus*), le brochet du Nord (*Esox lucius*), la truite arc-en-ciel (*Oncorhynchus mykiss*) et le saumon rouge (*Oncorhynchus nerka*)~~.

Famille	Nom scientifique	Nom vernaculaire
Esocidae	*Esox lucius*	brochet du Nord
Salmonidae	*Onchorynchus clarkii*	truite cutthroat
	Oncorhynchus keta	saumon chien
	Oncorhynchus kisutch	saumon coho
	Oncorhynchus masou	saumon du Japon
	Oncorhynchus mykiss	truite arc-en-ciel
	Oncorhynchus nerka	saumon rouge
	Oncorhynchus tshawytscha	saumon royal
	Salmo marmoratus	truite marbrée
	Salmo salar	saumon de l'Atlantique
	Salmo trutta	truite de mer
	Salvelinus alpinus	omble chevalier
	Salvelinus fontinalis	saumon de fontaine
	Salvelinus namaycush	omble du Canada

[…]

Annexe 38. Point 6.9. – Article 10.11.2. du chapitre 10.11. « Infection par le virus du tilapia lacustre »

CHAPITRE 10.11.

INFECTION PAR LE VIRUS DU TILAPIA LACUSTRE

[…]

Article 10.11.2.

Champ d'application

Les recommandations du présent chapitre s'appliquent aux espèces ci-après, satisfaisant aux critères permettant de les lister comme étant sensibles, conformément au chapitre 1.5. : ~~[*Oreochromis aureus*, *Oreochromis aureus* X *Oreochromis niloticus*, *Sarotherodon galilaeus*, tilapia du Mozambique (*Oreochromis mossambicus*), tilapia du Nil (*Oreochromis niloticus*) et *Oreochromis niloticus* x *Oreochromis mossambicus*, *Sarotherodon galilaeus*, Tilapia du Mozambique (*Oreochromis mossambicus*), *Oreochromis niloticus*, Tilapia zilli, *Barbonymus schwanenfeldii*, *Tristramella simonis* et *Oreochromis niloticus* X *Oreochromis aureus*] (à l'étude)~~.

Famille	Nom scientifique	Nom vernaculaire
Cichlidae	*Oreochromis aureus x O. niloticus*	[blue-Nile tilapia hybrid]
	Oreochromis mossambicus	tilapia du Mozambique
	Oreochromis niloticus	tilapia du Nil
	Oreochromis niloticus x O. mossambicus	[red hybrid tilapia]
	Sarotherodon galilaeus	[mango tilapia]

[…]

_____

Annexe 39. Point 6.10. – Articles 11.5.1. et 11.5.2. du chapitre 11.5. « Infection à *Perkinsus marinus* »

CHAPITRE 11.5.

INFECTION À *PERKINSUS MARINUS*

Article 11.5.1.

Aux fins *du Code aquatique*, l'expression « infection à *Perkinsus marinus* » désigne une infection causée exclusivement par l'*agent pathogène P. marinus* appartenant à la famille Perkinsidae.

Le *Manuel aquatique* contient des informations sur les méthodes de diagnostic.

Article 11.5.2.

Champ d'application

Les recommandations du présent chapitre s'appliquent aux espèces ci-après, satisfaisant aux critères permettant de les lister comme étant sensibles conformément au chapitre 1.5. : ~~à l'huître creuse américaine *(Crassostrea virginica)*,~~ ~~à l'huître du Pacifique (*Crassostrea gigas*)~~, ~~à l'huître de Suminoe (*Crassostrea ariakensis*)~~, ~~à *Mya arenaria*~~, ~~à *Macoma balthica*~~ ~~*Magallana* [Syn. *Crassostrea*] *ariakensis*,~~ ~~l'huitre creuse de Cortez (*Crassostrea corteziensis*)~~ et ~~l'huitre palmée (*Saccostrea palmula*)~~ ~~la praire (*Mercenaria mercenaria*)~~. ~~Ces recommandations concernent également toutes les autres *espèces sensibles* visées dans le *Manuel aquatique* lorsqu'elles font l'objet d'*échanges internationaux*.~~

Famille	Nom scientifique	Nom vernaculaire
Ostreidae	*Crassostrea corteziensis*	huitre creuse de Cortez
	Crassostrea virginica	huître creuse américaine
	Magallana [syn. *Crassostrea*] *ariakensis*	[Ariake cupped oyster]
	Saccostrea palmula	huitre palmée

[…]

SECTION 2.2.

DISEASES OF CRUSTACEANS

CHAPTER 2.2.0.

GENERAL INFORMATION

A. SAMPLING

1. Assessing the health status of the epidemiological unit

1.1. Sample material to be used for tests

Sample material and the number of samples to be collected depend on the specific disease or pathogen, the size of animals, the diagnostic method to be used and the objective of testing (i.e. surveillance of apparently healthy animals, presumptive diagnosis of clinically affected animals or confirmatory diagnosis of a suspect result from surveillance or presumptive diagnosis ~~of overt disease, detection of subclinical infection in apparently healthy animals or sampling for targeted surveillance to demonstrate freedom from infection with a specified pathogen~~). See the individual disease chapters in this *Aquatic Manual* for specific details of sample requirements.

1.2. Specifications according to crustacean populations

For details of animals to sample for a specific listed disease, see the relevant disease chapter in the *Aquatic Manual*. The design of a surveillance system for demonstrating disease-free status for a country, zone or compartment should be in accordance with the recommendations of the WOAH *Aquatic Code* Chapter 1.4. *Aquatic animal disease surveillance.*

Animals to be sampled are selected as follows:

i) Susceptible species should be sampled proportionately or following risk-based criteria for targeted selection of lots, epidemiological units or populations with a history of abnormal mortality or potential exposure events (e.g. replacement with stocks of unknown disease status).

ii) If more than one water source is used for production, animals from all water sources should be included in the sample collection.

iii) ~~For the study of presumptively diseased crustaceans select those animals that are moribund, discoloured, displaying abnormal behaviour, or otherwise abnormal.~~ If weak, abnormally behaving, discoloured or freshly dead (not decomposed) animals are present, such animals should be selected. If such animals are not present, animals should be selected in such a way that all epidemiological units of the farm or waterbody are proportionately represented in the sample.

~~iv) When sampling is aimed at assessing disease occurrence (e.g. estimation of disease prevalence), the preferred selection method is probability sampling.~~

1.3. Specifications according to clinical status

In clinical disease episodes, carefully selected quality specimens with representative lesions should be obtained from live ~~or~~ (including moribund) crustaceans. Collection of dead specimens during disease outbreaks should be avoided when possible, but recently dead samples may be suitable for some diagnostic assays provided they are not decomposed. When cultured or wild crustacean stocks are presenting clinical signs of an active disease that are consistent with, or suggestive of, any one of the WOAH-listed crustacean diseases, care should be taken to ensure

that the samples collected are preserved appropriately for the anticipated diagnostic tests (see sample preservation section for recommended methods). In situations other than when clinical disease episodes are investigated, for the WOAH-listed diseases it is highly recommended that the scheduling of sampling be planned (i.e. by farm schedule, season, etc.) so that the particular life-stage(s) are sampled at a time when the pathogen of concern is most likely to be detected. Disease-specific recommendations are provided in Section 3 *Sample selection, sample collection, transportation and handling* of the individual chapters.

~~Recently dead crustaceans may be suitable (depending on their condition) for certain diagnostic assays such as nucleic acid detection techniques.~~

1.4. Specifications according to crustacean size

See the individual disease chapters in this *Aquatic Manual* for specific details of sample requirements.

2. General processing of samples

2.1. Macroscopic examination

See disease-specific chapters in this *Aquatic Manual*.

2.2. Virological examination

Virological examination by virus isolation in cell culture ~~of crustaceans~~ is not ~~routinely~~ used for listed diseases of crustaceans. ~~*Macrobrachium rosenbergii* has been isolated in insect cell lines, but it is not a recommended method.~~

2.2.1. Transportation and antibiotic treatment of samples

~~Culture systems for crustacean viruses are not available; antibiotic treatment of samples is not required. For transportation of samples see Section 3 of disease-specific chapters in this *Aquatic Manual*.~~ Not applicable.

2.2.2. Virus isolation

~~For processing of tissues see Section 3 of disease-specific chapters in this *Aquatic Manual*.~~ Not applicable.

2.2.3. Treatment to neutralise enzootic viruses

Not applicable.

2.3. Bacteriological examination

Bacteriological examination of crustaceans is not routinely used for listed diseases, but it may be used for the strains of *Vibrio parahaemolyticus* (*Vp*$_{AHPND}$) that cause acute hepatopancreatic necrosis disease (AHPND). ~~and for can be isolated on standard bacteriological media. *Hepatobacter penaei*, the causative agent of necrotising hepatopancreatitis (NHP) has not been cultured and, because of its very small size, bacteriological examination may be limited to Gram staining.~~ See disease-specific chapters in this *Aquatic Manual* for identification methods.

2.4. Parasitic examination

Not applicable for currently listed diseases.

2.5. Fungal and other protists examination

See Chapter 2.2.2 *Infection with* Aphanomyces astaci (*Crayfish plague*).

B. MATERIALS AND BIOLOGICAL PRODUCTS REQUIRED FOR THE ISOLATION AND IDENTIFICATION OF CRUSTACEAN PATHOGENS

1. Crustacean viruses

1.1. Crustacean cell lines

Not applicable. There are currently no confirmed or documented crustacean cell lines.

1.2. Culture media

Not applicable.

1.3. Virus positive controls and antigen preparation

1.3.1. Virus nomenclature

In general, the virus nomenclature used in the disease-specific chapters follows the most recent taxonomy for viruses as given in the Report of the Committee on Taxonomy of Viruses (see: ICTV [ictvonline.org] for latest information). Also provided in the disease-specific chapters are the disease and virus names that are in common use by the shrimp/prawn farming industries, as well as the more common synonyms that have been used or are in current use.

1.3.2. Virus production for experimental purposes

As no cell lines (crustacean, arthropod, or vertebrate) are known that can be used to produce crustacean virus~~es,~~ stocks *in vitro*, infection of known susceptible host species (which are free ~~of~~ from infection ~~by~~ with the pathogenic agent in question) is the preferred method for virus production for experimental purposes or for the development of positive control material.

1.3.3. Virus preservation and storage

Infectivity of all of the WOAH-listed crustacean viruses can be preserved by freezing infected whole crustaceans or infected target tissues at –20°C for short-term storage, or at –80°C or lower for long-term storage.

2. Crustacean bacteria

2.1. Culture media

See Chapter 2.2.1. *Acute hepatopancreatic necrosis disease* for details.

2.2. Storage of cultures

Lyophilisation or storage at –70°C is recommended for long-term storage of bacterial cultures.

3. Crustacean parasites

3.1. Culture media

Not applicable for currently listed diseases.

3.2. Storage of cultures

Not applicable for currently listed diseases.

4. Crustacean fungi and protists

4.1. Culture media

See Chapter 2.2.2. *Infection with* Aphanomyces astaci (*crayfish plague*)

4.2. Storage of cultures

See Chapter 2.2.2.

5. Techniques

The available diagnostic methods that may be selected for diagnosis of the WOAH-listed crustacean diseases or detection of their aetiological agents are based on:

i) Gross and clinical signs.

ii) Direct bright-field, phase-contrast or dark-field microscopy with whole stained or unstained tissue wet-mounts, tissue squashes, and impression smears; and wet-mounts of faecal strands.

iii) Histology of fixed specimens.

iv) Bioassays of suspect or subclinical carriers using a highly susceptible host (life stage or species) as the indicator for the presence of the pathogen.

v) Antibody-based tests for pathogen detection using specific antisera, polyclonal antibodies (PAbs) or monoclonal antibodies (MAbs).

vi) Molecular methods (including sequencing):

DNA probes or RNA probes for *in-situ* hybridisation (ISH) assays with histological sections of fixed tissues;

Conventional and real-time PCR/RT-PCR and LAMP for direct assay with fresh tissue samples or with extracted DNA or RNA.

Pooling of samples from more than one individual animal for a given purpose ~~should only be~~ 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 crustaceans should be processed and tested individually. However, for eggs, larvae and postlarvae, pooling of ~~larger numbers~~ individuals ~~(e.g. ~150 or more eggs or larvae or 50 to 150 postlarvae depending on their size/age)~~ may be necessary to obtain sufficient sample material to run a diagnostic assay.

5.1. Antibody-based tests

See disease-specific chapters in this *Aquatic Manual*.

5.2. Direct microscopy

Samples for direct microscopic examination should be examined as soon as possible after collection. Use live specimens whenever possible, or use fresh, chilled, or fixed specimens when live specimens are not practical. If an adequate field laboratory is available, it should be used to process and examine samples near the site of collection.

5.3. Histological techniques

Only live (including moribund) specimens with clinical signs should be sampled for histology. Collect crustaceans by whatever means are available with a minimum of handling stress. Hold animals in a container appropriate for maintaining suitable water quality and supply adequate aeration to the container if the crustaceans are to be held for a short period of time before actual fixation.

5.3.1. Fixation

A general rule is that a minimum of ten volumes of fixative should be used for one volume of tissue sample (i.e. a 10 g sample of crustacean would require 100 ml of fixative).

i) Davidson's AFA (alcohol, formalin, acetic acid) fixative

Davidson's AFA fixative is recommended for most histological applications. The fixative is rapid, reduces autolytic changes in decapod crustaceans (i.e. especially in crustaceans in tropical and subtropical regions), and its acidic content decalcifies the cuticle. The formulation for Davidson's AFA is (for 1 litre):

330 ml 95% ethyl alcohol
220 ml 100% freshly made formalin (a saturated 37–39% aqueous solution of formaldehyde gas)
115 ml glacial acetic acid
335 ml tap water (for marine crustaceans, seawater may be substituted)
Store the fixative in glass or plastic bottles with secure caps at room temperature.

ii) Fixation procedures with Davidson's AFA

For larvae and postlarvae that are too small to be easily injected with fixative using a tuberculin syringe: Using a fine mesh screen or a Pasteur pipette, select and collect specimens. Immerse crustaceans selected for sampling directly in the fixative. Fix for 12–24 hours in fixative and then transfer to 70% ethyl alcohol for storage.

For juveniles that are too small to be injected: Select and collect specimens. Use a needle or fine-pointed forceps to incise the cuticle and immediately immerse crustaceans selected for sampling directly into the fixative. Fix for 12–24 hours in fixative and then transfer to 70% ethyl alcohol for storage.

For large juveniles and adults: to ensure proper fixation, kill the crustacean using a humane method, then immediately inject fixative (use 5–10% volume:weight). Fix for 12–24 hours in fixative and then transfer to 70% ethyl alcohol for storage.

The hepatopancreas (HP) should be injected first and at two or more sites, with a volume of fixative sufficient to change the HP to a white-to-orange colour (when Davidson's AFA is used); then inject fixative into adjacent regions of the cephalothorax, into the anterior abdominal region, and into the posterior abdominal region.

The fixative should be divided between the different regions, with the cephalothoracic region, specifically the HP, receiving a larger share than the abdominal region.

Immediately following injection, slit the cuticle with dissecting scissors, from the sixth abdominal segment to the base of the rostrum, being particularly careful not to cut deeply into the underlying tissue. The incision in the cephalothoracic region should be just lateral to the dorsal midline, while that in the abdominal region should be approximately mid-lateral.

For crustaceans larger than ~12 g: After injection of fixative, the body should then be transversely bisected, at least once, just posterior to the abdomen/cephalothorax junction, and (optional) again mid-abdominally.

For very large crustaceans (*e.g. lobsters, crabs, adult penaeids, adult Macrobrachium rosenbergii*, *some species and life stages of crabs, crayfish, etc.*): The organs of interest may be excised after injection of fixative. Completion of fixation of these tissue samples is then handled as outlined previously.

Following injection, incisions and bisection/trisection, or excision of key organs, immerse the specimen in the fixative (use 10:1 fixative:tissue ratio).

Allow fixation to proceed at room temperature for 24–72 hours depending on the size of crustacean being preserved. Longer fixation times in Davidson's AFA may be used to thoroughly decalcify the shell of crabs, lobsters, crayfish, etc.

Following fixation, the specimens should be transferred to 70% ethyl alcohol, where they can be stored for an indefinite period.

iii) Transport and shipment of preserved samples

~~As large volumes of alcohol should not be mailed or shipped, the following methods are recommended:~~ Remove the specimens from the 70% ethyl alcohol. For larvae, postlarvae, or small juveniles, use leak-proof, screw-cap plastic vials if available; if glass vials must be used, pack to prevent breakage. For larger specimens, wrap samples with white paper towels to completely cover (do not use raw or processed cotton). Place towel-wrapped specimens in a sealable plastic bag and saturate with 70% ethyl alcohol. Insert the label and seal the bag. Place the bag within a second sealable bag. Multiple small sealable bags can again be placed within a sturdy, crush-proof appropriately labelled container for shipment (for details see *Aquatic Code* Chapter 5.10 *Measures concerning international transport of aquatic animal pathogens and pathological material*).

5.4. Transmission or scanning electron microscopy

Electron microscopy (EM – transmission or scanning) is a valuable research tool for the study of disease in crustaceans. However, EM methods are not routinely used for diagnosis of the diseases listed by WOAH.

5.5. Use of molecular ~~and antibody-based~~ techniques for confirmatory testing and diagnosis

Molecular techniques, including the use of nucleic acid probes for *in-situ* hybridisation, conventional PCR and real-time PCR, have been developed for the identification of many pathogens of aquatic animals. Real-time PCR methods, in general, have high sensitivity and specificity and, following adequate validation, can be used for direct detection of ~~viral~~ nucleic acids ~~in samples prepared~~ extracted from crustacean tissue. ~~The~~ Molecular techniques can be used in direct surveillance of crustacean diseases in apparently healthy populations, if they have a high level of diagnostic sensitivity, as well as in the diagnosis of clinically affected animals.

When using PCR as a diagnostic method, the design of primers and probe, the use of positive and negative controls, as well as validation of the PCR method chosen are important. Real-time PCR is a powerful technique particularly for analysing relatively high numbers of samples (e.g. for surveillance) via high-throughput testing. Several nucleic acid probe and PCR protocols are included in this version of the *Aquatic Manual* as screening, diagnostic or confirmatory methods for crustaceans and can be undertaken as the standard method. Following real-time PCR-positive results, where possible, conventional PCR with sequencing of PCR products should be used for confirmation of pathogen identity.

As with all PCR protocols, optimisation may be necessary depending on the reagents, equipment and the plasticware used. PCR is prone to false-positive and false-negative results. False-positive results (negative samples giving a positive reaction) may arise from either product carryover from positive samples or, more commonly, from cross-contamination by PCR products from previous tests. False-negative results (positive samples giving a negative result) may lead to unwanted transmission of pathogens and biosecurity failure.

Each diagnostic sample~~s~~ should be tested in duplicate, i.e. by testing two aliquots. ~~and~~ Both aliquots must produce positive results for a sample to be deemed positive. In instances where a sample produces one positive and one negative result, these are deemed indeterminate and should be retested. In addition, the following controls should be run with each assay: negative extraction control (e.g. a tissue [or equivalent sample that is under test]) sample from a known uninfected animal; positive control (preferably, one that can be distinguished from the pathogen genomic sequence [e.g. an artificial plasmid], thus allowing detection of any cross-contamination leading to a false positive result); no template control (all reagents with water replacing the template); internal positive control (internal housekeeping gene). All controls should produce their expected results in order for the diagnostic test result to be valid.

To minimise the risk of contamination, aerosol ~~preventing~~ barrier pipette tips should be used for all sample preparation and PCR ~~preparation~~ steps. Additionally, all PCRs should be prepared in a clean area that is separate from the area where ~~the~~ nucleic acid extraction, amplification~~s~~ and gel electrophoresis are performed. Do not share equipment (e.g. pipettes, laboratory coats and consumables) between areas and, where possible, restrict access between areas. Contaminating PCR products can be carried on equipment, clothes, shoes, pens/marker pens and paper (e.g. workbooks). Also, ensure all work-tops and ~~air-flow~~ cabinets/hoods used for the extractions and PCR set-up are regularly cleaned and decontaminated. To ensure sample integrity, always store the samples (e.g. in a freezer or refrigerator) in a location ~~away~~ separate from the molecular biology laboratory and reagents.

Nested PCR involves two rounds of PCR and may be used to achieve increased sensitivity and specificity; however, it increases the risk of contamination. Contaminants from previous reactions can carry over and lead to false-positive results. Strict laboratory practices such as separate workspaces, dedicated equipment, and meticulous pipetting techniques are essential to mitigate this risk. In conclusion, nested PCR is not recommended for surveillance but may sometimes be used for confirmative studies.

5.5.1. Sample preparation and types

Samples should be prepared to preserve the nucleic acid of the pathogen and should be handled and packaged with the greatest care to minimise the potential for cross-contamination among the samples or target degradation before the assay can be performed. ~~Samples selected for nucleic acid-based or antibody-based diagnostic tests should be handled and packaged (in new plastic sample bags or bottles) with care to minimise the potential for cross-contamination among the sample set taken from different (wild or farmed) stocks, tanks, ponds, farms, etc.~~ A water-resistant label, with the appropriate data filled out, should be placed within each package or container for each sample set.

Some suitable methods for preservation and transport of samples taken for molecular ~~or antibody-based~~ tests are:

i) *Live specimens:* these may be processed in the field or shipped to the diagnostic laboratory for testing.
ii) *Haemolymph:* this tissue is the preferred sample for certain molecular ~~and antibody-based diagnostic~~ tests (see disease-specific chapters). Samples may be collected by needle and syringe through cardiac puncture, from the haemocoel (i.e. the ventral sinus in penaeids), or from a severed appendage, and immediately

transferred to a tube that is half full with ~~90–95%~~ 80% analytical grade ethanol or suitable nucleic acid preservative.

iii) *Iced or chilled specimens:* these are specimens that can be transported to the laboratory for testing within 24 hours. Pack samples in sample bags surrounded by an adequate quantity of wet ice or freezer bricks around the bagged samples in an insulated box and ship to the laboratory.

iv) *Frozen ~~whole~~ specimens:* select live specimens according to the criteria listed in disease-specific chapters in this *Aquatic Manual*. In situations where it is not possible to get the specimens to the laboratory alive, they may be quick ~~freeze~~ frozen in the field using crushed dry-ice or ~~freeze~~ frozen in ~~the~~ field laboratories using a mechanical freezer at –20°C or lower temperature. Prepare and insert the label into the container with the samples, pack samples with an adequate quantity of dry-ice in an insulated box, and ship to the laboratory.

v) *Alcohol-preserved samples:* in regions where the storage and shipment of frozen samples is problematic, ~~90–95%~~ 80% analytical grade ethanol may be used to preserve, store, and transport certain types of samples for molecular tests. Alcohol-preserved samples are generally not suitable for antibody-based tests. Whole crustaceans (any life stage provided the specimen is no larger than 2–3 g), excised tissues (i.e. pleopods) from large crustaceans, or haemolymph may be preserved in ~~90–95%~~ 80% analytical grade ethanol, and then packed for shipment according to the methods described in Section 5.3.1, paragraph iii (see chapter 5.10 of the *Aquatic Code* for additional details on the international transport of such samples).

vi) *Fixed tissues for* in-situ *hybridisation:* For this purpose, classic methods for preservation of the tissues are adequate. ~~Neutral-buffered formalin~~ Davidson's fixative is usually a good choice. Samples should be fixed for 24–48 hours; fixation for ~~over 24–~~more than 48 hours in Davidson's fixative should be avoided. Samples should be transferred to 80% analytical grade ethanol following Davidson's fixation ~~treatment~~.

5.5.2. Preservation of RNA and DNA in tissues

For routine diagnostic testing by PCR or RT-PCR, samples must be prepared to preserve the pathogen's nucleic acid. For most purposes, preservation of samples in analytical grade ethanol ~~alcohol~~ (80~~–90~~%) is the preferred method for subsequent molecular tests. Samples preserved in this way can be stored for up to 1 week at 4°C ~~for 1 month, at or 25°C for 1 week~~ or ~~indefinitely~~ for extended periods at –20°C or below. In addition, other products (e.g. nucleic acid preservatives, various lysis buffers, etc.) are acceptable and are commercially available for the same purpose.

5.5.3. Nucleic acid extraction

To isolate nucleic acids from tissues preserved in ethanol or nucleic acid preservative, simply remove the tissue from the fixative or preservative and treat it as though it was just harvested. Most fresh and preserved or fixed tissues can be homogenised (e.g. with a mortar and pestle or in bead-beating tubes) directly in the lysis or extraction buffer provided with commercially available DNA and RNA extraction kits. Commercial kits should be validated or undergo equivalence testing with current validated extraction procedures prior to routine use.

5.5.4. Preparation of slides for *in-situ* hybridisation

For *in-situ* hybridisation, fixed tissues that have been transferred to ~~70%~~ 80% analytical grade ethanol are embedded in paraffin according to standard histological methods. Sections are cut at a thickness of 5 µm and placed on aminoalkylsilane-coated slides, which are then baked overnight in an oven at 40°C. The sections are de-waxed by immersing in xylene for 10 minutes. This step is repeated once and then the solvent is eliminated by immersion in two successive absolute ethanol baths for 10 minutes each. The sections are then rehydrated by immersion in an ethanol series. The protocol may require a step of membrane permeabilisation enabling access to the target DNA. For this purpose, sections are treated with proteinase K (100 µg ml^{-1}) in TE buffer (Tris [50 mM], EDTA [10 mM]), at 37°C for 30 minutes. For in-situ hybridisation tests (see individual chapters for details), it is essential that both a known positive and a known negative slide be stained to eliminate false positive results due to non-specific staining/stain dropout, and false negative results due to errors in the staining protocol (Qadiri *et al*., 2019; Valverde *et al*., 2017). For further details see disease-specific chapters in this *Aquatic Manual*.

6. Additional information to be collected

Sample information should include the collector's name, organisation, date, time, and description of the geographical location of the place of origin. The geographical location of the place of origin of samples may be described as the name or location of the ~~sampling~~ site from which the sample has originated, or its geographical co-ordinates. There should also be

records that provide information to allow trace-backs on the sample movement from the ~~sample~~ site of origin to the storage facility or laboratory and within those facilities.

A history of the specimens should also be collected and should include species, age, weight, details of clinical signs including behavioural changes, as well as observations concerning any gross pathology which has been observed.

Information on the preservation method, storage location, and date and time of storage at each storage locker or freezer along with information on the storage temperature (continuously monitored is preferable) should be collected. This information should be tracked with a unique sample code for all samples. For laboratories, the date of receipt, storage location information, date of analysis, analysis notes, and report date should be maintained for all uniquely coded samples. These data will greatly facilitate the tracking of sample problems and provide assurance that the samples were properly handled.

See disease-specific chapters in this *Aquatic Manual* for recommendations on any additional information that may be required or that may assist the diagnostic laboratory in determining the most appropriate test(s) to be run for submitted samples.

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NB: FIRST ADOPTED IN 2000; MOST RECENT UPDATES ADOPTED IN 2012.

CHAPTER 2.2.2.

INFECTION WITH *APHANOMYCES ASTACI* (CRAYFISH PLAGUE)

1. Scope

Infection with *Aphanomyces astaci* means infection with the pathogenic agent *A. astaci*, Phylum Oomycota. The disease is commonly known as crayfish plague.

2. Disease information

2.1. Agent factors

2.1.1. Aetiological agent

Aphanomyces astaci is a water mould. The Oomycetida or Oomycota, are considered protists and are classified with diatoms and brown algae in a group called the Stramenopiles or Chromista.

Five groups (A–E) of *A. astaci* have been described based on random amplification of polymorphic DNA polymerase chain reaction (RAPD PCR) (Dieguez-Uribeondo *et al.,* 1995; Huang *et al.,* 1994; Kozubikova *et al.,* 2011). Additional geno- or haplotypes are still being detected using molecular methods (Di Domenico *et al*., 2021). Group A (the so called *Astacus* strains) comprises strains isolated from several European crayfish species. These strains are thought to have been in Europe for a long period of time. Group B (*Pacifastacus* strains I) includes isolates from several European crayfish species and from the invasive *Pacifastacus leniusculus* in Europe as well as Lake Tahoe, USA. Imported to Europe, *P. leniusculus* probably introduced this genotype of *A. astaci* and infected the native European crayfish. Group C (*Pacifastacus* strains II) consists of a strain isolated from *P. leniusculus* from Pitt Lake, Canada. Another strain (Pc), isolated from *Procambarus clarkii* in Spain, sits in group D (*Procambarus* strains). This strain shows temperature/growth curves with higher optimum temperatures compared with groups A and B (Dieguez-Uribeondo *et al*., 1995). *Aphanomyces astaci* strains that have been present in Europe for many years (group A strains) appear to be less pathogenic than strains introduced with crayfish imports from North America since the 1960s. North American host species spiny-cheek crayfish (*Orconectes limosus*) has been shown to be a carrier of Group E (Kozubíková *et al.,* 2011).

2.1.2. Survival and stability in processed or stored samples

Aphanomyces astaci is poorly resistant against desiccation and does not survive long in decomposing hosts. Any treatment of the crayfish (freezing, cooking, drying) will affect the survival of the pathogen (Oidtmann *et al.,* 2002). Isolation from processed samples is not possible, however they may be suitable for molecular methods used for pathogen detection.

2.1.3. Survival and stability outside the host

Outside the host *Aphanomyces astaci* is found as zoospores that remain motile for up to 3 days and form cysts that can survive for 2 weeks in distilled water. As *A. astaci* can go through three cycles of encystment and zoospore emergence, the maximum life span outside of a host could be several weeks. Spores remained viable in a spore suspension in clean water kept at 2°C for 2 months (Unestam, 1966). Survival time is probably shorter in natural waters.

For inactivation methods, see Section 2.4.5.

2.2. Host factors

2.2.1. Susceptible host species

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]

~~All stages of crayfish species native to Europe, including the noble crayfish (*Astacus astacus*) of north-west Europe, the white clawed crayfish (*Austropotamobius pallipes*) of south-west and west Europe, the related *Austropotamobius torrentium* (mountain streams of south-west Europe) and the slender clawed or Turkish crayfish (*Pontastacus leptodactylus*) of eastern Europe and Asia Minor are highly susceptible (e.g. Holdich *et al*., 2009). Australian species of crayfish are also highly susceptible. North American crayfish such as the signal crayfish (*Pacifastacus leniusculus*), Louisiana swamp crayfish (*Procambarus clarkii*) and *Faxonius* spp. are infected by *A. astaci*, but under normal conditions the infection does not cause clinical disease or death. All North American crayfish species investigated to date have been shown to be susceptible to infection, demonstrated by the presence of the pathogen in host cuticle (reviewed by Svoboda *et al*. 2017) and it is therefore currently assumed that this is the case for any other North American species.~~

~~The only other crustacean known to be susceptible to infection by *A. astaci* is the Chinese mitten crab (*Eriocheir sinensis*).~~

2.2.2. Species with incomplete evidence for susceptibility

[Under study]

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

The host species susceptible to infection with *A. astaci* fall largely into two categories: those ~~highly susceptible to infection with~~ that develop~~ment~~ ~~of~~ clinical disease and experience mortality~~-mortalities~~, and those that are infected ~~without associated~~ but do not display any significant clinical disease or experience mortality~~-mortalities~~. All life stages of susceptible species are considered susceptible to infection with *A. astaci*.

Species that develop clinical disease and experience mortality ~~mortalities~~ include the noble crayfish (*Astacus astacus*) of north-west Europe, the white clawed crayfish (*Austropotamobius pallipes*) of south-west and west Europe, the related *Austropotamobius torrentium* (mountain streams of south-west Europe) and the ~~slender clawed or Turkish~~ Danube crayfish (*Pontastacus leptodactylus*) of eastern Europe and Asia Minor (e.g. Holdich *et al*., 2009). Australian species of freshwater crayfish are also considered vulnerable to clinical disease and mortality ~~mortalities~~.

Species that can be infected but do not normally develop clinical disease include North American crayfish species such as the signal crayfish (*Pacifastacus leniusculus*), Louisiana swamp crayfish (*Procambarus clarkii*) and *Faxonius* spp. All North American crayfish species that have been investigated have been shown to be susceptible to infection, demonstrated by the presence of the pathogen in host cuticle (reviewed by Svoboda *et al*. 2017).

~~*Highly susceptible species:*~~ Clinical disease outbreaks caused by infection with *A. astaci* are generally known as 'crayfish plague' outbreaks. In such outbreaks, moribund and dead crayfish of a range of sizes (and therefore ages) can be found.

The only non-crayfish crustacean species known to be susceptible to infection by *A. astaci* is the Chinese mitten crab (*Eriocheir sinensis*) (Schrimpf *et al.,* 2014).

2.2.4. Distribution of the pathogen in the host

The tissue that becomes initially infected is the exoskeleton cuticle. Soft cuticle, as is found on the ventral abdomen and around joints, is preferentially affected. In ~~the highly susceptible~~ European crayfish species, which are prone to development of clinical disease, the pathogen often manages to penetrate the basal lamina located underneath the epidermis cell layer. From there, *A. astaci* spreads throughout the body primarily by invading connective tissue and haemal sinuses; however, all tissues may be affected.

In North American crayfish species, infection is usually restricted to the cuticle. Based on PCR results, the tailfan (consisting of uropods and telson) and soft abdominal cuticle appear to be frequently infected (Oidtmann *et al.,* 2006; Vralstad *et al.,* 2011).

2.2.5. Aquatic animal reservoirs of infection

North American crayfish species act mostly as reservoirs ~~carriers~~ of the infection without showing clinical signs. However, some strains of *A. astaci,* especially from group A, show lowered virulence, ~~thus~~ enabling ~~normally highly susceptible~~ European crayfish to act as reservoirs ~~carriers~~ as well (see review by Svoboda *et al*., 2017).

Colonisation of habitats~~, initially~~ by North American crayfish species carrying *A. astaci* ~~occupied by highly susceptible~~ is likely to result in an epizootic if crayfish species that are prone to expression of clinical disease are present ~~by North American crayfish species carrying *A. astaci* is likely to result in an epizootic among the highly susceptible animals~~.

2.2.6. Vectors

~~Transportation of finfish may facilitate the spread of *A. astaci* through the presence of spores in the transport water or co-transport of infected crayfish specimens (Alderman *et al*., 1987; Oidtmann *et al*., 2002). There is also circumstantial evidence of spread by contaminated equipment (e.g. nets, boots, clothing, traps) (Alderman *et al*., 1987).~~ None known.

2.3. Disease pattern

2.3.1. Mortality, morbidity, and prevalence

When the infection first reaches a naïve population of ~~highly susceptible~~ crayfish species that are prone to clinical disease, high levels of mortality are usually observed within a short space of time, ~~so that in and~~. In areas with high crayfish densities the bottoms of lakes, rivers and streams ~~are~~ become covered with dead and dying crayfish. A band of mortality will spread quickly from the initial outbreak site downstream, whereas upstream spread is slower. Lower water temperatures are associated with ~~slower~~ a lower rate of mortalities and a greater range of clinical signs in affected animals (Alderman *et al*., 1987). Observations from Finland suggest that at low water temperatures, noble crayfish (*Astacus astacus*) can be infected for several months without ~~the development of~~ any noticeable mortalities (Viljamaa-Dirks *et al*., 2013).

On rare occasions, single specimens of ~~the highly susceptible~~ species that are prone to clinical disease have been found after a wave of infection with *A. astaci* has gone through a river or lake. This is most likely to be due to lack of exposure of these animals during an outbreak (animals may have been present in a tributary of a river or lake or in a part of the affected river/lake that was not reached by spores, or crayfish may have stayed in burrows during the epizootic). However, low virulent strains of *A. astaci* have been described to persist in a waterway, kept alive by a weak infection in the remnant population (Viljamaa-Dirks *et al*., 2011). Although remnant populations of susceptible crayfish species remain in many European watersheds, the dense populations that existed 150 years ago are now heavily diminished (Souty-Grosset *et al*., 2006; Holdich *et al*., 2009). Populations of susceptible crayfish may re-establish, but once population density and geographical distribution is sufficient for susceptible animals to come into contact with spores, new outbreaks of infection with *A. astaci* and large-scale mortalities ~~will~~ may occur.

In ~~the highly susceptible~~ European crayfish species, which are prone to clinical disease, exposure to *A. astaci* spores usually leads to infection and eventually to death. Prevalence of infection within a population in the early stage of an outbreak may be low (few animals in a river population may be affected). However, the pathogen ~~is amplified~~ amplifies in affected animals and is subsequently released into the water; usually leading to 100% mortality in a contiguous population. The rate of spread from initially affected animals depends on several factors, one being water temperature. Therefore, the time from first introduction of the pathogen into a population to noticeable crayfish mortalities can vary greatly and may range from a few weeks to months. Prevalence of infection will gradually increase over this time and usually reach 100%. Data from a noble crayfish population in Finland that experienced an acute mortality event due to infection with *A. astaci* in 2001 suggest that in sparse noble crayfish populations, spread of disease throughout the host population may take several years (Viljamaa-Dirks *et al.,* 2011).

2.3.2. Clinical signs, including behavioural changes

~~Susceptible~~ species prone to clinical disease

Gross clinical signs are variable and depend on challenge severity and water temperatures. The first sign of an ~~epizootic~~ outbreak may be the appearance of crayfish during daylight (crayfish are normally nocturnal), some of which may show loss of co-ordination, falling onto their backs and remaining unable to right themselves. Occasionally, the infected animals can be seen trying to scratch or pinch themselves.

Often, however, the first sign of an outbreak may be the presence of large numbers of dead crayfish in a river or lake (Alderman *et al.,* 1987).

Infection with *A. astaci* may cause mass mortality of crayfish. However, investigation of mortality event should consider other causes such as environmental pollution (e.g. insecticides such as cypermethrin have been associated with initial misdiagnoses).

~~*North American crayfish*~~ *Susceptible* ~~-~~*species that do not normally develop clinical disease*

Infected North American crayfish may be subclinical ~~carriers~~ reservoirs. Controlled exposure to a highly virulent strain has resulted in mortality in juvenile stages of *Pacifastacus leniusculus* as well as behavioural alterations in adults (Thomas *et al.,* 2020).

2.3.3 Gross pathology

Susceptible ~~*S*~~*species prone to clinical disease*

Depending on a range of factors, the foci of infection in crayfish may be seen by the naked eye or may not be discernible despite careful examination. Infection foci are best viewed under a low power stereo microscope and are recognisable by localised whitening of the muscle beneath the cuticle. In some cases, a brown colouration of cuticle and muscle may occur, or hyphae may be visible in infected cuticles in the form of fine brown (melanised) tracks in the cuticle. Sites for examination include the intestinal soft ventral cuticle of the abdomen and tail, the cuticle of the perianal region, the cuticle between the carapace and abdomen, the joints of the pereiopods (walking legs), particularly the proximal joint, eyestalks and finally the gills.

~~*North American crayfish*~~ Susceptible ~~*S*~~*species that do not normally develop clinical disease*

Infected North American crayfish do not usually show signs of disease ~~can sometimes show melanised spots in their soft cuticle, for example, the soft abdominal cuticle and joints. These melanisations can be caused by mechanical injuries or infections with other water moulds and are non-specific~~. However, populations with high levels of infection can show abnormally high levels of cuticular damage in individual animals, such as missing legs and claws due to deteriorated joints.

2.3.4. Modes of transmission and life cycle

~~The main routes of spread of the pathogen are through 1) movement of infected crayfish, 2) movement of spores with contaminated water or equipment, as may occur during movements of finfish, or 3) through colonisation of habitats by invasive North American crayfish species.~~

~~The main route of spread of *A. astaci* in Europe between the 1960s and 2000 was through the active stocking of North American crayfish into the wild or escapes from crayfish farms. Subsequent spread occurs through expanding populations of invasive North American crayfish, accidental co-transport of specimens, and release of North American crayfish into the wild by private individuals (Holdich *et al.*, 2009).~~

Transmission from crayfish to crayfish occurs through the release of zoospores from an infected animal and attachment of the zoospores to naïve crayfish. The life cycle of *A. astaci* is simple with vegetative hyphae invading and ramifying through host tissues, eventually producing extramatrical sporangia that release amoeboid primary spores. These initially encyst, but then release a biflagellate zoospore (secondary zoospore). Biflagellate zoospores swim in the water column and, upon encountering a susceptible host, attach and germinate to produce invasive vegetative hyphae. The zoospores of *A. astaci* swim actively in the water column and have been demonstrated to show positive chemotaxis towards crayfish (Cerenius & Söderhäll, 1984). Zoospores are capable of repeated encystment and re-emergence, extending the period of their infectivity (Soderhall & Cerenius 1999). Growth and sporulation capacity is strain-and temperature-dependent (Dieguez-Uribeondo *et al*., 1995).

The main routes of spread of the pathogen are through 1) movement of infected crayfish, 2) movement of spores with contaminated water or equipment, or 3) through colonisation of non-native habitats by invasive North American crayfish species.

The main route of spread of *A. astaci* in Europe between the 1960s and 2000 was through the active stocking of North American crayfish into the wild or escapes from crayfish farms. Subsequent spread occurred through expanding populations of invasive North American crayfish, accidental co-transport of specimens, and release of North American crayfish into the wild by private individuals (Holdich *et al.,* 2009).

Transportation of finfish may facilitate the spread of *A. astaci* through the presence of spores in the transport water or co-transport of infected crayfish specimens (Alderman *et al*., 1987; Oidtmann *et al*., 2002). There is also circumstantial evidence of spread by contaminated equipment (e.g., nets, boots, clothing, traps) (Alderman *et al*., 1987).

2.3.5. Environmental factors

Under laboratory conditions, the preferred temperature range at which the *A. astaci* mycelium grows varies slightly depending on the strain. In a study, which compared several *A. astaci* strains that had been isolated from a variety of crayfish species, mycelial growth was observed between 4 and 29.5°C, with the strain isolated from *Procambarus clarkii* growing better at higher temperatures compared to the other strains. Sporulation efficiency was similarly high for all strains tested between 4 and 20°C, but it was clearly reduced for the non-*P. clarkii* strains at 25°C and absent at 27°C. In contrast, sporulation still occurred in the *P. clarkii* strain at 27°C. The proportion of motile zoospores (out of all zoospores observed in a zoospore suspension) was almost 100% at temperatures ranging from 4–18°C, reduced to about 60% at 20°C and about 20% at 25°C in all but the *P. clarkii* strain. In the *P. clarkii* strain, 80% of the zoospores were still motile at 25°C, but no motile spores were found at 27°C (Dieguez-Uribeondo *et al*., 1995).

Field observations show that outbreaks of infection with *A. astaci* occur over a wide temperature range, and at least in the temperature range 4–20°C. The rate of spread within a population depends on several factors, including water temperature. In a temperature range between 4 and 16°C, the speed of an epizootic is enhanced by higher water temperatures.

In buffered, redistilled water, sporulation occurs between pH 5 and 8, with the optimal range being pH 5–7. The optimal pH range for swimming of zoospores appears to be pH 6.0–7.5, with a maximum range between pH 4.5 and 9.0 (Unestam, 1966).

Zoospore emergence is influenced by the presence of certain salts in the water. $CaCl_2$ stimulates zoospore emergence from primary cysts, whereas $MgCl_2$ has an inhibitory effect. In general, zoospore emergence is triggered by transferring the vegetative mycelium into a medium where nutrients are absent or low in concentration (Cerenius *et al*., 1988).

2.3.6. Geographical distribution

In Europe the reports of large mortalities of crayfish go back to 1860. The reservoir of the original infections in the 19th century was never established. *Faxonius* (*Orconectes*) spp. were not known to have been introduced into Europe until the 1890s, but the post-1960s extensions are largely linked to more recent introductions of North American crayfish for farming (Alderman, 1996; Holdich *et al*. 2009). *Pacifastacus leniusculus* and *Procambarus clarkii* are now widely naturalised in many parts of Europe.

In recent years, crayfish plague has been reported in Asia and also in North- and South America (see e.g. references in Di Domenico *et al.* 2021). The distribution of *A. astaci* in North America is likely to be much wider than reported (Martín-Torrijos *et al.,* 2021).

~~Any geographical area where North American crayfish species were introduced must be considered as potentially infected if not proven otherwise. Lack of clinical disease in these carrier species may hamper the reliability in reporting the infection. For the highly susceptible species,~~ See WOAH WAHIS (https://wahis.woah.org/#/home) for recent information on distribution at the country level. ~~However, even high mortalities can go unnoticed in wild populations.~~

2.4. Biosecurity and disease control strategies

2.4.1. Vaccination

No vaccines are available.

2.4.2. Chemotherapy including blocking agents

No treatments are currently known ~~that can successfully treat the highly susceptible crayfish species, once infected~~.

2.4.3. Immunostimulation

No immunostimulants are currently known ~~that can successfully protect the highly susceptible crayfish species against infection and consequent disease due to *A. astaci* infection~~.

2.4.4. Breeding resistant strains

A few studies suggest that there might be differences in resistance between populations of ~~highly susceptible species~~ crayfish species that are prone to clinical disease (reviewed by Martín-Torrijos *et al*., 2017; Svoboda *et al.*, 2017). ~~The fact that North American crayfish generally do not develop clinical disease suggests that selection for resistance may be possible and laboratory studies using attenuated strains of *A. astaci* might be successful. However, there are currently no published data from such studies.~~

2.4.5. Inactivation methods

Aphanomyces astaci, both in culture and in infected crayfish, is inactivated by a short exposure to temperatures of 60°C or to temperatures of –20°C (or below) for 48 hours (or more) (Oidtmann *et al*., 2002). Sodium hypochlorite at 100 ppm, free chlorine and iodophors at 100 ppm available iodine, are effective for disinfection of contaminated equipment. Equipment must be cleaned prior to disinfection since organic matter decreases the effectiveness of disinfectants (Alderman & Polglase, 1985). Thorough drying of equipment (>24 hours) is also effective as *A. astaci* is not resistant to desiccation (Rennerfelt, 1936).

2.4.6. Disinfection of eggs and larvae

No information available.

2.4.7. General husbandry

If a ~~crayfish~~ farm for ~~highly susceptible~~ crayfish species that are prone to clinical disease is being planned, it should be carefully investigated whether North American crayfish species are in the vicinity of the planned site or present upstream. If North American crayfish are present, there is a high likelihood that susceptible farmed crayfish will eventually become infected.

In an endemic area where ~~the highly susceptible~~ species prone to expression of clinical disease are being farmed, the following biosecurity recommendations should be followed to avoid an introduction of *A. astaci* onto the site:

1. General biosecurity should be in place (e.g. controlled access to premises; disinfection of boots when entering the site; investigation of mortalities if they occur; introduction of live susceptible species or vectors ~~animals (crayfish, finfish)~~ only from sources known to be free from infection with *A. astaci*).

2. Prevent movements of potentially infected live or dead crayfish, potentially contaminated water, equipment or any other items that might carry the pathogen from an infected to an uninfected site holding susceptible species ~~should be prevented~~.

3. ~~If~~ Do not transfer~~s~~ of ~~finfish or crayfish are being planned, these should not come~~ susceptible species or vectors from streams or other waters that harbour potentially infected crayfish (either susceptible crayfish populations that are going through a current outbreak of infection with *A. astaci* or North American ~~carrier~~ crayfish species).

4. North American crayfish should not be brought onto the site.

5. Finfish obtained from unknown freshwater sources or from sources, where North American crayfish may be present or a current outbreak of infection with *A. astaci* may be taking place, must not be used as bait or feed for crayfish, unless they have been subject to a temperature treatment to kill *A. astaci* (see Section 2.4.5. *Inactivation methods*).

6. Any equipment that is brought onto site should be disinfected.

3. Specimen selection, sample collection, transportation and handling

3.1. Selection of populations and individual specimens

For a suspected outbreak of infection with *A. astaci* in a population of ~~highly susceptible~~ crayfish species ~~that are prone to clinical disease,~~ sampled crayfish should ideally consist of: a) live crayfish showing signs of disease, and b) live, apparently healthy crayfish. Freshly dead crayfish may also be suitable, although this will depend on their condition.

Live crayfish should be transported using insulated containers equipped with small holes to allow aeration. The temperature in the container should not exceed 16°C.

Crayfish should be transported in a moist environment ~~atmosphere~~, for example using moistened wood shavings/wood wool, newspaper, or grass/hay. Unless transport water is sufficiently oxygenated, live crayfish should not be transported in water, as they may suffocate.

Should only dead animals be found at the site of a suspected outbreak, freshly dead animals should be selected for diagnosis. Dead animals can either be: a) transported chilled (if they appear to have died only very recently), or, b) placed in non-methylated ethanol (minimum concentration 70%; see 3.5. *Preservation of samples for submission*), or c) placed in freezer at –20°C to avoid further decay and transported frozen.

When testing any population outside an acute mortality event for the presence of crayfish plague, as many individuals as possible should be inspected visually for signs of cuticular damage. Crayfish that have melanized spots or missing limbs should be selected in the first place for further analysis.

3.2. Selection of organs or tissues

~~In highly susceptible species that are prone to clinical disease,~~ The tissue recommended for sampling is the soft abdominal cuticle, which can be found on the ventral side of the abdomen. Any other soft part of the exoskeleton and eyestalks can be included as well.

If any melanised spots or whitened areas are detected, these should be included in the sampling. From diseased animals, samples should be aseptically collected from the soft abdominal cuticle. For identification of ~~carriers~~ reservoirs, samples should be aseptically collected from soft abdominal cuticle, and telson and uropods, separately.

In the North American crayfish species, sampling of soft abdominal cuticle, uropods and telson are recommended. Any other soft part of the exoskeleton can be included as well. If any melanized spots are detected, these should be included in the sampling.

3.3. Samples or tissues not suitable for pathogen detection

Autolysed material is not suitable for analysis.

3.4. Non-lethal sampling

A non-destructive sampling method that detects *A. astaci* DNA in the microbial biofilm associated with the cuticle of individual crayfish through vigorous scrubbing has been described (Pavic *et al.,* 2020), and could be considered in case of testing vulnerable populations.

If non-lethal tissue sample types differ from recommended tissues (see Section 3.2.), or from the tissue samples used in validation studies, the effect on diagnostic performance should be considered.

3.5. Preservation of samples for submission

The use of non-preserved crayfish is preferred, as described above. If transport of recently dead or moribund crayfish cannot be arranged, crayfish may be frozen or fixed in ethanol (minimum 70%). However, fixation may reduce test sensitivity. The crayfish:ethanol ratio should ideally be 1:10 (1 part crayfish, 10 parts ethanol).

For guidance on sample preservation methods for the intended test methods, see Chapter 2.2.0 *General information* (diseases of crustaceans)

3.5.1. Samples for pathogen isolation

The success of pathogen isolation depends strongly on the quality of samples (time since collection and time in storage). Isolation is best attempted from crayfish with clinical signs delivered alive (see Section 3.1.). Fresh specimens should be kept chilled and preferably sent to the laboratory within 24 hours of collection.

3.5.2. Preservation of samples for molecular detection

Tissue samples for PCR testing should be preserved in 70–90% (v/v) analytical/reagent-grade (undenatured) 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 2.2.0 *General information* (diseases of crustaceans).

3.5.3. Samples for histopathology

Standard sample collection, preservation and processing methods for histological techniques can be found in Section 2.2 of Chapter 2.2.0 *General information* (diseases of crustaceans).

3.5.4. Samples for other tests

Sensitive molecular methods can be used to detect *A. astaci* DNA directly from water samples (Strand *et al*. 2011, 2012). These methods require validation for diagnostic use.

3.6. Pooling of samples

Pooling of samples from more than one individual animal for a given purpose ~~should only be~~ is only recommended where robust supporting data on diagnostic sensitivity and diagnostic specificity have been evaluated and found to be suitable. The effect of pooling on diagnostic sensitivity has not been thoroughly evaluated, therefore, larger animals should be processed and tested individually. Small life stages such as PL, can be pooled to obtain the minimum amount of material for 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	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						+	+	NA				
Histopathology						+	+	NA				
~~Cell~~ Culture						+	+	NA				
Real-time PCR	++	++	++	1	++	++	++	1	++	++	++	1
Conventional PCR	+	+	+	1	++	++	++	1				
Conventional PCR followed by amplicon sequencing									+++	+++	+++	1
In-situ hybridisation												
Bioassay												
~~LAMP~~ Immunohistochemistry												
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); NA = not available. 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

Small pieces of soft cuticle excised from the regions mentioned above (Section 2.3.3 *Gross pathology*) and examined under a compound microscope using low-to-medium power will confirm the presence of aseptate fungal-like hyphae 7–9 µm wide. The hyphae can usually be found pervading the whole thickness of the cuticle, forming a three-dimensional network of hyphae in heavily affected areas of the cuticle. The presence of host haemocytes and possibly some melanisation closely associated with and encapsulating the hyphae give good presumptive evidence that the hyphae represent a pathogen rather than a secondary opportunist invader. In some cases, examination of the surface of such mounted cuticles will demonstrate the presence of characteristic *A. astaci* sporangia with clusters of encysted primary spores ~~(see Section 4.3 *Culture for isolation*)~~.

4.2. Histopathology

Unless the selection of tissue for fixation has been well chosen, *A. astaci* hyphae can be difficult to find in stained preparations. A histological staining technique, such as the Grocott silver stain counterstained with conventional haematoxylin and eosin, can be used to visualise *A. astaci* in tissues of crayfish species prone to clinical disease. However, such material does not prove that any hyphae observed are those of *A. astaci*, especially when the material comes from animals already dead by sampling.

See also Section 4.1 *Wet mounts*.

4.3. Culture for isolation

Isolation is not recommended as a routine diagnostic method (Alderman & Polglase, 1986; Cerenius *et al*., 1987; Viljamaa-Dirks, 2006). Test sensitivity and specificity of the cultivation method can be very variable depending on the experience of the examiner, but in general will be lower than the PCR. Isolation of *A. astaci* by culture from apparently healthy crayfish is challenging and molecular methods are recommended. A detailed description of this test is available from the Reference Laboratory[1].

4.4. Nucleic acid amplification

PCR assays should always be run with the controls specified in Section 5.5 '*Use of molecular and antibody-based techniques for confirmatory testing' and diagnosis* of Chapter 2.2.0 *General information* (diseases of crustaceans). Each sample should be tested in duplicate. Shrimp tissues may be used as negative controls.

Live crayfish can be killed using chloroform, electric current or by mechanical destroying the nerve cords. If live or moribund animals are not available, only recently dead animals should be used for DNA extraction. The soft abdominal cuticle is the preferred sample tissue for DNA extraction. Any superficial contamination should first be removed by thoroughly wiping the soft abdominal cuticle with wet (using autoclaved H_2O) clean disposable swabs. The soft abdominal cuticle is then excised and 30–50 mg of tissue is homogenised using standard physical methods ~~ground using a pestle and mortar.~~

Several PCR assays have been developed with varying levels of sensitivity and specificity. Two assays are described here. Both assays target the ITS (internal transcribed spacer) region of the nuclear ribosomal gene cluster within the *A. astaci* genome.

Extraction of nucleic acids

~~Numerous~~ Different kits and procedures can be used for nucleic acid extraction. The quality and concentration of the extracted nucleic acid is important and ~~should~~ can be checked using a suitable method as appropriate to the circumstances ~~using optical density or running a gel~~.

4.4.1. Real-time PCR

The following controls should be run with each assay: negative extraction control; positive control; no template control; internal PCR control.

Pathogen/ target gene	Primer/probe (5'–3')	Concentration	Cycling conditions[(a)]

[1] https://www.woah.org/en/what-we-offer/expertise-network/reference-laboratories/#ui-id-3

Method 1*: Vralstad *et al.,* 2009, ~~Strand, 2013~~; GenBank Accession No.: AM947024			
Aphanomyces ~~astacus~~ astaci & *A. fennicus*/ ITS	Fwd: AAG-GCT-TGT-GCT-GGG-ATG-TT Rev: CTT-CTT-GCG-AAA-CCT-TCT-GCT-A Probe: 6-FAM-TTC-GGG-ACG-ACC-C-MGBNFQ	500 nM 500 nM 200 nM	50 cycles of: 95°C/15 sec and ~~60~~58°C/~~30~~ 60 sec
Method 2: Strand *et al.*, 2023; GenBank Accession No.: AM947024			
Aphanomyces astacus astaci/ITS	Fwd: TAT-CCA-CGT-GAA-TGT-ATT-CTT-TAT Rev: GCT-AAG-TTT-ATC-AGT-ATG-TTA-TTT-A Probe: FAM-AAG-AAC-ATC-CCA-GCA-C-MGBNFQ	500 nM 500 nM 100 nM	50 cycles of: 95°C/15 sec and 60°C/30 sec

*These ITS-based methods have been found to give positive results for the species *Aphanomyces fennicus* (Viljamaa-Dirks & Heinikainen 2019).
[a]A denaturation step prior to cycling has not been included.

The absolute limit of detection of method 1 was reported as approximately 5 PCR forming units (= target template copies)~~, which is equivalent to less than one *A. astaci* genome~~ (Vralstad *et al*., 2009). Another study reported consistent detection down to 50 fg DNA using this assay (Tuffs & Oidtmann, 2011).

Analytical test specificity has been investigated (Tuffs & Oidtmann, 2011; Vralstad *et al*., 2009) and no cross-reaction was observed in these studies. However, a novel species, *Aphanomyces fennicus*, isolated from noble crayfish was reported in 2019 (Viljamaa-Dirks & Heinikainen, 2019) that gave a positive reaction in this test at the same level as *A. astaci*. Due to this problem in specificity, ~~A modified alternative method for~~ the assay ~~will be included once it has been published~~ has been modified according to the alternative method 2 (Strand *et al*., 2023 ~~manuscript in preparation~~).

Owing to the repeated discovery of new *Aphanomyces* strains, sequencing is required to determine the species of *Aphanomyces*~~.~~ in the case of ~~the non-negative~~ an amplification product in the real-time PCR assay result. This requires separate amplification of a PCR product ~~using primers ITS-1 and ITS-4~~ (see Section 4.5 *Amplicon sequencing*).

4.4.2. Conventional PCR

Pathogen/ target gene	Primer~~/probe~~ (5'–3')	Concentration	Cycling conditions[a]
Method 1*: Oidtmann *et al.,* 2006; GenBank Accession No.: AY310499; ~~Product~~ amplicon size: 569 bp			
Aphanomyces ~~astacus~~ astaci & *A. fennicus*/ ITS	Fwd: GCT-TGT-GCT-GAG-GAT-GTT-CT Rev: CTA-TCC-GAC-TCC-GCA-TTC-TG-	500 nM 500 nM	40 cycles of: 1 min/96°C, 1 min/59°C and 1 min/72°C

*This ITS-based method has been found to give positive results for the species *Aphanomyces fennicus* (Viljamaa-Dirks & Heinikainen 2019).
[a]A denaturation step prior to cycling has not been included.

Confirmation of the identity of the PCR product by sequencing is required as a novel species, *A. fennicus*, isolated from noble crayfish was reported in 2019 (Viljamaa-Dirks & Heinikainen, 2019) that gave a positive reaction in this assay.

The assay consistently detects down to 500 fg of genomic target DNA or the equivalent amount of ten zoospores submitted to the PCR reaction (Tuffs & Oidtmann, 2011).

4.4.3. Other nucleic acid amplification methods

Several genotype-specific molecular methods have been developed that, instead of requiring a pure growth as sample material like the RAPD-PCR assay, can be used to analyse crayfish tissue directly (Di Domenico *et al*., 2021; Grandjean *et al*., 2014; Makkonen *et al.,* 2018; Minardi *et al*., 2018; 2019). Detection of a known genotype group combined with a positive result by a recommended conventional or real-time PCR can be used as a confirmative

test in geographical areas where crayfish plague is known to be present. However, the current knowledge of the genotype variation is mostly limited to a few original host species and new genotypes or subtypes are expected to be found. Thus, the suitability of these methods is limited for initial excluding diagnosis or as confirmative tests in geographical areas not known to be infected.

PCR targeting mitochondrial DNA with *A. astaci* genotype specific primers have been shown to detect the known genotypes of *A. astaci*, but these assays may also provide positive results for some other oomycete genera (Casabella-Herrero *et al.,* 2021).

4.5. Amplicon sequencing

~~, and purified by excision from this gel.~~ Both DNA strands of the PCR product must be sequenced and analysed ~~and compared~~ in comparison with ~~published~~ reference sequences.

4.6. *In-situ* hybridisation

Not available.

4.7. Immunohistochemistry

Not available

4.8. Bioassay

No longer used for diagnostic purposes (see Cerenius *et al*., 1988).

4.9. Antibody- or antigen-based detection methods

Not available.

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

The recommended method for surveillance is real-time PCR utilising the modified assay by Strand *et al*. (2023 ~~manuscript in preparation~~).

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 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 suspect and confirmed cases 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. ~~It is recommended that all samples that yield suspect positive test results in an otherwise pathogen-free country or zone or compartment should be referred immediately to the WOAH Reference Laboratory for confirmation, whether or not clinical signs are associated with the case.~~ If a ~~laboratory~~ Competent Authority does not have the ~~capacity~~ 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.

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. Geographic 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

[2] For example transboundary commodities.

The presence of infection with *Aphanomyces astaci* shall be suspected if at least one of the following ~~criterion~~ criteria is met:

i) Positive result by real-time PCR

ii) Positive result by conventional PCR

6.1.2. Definition of confirmed case in apparently healthy animals

The presence of infection with *Aphanomyces astaci* is considered to be confirmed if the following criterion is met:

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

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 *Aphanomyces astaci* 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) Visual observation of hyphae indicative of *A. astaci* in wet mounts

iii) Observation of hyphae indicative of *A. astaci* in stained histological sections

iv) Culture and isolation of the pathogen

v) Positive result by real-time PCR

vi) Positive result by conventional PCR

6.2.2. Definition of confirmed case in clinically affected animals

The presence of infection with *Aphanomyces astaci* is confirmed if the following criterion is met:

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

6.3. Diagnostic sensitivity and specificity for diagnostic tests

The diagnostic performance of tests recommended for surveillance or diagnosis of infection with *Aphanomyces astaci* are provided in Tables 6.3.1. and 6.3.2 (~~none~~ no data are currently available for either). ~~This information can be used for the design of surveys for infection with *Aphanomyces astaci*, however, it should be noted that diagnostic performance is specific to the circumstances of each diagnostic accuracy study (including the test purpose, source population, tissue sample types and host species) and diagnostic performance may vary under different conditions.~~ 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

Test type	Test purpose	Source populations	Tissue or sample types	Species	DSe (*n*)	DSp (*n*)	Reference test	Citation
Real-time PCR	Distinguish between *A. astaci* and *A. finnicus*		Mycelium, tissue samples	*Astacus astacus*		Only detected *A. astacus*		Strand *et al.*, 2023

DSe = diagnostic sensitivity, DSp = diagnostic specificity, *n* = ~~samples~~ number of animals used in the validation study.

6.3.2. For surveillance of apparently healthy animals

Test type	Test purpose	Source populations	Tissue or sample types	Species	DSe (*n*)	DSp (*n*)	Reference test	Citation
Real-time PCR	Distinguish between *A. astaci* and *A. finnicus*		Tissue samples, environmental DNA	*Astacus astacus*		Only detected *A. astacus*		Strand *et al.*, 2023

DSe = diagnostic sensitivity, DSp = diagnostic specificity, *n* = ~~samples~~ number of animals used in the validation study.

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* *

NB: There is a WOAH Reference Laboratory for infection with *Aphanomyces astaci* (crayfish plague)
(please consult the WOAH web site for the most up-to-date list:
https://www.woah.org/en/what-we-offer/expertise-network/reference-laboratories/#ui-id-3).
Please contact the WOAH Reference Laboratories for any further information on
infection with *Aphanomyces astaci* (crayfish plague)

NB: FIRST ADOPTED IN 1995; MOST RECENT UPDATES ADOPTED IN 2017.

Annexe 53. Point 9.1.3. – Chapitre 2.2.6. « Infection par le nodavirus de Macrobrachium rosenbergii (maladie des queues blanches) »

CHAPTER 2.2.6.

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

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.

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).

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 *Macrobrachium satellite virus 1* of the family *Sarthroviridae.*

2.1.2. Survival and stability in processed or stored samples

Both viral pathogens (MrNV and XSV) are stable in processed or stored samples stored at –20 or –80°C. Storing the samples at –80°C is recommended for long-time storage and maintenance of pathogen virulence (Sahul Hameed & Bonami, 2012). The infected samples should be processed at low temperature to maintain the stability of the viruses. Viral inoculum prepared from infected prawn stored at –20°C caused 100% mortality in postlarvae (PL) of *M. rosenbergii* by immersion challenge (Qian *et al*., 2003; Sahul Hameed *et al*., 2004a). Ravi & Sahul Hameed (2016) found that MrNV in tissue suspensions was inactivated after exposure to 50°C for at least 5 min.

2.1.3. Survival and stability outside the host

Survival outside the host is not known.

2.2. Host factors

2.2.1. Susceptible host species

Species that fulfil the criteria for listing as susceptible to infection with MrNV according to Chapter 1.5. of the *Aquatic Animal Health Code* (*Aquatic Code*) are: giant river prawn (*Macrobrachium rosenbergii*).

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 MrNV according to Chapter 1.5. of the *Aquatic Code* are: white leg shrimp (*Penaeus vannamei*).

In addition, pathogen-specific positive polymerase chain reaction (PCR) results (but not active infection) have been reported in the following species:

Family	*Scientific name*	*Common name*
~~*Aeshnidae*~~	~~*Aeshna* sp.~~	~~dragonfly~~
Artemiidae	*Artemia* sp.	brine shrimps
~~*Belostomatidae*~~	~~*Belostoma* sp.~~	~~giant water bug~~
~~*Dytiscidae*~~	~~*Cybister* sp.~~	~~beetle~~
~~*Notonectidae*~~	~~*Notonecta* sp.~~	~~backswimmer~~
Palaemonidae	*Macrobrachium rude*	hairy river prawn
	Macrobrachium malcolmsonii	monsoon river prawn
Parastacidae	*Cherax quadricarinatus*	red claw crayfish
Penaeidae	*Penaeus japonicus*	kuruma prawn
	Penaeus indicus	Indian white prawn
	Penaeus monodon	giant tiger prawn

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

Experimental pathogenicity studies revealed that larvae, PL and early juveniles of *M. rosenbergii* are susceptible to MrNV/XSV, whereas adults are resistant (Gangnonngiwa *et al.,* 2020; Qian *et al*., 2003; Sahul Hameed *et al*., 2004a).

No mortality was observed either in naturally or experimentally ~~(~~MrNV/XSV~~)~~ infected subadult and adult prawns. Experimental studies confirmed vertical transmission from infected broodstock to PL (Sudhakaran *et al*., 2007a).

2.2.4. Distribution of the pathogen in the host

MrNV and XSV have been demonstrated in gill tissue, head muscle, heart, abdominal muscle, ovaries, pleopods and tail muscle, but not the hepatopancreas or eyestalk (Sahul Hameed *et al*., 2004a; Sri Widada *et al*., 2003).

2.2.5. Aquatic animal reservoirs of infection

~~One study has~~ Studies have indicated ~~the possibility~~ that marine shrimp may act as ~~a~~ reservoirs for MrNV and XSV and that these viruses maintain virulence in the shrimp tissue system (Senapin *et al.,* 2012; Sudhakaran *et al*., 2006).

2.2.6. Vectors

Aquatic insects such as dragonfly (*Aeshna* sp.), giant water bug (*Belostoma* sp.), beetle (*Cybister* sp.) and backswimmer (*Notonecta* sp.) may act as mechanical carriers for MrNV/XSV and are a potential transmission risk to cultivated *Macrobrachium rosenbergii* (Sudhakaran *et al.,* 2008). It is recommended to remove these insects from freshwater prawn culture systems, especially at larval-rearing centres. Sudhakaran *et al*. (2008) demonstrated RT-PCR positives from insects, and infected C6/36 insect cell line with tissue homogenates from the insects. Viral replication was confirmed through EM and RT-PCR, but transmission from insects to naïve shrimp was not demonstrated.

2.3. Disease pattern

2.3.1. Mortality, morbidity and prevalence

Larvae, PL and juveniles of *M. rosenbergii* are susceptible to infection with MrNV, which often causes high mortalities in these life stages. Mortality may reach a maximum in about 5 or 6 days after the appearance of the first clinical signs. Very few PL with infection with MrNV survive beyond 15 days in an outbreak, but PL that survive may grow to market size. Adults are resistant to infection with MrNV, but act as carriers (Qian *et al*., 2003;

Sahul Hameed *et al*., 2004a). Prevalence is variable from 10% to 100% in hatchery, nursery and grow-out systems (Arcier *et al*., 1999; Qian *et al*., 2003; Sahul Hameed *et al*., 2004a; 2004b).

2.3.2. Clinical signs, including behavioural changes

Infected PL become opaque and develop a whitish appearance, particularly in the abdominal region. The whitish discoloration appears first in the second or third abdominal segment and gradually diffuses both anteriorly and posteriorly. In severe cases, degeneration of telson and uropods may occur. Floating exuviae (moults) in the tanks appear abnormal and resemble 'mica flakes' (Arcier *et al*., 1999). The infected PL show progressive weakening of their feeding and swimming ability (Sahul Hameed *et al*., 2004a).

2.3.3. Gross pathology

Infection with MrNV is indicated by the whitish coloration of abdominal muscle.

2.3.4. Modes of transmission and life cycle

Transmission is vertical by trans-ovum and horizontal by the waterborne route (Qian *et al*., 2003; Sahul Hameed *et al*., 2004a; Sudhakaran *et al*., 2007a).

2.3.5. Environmental factors

Not available.

2.3.6. Geographical distribution

The disease was first reported in the ~~French West Indies~~ Caribbean (Arcier *et al*., 1999), and later in Asia-Pacific (Murwantoko *et al*., 2016; Owens *et al*., 2009; Qian *et al*., 2003; Saedi *et al*., 2012; Sahul Hameed *et al*., 2004b; Wang *et al*., 2008; Yoganandhan *et al*., 2006).

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

2.4. Biosecurity and disease control strategies

Preventive measures, such as screening of broodstock and PL, and good management practices may help to prevent infection with MrNV in culture systems. As the life cycle of *M. rosenbergii* is completed under controlled conditions, specific pathogen-free (SPF) broodstock and PL can be produced (Romestand & Bonami, 2003; Sri Widada *et al.,* 2003; Yoganandhan *et al.,* 2005).

2.4.1. Vaccination

Not available

2.4.2. Chemotherapy including blocking agents

No known chemotherapeutic agents are reported to treat MrNV-infected prawn.

2.4.3. Immunostimulation

The immunomodulatory effect of recombinant capsid protein and recombinant RNA-dependent RNA polymerase (RdRp) protein of MrNV has been studied and the protection of viral challenged post-larvae from MrNV infection has been demonstrated (Farook *et al*., 2014; NaveenKumar *et al*., 2021).

2.4.4. Breeding resistant strains

None reported.

2.4.5. Inactivation methods

A viral suspension treated with heat at 65°C for 2 hours destroyed infectivity of MrNV and XSV in challenge experiments (Qian *et al*., 2003). The viral inoculum exposed to UV irradiation for a period of 5 minutes and more was totally inactivated and failed to cause mortality in prawn PL ~~of prawn~~ (Ravi & Sahul Hameed, 2016).

2.4.6. Disinfection of eggs and larvae

Routine disinfection procedures followed for crustacean viral disease control are suggested.

2.4.7. General husbandry

MrNV is transmitted both horizontally and vertically in culture systems (Qian *et al*., 2003; Sahul Hameed *et al*., 2004a; Sudhakaran *et al*., 2007a). Good husbandry practices, such as proper disinfection of tanks and water may help to prevent infection. It is recommended to remove insects from freshwater prawn culture systems, especially at larval-rearing centres. Specific pathogen-free (SPF) broodstock and PL can be obtained from disease free populations or by RT-PCR screening and selection of negative broodstock (Romestand & Bonami, 2003; Sri Widada *et al.,* 2003; Yoganandhan *et al.,* 2005).

3. Specimen selection, sample collection, transportation and handling

3.1. Selection of populations and individual specimens

PLs are most suitable for detection of MrNV. PL showing clinical signs of disease can be sampled preferentially. Adults and juveniles can be sampled for MrNV however prevalence in these lifestages may be lower (see Section 2.3.1).

3.2. Selection of organs or tissues

The tissues most affected in moribund PLs/early juveniles are striated muscles of the abdomen, cephalothorax and tail. The whole PL body is preferred for detection of MrNV (Sahul Hameed *et al.,* 2004b; Sri Widada *et al.,* 2003; Yoganandhan *et al*., 2005). All organs of adult *M. rosenbergii* except eyestalks and the hepatopancreas, are best for screening the viruses by RT-PCR.

3.3. Samples or tissues not suitable for pathogen detection

Eyestalks and the hepatopancreas of adult prawns are not suitable (Sri Widada *et al.,* 2003; Sahul Hameed *et al.,* 2004a).

3.4. Non-lethal sampling

Pleopods (swimming legs) are a convenient source of RNA for non-destructive screening of MrNV in adult prawn (Sahul Hameed *et al.,* 2004a).

If non-lethal tissue sample types differ from recommended tissues (see Section 3.2.), or from the tissue samples used in validation studies, the effect on diagnostic performance should be considered.

3.5. Preservation of samples for submission

Infected larvae or PL with prominent signs of whitish muscle in the abdominal region are collected from disease outbreak areas. Samples are washed in sterile saline, transferred to sterile tubes, and transported to the laboratory. For general guidance on sample preservation methods for the intended test methods, see Chapter 2.2.0 *General information* (diseases of crustaceans).

3.5.1. Samples for pathogen isolation

Moribund or frozen PL samples can be used for isolation of viral pathogens using cell lines (C6/36 mosquito cell line (Sudhakaran et al., 2007b).

3.5.2. Preservation of samples for molecular detection

Infected samples stored at –80°C or samples preserved in 80% (v/v) analytical/reagent-grade (undenatured) ethanol should be used for RT-PCR for detection of MrNV (Sri Widada *et al.,* 2003; Sahul Hameed *et al.,* 2004b; Yoganandhan *et al*., 2005).

Standard sample collection, preservation and processing methods for molecular techniques can be found in Section B.5.5. of Chapter 2.2.0 *General information* (diseases of crustaceans).

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

Tissue samples for histopathology, immunohistochemistry or *in-situ* hybridisation should be fixed immediately after collection in neutral-buffered formalin or modified Davidson's fixative (Sri Widada *et al.,* 2003). The recommended ratio of fixative to tissue is 10:1. Standard sample collection, preservation and processing methods

for histological techniques can be found in Section 5.3. of Chapter 2.2.0 *General information* (diseases of crustaceans).

3.5.4. Samples for other tests

Not applicable

3.6. Pooling of samples

Pooling of samples from more than one individual animal for a given purpose ~~should only be~~ 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 animals should be processed and tested individually. Small life stages such as PL, can be pooled to obtain the minimum amount of material for virus isolation or molecular detection.

4. Diagnostic methods

The methods currently available for identifying infection 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 life stage.

Ratings against purposes of use. For each recommended assay a qualitative rating against 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, successful application by diagnostic laboratories, cost, timeliness, and sample throughput. For a specific purpose of use, assays are rated as:

+++ =	Most suitable methods – desirable performance and operational characteristics;
++ =	Suitable method(s) acceptable performance and operational characteristics under most circumstances;
+ =	Less suitable methods – performance or operational characteristics may significantly limit application;
Shaded boxes =	Not appropriate for this purpose.

Level of validation. 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). 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 for MrNV 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						++	++	NA				
Cell culture												
Real-time RT-PCR	+++	+++	+++	1	+++	+++	+++	1	+++	+++	+++	1
Conventional RT-PCR	++	++	++	1	+++	+++	+++	2				
Conventional RT-PCR followed by amplicon sequencing									+++	+++	+++	2
In-situ hybridisation						++	++	1		++	++	1
Bioassay												
LAMP	++	++	++	1	++	++	++	1				
Ab-ELISA												
Ag-ELISA					++	++	++	1				
Lateral flow assay					++	++	++	2				
Other methods[3]												

LV = level of validation, refers to the stage of validation in the WOAH Pathway (chapter 1.1.2); NA = not available; 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

None to date

4.2. Histopathology and cytopathology

The most affected tissue in infected PL is striated muscle of the cephalothorax, abdomen and tail. Histological features include the presence of acute Zenker's necrosis of striated muscles, characterised by severe hyaline degeneration, necrosis and muscular lysis. Moderate oedema and abnormal open spaces among the affected muscle cells are also observed, as is the presence of large oval or irregular basophilic cytoplasmic inclusion bodies in infected muscles (Arcier *et al.,* 1999; Hsieh *et al.,* 2006).

4.3. Cell culture for isolation

MrNV has been isolated in insect cell lines, but this is not a recommended method (Hernandez-Herrera *et al.,* 2007; Sudhakaran *et al.,* 2007b).

4.4. Nucleic acid amplification

PCR methods for MrNV and XSV are included in this section for completeness. However, the case definitions in Section 6 are based on detection methods for MrNV only.

PCR assays should always be run with the controls specified in Section 5.5. *Use of molecular techniques for confirmatory testing and diagnosis* of Chapter 2.2.0 *General information* (diseases of crustaceans). 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 RT-PCR

Real-time RT-PCR assay can be performed using the SYBR Green dye based on the method described by Hernandez-Herrera *et al*. (2007) or the TaqMan assay described by Zhang *et al*. (2006).

Pathogen / target gene	Primer/probe (5'–3')	Concentration	Cycling parameters[(a)]
Method 1: Hernandez-Herrera *et al.* (2007); GenBank Accession No.: AY222839)			
MrNv/RNA1	Fwd: AGG-ATC-CAC-TAA-GAA-CGT-GG Rev: CAC-GGT-CAC-AAT-CCT-TGC-G	500 nM 500 nM	40 cycles of: 95°C/15 sec, 60°C/5 sec and 72°C/10 sec
Method 2: Zhang *et al.* (2006); GenBank Accession No.: AY231436)			
MrNv/RNA1	Fwd: CAA-CTC-GGT-ATG-GAA-CTC-AAG-GT Rev: AGG-AAA-TAC-ACG-AGC-AAG-AAA-AGT-C Probe: FAM-ACC-CTT-CGA-CCC-CAG-CAA-TGG-TG-TAMARA	1000 nM 1000 nM 400 nM	50 cycles of: 94°C/30 sec and 58°C/30 sec
Method 3: Zhang *et al.* (2006); GenBank Accession No.: DQ174318)			
XSV	Fwd: AGC-CAC-ACT-CTC-GCA-TCT-GA Rev: CTC-CAG-CAA-AGT-GCG-ATA-CG Probe: FAM-CAT-GCC-CCA-TGA-TCC-TCG-CA-TAMARA	1000 nM 1000 nM 400 nM	50 cycles of: 94°C/30 sec and 58°C/30 sec

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

4.4.2. Conventional RT-PCR

The protocol for the conventional RT-PCR for detection of MrNV/XSV developed by ~~Sri Widada *et al.* (2003),~~ Sahul Hameed *et al.* (2004a; 2004b) and Sudhakaran *et al*. (2007a) is recommended. MrNV and XSV can be detected by conventional RT-PCR separately using a specific set of primers or these two viruses can be detected simultaneously using a single-tube one-step multiplex RT-PCR (Yoganandhan *et al*., 2005). ~~Conventional real-time RT-PCR is recommended in situations where high sensitivity is required.~~

Pathogen / target gene	Primer (5'–3')	Concentration	Cycling parameters[(a)]
Method 1: One step RT-PCR (~~Sri Widada *et al.*, 2003;~~ Sahul Hameed *et al*., 2004a, b; Sudhakaran *et al*., 2007a) GenBank Accession No.: AY222840 (MrNV) and AY247793 (XSV); amplicon size: 425 bp (MrNv) and 546 bp (XSV)			
MrNv	Fwd: GCG-TTA-TAG-ATG-GCA-CAA-GG Rev: AGC-TGT-GAA-ACT-TCC-ACT-GG	~~0.02 nM~~ 400 nM ~~0.02 nM~~ 400 nM	30 cycles of: 94°C/40 sec, 55°C/40sec and 68°C/60 sec
XSV	Fwd: CGC-GGA-TCC-GAT-GAA-TAA-GCG-CAT-TAA-TAA Rev: CCG-GAA-TTC-CGT-TAC-TGT-TCG-GAG-TCC-CAA	~~0.02 nM~~ 400 nM ~~0.02 nM~~ 400 nM	30 cycles of: 94°C/40 sec, 55°C/40 sec and 68°C/60 sec
Method 2: nested RT-PCR using above-mentioned primers as external primers (Sudhakaran *et al*., 2007a); amplicon size: 205 bp (MrNv) and 236 bp (XSV)			
MrNv	External primers: as for Method 1. Internal primers: Fwd: GAT-GAC-CCC-AAC-GTT-ATC-CT Rev: GTG-TAG-TCA-CTT-GCA-AGA-GG	~~0.02 nM~~ 1000 nM ~~0.02 nM~~ 1000 nM	30 cycles of: 94°C/60 sec, 55°C/60 sec and 72°C/60 sec
XSV	External primers: as for Method 1. Internal primers: Fwd: ACA-TTG-GCG-GTT-GGG-TCA-TA Rev: GTG-CCT-GTT-GCT-GAA-ATA-CC-3	~~0.02 nM~~ 1000 nM ~~0.02 nM~~ 1000 nM	30 cycles of: 94°C/60 sec, 55°C/60 sec and 72°C/60 sec
Method 3: Multiplex RT-PCR (Yoganandhan *et al*., 2005); GenBank Accession No.: AY222840 (MrNV) and AY247793 (XSV); amplicon size: 681 bp (MrNv) and 500 bp (XSV)			
MrNV	Fwd: GAT-ACA-GAT-CCA-CTA-GAT-GAC-C Rev: GAC-GAT-AGC-TCT-GAT-AAT-CC	~~0.02 nM~~ 400 nM ~~0.02 nM~~ 400 nM	30 cycles of: 94°C/40 sec, 55°C/40 sec and 68°C/60 sec
XSV	Fwd: GGA-GAA-CCA-TGA-GAT-CAC-G Rev: CTG-CTC-ATT-ACT-GTT-CGG-AGT-C	~~0.02 nM~~ 400 nM ~~0.02 nM~~ 400 nM	30 cycles of: 94°C/40 sec, 55°C/40 sec and 68°C/60 sec

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

4.4.3. Other nucleic acid amplification methods: Loop-mediated isothermal amplification (LAMP)

Haridas *et al*. (2010) have applied loop-mediated isothermal amplification (LAMP) for rapid detection of MrNV and XSV in the freshwater prawn. A set of four primers, two outer primers and two inner primers, have been designed separately for detection of MrNV and XSV.

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

The presence of MrNV in infected cells can be demonstrated in histological sections using a DIG-labelled DNA *in-situ* hybridisation probe specific for MrNV (Sri Widada *et al.,* 2003).

4.7. Immunohistochemistry

None developed.

4.8. Bioassay

Not used for diagnostic purposes.

4.9. Antibody- or antigen-based detection methods

4.9.1. ELISA

Antibody-based diagnostic methods for MrNV include the ELISA described by Romestand & Bonami (2003) or the triple-antibody sandwich (TAS) ELISA based on a monoclonal antibody (Qian *et al.,* 2006).

4.9.2. Lateral flow assay (LFA)

An antibody-based lateral flow assay (LFA) has been developed for the early detection of MrNV in the PL stage (Jamalpure *et al.*, 2021).

4.10. Other methods

None

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

Real-time RT-PCR is recommended for targeted surveillance to declare freedom from infection with MrNV.

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 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 suspect and confirmed cases 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.

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 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 MrNV shall be suspected if at least one of the following criteria is met:

i) Positive result by real-time RT-PCR

ii) Positive result by conventional RT-PCR

iii) Positive result by LAMP

[3] For example transboundary commodities.

6.1.2. Definition of confirmed case in apparently healthy animals

The presence of infection with MrNV is considered to be confirmed if the following criterion is met:

i) Positive result by real-time RT-PCR result and positive result by conventional RT-PCR and sequence analysis

6.2 Clinically affected animals

6.2.1. Definition of suspect case in clinically affected animals

The presence of infection with MrNV shall be suspected if at least one of the following criteria is met:

i) Clinical signs consistent with infection by MrNV

ii) Histopathology consistent with infection by MrNV

iii) Positive result by real-time RT-PCR

iv) Positive result by conventional RT-PCR

v) Positive result by *in situ* hybridisation

vi) Positive result by LAMP

vii) Positive result by Ag ELISA

viii) Positive result by lateral flow assay

6.2.2. Definition of confirmed case in clinically affected animals

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

i) Positive result by real time RT-PCR and positive result by conventional RT-PCR with sequence analysis

ii) Positive result by ISH followed by positive result by conventional RT-PCR with sequence analysis

iii) Positive result by ISH followed by positive result by real-time RT-PCR

6.3. Diagnostic sensitivity and specificity for diagnostic tests

The diagnostic performance of tests recommended for surveillance or diagnosis of infection with MrNV are provided in Tables 6.3.1. and 6.3.2 (no data are currently available). This information can be used for the design of surveys for infection with MrNV, however, it should be noted that diagnostic performance is specific to the circumstances of each diagnostic accuracy study (including the test purpose, source population, tissue sample types and host species) and diagnostic performance may vary under different conditions. 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

Test type	Test purpose	Source populations	Tissue or sample types	Species	DSe (*n*)	DSp (*n*)	Reference test	Citation
RT-PCR	Diagnosis	Clinically affected PL from hatchery and nursery	Whole post-larvae	*Macrobrachium rosenbergii*	100 (*n*=20)	100 (*n*=20)	Western blot or ELISA	~~Sri Widada *et al.* (2003);~~ Sahul Hameed *et al.* (2011)
Lateral flow immune-assay	Surveillance	PL from prawn hatcheries	Whole post-larvae	*Macrobrachium rosenbergii*	100 (*n*=80)	90 (*n*=80)	RT-PCR	Jamalpure *et al.* (2021)

DSe = diagnostic sensitivity, DSp = diagnostic specificity, *n* = ~~samples~~ number of animals used in the validation study,
RT-PCR: = reverse transcription polymerase chain reaction.

6.3.2. For surveillance of apparently healthy animals

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

DSe = diagnostic sensitivity, DSp = diagnostic specificity, *n* = *n* = ~~samples~~ number of animals used in the validation study,
RT-PCR: = reverse transcription polymerase chain reaction.

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* *

NB: There is a WOAH Reference Laboratory for infection with *Macrobrachium rosenbergii* nodavirus (white tail disease) (please consult the WOAH web site: https://www.woah.org/en/what-we-offer/expertise-network/reference-laboratories/#ui-id-3) any further information on infection with *Macrobrachium rosenbergii* nodavirus (white tail disease)

NB: FIRST ADOPTED IN 2009. MOST RECENT UPDATES ADOPTED IN 2017.

Annexe 54. Point 9.1.4. – Chapitre 2.2.9. « Infection par le génotype 1 du virus de la tête jaune »

CHAPTER 2.2.9

INFECTION WITH YELLOW HEAD VIRUS GENOTYPE 1

1. Scope

Infection with yellow head virus genotype 1 means infection with the pathogenic agent yellow head virus genotype 1 (YHV1) of the Genus *Okavirus* and Family *Roniviridae*.

2. Disease information

2.1. Agent factors

2.1.1. Aetiological agent

Yellow head virus genotype 1 (YHV1; species *Yellow head virus*) is one of eight known genotypes in the yellow head complex of viruses and is the only known genotype that causes yellow head disease. YHV1 forms enveloped, rod-shaped particles 40–50 nm × 150–180 nm (Chantanachookin *et al.,* 1993; Wongteerasupaya *et al*., 1995). Envelopes are studded with prominent peplomers projecting approximately 11 nm from the surface. Nucleocapsids appear as rods (diameter 20–30 nm) and possess a helical symmetry with a periodicity of 5–7 nm. Virions comprise three structural proteins (nucleoprotein p20 and envelope glycoproteins gp64 and gp116) and a ~26 kb positive-sense single-stranded RNA genome. The nucleotide sequence of the ORF1b region of the viral genome has been used to determine the phylogenetic relationships of YHV1 and other yellow head virus genotypes (Dong *et al*., 2017; Mohr *et al*., 2015; Wijegoonawardane *et al.,* 2008a).

YHV1, yellow head virus genotype 2 (YHV2; species *Gill-associated virus*) and yellow head virus genotype 8 (YHV8; species *Okavirus 1*) have been formally classified by the International Committee on Taxonomy of Viruses (Walker *et al*., 2021). Four other genotypes in the complex (YHV3–YHV6) occur commonly in healthy *Penaeus monodon* in East Africa, Asia and Australia and are rarely or never associated with disease (Walker *et al.,* 2001; Wijegoonawardane *et al.,* 2008a). Of the remaining two yellow head virus genotypes, YHV7 was detected in diseased *P. monodon* in Australia (Mohr *et al*., 2015) and YHV8 was detected in *P. chinensis* suspected of suffering from acute hepatopancreatic necrosis disease (Liu *et al*., 2014). There is evidence of genetic recombination between genotypes (Wijegoonawardane *et al.*, 2009).

2.1.2. Survival and stability in processed or stored samples

YHV1, identified by either transmission electron microscopy (TEM) (Nunan *et al.,* 1998), or molecular methods (Durand *et al.,* 2000; McColl *et al.,* 2004), has been detected in frozen commodity prawns with infectivity demonstrated by bioassay.

2.1.3. Survival and stability outside the host

YHV1 remains viable in aerated seawater for up to 72 hours (Flegel *et al.*, 1995b).

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 YHV1 according to Chapter 1.5 of the *Aquatic Animal Health Code* (*Aquatic Code*) are: ~~blue shrimp (*Penaeus stylirostris*), dagger blade grass shrimp~~

~~(*Palaemonetes pugio*), giant tiger prawn (*Penaeus monodon*), jinga shrimp (*Metapenaeus affinis*) and whiteleg shrimp (*Penaeus vannamei*).~~

Family	Scientific name	Common name
Palaemonidae	*Palaemonetes pugio*	dagger blade grass shrimp
Penaeidae	*Metapenaeus affinis*	jinga shrimp
	Penaeus monodon	giant tiger prawn
	Penaeus stylirostris	blue shrimp
	Penaeus vannamei	whiteleg shrimp

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 YHV1 according to Chapter 1.5 of the *Aquatic Code* are:

Family	Scientific name	Common name
Palaemonidae	*Palaemon serrifer*	carpenter prawn
	Palaemon styliferus	Pacific blue prawn
	Macrobrachium sintangense	Sunda river prawn
Parastacidae	*Cherax quadricarinatus*	red claw crayfish
Penaeidae	*Metapenaeus brevicornis*	yellow shrimp
	Penaeus aztecus	northern brown shrimp
	Penaeus duorarum	northern pink shrimp
	Penaeus japonicus	kuruma prawn
	Penaeus merguiensis	banana prawn
	Penaeus setiferus	northern white shrimp

In addition, pathogen-specific positive polymerase chain reaction (PCR) results have been reported in the

following species, but an active infection has not been demonstrated: ~~acorn barnacle (*Chelonibia patula*), blue crab (*Callinectes sapidus*), cyclopoid copepod (*Ergasilus manicatus*), gooseneck barnacle (*Octolasmis muelleri*), Gulf killifish (*Fundulus grandis*) and paste shrimp (*Acetes* sp.).~~

Family	Scientific name	Common name
Chelonibiidae	*Chelonibia patula*	acorn barnacle
Ergasilidae	*Ergasilus manicatus*	cyclopoid copepod
Fundulidae	*Fundulus grandis*	gulf killifish
Poecilasmatidae	*Octolasmis muelleri*	gooseneck barnacle
Portunidae	*Callinectes sapidus*	blue crab
Sergestidae	*Acetes sp.*	paste shrimp

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

Penaeus monodon are susceptible to YHV1 infection beyond PL15 (Khongpradit *et al.*, 1995). Lightner *et al*. (1998) YHV1 challenge caused disease in juveniles of *Penaeus aztecus, P. duorarum, P. setiferus,* and *P. vannamei* but postlarvae appeared resistant (Lightner *et al*. 1998). YHV1 infections are usually detected only when disease is evident, however infections have been detected in healthy wild populations of *P. stylirostris* (Castro-Longoria *et al.,* 2008). Natural YHV1 infections have been detected in *P. japonicus*, *P. merguiensis*, *P. setiferus*, *M. ensis*, and *P. styliferus* (Cowley *et al.,* 2002; Flegel *et al.,* 1995a; 1995b).

2.2.4. Distribution of the pathogen in the host

YHV1 targets tissues of ectodermal and mesodermal origin including lymphoid organ, haemocytes, haematopoietic tissue, gill lamellae and spongy connective tissue of the subcutis, gut, antennal gland, gonads, nerve tracts and ganglia (Chantanachookin *et al*., 1993; Lightner, 1996).

2.2.5. Aquatic animal reservoirs of infection

YHV1 was detected by reverse-transcription polymerase chain reaction (RT-PCR) in clinically normal wild *P. stylirostris* collected for surveillance purposes in the Gulf of California in 2003 (Castro-Longoria *et al*., 2008). ~~The infectious nature of the YHV1 detected was confirmed by experimental infections. There is also evidence that YHV1 can persist in survivors of experimental infection (Longyant *et al.*, 2005; 2006).~~

2.2.6. Vectors

There are no known vectors of YHV1.

2.3. Disease pattern

2.3.1. Mortality, morbidity and prevalence

In farmed *P. monodon*, YHV disease can cause up to 100% mortality within 3–5 days of the first appearance of clinical signs (Chantanachookin *et al.,* 1993). Mortalities can be induced by experimental exposure of *P. monodon* to YHV1 (Oanh *et al.,* 2011).

2.3.2. Clinical signs, including behavioural changes

Shrimp from late postlarvae (PL) stages onwards can be infected experimentally with YHV1. In cultured shrimp, infection can result in mass mortality occurring, usually in early to late juvenile stages. Moribund shrimp may exhibit a bleached overall appearance and a yellowish discoloration of the cephalothorax. However, these disease features are not particularly distinctive, and gross signs are not reliable even for preliminary diagnosis of YHV1.

Exceptionally high feeding activity followed by an abrupt cessation of feeding may occur within 2–4 days of the appearance of gross clinical signs of disease and mortality. Moribund shrimp may congregate at pond edges near the surface (Chantanachookin *et al.,* 1993).

2.3.3 Gross pathology

The yellow hepatopancreas of diseased shrimp may be exceptionally soft when compared with the brown hepatopancreas of a healthy shrimp (Chantanachookin *et al.,* 1993).

2.3.4. Modes of transmission and life cycle

YHV1 can be transmitted horizontally by ingestion of infected tissue, immersion in membrane-filtered tissue extracts, or by cohabitation with infected shrimp (Walker & Sittidilokratna, 2008). YHV1 replicates in the cytoplasm of infected cells in which long filamentous pre-nucleocapsids are abundant and virions bud into cytoplasmic vesicles in densely packed paracrystalline arrays for egress at the cytoplasmic membrane (Chantanachookin *et al.,* 1993).

2.3.5. Environmental factors

Elevated virus infection levels accompanied by disease can be precipitated by physiological stress induced by sudden changes in pH or dissolved oxygen levels, or other environmental factors (Flegel *et al*., 1997).

2.3.6. Geographical distribution

YHV1 has been reported in South-East Asia (Walker *et al.*, 2001). YHV1 has also been detected in *P. stylirostris* and *P. vannamei* in the Americas (Castro-Longoria *et al.,* 2008; Sanchez-Barajas *et al.*, 2009).

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 available.

2.4.2. Chemotherapy including blocking agents

No effective commercial anti-viral product is yet available.

2.4.3. Immunostimulation

A multi-target dsRNA for simultaneous inhibition of YHV1 and white spot syndrome virus demonstrated inhibition of the two viruses when administered to shrimp by injection (Chaimongkon *et al.,* 2020)

2.4.4. Breeding resistant strains

Not reported.

2.4.5. Inactivation methods

YHV1 can be inactivated by heating at 60°C for 15 minutes (Flegel *et al.*, 1995b). Little information is available on other inactivation methods but the virus appears to be susceptible to treatment with chlorine at 30 parts per million (0.03 mg ml^{-1}) (Flegel *et al.,* 1997).

2.4.6. Disinfection of eggs and larvae

Not reported.

2.4.7. General husbandry

The focus is to exclude YHV1 from entering production systems; for example, by using specific pathogen-free (SPF) stock, batch testing stock and biosecurity measures to reduce entry into culture systems.

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

For diagnosis during a disease outbreak, moribund shrimp collected from pond edges are the preferred source of material for examination. Apparently healthy shrimp should also be collected from the same ponds. For surveillance in populations of apparently healthy shrimp, life stages from PL stage 15 onwards can provide tissue sources useful for testing.

3.2. Selection of organs or tissues

In moribund shrimp suspected to be infected with YHV1, pleopods, gill and lymphoid organ are the most suitable sample tissues. For screening or surveillance of juvenile or adult shrimp that appear grossly normal, pleopods or gills are preferred.

3.3. Samples or tissues not suitable for pathogen detection

Not determined.

3.4. Non-lethal sampling

Haemolymph can be used for non-lethal sampling.

If non-lethal tissue sample types differ from recommended tissues (see Section 3.2.), or from the tissue samples used in validation studies, the effect on diagnostic performance should be considered.

3.5. Preservation of samples for submission

For guidance on sample preservation methods for the intended test methods, see Chapter 2.2.0 *General information* (diseases of crustaceans).

3.5.1. Samples for bioassay

The success of bioassay depends 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 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 70–90% (v/v) analytical/reagent-grade (absolute) 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 can be frozen at –20°C or below for 1 month or less; for long-term storage. –80°C is recommended.

Standard sample collection, preservation and processing methods for molecular techniques can be found in Section B.5.5. of Chapter 2.2.0 *General information* (diseases of crustaceans).

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

Standard sample collection, preservation and processing methods for histological techniques can be found in Section B.2.2 of Chapter 2.2.0 *General information* (diseases of crustaceans).

3.5.4. Samples for other tests

Not applicable.

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 can be pooled to obtain the minimum amount of material for 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	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				
Cell culture												
Real-time RT-PCR	~~++~~	~~+++~~	~~+++~~	~~1~~	~~++~~	~~+++~~	~~+++~~	~~4~~	~~++~~	~~+++~~	~~+++~~	~~4~~
Conventional RT-PCR	++	++	++	1	++	++	++	1				
Conventional PCR followed by amplicon sequencing									++	+++	+++	1
In-situ hybridisation						++	++	1				
Bioassay					+	+	+	1				
~~LAMP~~ Immunohistochemistry												
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

Fix the cephalothorax tissues of moribund shrimp suspected to be affected by YHV1 in Davidson's fixative, prepare tissue sections and stain with Meyer's haematoxylin and eosin (H&E) using standard histological procedures (Lightner, 1996). Examine tissues of ectodermal and mesodermal origin by light microscopy for the presence of moderate to large numbers of deeply basophilic, evenly stained, spherical, cytoplasmic inclusions approximately 2 µm in diameter or smaller (Chantanachookin *et al.,* 1993). Tissues of the lymphoid organ, stomach subcuticulum and gills are particularly informative.

Lymphoid organ spheroids are commonly observed in healthy *P. monodon* chronically infected with YHV1 or GAV and lymphoid organ necrosis often accompanies disease (Spann *et al.*, 1997). However, spheroid formation and structural degeneration of lymphoid organ tissue also result from infection by other shrimp viruses (Lightner, 1996).

4.3. Cell culture for isolation

Although primary shrimp cell culture methods are available, they are not recommended to isolate and identify YHV1 as a routine diagnostic method. No continuous cell lines suitable for YHV1 culture are available.

4.4. Nucleic acid amplification

PCR assays should always be run with the controls specified in Section 5.5 *Use of molecular and antibody-based techniques for confirmatory testing and diagnosis* of Chapter 2.2.0 *General information* (diseases of crustaceans). 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

~~Not available.~~

The protocol for the real-time RT-PCR for detection of YHV1 has been developed by the WOAH Reference Laboratory for YHV1. This assay is specific for genotype 1. Validation data are provided in the submitted validation report for diagnostic tests and confirm suitability to be recommended for inclusion in the *Aquatic Manual* (ADD LINK OR AGREED REFERENCE).

Pathogen/ target gene	Primer/probe (5'–3')	Concentration	Cycling conditions[(a)]
YHV1 /ORF1	YHV1-12-qF: AGT-CTA-CAG-TGC-TCT-GAT-CT YHV1-12-qR: GAT-TCT-TGA-AGC-GCA-TGA-GT YHV1-12-qPr: FAM-TCT-CAT-GTG/ZEN/TCA-TGA-TAT-TCT-CAA-GCG-AGT-IABkFQ	900 nM of each primer 250 nM of probe	Reverse transcription at 48°C/30 min 1 cycle 95°C/10 min 45 cycles of 95°C/15 sec and 60°C/60 sec

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

4.4.2. Conventional RT-PCR

Three RT-PCR protocols are described. For all conventional RT-PCR protocols, assignment to YHV genotype 1 can be achieved by nucleotide sequence analysis of the RT-PCR amplicon. Reference sequences for YHV1 include:

Protocol 1 is a 1-step RT-PCR that can be used to detect YHV1 in affected shrimp. The protocol is as described by Mohr *et al.* (2015) and adapted from Wongteerasupaya *et al.* (1997). This protocol will detect YHV1 but not GAV or any of the other genotypes currently recognised.

Protocol 2 is a more sensitive multiplex nested RT-PCR that can be used to differentiate YHV1 from GAV. The protocol is as described by Mohr *et al*. (2015) and adapted from Cowley *et al.* (2004). The first stage of the multiplex nested RT-PCR (primary RT-PCR) was designed to detect YHV1 and GAV but has been reported to also detect YHV7 (Mohr *et al*., 2015). Both the primary RT-PCR and the nested PCR detected the novel YHV genotype from China (People's Rep. of) (Liu *et al*., 2014). In the second PCR step, a 277 bp product indicates detection of YHV and a 406 bp product indicates detection of GAV. The presence of both 406 bp and 277 bp products indicates a dual infection with GAV and YHV1. The nested PCR can be run as two separate assays specific for YHV1 or GAV by omitting either the G6 or Y3 primer, respectively. **NOTE:** Due to reported problems with primer specificity for some emerging strains, all PCR products generated using protocol 2 should be sequenced to confirm the virus genotype.

Protocol 3 is a multiplex nested RT-PCR protocol that can be used for screening shrimp for any of the seven genotypes of the yellow head complex of viruses. The protocol is as described by Mohr *et al*. (2015) and adapted from Wijegoonawardane *et al.* (2008b). Two primers were designed to each site, one accommodating sequence variations amongst YHV1 isolates and the other variations amongst isolates of the other genotypes (Wijegoonawardane *et al.*, 2008b). It is not known whether this assay will detect ~~the~~ YHV8 ~~genotype recently detected in China (People's Rep. of)~~ (Liu *et al*., 2014).

Primer sequences

Pathogen / target gene	Primer (5'–3')	Concentration	Cycling parameters[a]
Protocol 1 (Wongteerasupaya *et al.,* 1997; GenBank Accession No.: FJ848675.1; amplicon size: 135 bp)			
YHV1 / ORF1b	10F: CCG-CTA-ATT-TCA-AAA-ACT-ACG 144R: AAG-GTG-TTA-TGT-CGA-GGA-AGT	180 nM 180 nM	40 cycles of 94°C/30sec, 58°C/45 sec, 68°C/45 sec,
Protocol 2 (Cowley *et al.,* 2004; GenBank Accession No.: FJ848675.1)			
YHV1 and GAV / ORF1b	Primary (Amplicon size: 794 bp) GY1: ~~5~~GAC-ATC-ACT-CCA-GAC-AAC-ATC-TG GY4: GTG-AAG-TCC-ATG-TGT-GTG-AGA-CG Nested for detection of YHV1 (Amplicon size: 277 bp) GY2: CAT-CTG-TCC-AGA-AGG-CGT-CTA-TGA Y3: ACG-CTC-TGT-GAC-AAG-CAT-GAA-GTT Nested for detection of GAV (Amplicon size: 406 bp) GY2: CAT-CTG-TCC-AGA-AGG-CGT-CTA-TGA G6: GTA-GTA-GAG-ACG-AGT-GAC-ACC-TAT	 180 nM 180 nM 360 nM 360 nM 360 nM 360 nM	35 cycles of 95°C/30 sec, 66°C/30 sec, and 68°C/45 sec
Protocol 3 (Wijegoonawardane *et al.,* 2008b; GenBank Accession No.: FJ848675.1)			
YHV1 to YHV7 / ORF1b	Primary (amplicon size: 359 bp) YC-F1ab pool: ATC-GTC-GTC-AGC-TAC-CGC-AAT-ACT-GC ATC-GTC-GTC-AGY-TAY-CGT-AAC-ACC-GC YC-R1ab pool: TCT-TCR-CGT-GTG-AAC-ACY-TTC-TTR-GC TCT-GCG-TGG-GTG-AAC-ACC-TTC-TTG-GC Nested (amplicon size: 147 bp) YC-F2ab pool: CGC-TTC-CAA-TGT-ATC-TGY-ATG-CAC-CA CGC-TTY-CAR-TGT-ATC-TGC-ATG-CAC-CA YC-R2ab pool: RTC-DGT-GTA-CAT-GTT-TGA-GAG-TTT-GTT GTC-AGT-GTA-CAT-ATT-GGA-GAG-TTT-RTT Mixed base codes: R(AG), Y(CT), M(AC), K(GT), S(GC), W(AT), H(ACT), B(GCT), V(AGC), D(AGT), N(AGCT).	 180 nM 180 nM 180 nM 180 nM 180 nM 180 nM 180 nM 180 nM	35 cycles of 94°C/45 sec, 60°C/45 sec, 68°C/45 sec, 35 cycles of 94°C/45 sec, 60°C/45 sec, 72°C/45 sec;

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

The Protocol 2 Y3 primer contains a mismatch for GAV but is specific for YHV1. The mismatch can cause false-positives with GAV in the nested PCR of protocol 2 where GAV generates an amplicon of very similar size to the expected size of the YHV1 amplicon. ~~For GAV, the 7th base from left (T) is substituted for C so that the primer sequence for GAV should be 5'-CAT-CTG-CCC-AGA-AGG-CGT-CTA-TGA-3', according to the sequence data of the GAV genome (database accession numbers: NC_010306.1 and AF227196.2).~~

The following controls should be run with each assay: negative extraction control; positive control; no template control; internal PCR control.

4.4.3. Other nucleic acid amplification methods

Not available.

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

The *in-situ* hybridisation (ISH) protocol of Tang *et al.* (2002) is suitable for detecting YHV1 or GAV (Tang & Lightner, 1999). To preserve viral RNA accessibility, fix tissues sampled from live shrimp in neutral-buffered, modified Davidson's fixative without acetic acid (RF-fixative) (Hasson *et al.*, 1997). To achieve good tissue preservation whilst also preserving RNA accessibility, normal Davidson's fixative can be used as long as the fixation time is limited to 24 hours (maximum of 48 hours). Detailed methods can be found in Tang *et al.* (2002) YHV-infected cells give a blue to purple-black colour against the brown counter stain. Positive controls of YHV-infected tissue and negative controls of uninfected shrimp tissue should be included. The digoxigenin-labelled DNA probe can be prepared by PCR labelling using the following primers:

YHV1051F: 5'-ACA-TCT-GTC-CAG-AAG-GCG-TC-3'

YHV1051R: 5'-GGG-GGT-GTA-GAG-GGA-GAG-AG-3'

4.7. Immunohistochemistry

Not applicable.

4.8. Bioassay

The bioassay procedure is based on that described by Spann *et al.* (1997), but similar procedures have been described by several other authors (e.g. Lu *et al.,* 1994). The bioassay should be conducted in susceptible shrimp that have been determined to be free from YHV complex viruses.

Suspect YHV1-infected samples should be maintained at 4°C or on ice. If necessary, the whole shrimp or the retained cephalothorax may be snap-frozen and stored at –80°C or in liquid nitrogen until required. Viral inoculum should be prepared as described by Spann *et al.* (1997).

Juvenile shrimp of a known susceptible species are injected with viral inoculum. Negative controls (buffer injected) and positive controls (known YHV1 positive material) treatment groups are required. Shrimp should be maintained separately to prevent cross-contamination between treatments. Observe the shrimp and record mortalities for at least 21 days or until the test and positive control groups reach 100% mortality.

Dead shrimp can be processed for PCR and sequence analysis. The surviving shrimp are processed for gross signs, histopathology, PCR and sequence analysis. A positive result is indicated by the detection of gross signs and characteristic histological lesions, and by PCR and amplicon sequence analysis. The negative control shrimp must remain negative for at least 21 days for gross or histological signs of infection with YHV1.

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

None has been successfully developed.

4.10. Other methods

None at present.

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

~~Nested~~ Real-time RT-PCR ~~(Protocol 3)~~ is recommended for demonstrating freedom from YHV1 in ~~an~~ apparently healthy populations. ~~Sequencing of any amplified PCR products is required to determine the YHV genotype. Two-step PCR negative results are required for YHV1.~~

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 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.

6.1. Apparently healthy animals or animals of unknown health status[4]

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 YHV1 shall be suspected if the following criterion is met:

i) Positive result by ~~a recommended~~ conventional RT-PCR ~~detection test~~

ii) Positive result by real-time RT-PCR

6.1.2. Definition of confirmed case in apparently healthy animals

The presence of infection with YHV1 is considered to be confirmed if the following criterion is met:

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

ii) ~~A positive result by conventional RT-PCR and identification of YHV1 by sequence analysis of the amplicon from each of two different RT-PCR methods followed by sequence analysis of the amplicons to identify YHV1~~

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 YHV1 shall be suspected if at least one of the following criteria is met:

i) Gross pathology or clinical signs consistent with YHV1 infection

ii) Histopathology consistent with YHV1 infection

iii) Positive result by real-time RT-PCR

iv) Positive result by conventional RT-PCR

v) Positive result by ISH

[4] For example transboundary commodities.

vi) Positive result by bioassay

6.2.2. Definition of confirmed case in clinically affected animals

The presence of infection with YHV1 is considered to be confirmed if the following criterion is met:

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

~~i) A positive result from each of two different RT-PCR methods targeting non-overlapping parts of the genome followed by sequence analysis of the amplicons to identify YHV1~~

6.3. Diagnostic sensitivity and specificity for diagnostic tests

The diagnostic performance of tests recommended for surveillance or diagnosis of infection with YHV1 are provided in Tables 6.3.1. and 6.3.2 (no data are currently available for either). ~~This information can be used for the design of surveys for infection with YHV1, however, it should be noted that diagnostic performance is specific to the circumstances of each diagnostic accuracy study (including the test purpose, source population, tissue sample types and host species) and diagnostic performance may vary under different conditions.~~ 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

Test type	Test purpose	Source populations	Tissue or sample types	Species	DSe (*n*)	DSp (*n*)	Reference test	Citation
YHV1 RT-qPCR	Diagnosis	Infected by co-habitation or feeding	Pleopods	*Penaeus monodon, P. merguiensis*	100% (n=130)	100% (n=130)	Real-time PCR	Validation report

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

6.3.2. For surveillance of apparently healthy animals

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

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

7. References

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* *

NB: There is a WOAH Reference Laboratory for infection with yellow head virus genotype 1 (please consult the WOAH web site: https://www.woah.org/en/what-we-offer/expertise-network/reference-laboratories/#ui-id-3). Please contact the WOAH Reference Laboratories for any further information on infection with yellow head virus genotype 1

NB: FIRST ADOPTED IN 1995 AS YELLOWHEAD DISEASE. MOST RECENT UPDATES ADOPTED IN 2019.

Annexe 55. Point 9.1.5. – Chaptire 2.2.X. « Infection par le virus 1 iridescent des décapodes »

CHAPTER 2.2.X.

INFECTION WITH DECAPOD IRIDESCENT VIRUS 1

1. Scope

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

2. Disease information

2.1. Agent factors

2.1.1. Aetiological agent

DIV1 is the only species of the genus *Decapodiridovirus* assigned to the subfamily *Betairidovirinae*, family *Iridovirus* (ICTV, 2023). DIV1 is a 150–158 nm, enveloped icosahedral double-stranded DNA virus, with a linear genome of 165 kb composed of 34.6% G + C content and 170–178 putative open reading frames (ORFs) (Li *et al*., 2017; Qiu *et al*., 2017; 2018a; Xu *et al*., 2016). Although *Cherax quadricarinatus* iridovirus (CQIV) (Xu *et al*., 2016) and shrimp haemocyte iridescent virus (SHIV) (Qiu *et al*., 2017) have been reported from the redclaw crayfish (*C. quadricarinatus*), and the whiteleg shrimp (*L. vannamei*), respectively, they are classified as different isolates (strains) within the DIV1 species.

2.1.2. Survival and stability in processed or stored samples

DIV1-infected cephalothoraxes are infectious after homogenisation, centrifugation, filtration and storage at –80°C (Qiu *et al*., 2022a; Xu *et al.,* 2016).

2.1.3. Survival and stability outside the host

Not available.

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 DIV1 according to chapter 1.5. *Aquatic Animal Health Code* (*Aquatic Code*) are: ~~fleshy prawn (*Penaeus chinensis*), gazami crab (*Portunus trituberculatus)*, giant river prawn (*Macrobrachium rosenbergii*), kuruma prawn (*Penaeus japonicus*), Oriental river prawn (*Macrobrachium nipponense*), red claw crayfish (*Cherax quadricarinatus*), red swamp crawfish (*Procambarus clarkii*), ridgetail prawn (*Palaemon carinicauda*), and whiteleg shrimp (*Penaeus vannamei*).~~

Family	**Scientific name**	**Common name**
Cambaridae	*Procambarus clarkii*	red swamp crawfish
Palaemonidae	*Macrobrachium nipponense*	Oriental river prawn
	Macrobrachium rosenbergii	giant river prawn
	Palaemon carinicauda	ridgetail prawn
Parastacidae	*Cherax quadricarinatus*	red claw crayfish
Penaeidae	*Penaeus japonicus*	kuruma prawn
	Penaeus vannamei	whiteleg shrimp
Portunidae	*Portunus trituberculatus*	swimming crab

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 DIV1 according to Chapter 1.5 of the *Aquatic Code* are: ~~giant tiger prawn (*Penaeus monodon*).~~

Family	Scientific name	Common name
Penaeidae	*Penaeus chinensis*	fleshy prawn
	Penaeus monodon	giant tiger prawn

In addition, pathogen-specific positive polymerase chain reaction (PCR) results have been reported in the following species, but an active infection has not been demonstrated: ~~channeled applesnail (*Pomacea canaliculata*), *Helice tientsinensis*, Japanese shore crab (*Hemigrapsus penicillatus*), *Macrobrachium superbum* and *Plexippus paykulli*.~~

Family	Scientific name	Common name
Ampullariidae	*Pomacea canaliculata*	channeled applesnail
Palaemonidae	*Macrobrachium superbum*	no common name
Salticidae	*Plexippus paykulli*	no common name
Varunidae	*Helice tientsinensis*	no common name
	Hemigrapsus penicillatus	Japanese shore crab

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

All live stages are potentially susceptible to infection; DIV1 has been detected in post-larvae (PL), juvenile and sub-adult stages of shrimp (*Penaeus vannamei*, *P. chinensis*, *Exopalaemon carinicauda*, *Macrobrachium nipponense, M. rosenbergii*, crayfish [*Cherax quadricarinatus*, *Procambarus clarkia*] and crab [*Portunus trituberculatus*]) as natural infection or by experimental (*per os*) exposure (Chen *et al*., 2019; Qiu *et al*.,-2018; 2019b; 2020b; 2021b; 2022b). Species with a positive DIV1 polymerase chain reaction (PCR) result, without an active infection include: *Penaeus monodon*, *Pomacea canaliculata, Macrobrachium superbum, Plexippus paykulli and Hemigrapsus penicillatus (*Qiu *et al.,* 2021; 2019a; 2022b; Srisala *et al.,* 2021).

2.2.4. Distribution of the pathogen in the host

The principal target tissues for DIV1 include lymphoid organ, haematopoietic tissues, as well as epithelia and haemocytes in gills, muscle, hepatopancreas, pereiopods, pleopods, uropods, and antenna (Qiu *et al*., 2017; 2019a; 2021a; Sanguanrut *et al*., 2021).

2.2.5. Aquatic animal reservoirs of infection

There is evidence that crustacean species may become reservoirs of DIV1 infection. DIV1 was detected in non-clinical adult wild giant tiger prawn (*P. monodon*) (Srisala *et al*., 2021), wild crabs (*Helis tientsinensis*, *Hemigrapsus penicillatus*) in drainage ditches (Qiu *et al*., 2022a), and *Macrobrachium superbum* in affected shrimp ponds (Qiu *et al*., 2019a).

Subclinical infection has been reported in gazami crab, *Portunus trituberculatus*, which is widely distributed in environmental waters in Asia and could be a potential source of DIV1 infection on shrimp farms (Qiu *et al.,* 2022a).

2.2.6. Vectors

There are no confirmed vectors of DIV1.

2.3. Disease pattern

2.3.1. Mortality, morbidity and prevalence

Mortality can be high (80–100%) after a natural infection with DIV1 and mostly reported in the adult stage of shrimp (Liao *et al.,* 2022) ~~in shrimp~~ and crayfish species, which has been confirmed by experimental infection through intramuscular injection or oral administration in *P. vannamei*, *Cherax quadricarinatus*, *Procambarus clarkii* and *Macrobrachium rosenbergii* (Qiu *et al*., 2017; 2019a; Xu *et al*., 2016). Experimental infection with DIV1 administered orally or by intramuscular injection resulted in 50% and 100% mortality, respectively, in the gazami crab (*Portunus trituberculatus*) (Qiu *et al*., 2022a).

In pathogenicity studies of crustacean species, mortalities rose more rapidly in *Litopenaeus vannamei* compared with *Cherax quadricarinatus* or *Procambarus clarkii* in experimental infections (Xu *et al*., 2016).

The prevalence of DIV1 infection was 15.5, 15.2, and 50% in *P. vannamei, P. chinensis*, and *M. rosenbergii,* respectively, in a survey of shrimp farms tested in the period 2014 to 2016 (Qiu *et al*., 2017).

2.3.2. Clinical signs, including behavioural changes

Clinical signs in affected whiteleg shrimp (*P. vannamei*) are reddish bodies, white atrophied hepatopancreas, soft shells and empty stomachs and intestines, while giant freshwater shrimp (*M. rosenbergii*) showed a white discoloration at the base of the rostrum (white head) and hepatopancreatic atrophy (Qiu *et al.,* 2017; 2019a). However, these disease signs are not always distinctive because the course of the disease varies in affected animals.

2.3.3 Gross pathology

See Section 2.3.2.

2.3.4. Modes of transmission and life cycle

Based on experimental and natural infections, DIV1 is thought to be transmitted horizontally by oral routes and contaminated water (Qiu *et al*., 2017; 2019a; 2022a; Xu *et al*., 2016).

2.3.5. Environmental factors

Temperature and co-culture play an important role in DIV1 infection. DIV1 has been detected in shrimp and crayfish reared at 16–32°C, but not at temperatures above 32°C in a 2017–2018 survey (Qiu *et al.,* 2018b; 2019b; 2020b; 2021b 2022b). In shrimp farm management, polyculture with different species of crustaceans increases the risk of DIV1 infection in farmed shrimp due to cross-species transmission (Qiu *et al*., 2019a; 2022a).

2.3.6. Geographical distribution

DIV1 has been reported in farmed shrimp and crayfish in the Asia-Pacific region (Qiu *et al*., 2017; Xu *et al*., 2016).

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

Not available.

2.4.2. Chemotherapy including blocking agents

Not available.

2.4.3. Immunostimulation

Not available.

2.4.4. Breeding resistant strains

Not available.

2.4.5. Inactivation methods

Not known.

2.4.6. Disinfection of eggs and larvae

Not available

2.4.7. General husbandry

Biosecurity practices can be used to reduce the risk of DIV1 infection. These includes PCR pre-screening of broodstock and larvae, PCR pre-screening of polychaetes and food organisms for broodstock and larvae,

disinfection of rearing water and farming equipment, controlled stocking density, and avoidance of polyculture with different crustacean species.

Using an experimental protocol of 15-day thermal treatment at 36°C combined with 15-day restoration treatment at 28°C, *P. vannamei* infected by intramuscular injection of DIV1 showed no clinical signs, no DNA replication, no histopathology and *in-situ* DIG-labelling, loop-mediated DNA amplification (ISDL) results, indicating DIV1 could ~~can~~ be eliminated from challenged shrimp after 36°C treatment (Guo *et al*., 2022).

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

For diagnosis during a disease outbreak, moribund and apparently healthy crustacean specimens of susceptible species (see Section 2.2.3) from the same ponds, especially in polyculture mode, are selected as samples for identification testing. Apparently healthy or even dead and dried samples from crustacean farms next to the affected farms can be used as sources of materials for examination (Qiu *et al*., 2019a). For surveillance in apparently healthy populations, all life stages of samples reared at 16–32°C should be suitable for testing (see Section 2.3.5)

Shrimp and crayfish that are 4–7 cm in body length provide the highest detection rate of DIV1 when used for examination (Qiu *et al*., 2018b ;2019b ;2020b; 2021b ;2022b).

3.2. Selection of organs or tissues

Suitable tissues for testing are lymphoid organ, haematopoietic tissues, muscle, gills, hepatopancreas, pereiopods, pleopods, uropods, and antennae (Qiu *et al*., 2017; 2019a; 2021a; Srisala *et al*., 2021). Quantitative virus analysis from different tissues of naturally infected *Macrobrachium rosenbergii* showed that muscle and hepatopancreas had lower virus load compared with that of the lymphoid organ, haematopoietic tissues, gills, pereiopods, pleopods, uropods and antennae (Qiu *et al*., 2019a).

3.3. Samples or tissues not suitable for pathogen detection

Autolytic and compound eyes samples are not suitable for PCR-based pathogen detection.

3.4. Non-lethal sampling

If non-lethal tissue sample types differ from recommended tissues (see Section 3.2.), or from the tissue samples used in validation studies, the effect on diagnostic performance should be considered.

3.5. Preservation of samples for submission

For guidance on sample preservation methods for the intended test methods, see Chapter 2.2.0 *General information* (diseases of crustaceans).

3.5.1. Samples for pathogen isolation

The success of pathogen isolation and 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 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 70–90% (v/v) analytical/reagent-grade (undenatured) 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 2.2.0 *General information* (diseases of crustaceans).

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

Standard sample collection, preservation and processing methods for histological techniques can be found in Section B.5.3 of Chapter 2.2.0 *General information* (diseases of crustaceans).

3.5.4. Samples for other tests

Not available

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 shrimp (or other decapod crustaceans) should be processed and tested individually. Small life stages such as larvae or PLs 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 [amend or shade in as relevant]	**Surveillance of apparently healthy animals**				**Presumptive diagnosis of clinically affected animals**				**Confirmatory diagnosis1 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				
Cell culture												
Real-time PCR	++	+++	+++	NA	+++	+++	+++	1	+++	+++	+++	1
Conventional PCR	++	++	++	NA	++	++	++	NA				
Conventional nested PCR followed by amplicon sequencing									+	+	+	1
Conventional PCR followed by amplicon sequencing									+++	+++	+++	1
In-situ hybridisation						++	++	1		+++	+++	1
Bioassay					+	+	+	NA				
LAMP	+	+	+	NA	+	+	+	NA				
Quantitative LAMP	++	++	++	NA	++	++	++	1				
Ag-ELISA												
RPA	++	++	++	NA	++	++	++	1				
Other methods[3]												

LV = level of validation, refers to the stage of validation in the WOAH Pathway (chapter 1.1.2); NA = not available;
PCR = polymerase chain reaction; LAMP = loop-mediated isothermal amplification
Ag-ELISA = antigen enzyme-linked immunosorbent assay; RPA = recombinase polymerase amplification
[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 relevant

4.2. Histopathology and cytopathology

Histopathological examination revealed pathognomonic dark eosinophilic cytoplasmic inclusion bodies in the karyopyknotic cells of haemopoietic tissues and lymphoid organs, and in the haemocytes of gills, pereopods and sinus of the hepatopancreas (Qiu *et al.,* 2017; 2019a), as well as cuticular epithelium under the cuticles (Chen *et al.,* 2019).

4.3. Cell culture for isolation

Not available.

4.4. Nucleic acid amplification

PCR assays should always be run with the controls specified in Section 5.5 '*Use of molecular and antibody-based techniques for confirmatory testing and diagnosis'* of Chapter 2.2.0 *General information* (diseases of crustaceans). 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

Table 4.4.1.1. Primers and probes (sequences) and cycling conditions for DIV1 real-time PCR

Target gene	Primer/probe (5'–3')	Concentration	Cycling parameters[a]
Method 1: Qiu *et al.,* 2018a; GenBank Accession No.: MF599468.1			
ATPase	SHIV-F: AGG-AGA-GGG-AAA-TAA-CGG-GAA-AAC SHIV-R: CGT-CAG-CAT-TTG-GTT-CAT-CCA-TG Probe: FAM-CTG-CCC-ATC-TAA-CAC-CAT-CTC-CCG-CCC-TAMRA	500 nM 200 nM	40 cycles of 95°C/100 sec and 60°C/30 sec
Method 2: Qiu *et al.,* 2020a; GenBank Accession No.: MF599468.1			
MCP	142F: AAT-CCA-TGC-AAG-GTT-CCT-CAG-G 142R: CAA-TCA-ACA-TGT-CGC-GGT-GAA-C Probe: FAM- CCA-TAC-GTG-CTC-GCT-CGG-CTT-CGG-TAMRA	500 nM 200 nM	40 cycles of 95°C/10 sec and 60°C/30 sec
Method 3: Gong *et al.,* 2021; GenBank Accession No.: MF599468.1			
ATPase	DIV1-F: AGG-AAA-GGA-AAC-GAA-AGA-AAT-TAT-ACC DIV1-R: GCT-TGA-TCG-GCA-TCC-TTG-A Probe: FAM-CAC-ATG-ATT-TGC-AAC-AAG-CTT-CCA-GCA-TAMRA	400 nM 200 nM	40 cycles of: 95°C/10 sec and 60°C/30 sec

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

4.4.2. Conventional PCR/nested PCR

Table 4.4.2.1. Primer sequences and cycling conditions for DIV1 PCR and nested PCR

Target gene	Primer (5'–3')	Concentration	Cycling parameters[a]
Method 1: Xu *et al.,* 2016; GenBank Accession No.: ; amplicon size: 103 bp			
MCP	CQIV-MCP-F: GAA-ACT-TTA-TGC-ACA-ATC-TTA-T CQIV-MCP-R: CCA-ATC-ATG-TTG-TCG-TAT-CC	NA	25 cycles of: 94°C/30 sec, 55°C/30 sec and 72°C/30 sec
Method 2: Qiu *et al.,* 2017; GenBank Accession No.: KY618040; amplicon size: 457 and 129 bp			
ATPase	Primary step: SHIV-F1: GGG-CGG-GAG-ATG-GTG-TTA-GAT SHIV-R1: TCG-TTT-CGG-TAC-GAA-GAT-GTA Nested PCR: SHIV-F2: CGG-GAA-ACG-ATT-CGT-ATT-GGG SHIV-R2: TTG-CTT-GAT-CGG-CAT-CCT-TGA	400 nM 400 nM	Primary and nested steps: 95°C/3 min; 35 cycles of 95°C/30 sec, 59°C/30 sec and 72°C/30 sec

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

4.4.3. Other nucleic acid amplification methods

Table 4.4.3 Primers and probes (sequences) for DIV1 LAMP, RPA and qLAMP

Method / Target gene	Primer (5'–3')	Concentration	Cycling parameters[a]/ method
Method 1: Chen *et al.,* 2019; GenBank Accession No.:xxx			
LAMP/ DNA-directed RNA polymerase II	SHIV-FIP (F1C + F2): TGG-GGT-TTC-ATA-TGG-GCA-AA T-GAT-TTT-AAG-AAT-GGA-AAG-ATC-CTA-TCA-GC SHIV-BIP (B1C + B2): AGG-AGA-AAA-GGT-TGG-ATT-GGT-TAC-TTT-TAC-TTC-TGT-TAC-TGC-GAT-GG SHIV-LF: GAG-AGG-CGT-GCA-ACT-TTC-TG SHIV-LB: TTT-GGC-ATT-GTC-TGC-TAC-AAT-TTC-C SHIV-F3: GAT-GGC-CAT-TCC-TTC-AAA-C SHIV-B3: AAA-ATA-GTC-ATC-CTG-AAA-TCC-T	1600 nM 1600 nM 800 nM 800 nM 200 nM 200 nM	60 cycles of: 60°C 85°C/5 min:
Method 2: Chen *et al.,* 2020; GenBank Accession No.:xxx			
RPA/ MCP	RPA-F : CAG-ATC-AGA-GCG-CAT-TCG-ATC-CCA-TAG-GCA-CCG-C RPA-R: CGT-AAG-AGA-ACA-TGT-GGT-ATC-CGG-TGA-GTT-CGG-G RPA- Probe: ATA-CGA-ATC-TTC-AGA-TCG-TAT-TCC-CGT-GA(FAM-dT)G(THF)C(BHQ1-dT)GCC-GAT-TAC-TTC-TC (phosphorylation)	400 nM 400 nM 120 nM	40 cycles of: 39°C/45 sec, and 39°C/15 sec:
Method 3: Gong *et al.,* 2021; GenBank Accession No.:xxx			
qLAMP/ ATPase	F3: GGC-TTG-GTA-TCT-TAT-TCA-GAG-AT B3: ATT-CAC-AAC-ATC-GTC-ACC-AT FIP: CTC-TTG-ATG-GAT-ACA-CTG-ATC-TTC-GGA-GCC-AGA-GAT-TGT-AAC-GG BIP: ATT-CAG-TAT-TCA-AGG-ATT-GGT-TCA-AAA-GTT-CTT-CCA-TCT-ACC-TCT-C LF: TTC-GGT-ACG-AAG-ATG-TAG-C LB: GAA-GAG-TAT-CCT-AAT-ATG-ACC-ATC-C	200 nM 200 nM 1600 nM 1600 nM 800 nM 800 nM	63°C/30 sec 40 cycles of: 63°C/60 sec:

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

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

In-situ hybridisation has been applied to paraffin sections to determine the specific location of DIV1 in target tissues by either DIG-labelled oligonucleotide probe or DIG-labelling-loop-mediated DNA amplification (ISDL) (Chen *et al*., 2019; ~~Xu *et al*., 2016~~ Sanguanrut *et al.,* 2021). ISDL is the preferred method to use because it is highly sensitive through simultaneous pathogen DNA amplification and labelling techniques, compared with routine probe-based *in-situ* hybridisation.

4.7. Immunohistochemistry

Not available.

4.8. Bioassay

Bioassay has application in presumptive diagnosis, but cost, accuracy, labour, timing, or other factors limit its application (Qiu *et al*., 2017; Xu *et al*., 2016).

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

Not available.

4.10. Other methods

Not available.

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

Any of the real-time PCR assays is recommended for surveillance to demonstrate freedom in apparently health populations.

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 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.

6.1. Apparently healthy animals or animals of unknown health status[5]

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 DIV1 shall be suspected if at least one of the following criteria is met:

i) Positive result by real-time PCR

ii) Positive result by conventional PCR,

iii) Positive result by LAMP

iv) Positive result by RPA

[5] For example transboundary commodities.

6.1.2. Definition of confirmed case in apparently healthy animals

The presence of infection with DIV1 is considered to be confirmed if at least one of the following criteria is met:

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

ii) Positive result by real-time PCR followed by conventional nested PCR and amplicon sequencing.

~~iii) A positive result from each of two different real-time PCR methods~~

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 DIV1 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) Positive result by real-time PCR

iii) Positive result by conventional PCR

iv) Positive result by LAMP

v) Positive result by RPA

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

vii) Positive result by *in-situ* hybridisation

6.2.2. Definition of confirmed case in clinically affected animals

The presence of infection with DIV1 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 real-time PCR and positive result by conventional nested PCR and amplicon sequencing

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

~~iv) A positive result from each of two different real-time PCR methods~~

6.3. Diagnostic sensitivity and specificity for diagnostic tests

The diagnostic performance of tests recommended for surveillance or diagnosis of infection with DIV1 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.

6.3.1. For presumptive diagnosis of clinically affected animals

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

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

6.3.2. For surveillance of apparently healthy animals

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

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

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* *

NB: There is a WOAH Reference Laboratory for infection with decapod iridescent virus 1
(please consult the WOAH web site:
https://www.woah.org/en/what-we-offer/expertise-network/reference-laboratories/#ui-id-3).
Please contact the WOAH Reference Laboratories for any further information on
infection with decapod iridescent virus 1

NB: FIRST ADOPTED IN 20XX.

SECTION 2.4.

DISEASES OF MOLLUSCS

CHAPTER 2.4.0.

GENERAL INFORMATION

A. SAMPLING

1. Assessing the health status of the epidemiological unit

1.1. Sample material to be used for tests

Sample material and the number of samples to be collected depend on the specific disease or pathogen, the size of animals and the objective of testing (i.e. surveillance of apparently healthy animals, presumptive diagnosis of clinically affected animals or confirmatory diagnosis of a suspect result from surveillance or presumptive diagnosis). See individual disease chapters in this *Aquatic Manual* for specific details of sample requirements.

1.2. Specifications according to mollusc populations

For details of animals to sample for a specific listed disease, see the relevant disease chapter in this *Aquatic Manual.* The design of a surveillance system for demonstrating disease-free status for a country, zone or compartment should be in accordance with the recommendations of the WOAH *Aquatic Code* Chapter 1.4. *Aquatic animal disease surveillance*.

The following factors should be considered when selecting animals to be sampled:

i) for apparently healthy populations, susceptible species should be sampled proportionately or following risk-based criteria for targeted selection of lots, epidemiological units or populations with a history of abnormal mortality or potential exposure events (e.g. stocking with animals of unknown disease status);

ii) If weak, abnormally behaving or freshly dead (not decomposed) animals are present, such animals should be selected. If such animals are not present, animals should be selected in such a way that all epidemiological units of the farm or waterbody are proportionately represented in the sample;

iii) if more than one water source is used for production, animals from all water sources should be included in the sample.

1.3. Specifications according to clinical status

In addition to sampling of target tissues, other organs showing macroscopic abnormalities or lesions should also be sampled. For disease outbreaks, at least ten diseased or moribund molluscs should be sampled for testing. Parallel samples (n > 10) from apparently normal animals in the same production region should also be collected. Collection of dead specimens during disease outbreaks should be avoided when possible, but recently dead samples may be suitable for some diagnostic assays provided the animals are not decomposed. Disease-specific recommendations are provided in Section 3 *Sample selection, sample collection, transportation and handling* of the individual chapters.

1.4. Specifications according to mollusc size

For the WOAH-listed diseases it is recommended that the scheduling of sampling be planned (i.e. by farm schedule, season, etc.) so that the particular life-stage(s) are sampled at a time when the pathogen of concern is most likely to be detected.

1.4.1. For the listed parasites

Juveniles below 1.5 cm: sample the entire animal but remove the shell when possible or proceed with a decalcification protocol. When animals are too small for individual analyses, analyses can be performed on pools of several animals.

Juveniles 1.5–3 cm: sample the entire mollusc and cut in half sagittally. Keep one half of the animal for histological analyses and the other half for molecular analyses.

Molluscs over 3 cm: take a cross-section of the body, passing through the mantle, gills, digestive gland and gonads for histological analyses. Keep the remaining tissues for molecular analyses.

1.4.2. For infection with *Xenohaliotis californiensis*

For abalone ≥20 mm, excise several 3–5 mm cross sections containing posterior oesophagus (postoesophagus), digestive gland, and foot muscle.

1.4.3. For abalone herpesvirus infections

Sample as outlined in Section 1.4.2 above with the addition of a cross section of the head to obtain the cerebral ganglion and removal of several sections of the foot and adductor muscle complex including one section 0.25–1.0 cm (distance depends on abalone maximum length) posterior to the head to obtain the pedal ganglion. In addition, a longitudinal section from the anterior pedal ganglion to the posterior portion of the pedal musculature should be taken.

2. General processing of samples

Sampled molluscs should be delivered alive to the diagnostic laboratory. The laboratory should be informed of the estimated time of arrival of the sample so the required materials to process the molluscs can be prepared before receipt of the samples.

Mollusc samples should be packed appropriately in order to keep them alive. Required samples should be shipped as soon as possible after collection from the water. Unless otherwise specified, moribund animals should be sent on ice (but not frozen) to reduce sample decomposition.

For samples that cannot be delivered live to the diagnostic laboratory, specimens should be fixed on site as recommended in the following sections of this chapter or the relevant disease chapters of this *Aquatic Manual*. While this may be suitable for subsequent histology, transmission electron microscopy examination or PCR analyses for example, other techniques, such as fresh smears, tissue imprints, routine bacteriology, mycology or Ray's fluid thioglycollate culture of *Perkinsus* spp., cannot be performed on such samples. Diagnostic needs and sample requirements should be discussed with the diagnostic laboratory prior to collection of the sample.

2.1. Macroscopic examination

The gross observation of molluscs should target, as far as possible, animal behaviour, shell surface, inner shell and soft tissues.

It is often difficult to observe the behaviour of molluscs in open systems. However, observation of molluscs in certain rearing facilities, such as broodstock in tanks and larvae in hatcheries, can provide useful indications of disease-related behavioural changes. If signs are noted (e.g. pre-settlement of larvae on the bottom, food accumulation in tanks, signs of weakening, etc.), samples may be examined for gross signs, including observation under a dissecting microscope for abnormalities and deformities, fouling organisms, and fixed for further processing as recommended below. For adults and juveniles, signs of weakening may include gaping, accumulation of sand, mud and debris in the mantle and on the gills, mantle retraction away from the edge of the shell, decreased activity (scallop swimming, clam burrowing, abalone grazing), etc. The righting reflex of abalone after being inverted does not occur in weakened animals, and it is a good indicator of weakness. Mortality in open systems should be monitored for patterns of losses, and samples should be collected for further analysis. Environmental factors, pre- and post-mortality, should be recorded.

Even under culture conditions, the shells of molluscs may not be clean and fouling organisms are normal colonists of mollusc shell surfaces. Organisms such as barnacles, limpets, sponges, polychaete worms, bivalve larvae, tunicates, bryozoans, etc., do not normally threaten the health of molluscs. Culture systems, such as suspension and shallow water culture, can even increase exposure to fouling organisms and shells may become covered by other animals and plants. This can affect health directly by impeding shell opening and closing or indirectly through competition for food resources. Signs of weakening associated with heavy fouling should be a cause for concern rather than fouling itself. Shell damage by boring organisms, such as sponges and polychaete worms, are usually benign, but under certain conditions may reach proportions that make the shell brittle or pierce through to the soft tissues. This degree of shell damage can weaken the mollusc and render it susceptible to pathogen infections. Shell deformities (shape, holes in the surface), fragility, breakage or repair should be noted, but may not be indicative of a disease concern. Burrowing epibionts may cause deformities and weaken the shell(s). Abnormal coloration and smell may indicate a possible soft-tissue infection that may need to be examined at a laboratory.

The molluscs should be opened carefully so as not to damage the soft tissues, in particular the mantle, gills, heart and digestive gland. The presence of fouling organisms on the inner shell surface is a clear indication of weakness. The inner surface of the shell is usually smooth and clean because of mantle and gill action. Perforation of the inner surface may occur but can be sealed off by the deposition of additional conchiolin and nacre. This may result in formation of mud- or water-filled blisters. Blisters may also form over superficial irritants such as foreign bodies. The degree of shell perforation can be determined by holding the shell up to a strong light. Where abnormalities occurring within the matrix of the shell warrant further investigation, freshly collected specimens can be brought intact to the laboratory or fixed for subsequent decalcification, as required. The appearance of the soft tissues is frequently indicative of the physiological condition of the animal. Soft tissues should be examined for the presence of abscess lesions, pustules, tissue discoloration, pearls, oedema, overall transparency or wateriness, gill deformities, etc., and, when found in association with weak or dying animals. Abnormalities and lesions of the tissues should be noted and recorded, as well as any shell deformities, shell-boring organisms and conspicuous mantle inhabitants. Levels of tissue damage should be recorded and samples of affected and unaffected animals collected for laboratory examination as soon as possible.

2.2. Virological examination

See Chapter 2.4.1. Infection with abalone herpesvirus for specific details.

2.3. Bacteriological examination

See Chapter 2.4.7. Infection with *Xenohaliotis californiensis* for specific details.

2.4. Parasitic (protists) examination

See Chapters 2.4.2 to 2.4.6. Infections with listed protists for specific details.

2.5. Fungal examination

Not applicable for currently listed diseases.

B. MATERIALS AND BIOLOGICAL PRODUCTS REQUIRED FOR THE ISOLATION AND IDENTIFICATION OF MOLLUSC PATHOGENS

1. Mollusc viruses

1.1. Mollusc cell lines

Not applicable. There are currently no confirmed or documented mollusc cell lines suitable for virus isolation.

1.2. Culture media

Not applicable.

1.3. Virus positive controls and antigen preparation

1.3.1. Virus nomenclature

In general, the virus nomenclature used in the disease-specific chapters follows the most recent taxonomy for viruses as given in the Report of the Committee on Taxonomy of Viruses (see: ICTV [ictvonline.org] for latest information).

1.3.2. Virus production for experimental purposes

As no cell lines are known that can be used to produce mollusc virus stocks, infection of known susceptible host species (which are free of infection with the pathogenic agent in question) is the preferred method for virus production for experimental purposes or for the production of positive control material.

1.3.3. Virus preservation and storage

Infectivity of all of the WOAH-listed mollusc viruses can be preserved by freezing infected whole molluscs or infected target tissues at –20°C for short-term storage, or at –80°C or lower for long-term storage.

2. Mollusc bacteria

Not applicable. There is currently no developed procedure to cultivate *Xenohaliotis californiensis*.

3. Mollusc parasites (protists)

3.1. Culture media

See Chapters 2.4.5 Infection with *Perkinsus marinus* and 2.4.6 Infection with *Perkinsus olseni* for details.

3.2. Storage of cultures

Perkinsus spp. cultures in the exponential phase of growth can be pelleted by centrifugation and cryopreserved by resuspending the pellet in 40% DMEM Ham's F-12 (1:1) culture medium with 10% glycerol and 50% FBS and freezing them using standard procedures.

4. Mollusc fungi

4.1. Culture media

Not applicable for currently listed diseases.

4.2. Storage of cultures

Not applicable for currently listed diseases.

5. Techniques

The available diagnostic methods that may be selected for diagnosis of the WOAH-listed mollusc diseases or detection of their aetiological agents are based on:

i) Gross and clinical signs.

ii) Direct bright-field, phase-contrast or dark-field microscopy with whole stained or unstained tissue wet-mounts, tissue squashes, and impression smears.

iii) Histology, *in-situ* hybridisation and electron microscopy of fixed specimens.

iv) Culture methods where applicable.

v) Molecular methods (including sequencing): Conventional and real-time PCR and LAMP for direct assay with fresh, frozen or ethanol fixed-tissue samples or with extracted DNA.

Bioassays of suspect or subclinical carriers using a highly susceptible host (life stage or species) may also be used as an indicator for the presence of the pathogen.

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 molluscs should be processed and tested individually. However, for eggs, larvae and postlarvae, pooling of individuals may be necessary to obtain sufficient sample material to run a diagnostic assay.

5.1. Gross and clinical signs

Macroscopic examination of gross and clinical signs reveals non-specific signs only (e.g. gaping in bivalves or general weakness of the foot muscle in abalone), and mortality may be caused by several disease agents or physiological problems, such as loss of condition following spawning. To obtain a definitive diagnosis further investigation is required and this can only be determined using a range of other techniques including histology/electron microscopy and molecular techniques such as PCR and gene sequence analysis.

5.2. Direct microscopy

Samples for direct microscopic examination should be examined as soon as possible after collection. Use live specimens whenever possible, or use fresh, chilled, or fixed specimens when live specimens are not practical. If an adequate field laboratory is available, it should be used to process and examine samples near the site of collection.

5.3. Histological techniques

Live moribund animals or freshly dead (within minutes) animals provide the optimal tissues for examination. Due to tissue lysis that occurs during the freeze-thaw cycle, frozen samples are not appropriate for histology. Should a delay between animal mortality and sampling occur, it is recommended that animals be stored intact on ice or in a refrigerator.

To obtain a sample that includes all the major tissues, a section should be taken to include digestive gland, gills, gonad, mantle and palps, where possible. For large specimens, it may be necessary to take several sections to include all the important tissues. Tissue preparation for examination by light microscopy involves several steps, including tissue fixation, dehydration, impregnation and embedding of samples, preparation of sections, staining and mounting of slides.

5.3.1. Tissue fixation

Tissue fixation is required to maintain the morphology of the tissues and to prevent post-sampling necrosis. Recommended fixatives used for the study of marine molluscs are Davidson's solution, Carson's solution and 10% formalin in filtered sea water. The ratio of fixative to tissue volume should be at least 10:1 to ensure good fixation.

Davidson's solution:

1 µm filtered sea water	1200 ml
95% Alcohol	1200 ml
35–40% Formaldehyde[6]	800 ml
Glycerol	400 ml
Glacial acetic acid	10% (add just prior to use)

Carson's solution:

$NaH_2PO_4.2H_2O$	23.8 g
Sodium hydroxide (NaOH)	5.2 g
Distilled water	900 ml
40% Formaldehyde[1]	100 ml
Adjust the pH to 7.2–7.4	

10% formalin in filtered sea water solution:

1 µm filtered sea water	900 ml

[6] A saturated 37–39% aqueous solution of formaldehyde gas.

35–40% Formaldehyde[1] 100 ml

These solutions allow tissue structure to be preserved and different histochemical methods to be used including for *in-situ* hybridisation with DNA probes. Over-fixation (over 24–48 hours) should be avoided. After fixation, the specimens should be transferred to 70% ethyl alcohol, where they can be stored indefinitely. Davidson's solution is normally used because it provides better preservation of the cell nuclei. Carson's solution or 10% formalin in seawater can be used to examine tissues by electron microscopy. As electron microscopy can be a valuable aid in diagnosing or confirming infections in bivalve molluscs, fixing some samples (particularly the smaller ones) with glutaraldehyde, as described in Section B.5.4.1 of this chapter, may be considered, and will provide electron micrographs of the highest quality. It is recommended that a representative portion of the mollusc is fixed in Davidson's solution, while another representative portion is fixed in Carson's solution for further examination to ensure that all tissues/organs are fixed in both fixatives. If neither is available, 10% formalin buffered with filtered seawater will suffice.

For transport and shipping, see *Aquatic Code* Chapter 5.10 *Measures concerning international transport of aquatic animal pathogens and pathological material*.

5.3.2. Dehydration, impregnation and embedding of the samples

The fixed samples are transferred through a series of graded alcohols (70–95% [v/v]) before final dehydration in absolute ethanol. The alcohol contained in the tissues is next eliminated by immersing them in xylene. The tissues are then impregnated with paraffin, which is soluble in xylene, at 60°C. These steps are often carried out automatically using a tissue processing machine. Should processing be delayed, fixed tissues may be stored in 70% ethanol.

Histological blocks are produced by letting the tissues cool in moulds filled with paraffin on a cooling table.

5.3.3. Preparation of the sections

After the blocks have cooled and the paraffin has solidified, histological sections of about 2–5 µm are cut using a microtome. The sections are recovered on histological slides, drained and dried for up to 1 hour at 40–42°C or overnight at room temperature.

5.3.4. Staining and mounting the slides

Before staining, the paraffin is removed from the sections by immersing them in xylene or equivalent clearing solution for 10–20 minutes. This is repeated once and then the solvent is eliminated by immersion in two successive absolute ethanol baths for 10-minute periods each, and they are then rehydrated through a descending series of ethanol baths (for example 95%, 70%, 50%, 30%, 10 minutes each) with a final immersion in tap water for 10 minutes. Different topographical or histochemical staining techniques can then be performed.

When haematoxylin–eosin (H&E) stain is used (haematoxylin or equivalent), nuclear and basophilic structures stain a blue-to-dark-purple colour, the endoplasmic reticulum stains blue, while the cytoplasm takes on a grey colour. The acid dye eosin stains the other structures pink. This staining technique is simple and reproducible and, although it only allows a limited differentiation of cell structures, it is possible to detect any abnormalities in tissue and cellular structure. Other techniques may be applied to demonstrate particular structures or features as required (e.g. trichrome for connective tissue and cytoplasmic granules).

5.4. Transmission electron microscopy methods

Transmission electron microscopy can be used as part of the diagnostic procedures for diseases of molluscs.

Fixation for electron microscopy should be done immediately after the animal has been killed and before fixation for histology. Only samples taken rapidly from live animals will be of any use. The preparation of samples for electron microscopy involves the following steps: tissue fixation, decalcification of the samples (when necessary), dehydration, impregnation and embedding of the samples, preparation and counterstaining of the sections.

5.4.1. Tissue fixation

For tissues that are to be examined by electron microscopy, it is important that the fixation be performed correctly in order to cause as little damage as possible to the ultrastructure. The specimens are cut such that their dimensions do not exceed 1–2 mm. This small size allows rapid penetration of the various solutions into the tissue sample.

Fixation is carried out directly in 3% glutaraldehyde for 1–4 hours. The samples are washed in buffer three times, then post-fixed in 1% osmic acid (aqueous OsO_4) and washed twice again in buffer. Various formulations of glutaraldehyde fixative and buffers work equally well.

In order to cause as little damage as possible to the ultrastructure, the samples are treated with solutions that have an osmolarity close to that of the tissues. Thus, mollusc tissues are treated with solutions with an osmolarity of approximately 1000 mOsm. The osmolarity of the solutions is adjusted with artificial sea salts or NaCl. Alternatively, the glutaraldehyde can be formulated with 0.22 µm filtered seawater, and filtered seawater used for subsequent washes.

Sodium cacodylate	0.4 M: 8.6 g in 100 ml of distilled water
Sodium chloride	10% in distilled water
Cacodylate buffer, pH 7.4:	
1000 mOsm	
Sodium cacodylate	50 ml from 0.4 M stock solution
NaCl	20 ml from 10% stock solution
Distilled water	30 ml
Adjust the pH to 7.4	
3% Glutaraldehyde:	
1000 mOsm	
25% glutaraldehyde	2.5 ml
0.4 M sodium cacodylate	5 ml
10% NaCl	3.5 ml
Distilled water	9 ml
1% Osmic acid:	
1000 mOsm	
4% Osmic acid	1 volume
0.4 M sodium cacodylate	1 volume
NaCl	1 volume from 10% stock solution
Distilled water	1 volume
5% ethylene diamine tetra-acetic acid (*EDTA*)*:*	
Disodium EDTA	5 g
Cacodylate buffer	100 ml

EDTA dissolves when the pH is above 8. When the solution becomes clear adjust the pH to 7.4 by adding concentrated HCl.

If the samples have been previously fixed and stored in Carson's solution, they should be washed several times in a bath of buffer before fixation with 3% glutaraldehyde.

5.4.2. Dehydration, impregnation and embedding of the samples

The samples are dehydrated in successive baths of ethanol: 70% ethanol once, 95% ethanol twice, absolute ethanol three times. The dehydration is completed by two baths of propylene oxide, which allows subsequent impregnation with Epon.

The samples are impregnated progressively. After a first bath in a mixture of polypropylene oxide–Epon (50/50), the samples are placed in a bath of Epon. The longer the incubation, the better the impregnation of the tissues.

Embedding is carried out by placing the samples in moulds filled with Epon resin. A label identifying the sample is included in each block and the blocks are then placed at 60°C (the temperature at which Epon resin polymerises) for 48 hours.

5.4.3. Preparation of the sections and the counterstaining

The blocks are cut to appropriate sizes with a razor blade and, using an ultra- microtome, semi-thin sections (0.5–1 µm) are cut and placed on glass slides. These will be used to monitor the quality of the samples by light microscopy and to locate the areas of interest on the section.

The semi-thin sections are stained at 90–100°C with 1% toluidine blue solution. After drying, the slides are mounted under cover-slips with a drop of synthetic resin and observed using the light microscope.

Ultra-thin sections 80–100 nm thick are placed on mesh copper grids for electron microscopy. Uranyl acetate and lead citrate are used to counterstain the ultra-thin sections.

5.5. Use of molecular techniques for surveillance, confirmatory testing and diagnosis

Molecular techniques, including the use of nucleic acid probes for *in-situ* hybridisation, conventional PCR and real-time PCR, have been developed for the identification of many pathogens of aquatic animals. Real-time PCR methods, in general, have high sensitivity and specificity and, following adequate validation, can be used for direct detection of pathogen nucleic acids in samples prepared from mollusc tissues. These techniques can be used in direct surveillance of apparently healthy populations, if they have a high level of diagnostic sensitivity, as well as in the diagnosis of clinically affected animals.

When using PCR as a diagnostic method, the design of primers and probe, the use of positive and negative controls, as well as validation of the PCR method chosen are important. Real-time PCR is a powerful technique particularly for analysing relatively high numbers of samples (e.g. for surveillance) via high-throughput testing. Several nucleic acid probe and PCR protocols are included in this version of the *Aquatic Manual* as screening, diagnostic or confirmatory methods for molluscs and can be undertaken as the standard method. Following real-time PCR-positive results, where possible, conventional PCR with sequence analysis of PCR products should be used for confirmation of pathogen identity.

As with all PCR protocols, optimisation may be necessary depending on the reagents, equipment and the plasticware used. PCR is prone to false-positive and false-negative results. False-positive results (negative samples giving a positive reaction) may arise from either product carryover from positive samples or, more commonly, from cross-contamination by PCR products from previous tests. Therefore, each assay and tissue extraction should include a negative control to rule out contamination. False-negative results (positive samples giving a negative result) may lead to unwanted transmission of pathogens and biosecurity failure. Therefore, each assay (and ideally each tissue extraction) should include positive controls to ensure the assay performed correctly. Additionally, mollusc tissues are known to potentially contain PCR inhibitors. It is therefore recommended to check for the presence of PCR inhibitors in DNA extracts to avoid false negative results.

To minimise the risk of contamination, aerosol barrier pipette tips should be used for all sample preparation and PCR steps. Additionally, all PCRs should be prepared in a clean area that is separate from the area where the nucleic acid extraction, amplification and gel electrophoresis are performed. Do not share equipment (e.g. laboratory coats and consumables) between areas and, where possible, restrict access between areas. Contaminating PCR products can be carried on equipment, clothes, shoes and paper (e.g. workbooks). Also, ensure all work-tops and air-flow hoods/cabinets used for the extractions and PCR set-up are regularly cleaned and decontaminated. To ensure sample integrity, always store the samples (e.g. in a freezer or refrigerator) in a location separate from the molecular biology laboratory and reagents.

Nested PCR involves two rounds of PCR and may be used to achieve increased sensitivity and specificity; however, it increases the risk of contamination. Contaminants from previous reactions can carry over and lead to false-positive results. Strict laboratory practices such as separate workspaces, dedicated equipment, and meticulous pipetting techniques are essential to mitigate this risk. In conclusion, nested PCR is not recommended for surveillance but may sometimes be used for confirmative studies.

5.5.1. Sample preparation

Samples should be prepared to preserve the nucleic acid of the pathogen and should be handled and packaged with the greatest care to minimise the potential for cross-contamination amongst the samples or target

degradation before the assay can be performed. A water-resistant label, with the appropriate data filled out, should be placed within each package or container for each sample set. Use of household permanent markers should be avoided as their ink dissolves in ethanol and may result in loss of the sample label. Use pencil or histology pens only to label vials or jars.

Some suitable methods for preservation and transport of samples taken for molecular tests are:

i) *Live, iced specimens or chilled specimens:* for specimens that can be rapidly transported to the laboratory for testing within 24 hours, pack samples in sample bags in an insulated box containing a cold pack and ship to the laboratory. Note: cold packs should not be in direct contact with the animals to avoid freezing some parts of the tissues if histological analyses are also planned on the samples (histology cannot be performed on frozen tissues).

ii) *Frozen whole specimens: s*elect live specimens according to the purpose of sampling, quick freeze in the field using crushed dry-ice, or freeze in a field laboratory using a mechanical freezer at –20°C or lower temperature. Prepare and insert the label into the container with the samples, pack samples with an adequate quantity of dry-ice in an insulated box, and ship to the laboratory.

iii) *Alcohol-preserved samples:* 80% analytical grade ethanol (i.e. methanol-free ethanol) can be used to preserve, store, and transport mollusc tissues. Tissues should be fully immersed in ethanol. Shipment can be performed at room temperature .

iv) *Fixed tissues for* in-situ *hybridisation:* for this purpose, classic methods for preservation of the tissues for histology are adequate. Davidson's solution is usually a good choice for later use of molecular probes (See Section B.5.3). For DNA, specifically, over-fixation (more than 48 hours) should be avoided.

5.5.2. Preservation of DNA in tissues

For routine diagnostic testing by PCR, samples must be prepared to preserve the pathogen's nucleic acid. For most purposes, preservation of samples in analytical grade ethanol (80%) at room temperature is the preferred method for subsequent molecular tests. Samples preserved in this way can be stored for up to 1 week at 4°C or 25°C for 1 week or for extended periods at –20°C or below. In addition, other products (e.g. nucleic acid preservatives, various lysis buffers, etc.) are acceptable and are commercially available for the same purpose.

5.5.3. Nucleic acid extraction

To isolate nucleic acids from tissues preserved in ethanol or other preservative, simply remove the tissue from the fixative or preservative, press the tissues on absorbent paper to remove the excess of ethanol and let the ethanol evaporate, then treat it as fresh or frozen samples. Most fresh and preserved or fixed tissues can be homogenised (e.g. with a mortar and pestle or in bead-beating tubes) directly in the lysis or extraction buffer provided with commercially available DNA and RNA extraction kits. Commercial kits should be validated or undergo equivalence testing with current validated extraction procedures prior to routine use.

5.5.4. Preparation of slides for *in-situ* hybridisation

For *in-situ* hybridisation, molluscs are fixed and embedded in paraffin, according to the methods described above for histology. Sections are cut at 5 µm thick and placed on aminoalkylsilane-coated slides, which are then dried overnight at room temperature or in an oven at 40°C. The sections are dewaxed by immersing in xylene for 10 minutes. This step is repeated once and then the solvent is eliminated by immersion in two successive absolute ethanol baths for 10 minutes each. The sections could be rehydrated by immersion in a descending ethanol series. The protocol may require a step of membrane permeabilisation enabling access to the target DNA. For this purpose, sections are treated with proteinase K (100 µg ml^{-1}) in TE buffer (Tris [50 mM], EDTA [10 mM]), at 37°C for 10–30 minutes in a humid chamber. Slides are dehydrated by immersion in one or several ethanol series and then air-dried. For *in-situ* hybridisation tests (see individual chapters for details), it is essential that both a known positive and a known negative slide be stained to eliminate false positive results due to non-specific staining/stain dropout, and false negative results due to errors in the staining protocol. It is also recommended to test non-specific ISH probes (e.g. "universal" 18s probes) on tested samples to check if the material is suitable for ISH analyses.

For further details see disease-specific chapters in this *Aquatic Manual*.

6. Additional information to be collected

Sample information should include the collector's name, organisation, date, time, and description of the geographical location of the place of origin. The geographical location of the place of origin of samples may be described as the name or location of the site from which the sample has originated, or its geographical co-ordinates. There should also be records that provide information to allow trace-backs on the sample movement from the site of origin to the storage facility or laboratory and within those facilities.

Samples should be accompanied with background information, including the reason for submitting the sample (surveillance, abnormal mortality, abnormal growth, etc.), gross observations and associated environmental parameters, approximate prevalence and patterns of mortality, origin and nature of the molluscs (species, age, whether or not the samples are from local mollusc populations or stocks transferred from another site, date of transfer and source location, etc.). This information should identify possible changes in handling or environmental conditions that could be a factor in mortality in association, or not, with the presence of infectious agents

Information on the preservation method, storage location, and date and time of storage at each storage locker or freezer along with information on the storage temperature (continuously monitored is preferable) should be collected. This information should be tracked with a unique sample code for all samples. For laboratories, the date of receipt, storage location information, date of analysis, analysis notes, and report date should be maintained for all uniquely coded samples. These data will greatly facilitate the tracking of sample problems and provide assurance that the samples were properly handled.

See disease-specific chapters in this *Aquatic Manual* for recommendations on any additional information that may be required or that may assist the diagnostic laboratory in determining the most appropriate test(s) to be run for submitted samples.

7. Key references for further reading

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NB: First adopted in 1997. Most recent updates adopted in 2012.

Annexe 57. Point 9.2.2. – Chapitre 2.4.1. « Infection par l'herpèsvirus de l'ormeau »

CHAPTER 2.4.1.

INFECTION WITH ABALONE HERPESVIRUS

1. Scope

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

2. Disease information

2.1. Agent factors

2.1.1. Aetiological agent

Aurivirus haliotidmalaco1 ~~AbHV-1~~ is the aetiological agent of abalone viral ganglioneuritis (AVG); for the purpose of this chapter, the agent will be referred to as AbHV. AVG is a contagious disease of abalone species in Australia (Ellard *et al.,* 2009; Hooper *et al.,* 2007), China (People's Rep. of) (Gu *et al*., 2019; Wang *et al.,* 2004) and Chinese Taipei (Chang *et al.,* 2005). Comparison of nucleotide sequences of the Victorian isolate of AbHV-~~1~~ and ostreid herpesvirus-1 (Davison *et al.,* 2009; Le Deuff & Renault, 1999) over common coding regions identified similarities ranging from 19% to 53%, indicating that these viruses share a low level of sequence similarity (Savin *et al.,* 2010). AbHV-~~1~~ has been assigned as a second member of the *Malacoherpesviridae* (ICTV, 2022). Complete genome sequences of isolates demonstrated that there are at least five genetic variants of AbHV-~~1~~ within Australia (Cowley *et al*., 2012; Corbeil *et al*., 2016) and one Chinese Taipei strain (Chang *et al*., 2005). More recent analysis demonstrated that the Chinese strain represents a further variant (Bai *et al*., 2019b).

Purified AbHV-~~1~~ particles (Tan *et al.,* 2008) observed by transmission electron microscopy are enveloped and icosahedral with electron dense cores and 100–110 nm in diameter. The intranuclear location of AbHV-~~1~~ particles, their size and ultrastructure are characteristic of members of the *Herpesviridae*. Isopycnic gradient centrifugation (in potassium tartrate and caesium chloride density gradients) indicated a virus particle buoyant density of 1.17–1.18 g ml^{-1} (Tan *et al.,* 2008).

2.1.2. Survival and stability in processed or stored samples

Virus derived from tissue obtained from experimentally infected abalone that had been homogenised in sterile EMEM Gibco) containing 10% fetal bovine serum, centrifuged (1500 **g** for 20 minutes at 4°C), filtered (0.22 µM) and stored as 250 µl aliquots in liquid nitrogen remains infectious for at least 21 months (Corbeil *et al*., 2012b).

2.1.3. Survival and stability outside the host

Experimental studies (Corbeil *et al*., 2012b) demonstrated that AbHV-~~1~~ remained infectious for up to 5 days when held in seawater at 4°C and for only 1 day at 15°C.

2.2. Host factors

Acute disease was first reported in farmed *Haliotis diversicolor supertexta* in Chinese Taipei (Chang *et al*., 2005). Subsequently, disease outbreaks occurred in both farmed and wild abalone populations in Australia in all age classes of *H. rubra*, *H. laevigata*, and their hybrids (Hooper *et al.,* 2007). AbHV-~~1~~ is also suspected to be the aetiological agent of an epizootic disease that devastated the abalone aquaculture industry in southeastern China (People's Rep. of) starting in 1999 and continuing through the early 2000s (Gu *et al*., 2019; Wei *et al*., 2018; Wu & Zhang, 2016). Interestingly, New Zealand päua (*H. iris*) was highly resistant to experimental infection (Corbeil *et al*., 2017).

2.2.1. Susceptible host species

Species that fulfil the criteria for listing as susceptible to infection with AbHV~~-1~~ according to Chapter 1.5. of the *Aquatic Animal Health Code* (*Aquatic Code*) are: ~~small abalone (*Haliotis diversicolor*), Greenlip abalone (*Haliotis laevigata*), Blacklip abalone (*Haliotis rubra*) and hybrids of Greenlip × Blacklip abalone (*Haliotis laevigata x Haliotis rubra*).~~

Family	Scientific name	Common name
Haliotidae	*Haliotis diversicolor*	small abalone
	Haliotis laevigata	greenlip abalone
	Haliotis rubra	blacklip abalone
	Haliotis laevigata × H. rubra	hybrid of greenlip × blacklip 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 AbHV~~-1~~ according to Chapter 1.5 of the *Aquatic Code* are: none ~~Japanese abalone (*Haliotis discus*) and Rainbow abalone (*Haliotis iris*)~~.

In addition, pathogen-specific positive polymerase chain reaction (PCR) results have been reported in the following species, but no active infection has been demonstrated: ~~none.~~

Family	Scientific name	Common name
Haliotidae	*Haliotis discus*	Japanese abalone
	Haliotis iris	rainbow abalone

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

All age classes of *H. diversicolor, H. rubra*, *H. laevigata*, and hybrids of *H. rubra* × *H. laevigata* appear to be highly susceptible to disease (Corbeil 2020; Gu *et al.,* 2019).

2.2.4. Distribution of the pathogen in the host

The major histopathological lesion identified in abalone affected with AVG is ganglioneuritis: inflammation confined to neural tissue. The cerebral, pleuropedal and buccal ganglia can be affected as well as the cerebral commissure and associated peripheral nerves (Bai *et al*., 2019a; Chang & Handlinger, 2022; Hooper *et al.,* 2007). The Chinese variant is also able to infect and replicate in haemocytes of *H. diversicolor* (Bai *et al.,* 2020)

2.2.5. Aquatic animal reservoirs of infection

No information available.

2.2.6. Vectors

No information available.

2.3. Disease pattern

Outbreaks of AVG in both farmed and wild abalone populations in Australia are associated with the rapid onset of high mortality rates (up to 90%) in all age classes (Corbeil *et al.,* 2010). Similarly, in Chinese Taipei, during the epizootic in cultured abalone (the water temperature was 16–19°C), both adult and juvenile abalone suffered from the disease, with cumulative mortalities of 70–80%. It was reported that death of all of the abalone in a pond could occur within 3 days of the onset of clinical signs (Chang *et al.,* 2005). A similar disease pattern occurred with experimental infections (Chang *et al.,* 2005; Crane *et al.,* 2009).

2.3.1. Mortality, morbidity and prevalence

In on-farm epizootics in Australia cumulative mortality in all age classes can reach >90%. In experimental trials, 100% mortality can occur within 5 days post-exposure. Most abalone that display gross signs are likely to die within 1–2 days.

In Australia, and similarly in Chinese Taipei, an outbreak of AVG is associated with a rapid rise in mortality rate (up to 90% or more). Affected abalone demonstrating clinical signs (e.g. curling of the foot) are likely to die within 1 day of showing these signs. Ganglioneuritis is observed in sections of neural tissue by light microscopy and confirmation of the presence of AbHV~~-1~~ is obtained by real-time PCR or *in-situ* hybridisation

(Crane *et al.,* 2016). The precise prevalence of AVG in wild abalone populations in Australian waters is unknown. The first epidemiological study undertaken in China (People's Rep. of), using real-time PCR (Gu *et al*., 2019), revealed a detection rate of 27–30% in abalone (*H. diversicolor* and *H. discus hannai*) farms with both healthy and diseased abalone.

2.3.2. Clinical signs, including behavioural changes

AVG outbreaks in both farmed and wild abalone were associated with high mortality rates (up to 90% on farm). Clinically, abalone may demonstrate one or more of the following signs: irregular peripheral concave elevation of the foot; swollen and protruding mouth parts; eversion of the radula; minimal movement of the pedal muscle; excessive mucus production; absence of the marked extension of the foot shown in the righting reflex when healthy abalone are turned onto their backs; reduced pedal adhesion to the substrate. In Tasmania, abalone affected by AVG in processing plants exhibited 'hard foot' or tetany, excessive mucus production, abnormal spawning and 'bloating' (Ellard *et al.,* 2009). These facilities also experienced much lower morbidity and mortality rates than reported on farms or in wild abalone in Victoria, Australia. Similar signs have been reported for an abalone disease epizootic in Chinese Taipei (Chang *et al.,* 2005).

AVG is normally an acute disease, with abalone dying within 1–2 days of demonstrating gross signs of the disease. Wild harvested abalone held in live-holding facilities in Tasmania have previously exhibited slower onset of clinical signs and mortality. Some Tasmanian wild caught abalone have previously tested positive for AVG using real-time PCR without overt clinical or histological signs.

2.3.3 Gross pathology

Abalone that are loosely attached to the substrate owing to weakness or abnormalities of the pedal muscle should be selected for sampling. If this gross pathology is caused by acute AVG, it is likely that these abalone will die within 1–2 days.

2.3.4. Modes of transmission and life cycle

Horizontal transmission (Bai *et al.,* 2019a; Chang *et al.,* 2005; Crane *et al.,* 2009) has been demonstrated experimentally by:

1. exposing healthy abalone to water containing diseased abalone in the same tank without direct contact between the diseased and healthy abalone;
2. placing healthy abalone in water that was previously inhabited by diseased abalone.

In all cases, 100% mortality was observed with a preclinical period of 1–2 days following exposure and then mortality commenced until 100% mortality occurred within 2–5 days post-infection.

2.3.5. Environmental factors

In Australia, the initial outbreak of AVG occurred on a farm during summer 2005/2006 and subsequently appeared to spread to wild populations, which experienced mortality throughout the following year i.e. during all seasons. All experimental infections to date have been carried out in the temperature range 15–18°C. In Chinese Taipei, during the reported epizootic, the water temperature was 16–19°C, and experimental infections were carried out at 17–20°C. In China (People's Rep. of), natural infections were only detected at water temperatures below 23°C (Gu *et al*., 2019). How temperature affects viral replication and onset of disease has yet to be determined. The possible effects of changes in other environmental factors such as salinity and dissolved oxygen are unknown.

2.3.6. Geographical distribution

Reported in Asia-Pacific.

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

No data available.

2.4.3. Immunostimulation

No data available.

2.4.4. Breeding resistant strains

No data available.

2.4.5. Inactivation methods

AbHV-1 was inactivated by treatment with 50 ppm of the iodophor Buffodine® as well as a 1% solution of the non-ionic surfactant Impress®. Calcium hypochlorite (1.5 ppm) treatment also inactivated the virus (Corbeil *et al*., 2012b).

2.4.6. Disinfection of eggs and larvae

No data available.

2.4.7. General husbandry

To date, experimental data indicates that AbHV-1 is highly virulent. Practices that could be implemented to reduce the severity of the disease have not been identified. It is interesting to note that, in contrast to the situation in Victoria, Australia, clinical disease has not been reported in wild abalone populations in Tasmania, Australia. Disease outbreaks in processing plants in Tasmania suggest that stress factors may influence expression of subclinical infection.

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

At the first signs of increased numbers of abalone appearing to be weak or behaving abnormally, or sudden onsets of unexplained mortality, live moribund individuals should be selected for sampling. If moribund or freshly dead abalone are not available, samples of overtly normal abalone from all parts of the farm, and representing all age classes, should be selected for sampling.

3.2. Selection of organs or tissues

Neural tissue that includes the cerebral, pleuropedal and buccal ganglia.

3.3. Samples or tissues not suitable for pathogen detection

To date, lesions have not been detected consistently in non-neural tissues.

3.4. Non-lethal sampling

Not available.

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 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 70–90% (v/v) analytical/reagent-grade (undenatured) 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

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

Not applicable.

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 larvae can be pooled to obtain the minimum amount of material for 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	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												
Imprints												
~~Histopathology~~		~~+~~	~~+~~	~~NA~~		~~++~~	~~++~~	~~2~~		~~++~~	~~++~~	~~2~~
Transmission electron microscopy						+	+	NA		~~+~~	~~+~~	~~NA~~
Real-time PCR		+++	+++	2		+++	+++	2		+++	+++	2
Conventional PCR						++	++	2				
Conventional PCR followed by amplicon sequencing										+++	+++	2
In-situ hybridisation						++	++	NA		++	++	NA
Bioassay						+	+	NA				
LAMP												
Ab-ELISA												
Ag-ELISA												
Other antigen detection methods												
Other methods												

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

4.1. Wet mounts

Not applicable.

4.2. Electron microscopy/cytopathology

Transmission electron microscopy is not a routine diagnostic method but can be used to confirm the presence of viral particles in infected ganglia. AbHV~~-1~~ particles are icosahedral with electron dense cores and a diameter of 100–110 nm. The intranuclear location of the particles and their ultrastructure are characteristic of members of the *Herpesviridae* (Tan *et al.,* 2008).

Tissue samples (containing pleuropedal ganglion) for examination by electron microscopy should be fixed using 2.5% (v/v) glutaraldehyde and 2–4% (v/v) paraformaldehyde in 0.1 M cacodylate buffer and post-fixed in 1% (w/v) osmium tetroxide, washed in reverse osmosis water (3 × 5 minutes), dehydrated in a graded series of 'analytical grade' ethanol (70%, overnight at 4°C; 95%, 20 minutes; 100%, 3 × 20 minutes), infiltrated in 100% Spurr's resin (overnight) and then embedded in Spurr's resin.

4.3. Histopathology

Neural tissue (cerebral, pleuropedal and buccal ganglia, branches of the pedal nerve and peripheral nerves) is the prime target and should be sampled, fixed ~~(using 10% formalin)~~ and processed using standard procedures, and stained with haematoxylin and eosin for histological examination as specified in Chapter 2.4.0.

Abalone affected with AVG demonstrate inflammation (increased infiltration by haemocytes) and necrosis confined to neural tissue (cerebral, pleuropedal and buccal ganglia, branches of the pedal nerve and peripheral nerves) as observed in histological sections of neural tissue stained with haematoxylin and eosin and examined by light microscopy (Chang & Handlinger, 2022; Ellard *et al.,* 2009; Hooper *et al.,* 2007).

4.4. Nucleic acid amplification

PCR assays should always be run with the controls specified in Section B.5.5 *Molecular methods* of Chapter 2.4.0 *General information* (diseases of molluscs). An 18S rDNA real-time PCR can be used to validate nucleic acid extraction and integrity, and the absence of PCR inhibitors (Crane *et al.,* 2016). 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

Following validation of the real-time PCR test targeted to ORF49 (Corbeil *et al.,* 2010), the discovery of genotypic variants in Australia not recognised by this test necessitated other real-time PCR tests to be developed based on more conserved regions of the viral genome. Real-time PCR tests targeted to ORF49 and ORF66 have been used extensively in disease investigations and the accumulated data have been used in test validation (Caraguel *et al*., 2019). For the detection of all genetic variants, the ORF49 and ORF66 real-time PCR tests should be run in parallel, and infection with AbHV can be confirmed by a positive result from either of the two tests. Each of these tests can be multiplexed with an 18S rDNA real-time PCR test, used to validate nucleic acid extraction and integrity, and the absence of PCR inhibitors (Crane et al., 2016).

***Primers and probes* (*sequences*)**

Pathogen/ target gene	**Primer/probe (5'–3')**	**Concentration**	**Cycling parameters[(a)]**
Crane *et al.,* 2016; GenBank Accession No.: MW412419.1			
AbHV ORF49	ORF49F1: AAC-CCA-CAC-CCA-ATT-TTT-GA ORF49R1: CCC-AAG-GCA-AGT-TTG-TTG-TT 49Prb1: 6FAM-CCG-CTT-TCA-ATC-TGA-TCC-GTG-G-TAMRA	300 nM 300 nM 100 nM	50 cycles of: 95°C/3 sec and 62°C/30 sec

Crane *et al.,* 2016; GenBank Accession No.: MW412419.1			
AbHV ORF66	ORF66F1: TCC-CGG-ACA-CCA-GTA-AGA-AC ORF66R1: CAA-GGC-TGC-TAT-GCG-TAT-GA 66Prb1: 6FAM-TGG-CCG-TCG-AGA-TGT-CCA-TG-TAMRA	300 nM 300 nM 100 nM	50 cycles of: 95°C/3 sec and 60°C/30 sec

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

4.4.2. Conventional PCR

Conventional PCR may also be used for detection of AbHV-~~1~~ in tissue samples. Nucleic acid is extracted as described above. The AbHV1617 PCR has been shown to generate amplicons of various length (522bp to 588bp) depending on the AbHV-~~1~~ isolate. Thus, it is potentially useful for epidemiological studies and to confirm positive real-time PCR results (Crane *et al*., 2016). A second PCR targeting the Taiwanese AbHV-~~1~~ DNA polymerase gene has also been developed (Chen *et al*., 2012). The primer sequences for the two tests are detailed below.

Primer sequences

Pathogen / target gene	**Primer (5'–3')**	**Concentration**	**Cycling parameters[(a)]**
Method 1: Crane *et al.,* 2016; GenBank Accession No.:MW412419.1 amplicon size: 522–588 bp (depending on genetic variant)			
AbHV	AbHV-16: GGC-TCG-TTC-GGT-CGT-AGA-ATG AbHV-17: TCA-GCG-TGT-ACA-GAT-CCA-TGT-C	360 nM 360 nM	40 cycles of: 94°C/30 sec and 52°C/30 sec
Method 2: Chen *et al*., 2012; GenBank Accession No.: HQ317456; amplicon size: 606 bp			
AbHV	40f: TCC-ATC-GAG-ATT-CCC-AGT-TC 146r: ACG-CCA-CCC-TGT-ATA-ACG-AG	400 nM 400 nM	35 cycles of: 94°C/60 sec and 52°C/60 sec

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

4.4.3. Other nucleic acid amplification methods

A loop-mediated isothermal amplification assay for rapid and sensitive detection of AbHV-~~1~~ has been developed that is 100-fold more sensitive than conventional PCR (Chen *et al*., 2014) but is not widely used because of false positive and false negative results.

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

In-situ hybridisation localises AbHV-~~1~~-infected cells within the neural tissue which, on histological examination, demonstrates ganglioneuritis typified by an inflammatory change with increased cellularity involving mainly haemocytes and glial cells, and cell necrosis in the affected nerves (Mohammad *et al.,* 2011).

The *in-situ* hybridisation (ISH) procedure uses a digoxygenin (DIG)-labelled DNA probe to detect AbHV-~~1~~ in formalin-fixed, paraffin-embedded (FFPE) tissue sections and is described in Crane *et al.* (2016).

4.7. Immunohistochemistry

Not applicable.

4.8. Bioassay

A bioassay is not normally required for routine diagnosis. However, when there is a suspect case due to the presence clinical signs and/or histopathology but molecular tests yield negative results, a bioassay (Corbeil *et al*., 2012a) can

be used for confirmation of the presence of a previously unknown genetic variant. Homogenised and clarified neural tissue is used as inoculum and injected (i.m.) into the foot of known uninfected susceptible abalone host species. The inoculated abalone are monitored for clinical signs such as loss of adhesion to the substrate and then samples taken for histology, molecular analyses and electron microscopy. If presence of a herpesvirus is confirmed by electron microscopy further investigation such as whole genome sequencing should be initiated.

4.9. Antibody- or antigen-based detection methods

None currently available.

4.10. Other methods

None.

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

The real-time PCR assays targeting ORF49 and ORF66 performed in parallel is recommended for surveillance to demonstrate freedom in apparently health populations (Caraguel *et al.*, 2019).

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 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.

6.1. Apparently healthy animals or animals of unknown health status[7]

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 abalone herpesvirus shall be suspected if at least one of the following criteria is met:

i) Positive result by a real-time PCR

ii) Positive result by conventional PCR

~~Histopathological changes consistent with the presence of the pathogen~~

6.1.2. Definition of confirmed case in apparently healthy animals

The presence of Infection with abalone herpesvirus is considered to be confirmed if one of the following criteria are met:

i) Positive results by real-time PCR and positive result by conventional PCR followed by sequence analysis of the amplicon

ii) Positive results by *in-situ* hybridisation and positive result by conventional PCR followed by sequence analysis of the amplicon

[7] For example transboundary commodities.

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 abalone herpesvirus 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) Positive result by a real-time PCR

iii) Positive result by conventional PCR

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

v) Positive result of a bioassay

6.2.2. Definition of confirmed case in clinically affected animals

The presence of infection with abalone herpesvirus is considered to be confirmed if one of the following criteria are met:

i) Positive results by real-time PCR and by conventional PCR followed by sequence analysis of the amplicon

ii) Positive results by *in-situ* hybridisation and by conventional PCR followed by sequence analysis of the amplicon

6.3. Diagnostic sensitivity and specificity for diagnostic tests

The diagnostic performance of tests recommended for surveillance or diagnosis of infection with abalone herpesvirus are provided in Tables 6.3.1. and 6.3.2. This information can be used for the design of surveys for infection with abalone herpesvirus, however, it should be noted that diagnostic performance is specific to the circumstances of each diagnostic accuracy study (including the test purpose, source population, tissue sample types and host species) and diagnostic performance may vary under different conditions. 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

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 the wild and processing plants	Pleuropedal ganglion or pedal nerve cords	*Haliotis rubra*	100 (48)	100 (48)	Histopathology	Corbeil *et al.*, 2010
Conventional PCR								
~~Histopathology~~								

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

6.3.2. For surveillance of apparently healthy animals

Test type	Test purpose	Source populations	Tissue or sample types	Species	DSe (*n*)	DSp (*n*)	Reference test	Citation
Real-time PCR	Surveillance	Naturally AbHV~~-1~~ infected wild and farmed populations;	Pleuropedal ganglion or pedal nerve cords	*Haliotis laevigata*; *H. rubra*; *H. laevigata* x	90.1 (1452)	97.7 (1452)	Histopathology	Caraguel *et al.*, 2019

		AbHV~~-1~~ free populations		*H. rubra* hybrids				
Histopathology	Surveillance	Naturally AbHV-1 infected wild and farmed populations; AbHV-1 free populations	Pleuropedal ganglion or pedal nerve cords	*Haliotis laevigata*; *H. rubra*; *H. laevigata* x *H. rubra* hybrids	6.3 (1452)	100 (1452)	real-time PCR	Caraguel *et al.,* 2019

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

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NB: There is a WOAH Reference Laboratory for infection with abalone herpesvirus
(please consult the WOAH web site:
https://www.woah.org/en/what-we-offer/expertise-network/reference-laboratories/#ui-id-3).
Please contact WOAH Reference Laboratories for any further information on infection with abalone herpesvirus

NB: FIRST ADOPTED IN 2012.

Annexe 58. Point 9.2.3. – Chapitre 2.4.4. « Infection à *Marteilia refringens* »

CHAPTER 2.4.4.

INFECTION WITH *MARTEILIA REFRINGENS*

1. Scope

Infection with *Marteilia refringens* means infection with the pathogenic agent *M. refringens* (including O and M types) of the Family *Marteiliidae.*

2. Disease information

2.1. Agent factors

2.1.1. Aetiological agent

Marteilia refringens is a protozoan parasite of the Family *Marteiliidae* (Cavalier-Smith & Chao, 2003; Feist *et al.*, 2009) infecting the digestive system of several bivalve species and inducing physiological disorders and eventual death of the animal (Alderman, 1979; Grizel *et al*., 1974). Two types of *M. refringens* (Grizel *et al*., 1974), types O and M, were defined by Le Roux *et al.* (2001). Although more recent results suggest that *M. refringens* should be distinguished from *M. pararefringens* (previously *M. maurini* or *M. refringens* type M) (Kerr *et al.*, 2018), a larger set of samples is required to properly define both species and most available data in the literature do not allow differentiation of *M. refringens* type O (= *M. refringens* in Kerr *et al.*, 2018) or *M. refringens* type M (= *M. pararefringens* in Kerr *et al.*, 2018) to be made.

2.1.2. Survival and stability in processed or stored samples

No information available

2.1.3. Survival and stability outside the host

After its release from the European flat oyster (*Ostrea edulis*), *M. refringens* can survive at least 20 days in seawater and faeces. Parasite survival seems improved in faeces compared with seawater (Mérou *et al.*, 2022).

2.2. Host factors

2.2.1. Susceptible host species

Species that fulfil the criteria for listing as susceptible to infection with *Marteilia refringens* according to Chapter 1.5. of the *Aquatic Animal Health Code* (*Aquatic Code*) are: ~~blue mussel (*Mytilus edulis*), dwarf oyster (*Ostrea stentina*), European flat oyster (*Ostrea edulis*), European razor clam (*Solen marginatus*), golden mussel (*Xenostrobus securis*), Mediterranean mussel (*Mytilus galloprovincialis*) and striped venus clam (*Chamelea gallina*).~~

Family	Scientific name	Common name
Ostreidae	Ostrea edulis	European flat oyster
	Ostrea stentina	dwarf oyster
Mytilidae	Mytilus edulis	blue mussel
	Mytilus galloprovincialis	Mediterranean mussel
	Xenostrobus securis	golden mussel
Solenidae	Solen marginatus	European razor clam
Veneridae	Chamelea gallina	striped venus clam

Additionally, a copepod species (*Paracartia grani*) has been found to meet the criteria for listing as susceptible to infection with *M. refringens* and is considered an intermediate host.

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 *M. refringens* according to Chapter 1.5 of the *Aquatic Code* are: ~~Chilean flat oyster (*Ostrea chilensis*), a copepod (*Paracartia latisetosa*) and Japanese flat oyster (*Ostrea denselamellosa*).~~

Family	Scientific name	Common name
Acartiidae	Paracartia latisetosa	no common name
Ostreidae	Ostrea chilensis	Chilean flat oyster
	Ostrea denselamellosa	Japanese flat 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: ~~Cortez oyster (*Crassostrea corteziensis*), grooved carpet shell (*Ruditapes decussatus*), Pacific cupped oyster (*Magallana* [syn. *Crassostrea] gigas*) and zooplankton (*Acartia discaudata, Centropages typicus, Euterpina acutifrons*, unidentified *Oithona* sp., *Penilia avirostris).*~~

Family	Scientific name	Common name
Acartiidae	Acartia discaudata	no common name
Achidiidae	Euterpina acutifrons	no common name
Centropagidae	Centropages typicus	no common name
Oithonidae	Oithona sp.	no common name
Ostreidae	Magallana [syn. Crassostrea] gigas	Pacific cupped oyster
	Crassostrea corteziensis	Cortez oyster
Sididae	Penilia avirostris	no common name
Veneridae	Ruditapes decussatus	grooved carpet shell

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

Marteilia refringens usually causes clinical infection in the European flat oyster, *O. edulis* (Berthe *et al.*, 2004; Grizel *et al.*, 1974). In flat oysters and mussels, prevalence and infection intensity are generally higher in individuals 2 years old or older (Audemard *et al.*, 2001; Villalba *et al.*, 1993b).

2.2.4. Distribution of the pathogen in the host

Marteilia refringens infects the digestive tract. Young plasmodia are mainly found in the epithelium of labial palps, oesophagus and the stomach (Grizel *et al*., 1974). Sporulation takes place in the digestive gland tubules and ducts. Propagules are released into the lumen of the digestive tract and shed into the environment in faeces (Audemard *et al.,* 2002; Berthe *et al*., 2004; Mérou *et al.*, 2022).

2.2.5. Aquatic animal reservoirs of infection

Infected flat oysters, *O. edulis,* and mussels, *Mytilus edulis* and *M. galloprovincialis,* might not exhibit clinical signs or mortality, however they can release parasite sporangiospores (Arzul *et al.,* 2014; Mérou *et al.,* 2023).

2.2.6. Vectors

None known.

2.3. Disease pattern

2.3.1. Mortality, morbidity and prevalence

Infection is lethal for oysters: a 50–90% mortality rate is usually reported during summer and autumn and is associated with sporulation of the parasite (Grizel, 1985; Grizel *et al*., 1974). Similarly, morbidity is higher during warmer periods. Mussels are less affected by infection but mortalities up to 40% were reported in

impacted areas (Berthe *et al*., 2004; Villalba *et al*., 1993b) and naïve mussels presented 100% mortality after being cultured for 6 months in an infected area (Thébault *et al.,* 1999).

Prevalence is highly variable – up to 98% in *O. edulis*. Higher prevalence is expected depending on farming practices and in areas where potential hosts have had more than 1 year of exposure to infection (Berthe *et al*., 2004; Grizel, 1985). Prevalence usually peaks in summer whereas the parasite is usually absent or found at lower infection intensity in winter and early spring (Audemard *et al.*, 2001; Mérou *et al.*, 2023). An additional prevalence peak in spring has been reported in several studies (Arzul *et al.*, 2014; Boyer *et al.*, 2013; Carrasco *et al.*, 2007; Mérou *et al.*, 2023).

2.3.2. Clinical signs, including behavioural changes

Clinical signs include dead or gaping molluscs (Grizel, 1985; Grizel *et al*., 1974) but are not specific for infection with *M. refringens* and could be indicative of other infections.

2.3.3 Gross pathology

Pale digestive gland, thin watery flesh, mantle retraction and reduced growth rate were reported for infected flat oysters (Berthe *et al*., 2004; Grizel, 1985; Grizel *et al*., 1974), although these gross signs are not specific for infection with *M. refringens*. Reduced growth rate and inhibition of gonad development were reported for infected mussels (Villalba *et al*., 1993a).

2.3.4. Modes of transmission and life cycle

Horizontal transmission of *M. refringens* occurs, probably via an intermediate host (Audemard *et al.,* 2002; Carrasco *et al.,* 2008b). The parasite could be experimentally transmitted from *O. edulis* and *M. galloprovincialis* to the copepod *Paracartia grani* (Audemard *et al.,* 2002; Carrasco *et al.,* 2008b). Transmission from *P. grani* to *O. edulis* or *M. galloprovincialis* has not been demonstrated experimentally (Audemard *et al.,* 2002; Carrasco *et al.,* 2008b). In oysters, the early stages of disease occur in the oesophagus, stomach, palps and even gill epithelia. It is thought that initial infection occurs via feeding currents. In mussels, the early stages have been observed in the epithelium of the gills, mantle, stomach and primary digestive tubules (Carrasco *et al.,* 2008a).

The life cycle of *M. refringens* is suspected to be indirect and may include *P. grani* (Audemard *et al.,* 2001; 2002), at least in pond systems. Other species (see Sections 2.2.5 and 2.2.6) might be involved as reservoirs or vectors in the *M. refringens* life cycle but their role has not been demonstrated).

The detection of *M. refringens* DNA in plankton, particularly nanoplankton, and in the benthos, suggests their involvement in the parasite life-cycle including transmission and storage or possible overwintering, respectively (Mérou *et al.*, 2023).

2.3.5. Environmental factors

The threshold temperature for parasite sporulation and transmission is 17°C. This temperature is common in estuaries or bays where prevalence is usually higher in the upper parts of the water column (Audemard *et al.,* 2001; Berthe *et al*., 2004; Carrasco *et al.,* 2007; Grizel, 1985). Infection with *M. refringens* is seldom observed in open sea waters (Grizel, 1985). High salinity and water renewal could be detrimental to *M. refringens* development and transmission, although these parameters appear to be less significant than temperature (Audemard *et al.,* 2001).

Parasite DNA detection in pelagic compartments was found higher when temperature, salinity and cholorophyll-a were higher (Mérou *et al.*, 2023).

2.3.6. Geographical distribution

Reported in Europe and North Africa.

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

None.

2.4.3. Immunostimulation

None.

2.4.4. Breeding resistant strains

None.

2.4.5. Inactivation methods

No data available.

2.4.6. Disinfection of eggs and larvae

No data available.

2.4.7. General husbandry

Stocking at low density or in association with resistant mollusc species, such as *Crassostrea gigas,* has been shown to be effective (Grizel, 1985). Stocking bivalves in deep zones exposed to currents seems to limit the transmission of the parasite. Considering the possible presence of the parasite in the sediment (Mérou *et al.*, 2023), maintaining bivalves at distance from the bottom should limit the number of infected animals.

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) of species referred to in Section 2.2.1., should be sampled preferentially, to increase the chances of finding infected bivalves. For histology, only live (including moribund) bivalves should be sampled.

Sampling of bivalves should be organised when prevalence is known to be at a maximum. When such data are not available in a particular ecosystem, sampling should preferably be carried out when temperature reaches the yearly maximum (Audemard *et al.,* 2001; Carrasco *et al.,* 2007).

3.2. Selection of organs or tissues

A 3–5 mm thick section of tissues including gills and digestive mass is used for diagnosis of *M. refringens* infection by histology and PCR. A piece of digestive gland is preferred for imprints.

3.3. Samples or tissues not suitable for pathogen detection

Tissues other than gills and digestive mass are not suitable.

3.4. Non-lethal sampling

Examination of fresh samples of faeces collected from potentially infected bivalves using light microscopy is possible although this approach has not been validated (See Section 4.1)

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 success of pathogen isolation and 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 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 histological 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

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.

Performances of diagnostic methods applied on pools have not been evaluated.

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						+	+	NA				
Tissue imprints		++	++	NA		+++	+++	NA				
Histopathology		++	++	2		+++	+++	NA				
Transmission electron microscopy					+	++	++	NA	~~+~~	~~++~~	~~++~~	~~NA~~
Real-time PCR	+++	+++	+++	3	+++	+++	+++	NA	+++	+++	+++	NA
Conventional PCR	++	++	++	2	+++	+++	+++	NA				
Conventional PCR followed by amplicon sequencing									+++	+++	+++	NA
In-situ hybridisation									+	+++	+++	NA
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

Samples to be taken consist of gaping bivalves ~~oysters/mussels~~ or freshly dead bivalves ~~oysters/mussels~~.

Squash a piece of digestive gland on a glass slide. Observations are then made at ×400 magnification and can potentially show refringent granules in mature sporangia.

Marteilia species are indicated by the presence of large (9–30 µm) spherical bodies containing thick wall structures.

4.2. Imprints

In moderate and advanced infections, digestive gland imprints are prepared.

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

*A*fter drying tissues on absorbent paper, several imprints are made on a glass slide. Slides are air-dried, fixed and stained using a commercially available blood-staining kit, in accordance with the manufacturer's instructions; fixation can be done using methanol or absolute ethanol. After rinsing in tap water and drying, the slides are mounted with a cover-slip using an appropriate synthetic resin. Slides are observed first at ×200 magnification and then under oil immersion at ×1000 magnification.

The observation of cells with a range in size of 5–8 µm diameter in the early stages of development and up to 30–40 µm during sporulation, may indicate infection with *Marteilia refringens.* The cytoplasm stains basophilic, whereas the nucleus stains eosinophilic. Pale halos around large, strongly stained (refringent) granules and, in larger cells, cell-within-cell arrangements are observed. In advanced stages, eight secondary cells can be observed in the primary cells and four spores in each secondary cell (Berthe *et al*., 2000; 2004; Grizel *et al*., 1974).

4.3. Histopathology

Samples to be taken consist of live or moribund bivalves.

*S*ections of tissues that include gills, digestive gland, mantle and gonad 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 ×1000.

Specificity and sensitivity: values of diagnostic sensitivity and specificity for histology were estimated at 70% and 99%, respectively (Thébault *et al*., 2005).

The observation of cells ranging in size from 4 to 40 µm may be indicative of infection with *Marteilia refringens*. Young stages (uninucleated primary cells) are mainly found in the apical part of the epithelium of labial palps, stomach and sometimes in the digestive tubules. Sporulation involves divisions of cells within cells and generally takes place in the digestive gland tubules and ducts. Refringent granules appear during sporulation but are not observed in early stages. The cytoplasm stains basophilic, whereas the nucleus stains eosinophilic. The granules can range from deep orange to deep red; *M. refringens* can sometimes be observed in other organs including gill and mantle connective tissues (Carrasco *et al.*, 2015; Grizel *et al.*, 1974).

Marteilia refringens is slightly different from other *Marteilia* species including *M. sydneyi* or *M. octospora.* Recognition criteria are mainly based on the number of secondary and tertiary cells (respectively 8 and 4 for *M. refringens*). Although *M. christenseni* and *Eomarteilia granula* display the same number of secondary and tertiary cells as *M. refringens,* they infect different host species in different geographic zones.

4.4. Transmission electron microscopy

A small-sized piece of digestive gland (1–2 mm) should be fixed in an appropriate fixative for at least 1 hour and then processed as described in Section B.5.4 *Transmission electron microscopy methods* of Chapter 2.4.0 *General information* (diseases of molluscs).

The presence of parasites within the epithelia of the digestive gland or the stomach may be indicative of infection with *Marteilia refringens*. Different parasite stages can be observed (Grizel *et al.,* 1974; Longshaw *et al*., 2001). The first stage (= primary cell) is uninucleated but is often observed presenting a single secondary cell within it. Secondary cells result from a series of divisions within the primary cells and include eight presporangia. These presporangia

(=secondary cells) divide and contain four-spore primordia (= tertiary cells). Spore primordia cleave internally to produce mature spores. Mature spores consist of three sporoplasms, one inside the other, the outermost one containing haplosporosomes.

4.5. Nucleic acid amplification

Samples to be taken consist of tissues of digestive gland and gills from live or freshly dead molluscs.

PCR assays should always include the controls specified in Section B.5.5 *Molecular methods* of 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 resolves most cases of PCR inhibition).

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.

4.5.1. Real-time PCR

Two multiplex real-time PCR assays targeting the ITS (internal transcribed spacer) gene have been developed for the specific detection and discrimination of *M. refringens* type O and type M (Carrasco *et al*., 2017; EURL, 2023).

Additionally, a multiplex real-time PCR assay targeting the 18S gene allows the concomitant detection of *M. refringens* and *Bonamia* spp. parasites (Canier *et al.*, 2020). However, validation tests showed that this PCR assay is less specific and also amplifies *M. cochillia* and to a lesser extent *M. sydneyi*.

***Primers and probes* (*sequences*)**

Pathogen/ target gene	Primer/probe (5'–3')	Concentration	Cycling parameters[(a)]
Method 1: Carrasco *et al.* (2017); GenBank Accession No.: MH304865.1			
M. refringens types O and M ITS	Fwd Mare-F: YCA-GGC-GAG-TGC-TCT-CGT-T Rev Mare-R: TGA-TCT-GAT-ATT-ATT-CAG-CTG-TTC-GA Probe Mare-O: CCT-TTC-CCC-GAC-GGC (VIC MGB-NFQ) Probe MareM: GCT-TGC-CCT-ACG-GCC (FAM MGB-NFQ)	400 nM 400 nM 80 nM 80 nM	50 cycles of: 95°C/3 sec and 60°C/30 sec
Method 2: EURL (2023); GenBank Accession No.: MH304863.1			
M. refringens types O and M ITS	Fwd TaqMar-F: GTG-TTC-GGC-ACG-GGT-AGT Rev TaqMar-R: TGA-TCT-GAT-ATT-ATT-CAG-CTG-TTC-G TaqProb-O: GCC-CTT-TCC-CCG-ACG-GCC-G (FAM-BHQ-1) TaqProb-M: GCG-CTT-GCC-CTA-CGG-CCG-TGC (HEX-BHQ-1)	100 nM 300 nM 250 nM 250 nM	40 cycles of: 95°C/30 sec and 60°C/1 min
Method 3: Canier *et al.* (2020); GenBank Accession No.: MH342044.1			
M. refringens Also amplifies *M. cochillia* and *M. sydneyi* 18S	Fwd Mar_18S_F: ACG-ATC-AAA-GTG-AGC-TCG-TG Rev Mar_18S_R: CAG-TTC-CCT-CAC-CCC-TGA-T Probe Mar_18S_IN: GCA-TGG-AAT-CGT-GGA-ACG-GG (FAM-BHQ-1)	400 nM 400 nM 300 nM	40 cycles of: 95°C/15 sec and 60°C/1 min

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

4.5.2. Conventional PCR

PCR primers are available that target the ITS1 (internal transcribed spacer) region (Le Roux *et al*., 2001), 18S gene (Le Roux *et al*., 1999) and the IGS (rDNA intergenic spacer) region (López-Flores *et al*., 2004).

Primer sequences

Pathogen/ target gene	Primer (5'–3')	Concentration	Cycling parameters[(a)]
Method 1: Le Roux *et al.* (2001); GenBank Accession No.: MH329403.1; amplicon size 412 bp			
M. refringens types M and O Also amplifies *M. cochillia* ITS-1	Fwd Pr4 (M2A): CCG-CAC-ACG-TTC-TTC-ACT-CC Rev Pr5 (M3AS): CTC-GCG-AGT-TTC-GAC-AGA-CG	1000 nM 1000 nM	30 cycles of: 95°C/1 min and 55°C/1 min and 72°/1 min
Method 2: Lopez-Flores *et al.* (2004) (nested PCR) ; GenBank Accession No.: MH356753.1; amplicon size [525bp & 358 bp]			
M. refringens types M and O Also amplifies *M. cochillia* and possibly other species IGS	PCR1 Fwd MT1: GCC-AAA-GAC-ACG-CCT-CTA-C Rev MT2: AGC-CTT-GAT-CAC-ACG-CTTT PCR2 Fwd MT-1B: CGC-CAC-TAC-GAC-CGT-AGC-CT Rev MT-2B: CGA-TCG-AGT-AAG-TGC-ATG-CA	1000 nM 1000 nM 1000 nM 1000 nM	PCR 1 I30 cycles of: 95°C/1 min and 55°C/1 min and 72°/1 min PCR2 25 cycles of: 95°C/30 sec and 60°C/30 sec and 72°/30 sec
Method 3: Le Roux *et al.* (1999); GenBank Accession No.: MH342044.1; amplicon size [266bp or 700 bp]			
***Marteilia* spp. amplifies *M. refringens* types M and O, *M. cochillia*, and possibly other species** **18S**	**Fwd SS2: CCG-GTG-CCA-GGT-ATA-TCT-CG** **(Rev SAS1: TTC-GGG-TGG-TCT-TGA-AAG-GC)** **Or** **Rev SAS2: CGA-ACG-CAA-ATT-GCG-CAG-GG**	**1000 nM** **1000 nM** **1000 nM**	**30 cycles of: 95°C/1 min and 55°C/1 min and 72°/1 min**

[(a)]A denaturation step prior to cycling has not been included.
Note: according to the alignment of available sequences
Rev SAS1 primer sequence should be: TTC-GG-TGG-TCT-TGA-AAG-GC

4.5.3. Other nucleic acid amplification methods

A loop-mediated isothermal amplification (LAMP) assay for the detection of *M. refringens* has been developed, but is not validated (Xie *et al.*, 2012).

4.6. Amplicon sequencing

The size of the PCR amplicon is verified by agarose gel electrophoresis and purified by excision from this gel for sequence analysis. Obtained sequences are compared with published sequences.

Sequencing is recommended as one of the final steps for confirmatory diagnostic. Targeted regions are the SSU rDNA (except 18S PCR SS2/SAS1), ITS1 and IGS (intergenic spacer). Although sequences are available in the public gene banks, it is recommended to refer such cases to the appropriate WOAH Reference Laboratory.

4.7. *In-situ* hybridisation

Le Roux *et al.* (1999) developed an ISH genus-specific method targeting the 18S gene. This method allows the detection of all currently known *Marteilia* species. It has been validated against histology for the detection of *M. refringens* (Thébault *et al.*, 2005).

Two other ISH assays have been developed, one targeting the ITS1 (internal transcribed spacer) region (Le Roux *et al*., 2001) and the other targeting the IGS (intergenic spacer) region (Lopez-Flores *et al*., 2008a; 2008b). These assays allow the detection of *M. refringens* type O and type M.

Samples to be taken consist of live or gaping molluscs.

Technical procedure:

Reference	Pathogen/target gene	ISH probe	Probe size
Le Roux et al. (1999)	Marteilia spp. 18S	Digoxigenin-labelled PCR product obtained with SS2/SAS1 primers	266 bp
Le Roux et al. (2001)	M. refringens types M and O ITS1	Digoxigenin-labelled PCR product obtained with Pr4/Pr5 primers	412 bp
Lopez-Flores et al. (2004)	M. refringens types M and O IGS	Digoxigenin-labelled PCR product obtained with MT-1B/MT-2B primers	358 bp

The first steps follow the recommendations described in Section B.5.5.4. of Chapter 2.4.0 *General information* (diseases of molluscs). For hybridisation, sections are incubated with 100 µl of hybridisation buffer (4 × SSC [standard saline citrate], 50% formamide, 1 × Denhardt's solution, 250 µg ml^{-1} yeast tRNA, 10% dextran sulphate) containing approx. 10 ng (2 to 5µl µl of digoxigenin-labelled probe prepared by conventional PCR as described above (section 4.5.2; Le Roux *et al.*, 1999; 2001, Lopez-Flores *et al*., 2004; 2008a; 2008b). Sections are covered with *in-situ* plastic cover-slips and placed on a heating block at 94°C for 5 minutes. Slides are then cooled on ice for 1 to 5 minutes before overnight hybridisation at 42°C in a humid chamber. Sections are washed twice for 5 minutes in 2 × SSC at room temperature, and once for 10 minutes in 0.4 × SSC at 42°C. The detection steps are performed according to the manufacturer's instructions. The slides are then rinsed with appropriate buffer. The sections are counter-stained with Bismarck Brown Yellow, rinsed in tap water, immersed in 95% and then 100% ethanol, 30 seconds for each, rinsed in Xylene (10–30 seconds), and cover-slips are applied using an appropriate mounting medium.

Positive/negative controls: inclusion of the following controls is compulsory. 1) Infected host positive control; 2) non-specific ISH (18S) on samples as an internal positive control. 3) No probe ISH negative control; 4) Uninfected host negative control. Positive controls are available on request from the WOAH Reference Laboratory.

4.8. Immunohistochemistry

Not available.

4.9. Bioassay

Not available.

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

Not currently available or used for diagnostic purposes but monoclonal antibodies have been developed (Berthe *et al*., 2004). These antibodies did not cross-react with *M. sydneyi*.

4.11. Other methods

None available.

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

Real-time PCR is recommended for targeted surveillance to declare freedom from infection with *M. refringens*.

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 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.

6.1. Apparently healthy animals or animals of unknown health status[8]

Apparently healthy populations may fall under suspicion, and therefore be sampled, if there is an epidemiological link 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 *M. refringens* shall be suspected if at least one of the following criteria is met:

i) Positive result by a recommended molecular detection test

ii) Visual observation of the pathogen by microscopy

6.1.2. Definition of confirmed case in apparently healthy animals

The presence of infection with *M. refringens* is considered to be confirmed if the following criterion is met:

i) positive result by real-time PCR and conventional PCR followed by sequence analysis

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 *M. refringens* shall be suspected if at least one of the following criteria is met:

i) Positive result by wet mounts

ii) Positive result by tissue imprints

iii) Positive result by histopathology

iv) Positive result by real-time PCR

v) Positive result by conventional PCR

6.2.2. Definition of confirmed case in clinically affected animals

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

i) positive result by real-time-PCR and conventional PCR followed by sequence analysis

ii) positive result by species-specific ISH and conventional PCR followed by sequence analysis

iii) Positive result of real-time PCR followed by species-specific ISH

6.3. Diagnostic sensitivity and specificity for diagnostic tests

The diagnostic performance of tests recommended for surveillance or diagnosis of infection with *M. refringens* are provided in Tables 6.3.1. (no data are currently available) and 6.3.2. This information can be used for the design of surveys for infection with *M. refringens*, however, it should be noted that diagnostic performance is specific to the circumstances of each diagnostic accuracy study (including the test purpose, source population, tissue sample types and host species) and diagnostic performance may vary under different conditions. Data are only presented where

[8] For example transboundary commodities.

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 [under study]

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

DSe = diagnostic sensitivity, DSp = diagnostic specificity, *n* = ~~samples~~ number of animals used in the validation study.

6.3.2. For surveillance of apparently healthy animals

Test type	Test purpose	Source populations	Tissue or sample types	Species	DSe (*n*)	DSp (*n*)	Reference test	Citation
Histology	Surveillance	Field samples from France and Netherlands, representative of three different levels of prevalence (free, mild, high)	Section of tissues including visceral mass	*Ostrea edulis* ~~Flat oysters~~	70% (200)	99% (200)	*In-situ* hybridisation (18S probe) Bayesian analyses	Thébault *et al.*, 2005
In-situ hybridisation (18S probe)	Surveillance	Field samples from France and Netherlands, representative of three different levels of prevalence (free, mild, high)	Section of tissues including visceral mass	*Ostrea edulis* ~~Flat oysters~~	90% (200)	99% (200)	Histology Bayesian analyses	Thébault *et al.*, 2005
Real-time PCR (Canier *et al.*, 2020)	Surveillance	Field samples from the three main producing areas in France, representative of three different levels of prevalence (free, low, high)	Gills and digestive gland tissues	*Ostrea edulis* ~~Flat oysters~~	87,2% (386)	98,4% (386)	Conventional PCR (Le Roux *et al.*, 2001) Bayesian analyses	Canier *et al.*, 2020
Conventional PCR (Le Roux *et al.*, 2001)	Surveillance	Field samples from the three main producing areas in France, representative of three different levels of prevalence (free, low, high)	Gills and digestive gland tissues	*Ostrea edulis* ~~Flat oysters~~	60.7% (386)	99.9% (386)	Real-time PCR (Canier *et al.*, 2020) Bayesian analyses	Canier *et al.*, 2020

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

7. References

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* *

NB: There is a WOAH Reference Laboratory for infection with *Marteilia refringens*
(please consult the WOAH web site:
https://www.woah.org/en/what-we-offer/expertise-network/reference-laboratories/#ui-id-3).
Please contact WOAH Reference Laboratories for any further information on infection with *Marteilia refringens*

NB: FIRST ADOPTED IN 1995 AS MARTEILIOSIS. MOST RECENT UPDATES ADOPTED IN 2012.

Annexe 59. Point 9.2.4. – Section 2.2.1. et 2.2.2. du chapitre 2.4.5. « Infection à *Perkinsus marinus* »

CHAPTER 2.4.5.

INFECTION WITH *PERKINSUS MARINUS*

[…]

2.2. Host factors

2.2.1. Susceptible host species

~~Eastern oyster, *Crassostrea virginica*; Pacific oyster, *C. gigas;* suminoe oyster, *C. ariakensis;* mangrove oyster, *C. rhizophorae*; Cortez oyster, *C. corteziensis* (Andrews 1996; Calvo *et al.,* 1999; Calvo *et al.,* 2001; Villalba *et al.,* 2004; Cáceres-Martínez *et al.,* 2008); softshell clam, *Mya arenaria*; Baltic macoma, *Macoma balthica* (Dungan *et al.,* 2007).~~

Species that fulfil the criteria for listing as susceptible to infection with *Perkinsus marinus* according to Chapter 1.5. of the *Aquatic Animal Health Code* (*Aquatic Code*) are: ~~American cupped oyster (*Crassostrea virginica*), Ariake cupped oyster (*Magallana* [Syn. *Crassostrea*] *ariakensis*), Cortez oyster (*Crassostrea corteziensis*) and palmate oyster (*Saccostrea palmula*).~~

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. ~~Susceptible stages of the host~~ Species with incomplete evidence for susceptibility

~~All stages after settlement are susceptible.~~

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: ~~Gasar cupped oyster (*Crassostrea tulipa*), mangrove cupped oyster (*Crassostrea rhizophorae*), and Pacific cupped oyster (*Magallana* [Syn. *Crassostrea*] *gigas*).~~

Family	Scientific name	Common name
Ostreidae	*Crassostrea gasar*	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: ~~Columbia black oyster (*Crassostrea columbiensis*), soft shell clam (*Mya arenaria*), and stone oyster (*Striostrea prismatica*).~~

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

[…]

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