

Diagnosis by histopathology of *Haplosporidium* sp. in oysters

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Editions

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1. Scope

This procedure explains the diagnostic test used for the protistan *Haplosporidium* sp. presumptive diagnosis in the oysters *Crassostrea virginica* and *Crassostrea gigas* after histological processing of samples.

2. References

- OIE. Manual of Diagnostic Tests for Aquatic Animals (last edition).
- Howard, D.W., E.J. Lewis, B.J. Keller, and C.S. Smith (2004). Histological techniques for marine bivalves mollusks and crustaceans. NOAA Tech. Memo. NOS NCCOS 5, 218 p.
- Burreson E.M. & Ford S.E. (2004). A review of recent information on the Haplosporidia, with special reference to *Haplosporidium nelsoni* (MSX disease). *Aquat. Living Resour.*, **17**, 499-517.
- Bower, S.M. (2007): Synopsis of Infectious Diseases and Parasites of Commercially Exploited Shellfish: *Haplosporidium nelsoni* (MSX) of Oysters. URL: http://www.pac.dfo-mpo.gc.ca/sci/shelldis/pages/hapneloy_e.htm
- Bower, S.M. (2007): Synopsis of Infectious Diseases and Parasites of Commercially Exploited Shellfish: *Haplosporidium costale* (SSO) of Oysters. URL: http://www.pac.dfo-mpo.gc.ca/sci/shelldis/pages/hcoy_e.htm

3. General information

Two species are known to infect oysters in North America, Asia and Europe:

Haplosporidium nelsoni: Susceptible host species of *Haplosporidium nelsoni*: *Crassostrea virginica* and *C. gigas*. The main infected area extends from Florida, USA north to Nova Scotia, Canada. *H. nelsoni* has also been observed in *C. gigas* in California (USA), Korea, Japan and France.

The disease is restricted to salinities over 15 ppt (*H. nelsoni* cannot survive below 10 ppt), rapid and high mortalities in *C. virginica* occur at 18-20 ppt (parasite proliferation is greatest above 20 ppt). There is some evidence that water temperatures exceeding 20 °C may cause the disease to disappear.

Haplosporidium costale: Susceptible host species of *Haplosporidium costale*: *Crassostrea virginica* with rare infections in *C. gigas*. Main infected area : from New York to Virginia (USA), in high salinity waters (more than 25 ppt). Plasmodia of *H. costale* (but no spores) have been detected at low prevalence in *C. virginica* from the south-east coast of Canada.

4. Equipment and environmental conditions

Binocular microscope for histological and cytological examination, equipped with different normal dry objectives (10X, 20X, 40X and/or 60X) and a 100X (oil) objective with immersion oil. Microscope should be set with [Koehler](#) illumination technique.

5. Operating procedure

5.1. Histological examination

We describe here the two most important species observed in oysters: *Haplosporidium nelsoni* and *H. costale*

5.1.1. *Haplosporidium nelsoni*

The disease is usually systemic with the pathogens spreading to all tissues via haemolymph sinuses, but early infections are localized to the digestive gland and intestine and to the gills. Multi-nucleated eosinophilic plasmodia (4 to 50 µm) can be seen extracellularly in connective tissue of all these organs. Sporocysts (20-50 µm in diameter) and spores (4-6 by 5-8 µm) with an operculum occur only in the epithelium of the digestive tubules of *Crassostrea virginica*. In *C. gigas* spores may be found in other tissues. Plasmodia of *H. nelsoni* are not distinguishable from *H. costale* plasmodia except when spores are visible.

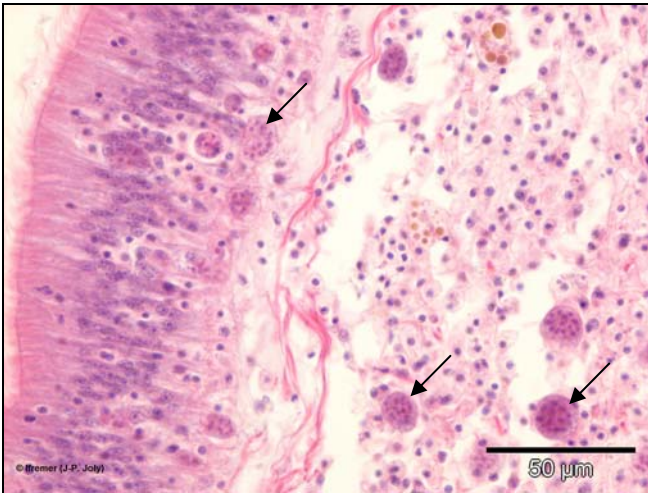


Figure 1 : Plasmodia of *Haplosporidium nelsoni* in connective tissue and intestine epithelium of *Crassostrea virginica*, H&E staining.

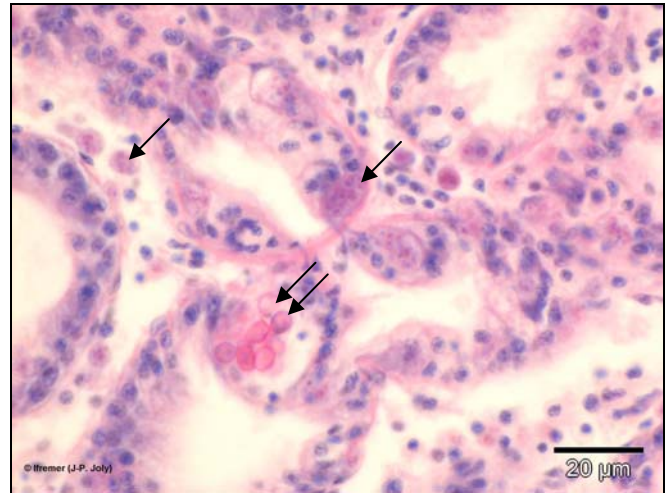


Figure 2 : Plasmodia (arrows) and spores (double arrow) of *Haplosporidium nelsoni* in connective tissue and digestive diverticula epithelium of *Crassostrea virginica*. H&E staining.

5.1.2. *Haplosporidium costale*

Multinucleate plasmodia (~ 10 µm in diameter) can be observed in the connective tissue. Plasmodia develop into sporocysts with spore walls forming around the nuclei. Spores have an operculum but are smaller than *H. nelsoni* (2.6 µm by 3.1 µm). Unlike *H. nelsoni* sporulation occurs throughout the connective tissue of the digestive gland, mantle and gonads, but not in the epithelia of the digestive tubules.

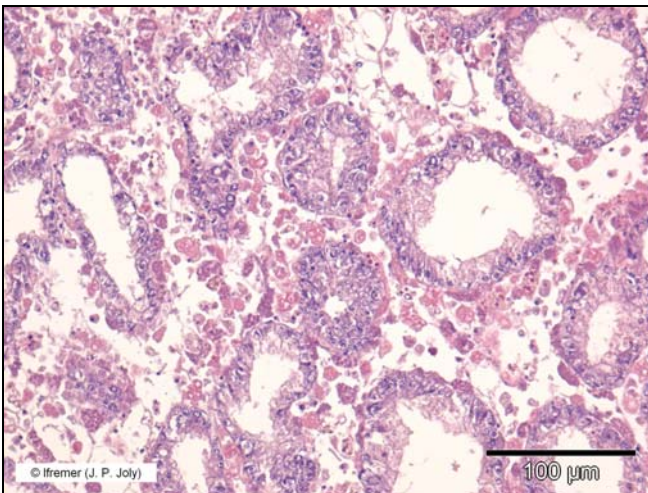


Figure 3 : Digestive gland of a *Crassostrea virginica* heavily infected by *Haplosporidium costale* with many plasmodia and sporocysts in the partially destroyed connective tissue, H&E staining.

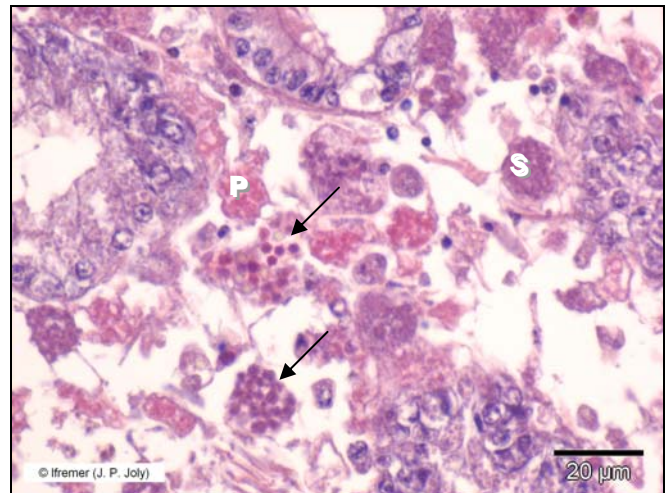


Figure 4 : Higher magnification of figure 3. Note the plasmodia (P), the sporocysts (S) and the spores (arrows) in the connective tissue, H&E staining.

5.2. Results statement

Results are expressed **qualitatively** (infected = **positive** / non-infected = **negative**) and only at the genus level in histology (i.e *Haplosporidium* sp.). They can also be expressed according to **semi-quantitative** scales (*high, medium or low infection*).