

Diagnosis by histo-cytopathology of *Mikrocytos* sp. in oysters

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Editions

Edition	Date	Updated part
1	22/04/09	Creation
2	26/07/10	Modification of § 2, 3, 5 and 5.3

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1. Scope

This procedure explains the tests used for the protistan *Mikrocytos* sp. presumptive diagnosis in the Pacific oyster *Crassostrea gigas*, the Eastern oyster *Crassostrea virginica*, the European flat oyster *Ostrea edulis* and the Pacific flat oyster *Ostrea conchaphila* following histological or cytological processing of samples.

2. References

- Council Directive [2006/88/EC](#) of 24 October 2006 on animal health requirements for aquaculture animals and products thereof, and on the prevention and control of certain diseases in aquatic animals.
- OIE. Manual of Diagnostic Tests for Aquatic Animals (2006 edition).
- Bower S.M., Hervio D. & Meyer G.R. (1997). Infectivity of *Mikrocytos mackini*, the causative agent of Denman Island disease in Pacific oysters, *Crassostrea gigas*, to various species of oysters. Dis. Aquat. Org., 29: 111-116.
- Friedman, C.S., H.M. Brown, T.W. Ewing, F.J. Griffin and G.N. Cherr. 2005. Pilot study of the Olympia oyster *Ostrea conchaphila* in the San Francisco Bay estuary: description and distribution of diseases. Diseases of Aquatic Organisms 65: 1-8.
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- Wang, Z., Y. Liang and X. Lu. 2010. Use of histopathology, PCR and *in situ* hybridization methods to detect the parasite *Mikrocytos* sp. in Pacific oyster *Crassostrea gigas* from the northern coast of the Yellow Sea, China. Aquatic Living Resources 23: 125-130.
- Bower, S.M. (2010): Synopsis of Infectious Diseases and Parasites of Commercially Exploited Shellfish: *Mikrocytos mackini* (Denman Island Disease) of Oysters. URL: http://www.pac.dfo-mpo.gc.ca/sci/shelldis/pages/mikmacoy_e.htm

3. General information

Mikrocytos mackini is an eukaryotic protistan of uncertain taxonomy parasite of oysters from the West coast of North America. *Crassostrea gigas* seems to be more resistant to the disease than the other oyster species (*C. virginica*, *Ostrea edulis*, *O. conchaphila*) challenged experimentally under laboratory and field conditions (Bower et al. 1997). Phylogenetic analysis shows that *M. mackini* is not affiliated to the Haplosporidian group including *Bonamia* spp. although very similar in shape to the *Bonamia* group parasites.

Characteristic of the disease is a focal intracellular infection of vesicular connective tissue cells which results in haemocyte infiltration and tissue necrosis that can be observed as yellow-green pustules on the mantle. Severe infections appear to be restricted to older oysters (over 2 years) and mortalities (often about 30% of older oysters *C. gigas* reared at low tide levels) occur predominantly in April and May after a 3-4 month period when temperatures are below 10 °C. Approximately 10% of infected *C. gigas* appear to recover. Presently *Mikrocytos mackini* seems to be the only representative of the genus *Mikrocytos*.

Mikrocytos-like protists have also been detected in *Ostrea conchaphila* from San Francisco Bay (Friedman et al. 2005), in *Ostrea edulis* from the Atlantic coast of Canada (Gagné et al. 2008) and in *Crassostrea gigas* from the Yellow Sea in China (Wang et al. 2010). Genetic analysis showed that the last two protists are closely related to *M. mackini*.

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

Note: The following descriptions refer to *Mikrocytos mackini* observation in the Pacific oyster *C. gigas*.

5.1. Imprints examination

Use Wright, Wright-Giemsa or equivalent stain (e.g., Hemacolor®). Stained slides with tissue imprints (mantle, palps, adductor muscle and specially tissue with yellow-green pustules) must be observed first with 10X or 20X dry objectives to find areas with parasites and host haemocytes and vesicular connective tissue cells (Figure 1). Using the 40X or 60X and 100X objectives, look for the presence of small protozoa preferably on the periphery of the imprint (Figure 2).

Mikrocytos mackini appears as small spherical or ovoid organisms (2-4 µm wide) usually free or sometimes inside vesicular cells. The parasites show a basophilic cytoplasm and an eosinophilic nucleus. Binucleated cells can be observed. Parasites can also be found inside haemocytes. Shape of the cells can also vary from ovoid to triangular or fusiform (Figure 2).

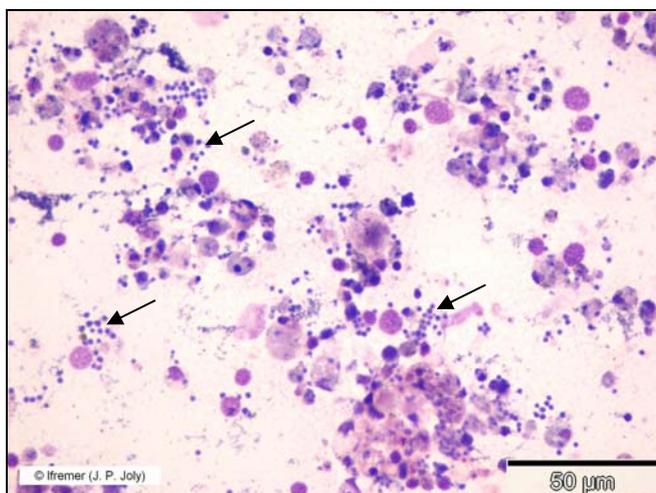


Figure 1 : Palps imprint of a heavily infected Pacific oyster *Crassostrea gigas* from British Columbia, Canada. Hemacolor® staining (X 40 objective).

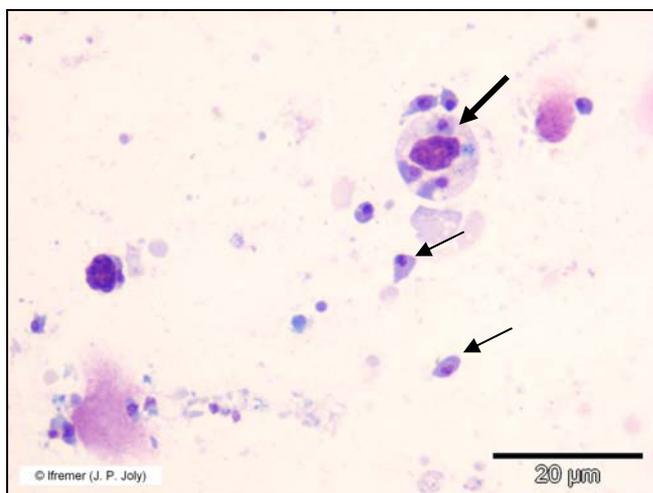


Figure 2 : Imprint of *Crassostrea gigas* adductor muscle tissue showing free *M. mackini* cells (thin arrows) and 3 parasites (thick arrow) inside a haemocyte, Hemacolor® staining (X100 objective).

5.2. Histological examination

Mikrocytos mackini can be detected as intracellular spherical protozoa 2-4 µm in diameter in the vesicular connective tissue cells surrounding the digestive gland (Figure 3 and Figure 4) and in the palps and the mantle. It can also be observed in the circulatory system sometimes inside haemocytes. Parasites can be more easily found in tissue associated with intense haemocyte infiltration (appearing as yellow-green pustules on the mantle during gross observation). In heavy infected oysters *Mikrocytos mackini* can be seen in all the host tissues, particularly in *Crassostrea virginica* a species more susceptible to the disease. This parasite can also be observed in muscle cells and connective tissue of the adductor muscle.

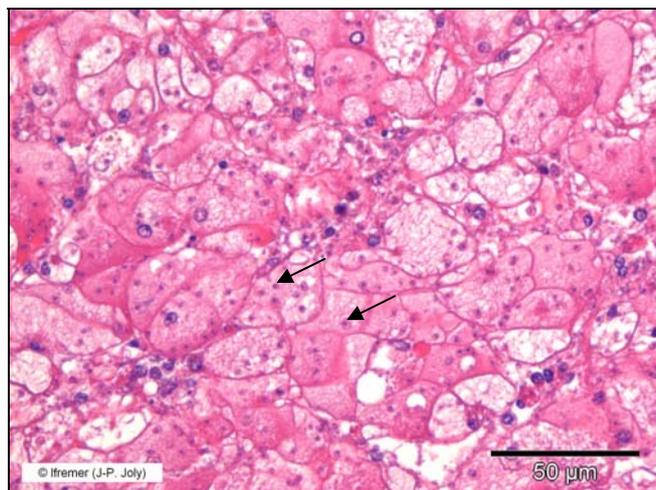


Figure 3 : Numerous *Mikrocytos mackini* parasites infecting vesicular connective tissue cells surrounding the digestive gland of a Pacific oyster *Crassostrea gigas*. H&E staining (X40 objective).

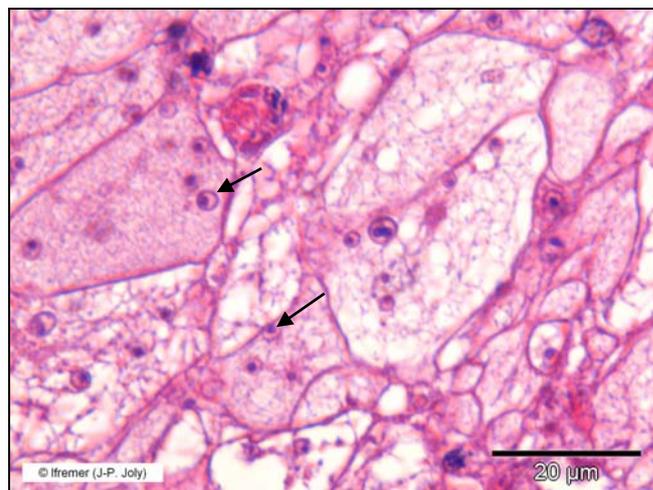


Figure 4 : *Mikrocytos mackini* cells (arrows) in the vesicular connective tissue of a Pacific oyster *Crassostrea gigas*. H & E staining (X100 objective).

5.3. Results statement

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