Mikrocytosis of bivalve molluscs

Ryan B. Carnegie and Gary R. Meyer

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International Council for the Exploration of the Sea
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H. C. Andersens Boulevard 44–46
DK-1553 Copenhagen V
Denmark
Telephone (+45) 33 38 67 00
Telefax (+45) 33 93 42 15
www.ices.dk
info@ices.dk

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Contents

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1 Disease name

Mikrocytosis of bivalve molluscs, Denman Island disease of oysters.

2 Aetiological agent

The disease is caused by protozoan parasites in the genus *Mikrocytos*, of the supergroup Rhizaria (Burki *et al.*, 2013). *Mikrocytos* species are 'microcell' parasites that are typically spherical in shape, ranging from 2 to 5 μm in diameter; usually uninucleate, with nuclei that can be central or eccentric in position depending on the species. They are superficially similar to *Bonamia* spp. parasites that also infect oysters. However, unlike *Bonamia* parasites they are amitochondriate and lack haplosporosomes, and are typically observed infecting vesicular connective tissue cells or myocytes (Figures 1 and 2) rather than haemocytes as *Bonamia* species do specifically (Hine *et al.*, 2001, Carnegie and Cochennec-Laureau 2004, Garcia *et al.*, 2018).

To date, five *Mikrocytos* species have been described from bivalve molluscs. The type species, *Mikrocytos mackini* (Farley *et al.*, 1988), is the agent of the historically significant Denman Island disease of Pacific oysters, *Crassostrea gigas*, in British Columbia, Canada (Quayle, 1961), occurring also in the USA from Washington State to California (Elston *et al.*, 2012, 2015). *Mikrocytos boweri* was also described from British Columbia, infecting the Olympia oyster, *Ostrea lurida* (Abbott *et al.*, 2014). *Mikrocytos mimicus* was described from *C. gigas* from Norfolk, United Kingdom (Hartikainen *et al.*, 2014). Two species, *Mikrocytos veneroïdes* and *Mikrocytos donaxi*, were described from the wedge clam *Donax trunculus* along the Atlantic coast of France (Garcia *et al.*, 2018).

Additional, undescribed *Mikrocytos* parasites have been detected in other hosts and geographic locations, their identities awaiting definitive determination. Parasites observed earlier in flat oysters, *Ostrea edulis*, from Nova Scotia, Canada, displaying mortality after transfer to quarantine in France (Gagné *et al.*, 2008), and in *C. gigas* from the Yellow Sea, China (Wang *et al.*, 2010), have SSU rDNA sequences suggesting an affinity to *M. boweri*. A *Mikrocytos* infecting the Manila clam *Ruditapes philippinarum* in Spain (Ramilo *et al.*, 2014, Garcia *et al.*, 2018) has an SSU rDNA sequence similar to *M. mimicus*.

While these *Mikrocytos* species are only known to infect bivalve mollusc hosts, a parasite species sister to *M. mackini*, *Mikrocytos mytilicoli*, was described infecting *Mytilicola intestinalis*, the gut symbiont copepod of *Mytilus galloprovincialis*, in Galicia, Spain (Darriba *et al.*, 2020). Association of a member of the *Mikrocytos* genus specifically with crustacean hosts raises the question of whether additional species may infect crustaceans when not infecting molluscs. A related genus, *Paramikrocytos*, was erected to accommodate a parasite detected in the European edible crab *Cancer pagurus* (Thrupp *et al.*, 2013, 2015), designated *Paramikrocytos canceri* (Hartikainen *et al.*, 2014).

3 Susceptible species

The best-studied *Mikrocytos* species, *M. mackini*, is known to infect other oysters besides *C. gigas*, including the eastern oyster *Crassostrea virginica*, *O. edulis*, and *O. lurida* (Bower *et al.,* 1997, the authors identifying *O. lurida* as *O. conchaphila* following the convention of the time), and Kumamoto oyster *Crassostrea sikamea* (Elston *et al.,* 2012). These alternative species may be comparably susceptible to disease and mortality as *C. gigas*, if not more susceptible. Experimental challenges have shown that juvenile Geoduck clams, *Panope abrupta*, and Manila clams are resistant to infection with *M. mackini* (Bower *et al.,* 2005; Meyer *et al.,* 2008).

Limited perspective on other *Mikrocytos* species suggests some spectrum of specificity beyond one bivalve host in at least two cases. Identification of DNA sequences similar to *M. boweri* in *O. edulis* (Gagné *et al.,* 2008) and *C. gigas* (Wang *et al.,* 2010) suggests that other species besides *O. lurida* (Abbott *et al.,* 2014) may be susceptible to *M. boweri*. Detection of an *M. mimicus*-like SSU rDNA sequence in a clam, *R. philippinarum* (Ramilo *et al.,* 2014, Garcia *et al.,* 2018), suggests the potential susceptibility of a broad range of bivalve molluscs.

Mikrocytos veneroïdes and *M. donaxi* are only known to infect wedge clam *D. trunculus*.

4 Geographical distribution

Particularly relative to other diseases of molluscs, mikrocytosis is notably restricted to cooler temperate marine systems: along the western coast of North America from British Columbia to California (Farley *et al.,* 1988; Elston *et al.,* 2012, 2015); along the western European coast from the United Kingdom to Spain (Hartikainen *et al.,* 2014; Ramilo *et al.,* 2014; Garcia *et al.,* 2018), and potentially locally in Nova Scotia, Canada (Gagné *et al.,* 2008) and the northern Yellow Sea, China (Wang *et al.,* 2010). It is not difficult to imagine the vast translocation of *C. gigas* from northern waters of Asia in the 20th century playing some role in shaping the distribution of mikrocytosis, especially as caused by *M. mackini* (Abbott *et al.,* 2011), in the same way that it likely shaped the distribution of haplosporidiosis caused by *Haplosporidium nelsoni* and perhaps *Haplosporidium costale*. Hypotheses concerning *Mikrocytos* origins are as yet untested, and will need to await better insight into the intraspecific genetic diversity of these pathogens.

5 Disease aetiology

Mikrocytos mackini is directly transmissible among hosts (Hervio *et al.,* 1996), and we assume direct transmission is typical of mikrocytosis more generally. Mikrocytosis caused by *M. mackini* is observed typically in spring, disease and mortality peaking from March to May (Bower, 1988; Abbott and Meyer, 2014). Based on experimental results, Hervio *et al.,* (1996) suggested that infections by *M. mackini* acquired in spring are limited in their development during summer, when seawater temperatures exceed 15°C; but that infections begin to progress when temperatures cool in the fall, to become patent by winter and the following spring. Four or five months of temperatures 10°C or lower are essential for expression of the disease. The epithelium of the digestive tract, including stomach and digestive gland, appear to be a primary portal of entry (Bower *et al.,* 2005). Disease and mortality tend to be focused in larger, older oysters, with oysters less than two years old less affected (Quayle, 1988; Bower *et al.,* 1997), although younger oysters can be infected (Bower *et al.,* 2005).

We might assume aetiology of mikrocytosis caused by other *Mikrocytos* species to be similar, but the validity of this assumption is uncertain, with data limited for other parasites. *Mikrocytos boweri* may display seasonality similar to *M. mackini* in its infection of *O. lurida*, in which Abbott *et al.* (2014) observed a histological prevalence of 2/40 oysters in April 2012 sampling but 0/60 in July. However, the available data do not provide a robust test of this. The disease and mortality caused by *M. veneroïdes* and *M. donaxi* clearly occurred in summer and early autumn (Garcia *et al.,* 2018).

6 Significance

The appearance of mikrocytosis in *C. gigas* in British Columbia, caused by *M. mackini* and known as Denman Island disease (Quayle 1961; Farley *et al.,* 1988), was one of the major mollusc disease events of the last century. The disease caused mortality exceeding 50% in oysters cultured at lower tide elevations in this initial emergence, the mortality so sudden and extensive that "meats were scattered among the oysters on the bed" (Quayle, 1961). Mikrocytosis caused mortality is far lower in endemic areas today, and infection of *C. gigas* by ostreid herpesvirus OsHV-1 microvariants has overshadowed mikrocytosis as a Pacific oyster disease concern in recent years (Segarra *et al.,* 2010). Mikrocytosis caused by *M. mackini*, however, remains an important disease threat to Pacific oyster culture worldwide, given the global importance of this industry and the potentially acute susceptibility of oyster populations in many places. Even where mortality impacts are reduced, the lesions characteristic of mikrocytosis can be economically impactful by reducing the marketability of product (Bower, 1988; Elston *et al.,* 2015).

Mikrocytos veneroides and *M. donaxi* were detected during high mortality events of wedge clams in France (Garcia *et al*., 2018). The significance and impacts of *M. mimicus* and *M. boweri* are not clear.

7 Clinical signs and pathology

In mikrocytosis caused by *M. mackini* as well as *M. mimicus* in *C. gigas*, infection is marked by the presence of greenish pustules in the mantle, body wall, labial palps, or adductor muscle (Figure 1.a) (Quayle, 1961; Bower, 1988; Farley *et al*., 1988; Hartikainen *et al.,* 2014). Such gross lesions are not apparent in infection by *M. boweri*, *M. veneroïdes*, and *M. donaxi* (Abbott *et al.,* 2014; Garcia *et al.,* 2018). Microscopically, these lesions represent areas of intense haemocyte infiltration and often necrosis (Figure 1.b), with *Mikrocytos* parasites evident within vesicular connective cells or myocytes at the periphery of the lesions (Figures 1.c and 1.d), and to some extent in haemocytes or free within the lesions (Farley *et al.,* 1988; Hervio *et al.,* 1996; Abbott *et al.,* 2014). Presentation of *M. veneroïdes* and *M. donaxi* as observed has differed only in the reduced degree of focal haemocyte infiltration in response to infection, and in occasional infection of neuronal ganglia and nerves (Garcia *et al.,* 2018). All *Mikrocytos* species are morphologically very similar (Figure 2) and species identification requires molecular diagnostics and analysis, especially in situations where *Mikrocytos* parasites are detected within new hosts or from new geographic locations.

Mikrocytos parasites are revealed to be far more abundant in host tissues when visualized using *in situ* hybridization with DNA probes, and can be observed in additional tissues, including the epithelium of digestive tubules and stomach (Bower *et al.,* 2005; Meyer *et al.,* 2005).

8 Control measures and legislation

Control of mikrocytosis is focused primarily on preventing the transfer or introduction of infected animals to areas free of the pathogens. Infection with *M. mackini* is a reportable disease in Canada (Canadian Animal Health Surveillance System, 2021) and notifiable to the Commission and Member States of Europe per Annex II to Regulation (EU) 2016/429. Infection with *M. mackini* was removed from the list of diseases notifiable to the World Organisation for Animal Health (OIE), although it continues to satisfy all criteria for listing: international spread of the agent with aquaculture commerce is likely; many countries may demonstrate country or zone freedom from the disease in susceptible *C. gigas*; a precise case definition is available and a reli-able means of detection and diagnosis exists; and the disease has been shown to affect the health of cultured *C. gigas* at the level of a country or zone resulting in significant consequences, in-cluding production losses and mortality at a zone or country level (OIE, 2019).

Aquaculturists in *M. mackini*-endemic areas have long realized that "the greatest infection occurs at or near the lowest tide level", so mikrocytosis impacts could be mitigated by moving oysters higher in the intertidal before March, and not planting in the lower intertidal before June (Bower, 1988). Because *M. mackini* is generally more prevalent in larger, older oysters, marketing at smaller size and removing older oysters can also help to minimize disease impacts.

9 Diagnostic methods

Mikrocytosis has historically been detected using standard histopathological analysis of haematoxylin and eosin-stained tissue sections (Meyer *et al.*, 2005). The very small size of the parasites and the sharply focal nature of mikrocytosis makes detection of light infections challenging, particularly when gross lesions are absent. Conventional (Carnegie *et al.*, 2003) and quantitative (Polinski *et al.*, 2015, 2021) PCR assays have been designed for more sensitive detection of *M. mackini*, although the Carnegie *et al.*, (2003) assay may cross-react with other *Mikrocytos* species (Abbott and Meyer, 2014). PCR assays suitable for specific detection of other *Mikrocytos* species have not yet been developed. *In situ* hybridization assays designed for *M. mackini* (Carnegie *et al.*, 2003; Meyer *et al.*, 2005) may be generally applicable to congeneric species, although this remains to be empirically determined. SSU rDNA sequencing is useful for species identification and discrimination, and is straightforward using existing PCR assays (Abbott and Meyer, 2014).

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11 Figures

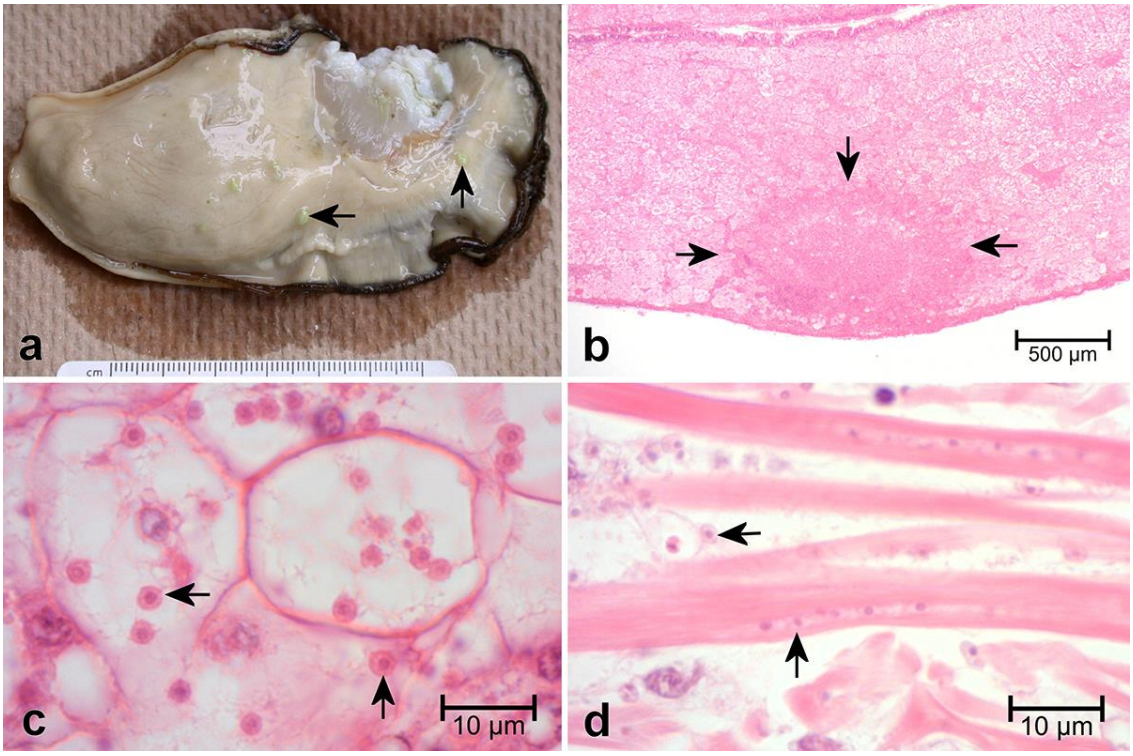

Figure 1. Mikrocytosis in the Pacific oyster *Crassostrea gigas*. (a) Macroscopic green pustules (arrows) in the mantle of an adult *C. gigas* typical of infection with *Mikrocytos mackini*. (b to d) Histological tissue sections of *C. gigas* stained with haemotoxylin and eosin. (b) Low magnification showing a focal lesion (arrows) within the mantle tissue. (c) High magnification showing *M. mackini* cells (arrows) within the vesicular connective tissue. (d) High magnification showing *M. mackini* cells (arrows) within the myocytes of the adductor muscle and also free in the haemal spaces.

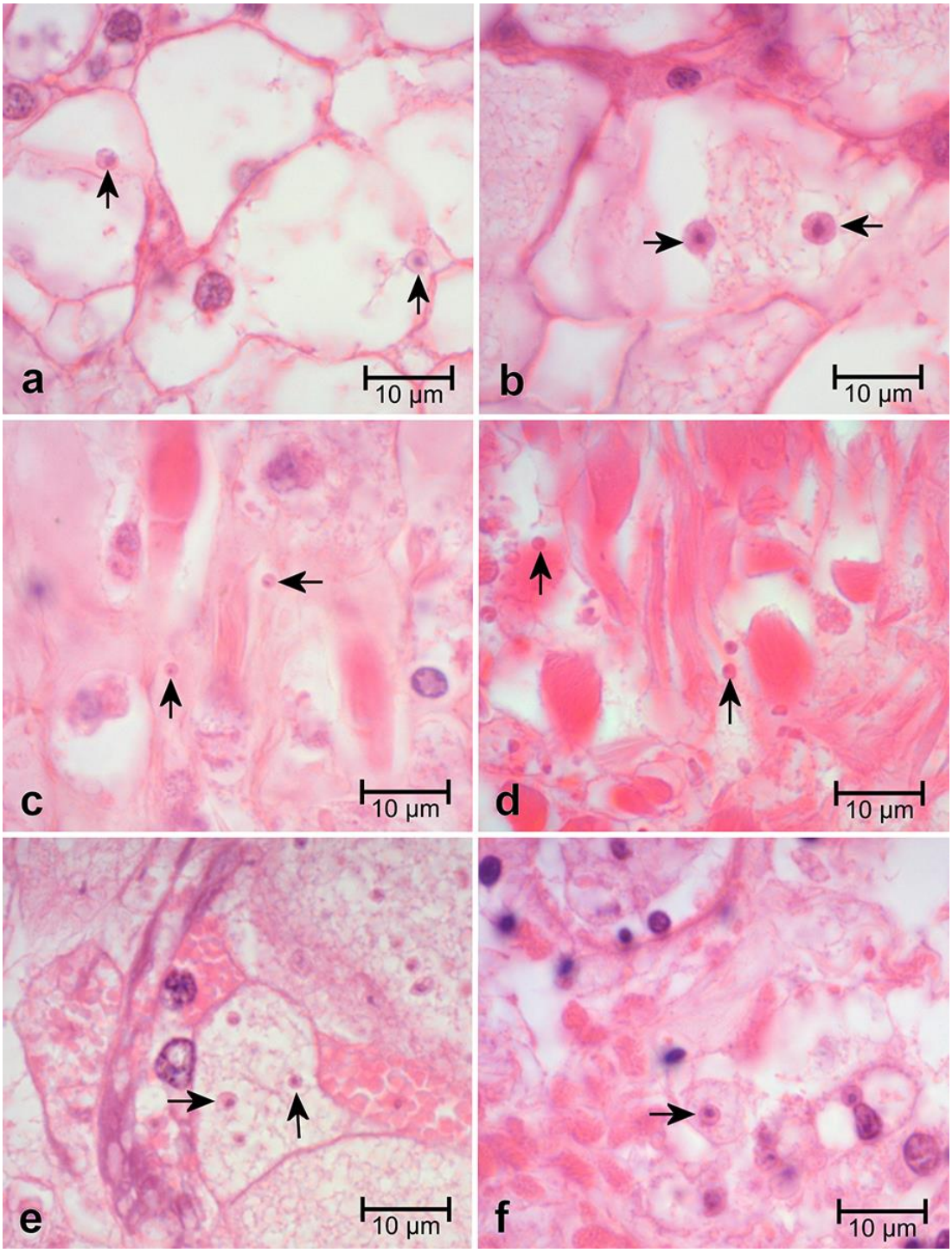

Figure 2. Histological tissue sections stained with haemotoxylin and eosin showing *Mikrocytos* species (arrows) in variety of different hosts. (a) *M. boweri* within in the connective tissue of *Ostrea lurida*. (b) *M. mimicus* within in the connective tissue of *Crassostrea gigas*. (c) *M. donaxi* between myocytes of *Donax trunculus*. (d) *M. veneroïdes* between myocytes of *Donax trunculus*. (e) *M. mytilicoli* within in the connective tissue of *Mytilicola intestinalis*. (f) A *Mikrocytos*-like parasite in the connective tissue of *Ruditapes philippinarum*.

12 Author contact details

Ryan B. Carnegie
Virginia Institute of Marine Science, William & Mary
PO Box 1346
1370 Greate Road
Gloucester Point, Virginia 23062
United States of America
carnegie@vims.edu

Gary R. Meyer
Fisheries and Oceans Canada
Pacific Biological Station
Aquatic Animal Health Laboratory
3190 Hammond Bay Road
Nanaimo, British Columbia V9T 6N7
Canada
Gary.Meyer@dfo-mpo.gc.ca