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First report of signs of infection by *Coccomyxa*-like algae in wild blue mussels, *Mytilus* spp., in the Gulf of Maine (USA, Maine)

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Abstract

In August 2019, visual inspection of intertidal zones of the Gulf of Maine (ME, USA) revealed young and adult wild blue mussels, *Mytilus* spp., in Alley Bay (Jonesport area) with the distinctive L-shaped shell deformity (LSSD) and green spots (GS) in the mantle and adductor muscle. LSSD is a characteristic sign of current or previous mussel infection by photosynthetic unicellular alga from the group *Coccomyxa*, while GS are algal colonies. Based on these findings, this study represents the first report of infection signs by pathogenic *Coccomyxa*-like algae in mussels from the coastal waters of the Northeastern United States, providing a base for future large scale monitoring of the alga in the region.

KEYWORDS

algae, Blue mussels, *Coccomyxa* sp., Gulf of Maine, infection

1 | INTRODUCTION

Among microbes that can be found associated with bivalve mollusks only pathogenic unicellular algae *Coccomyxa* spp. cause the formation of L-shaped shell deformity (LSSD) on the posterior shell edge (Figures 1 and 2) in several species of mytilid mussels (Zuykov, Anderson, & Pelletier, 2018). Previous investigations of blue mussels, *Mytilus edulis* L., *M. trossulus* Gould and their hybrids, infected with *Coccomyxa* sp. (GenBank accession number KJ372210) from the Estuary and the Gulf of St. Lawrence, Canada (Zuykov et al., 2014; Zuykov et al., 2018; Zhao et al., 2019; Zuykov et al., 2020) have demonstrated the following facts which are important to note for further discussion:

(1) LSSD only occurs if the mussel is heavily infected with *Coccomyxa* algae.
(2) LSSD degree (value of parameter "d" as defined on Figures 1c and 2b,c) does not depend on mussel age.
(3) The core of LSSD is built with extra shell material.
(4) Significant proportion of mussels with LSSD will show green spots (GS) in the mantle and adductor muscle.
(5) In studied mussel beds, the number of individuals with LSSD usually exceeded several dozen animals per square meter.

With that said, an observation of LSSD is well suitable for reliable and rapid monitoring of *Coccomyxa*-infected mussels on large spatial areas. This field-friendly technique does not require the collection and transportation of a great number of animals.

To date, there are no monitoring programs targeting pathogenic *Coccomyxa* alga in bivalves. Circa fifteen papers reported that infected mussels are known from a few sites in coastal waters of Spain, Norway, Denmark, Russia, Falkland Islands and Canada (Mortensen, Harkestad, Stene, & Renault, 2005; Zuykov et al., 2018). Nevertheless, LSSD, green spots and the presence of pearls in infected tissues make infected mussels non-attractive for harvesting. In this framework, the contribution of cultured and wild-caught blue mussels into coastal economy differs between regions on the Atlantic coast of North America. If the majority of mussels harvested in Québec and Canada Maritime Provinces comes from aquaculture, "most of the landings in Maine are from wild

mussel beds" (maine.gov, 2019). Therefore, the control of diseases, pathogens, and parasites of both natural and cultivated populations of mussels require equal attention (Coen & Bishop, 2015).

The aim of this paper was to determine whether distribution of harmful *Coccomyxa*-like algae in wild mytilid mussels, *Mytilus* spp., is limited to Canadian waters or if the disease is present in more southern locations, that is along the Atlantic coast of the USA (state of Maine).

2 | MATERIALS AND METHODS

2.1 | Sampling sites and host organism

Field examination of hundreds of intertidal mytilid mussels, *Mytilus* spp., has been carried out in early August 2019 at low tide (from −0.06 m to −0.4 m) in four sampling sites representing two ecologically different environments in the Gulf of Maine (Figure 1a): (a) not affected by commercial fishing or aquaculture activities, that is Cape Arundel (site no. 1) and Seawall Acadia National park, Mt. Desert Island (site no. 2); and (b) dock leg area of small-scale fishing boats with numerous cage aquaculture facilities, that is Jonesport (site no. 3) and northern part of Great Wass Island (site no. 4). Mussels, variously aged, were found attached to rocks and/or to be partly burrowed into the muddy or sandy bottom near the shoreline; the depth of water in sampling sites at high tide is up to 2 m.

2.2 | Identification of *Coccomyxa*-infected mussels in field conditions

The main attention was paid to the observation of shell morphology. The presence of LSSD, that is thickened PSE that forms parameter "d" (Figures 1c,f and 2b,c), was easy to distinguish from the sharpened PSE which commonly occurs in mussels (Figures 1c,e and 2a) even without animal handling. However, alternating growth increments and growth stops on the shell edge, which frequently occur in nearshore mussels, can be misidentified as LSSD. To overcome this problem, attention has to be paid to the direction of shell growth on the posterior shell edge (Figure 1d). Shells of uninfected mussels grow always from umbo, even if the posterior edge thickened due to micro-growth increments (Figure 1d-*a*′,″). The latter may appear under the influence of tides, storms, circatidal rhythm, temperature, seasons of growth, etc. (for ref Richardson, 1989). Shells of infected mussels, in the ventral or dorsal views, will have a heart-like shape, because the shell growths toward umbonal area (Figure 1d-*b*′). The presence of extra shell material on the PSE was studied on shells, obtained after necropsy of eight mussels (see below). Shell fragments were cut with the use of cordless Dremel® rotary tool with a diamond cutting wheel and embedded in the acrylic resin (Lang Dental Jet™ Denture Repair package); subsequently plastic tablets were polished (Buehler® grinding paper) and shown here on Figure 1e,f. Nevertheless, the presence of LSSD may indicate both current and prior infection with

FIGURE 1 (a) Map of study area showing positions of sites where mytilid mussels, *Mytilus* spp., were observed, 1—Cape Arundel (43°20′44″N, 70°27′31″W), 2—Seawall Acadia National park, Mt. Desert Island (44°14′30″N, 68°17′57″W), 3,4—dock leg area of small-scale fishing boats: 3—in Jonesport (44°31′40″N, 67°36′54″W) and 4—in the northern part of Great Wass Island (44°30′57″N, 67°35′25″W). (b, c) Site 4, where mussels with and without L-shaped shell deformity (LSSD) habit on bottom sediment; parameter "d"—thickening of the posterior shell edge. (d) Diagram showing the direction of growth (DOG) of shells in uninfected and *Coccomyxa* -infected mussels. (e, f) Shells in section near posterior shell edge (PSE), mussels are from site 4: e, without LSSD, f, with LSSD; pr, prismatic layer; na, nacreous layer; scale bars 5 mm [Colour figure can be viewed at wileyonlinelibrary.com]

Coccomyxa (because some individuals might fight off and eliminate the pathogen; Zuykov et al., 2020). Therefore, the presence of GS (the pathognomonic sign of the infection with the alga) need to be

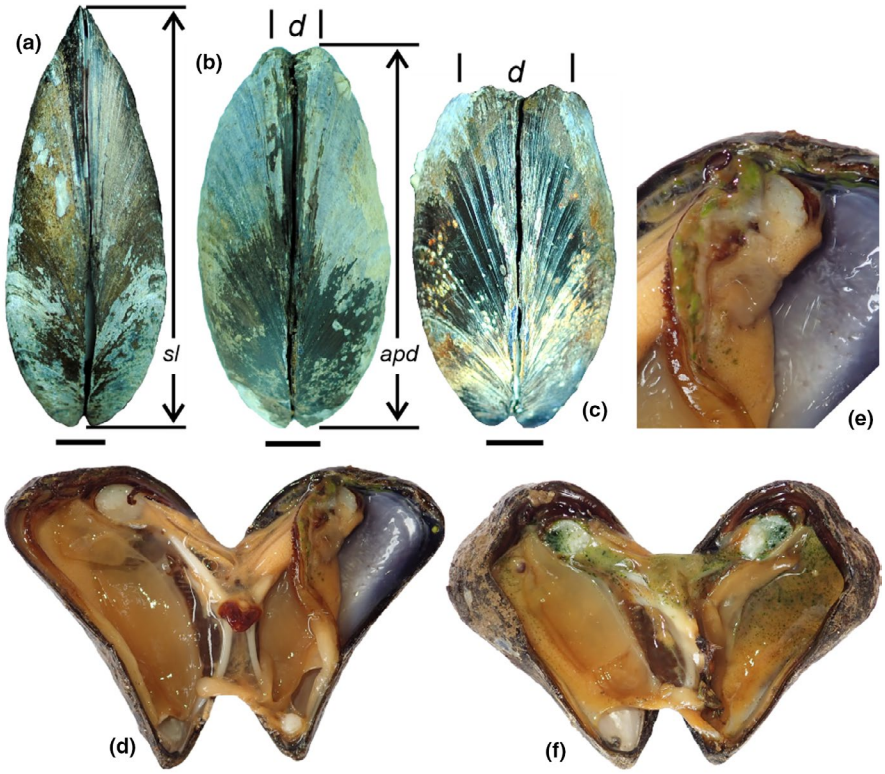

FIGURE 2 Shells and soft tissues of mytilid mussels, *Mytilus* spp., from the Great Wass Island (site 4), Maine, USA. (a) Shell without L-shaped shell deformity, LSSD; (b, c) shells with LSSD in different degrees (see difference in parameter "d"). (d-f) Soft tissues with green spots (presumptive colonies of *Coccomyxa* algae): (d, e) "lightly" infected mussel, green spots are only along the mantle posterior edge; (f) "heavily" infected mussel. Sl, shell length; APD, maximal anterior-posterior dimension. Scale bars 10 mm [Colour figure can be viewed at wileyonlinelibrary.com]

observed in individuals with LSSD from each sampling site. Further, the outer mantle surface is always characterized by a slightly wider spatial distribution of GS than the inner surface (Figure 2d,e); after necropsy, outer mantle near the posterior shell edge needs to be carefully examined. Additionally, haemolymph and extrapallial fluid in mussels infected with *Coccomyxa* is characterized by slightly greenish colour due to the presence of a high number of algal cells.

3 | RESULTS

In the sites no. 1 and no. 2, sparse mussels with shells length up to 50–60 mm do not show LSSD. Mussels displaying LSSD were found in abundance in sites no. 3 and no. 4 (Figure 1b,c). Organic periostracum of many individuals (with and without LSSD) was eroded to show underlying mineral layers (Figure 1c). The maximal shell length or anterior-posterior dimension for animals with LSSD (Figure 2a,b) was 70 mm; parameter "d" differs between individuals, and in a couple of mussels, it reached 24 mm (this is among the largest known values that were earlier documented from the Canada Atlantic region). Visual assessments estimated that mussels with and without LSSD occurred in similar abundance in both sites. In total, six (three from each site) randomly selected mussels with LSSD were shucked, macroscopically examined and photographed. The same manipulations, for comparative study, were also made with two mussels that did not exhibit LSSD.

Only in individuals with LSSD: (a) PSE exhibited the extra shell material (Figure 1f), (b) GS were visible in soft tissues of four mussels only along the mantle posterior edge (Figure 2d,e), and in two mussels GS covered nearly whole mantle surface (Figure 2f), (c) GS were visible inside of posterior adductor muscle, and (d) the colour of haemolymph leaking from the adductor muscle after dissection was slightly greenish.

4 | DISCUSSION

Herein, we conclude, for the first time, that wild mytilid mussels, *Mytilus* spp., from the intertidal zone of the Gulf of Maine (ME, USA) shown signs of infection with pathogenic *Coccomyxa*-like algae. This is remarkable because macroscopic examination rarely gives indicative information or pathognomonic signs which can promote the identification of pathogens/diseases in bivalve mollusks (Zannella et al., 2017). Further, data reported in Rodríguez et al. (2008) and Zuykov et al. (2014), enable us to propose that *Coccomyxa* algae responsible for mussel infection in the North and in the South Atlantic Ocean may belong to the same taxon.

One of the major aims of follow-up studies should be the precise identification of both the pathogen and the host from the Gulf of Maine. Further, a more precise spatial distribution of infected mussels, that is the scale of infection in mussel populations, in the Gulf of Maine should be determined, which can be facilitated by the development of in situ observations of mussels with LSSD using submersible cameras deployed from small vessels.

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CONFLICT OF INTEREST

The authors declare that they have no competing interests.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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