

Intracellular Ciliates of cultured Mediterranean mussel (*Mytilus galloprovincialis*) in the Gulf of Trieste in Slovene Adriatic Sea

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Abstract

Mediterranean mussels (*Mytilus galloprovincialis*) are reared at three locations in the Slovene Sea. From two established sampling site, i.e., Seca and Strunjan, 154 adult cultured mussels were collected in May and July 2004. No mortalities occurred at either sampling sites and no abnormalities were seen during macroscopic examination of collected mussels. Intracellular ciliates were detected in digestive glands in 21.4% of mussels. A mild infection was noted in 94% and moderate infection in 6% of mussels. No inflammation occurred in any of infected mussels and only focal necrosis of digestive glands was noticed in some of infected samples.

Introduction

The Gulf of Trieste lies at the very north end of The Adriatic Sea. The part of the gulf is the Slovene Sea, which is bordered with the line between Grado and Savudrija and the coastline. The entire Slovene coast is 46 km long and is made of Eocene sedimentary flysch (Richter, 2005; Lipej, 2004). The temperature varies considerably. During the summer the sea can heat up to 30°C and the coastline can even freeze during very cold winters. Many large rivers, groundwater and underwater springs called "brojnice" (Richter, 2005) have a strong influence on salinity, which fluctuates from 20 ppt after abundant rainfall to 38 ppt during the late summer and winter (Richter, 2005; Lipej, 2004). The difference between low and high tides can reach more than 180 cm and is the largest in

the Adriatic. The deepest point of the Gulf is 39.9 m deep (Richter, 2005).

One hundred and six taxons of Mollusca and 38 taxons of Bivalvia have been noted in the Slovene Sea (Lipej, 2004), but only Mediterranean mussels (*Mytilus galloprovincialis*) are reared at three locations, i.e., Seca, Strunjan and Debeli rtic. Mussel seeds (ca 2 cm long) are bought or collected from natural beds, put in nets and cultured on ropes, which are hanging from rafts. They reach commercial size after 14 to 18 months and are up to 7 cm long at the time. The constant income of sweet water, rich in minerals, makes the mussels of good taste and very high quality (Bojc, 2004).

Entering the European Union (EU) and respecting their legislation we were obliged

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to establish the system of surveillance of shellfish farms and detection of marteiliosis in Mediterranean mussels, the only selfish species cultured in The Slovene Sea.

Ciliates are protozoan and belong to the phylum *Ciliophora*. Many of them are associated with marine bivalves, particularly those of the classes *Kinetofragminophorea*, *Oligohymenophorea* and *Polyhymenophorea*. Ciliates are ubiquitous and have been reported in every mollusc species (Chollet et al., 2003). Most of them are extracellular and found in lumen of the digestive gland tubules in the intestine and in the gland digestive ducts (*Ancistrocoma*-like), others are attached to or located near the gills, mantle and labial palps (*Sphenophrya*-like, *Trichodina sp.*, *Ancistrum*-like and *Ancistrocoma*-like). Some ciliates are also intracellular (*Sphenophrya*-like and *Rhynchodida*-like) (Chollet et al., 2003). Most of them are harmless and commensals, but intracellular ciliates can cause xenoma (*Sphenophrya*-like) (Chollet et al., 2003) of the gills of *Mytilus edulis* (McGladdery & Bower, 2002) and disruption of the digestive tubule epithelia (*Rhynchodida*-like) (McGladdery & Bower, 2002).

Mytilus edulis, *Mytilus trossulus* and *Mytilus galloprovincialis* are often infected with *Rhynchodida*-like ciliates, which are also known as mussel protozoan X (MPX) or intracellular ciliates of mussels or digestive gland ciliates (Bower et al., 1994). They are spindle-shaped and measure 9-16 μm \times 4.5-6 μm . Although they may disrupt digestive tubule epithelia, no mortalities have been linked with the infection. The reported prevalence is over than 40% in cultured *Mytilus galloprovincialis* in Spain (Villalba et al., 1997), 4% in wild *Mytilus*

edulis on the east coast of the United States (Figueras et al., 1991) and less than 1% on the German coast (McGladdery & Bower, 2002).

In our study we are trying to give some data about the intracellular ciliates, found in the digestive glands of cultured Mediterranean mussels from Slovene Adriatic Sea. This is the first report of intracellular bivalve parasites in the region.

Material and methods

154 live adult blue mussels (*Mytilus galloprovincialis*), from 5 to 7 cm long, were collected from two sample sites: 105 in Strunjan and 49 in Seca, 61 at the beginning of May and 93 at the end of July 2004. The mussels were sampled from a depth of 3 m and the temperature of the water was measured at each sampling. Mussels were put in a cool bag immediately after sampling in order to keep them alive and transported to a diagnostic laboratory within 2 hours after sampling. On opening, mussels were inspected for any abnormalities or lesions.

The flesh was removed from the shell and a standard section through the visceral mass was performed. One sample from each mussel was taken. Samples were put in 10 % formalin solution for 24 hours at the room temperature. Steaks were processed to wax blocks using standard histological techniques and cut in 3 μm sections. They were stained with haematoxylin and eosin (H&E) and examined by light microscopy for the presence of ciliates.

H&E stained sections were scored on a four-point scale due to the severity of infection: 0, no ciliate; 1, mild infection – less than twenty

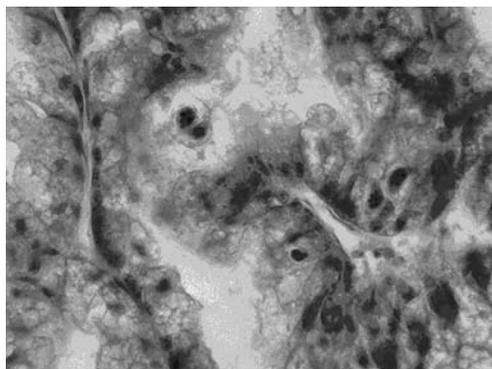


Figure 1. Ciliates in epithelial cells of the digestive glands in *Mytilus galloprovincialis*, x 400.

ciliates per section; 2, moderate infection – from twenty to fifty ciliates per section; and 3, severe infection – more than fifty ciliates per section.

Results

In the Slovene Sea there are three locations of shellfish farms, i.e., Seca, Strunjan and Debeli rtič, where 201 tons of Mediterranean mussels (*Mytilus galloprovincialis*) are produced per year. With regard to the EU legislation demands two sampling sites have been established in the Slovene Sea: Seca and Strunjan.

Water temperature was the same at both sampling sites: 19.3°C in May and 23.7°C in July. No mortality was detected at either sampling sites and no abnormalities or lesions were detected during the macroscopic inspection of mussels.

The microscopic examination revealed digestive glands ciliates in 21.4% (n=33) of mussels, 20.4% (n=10) in Seca and 21.9% (n=23) in Strunjan. The prevalence of infection was 24.6% (n=15) in mussels, collected in early May, and 19.4% (n=18) in mussels, collected

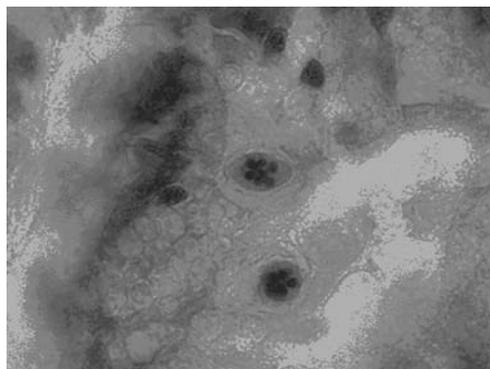


Figure 2. Ciliates in epithelial cells of the digestive glands in *Mytilus galloprovincialis*, x 1000.

in late July. A mild infection was determined in 94% (n=31) and moderate infection in 6% (n=2) of mussels.

Ciliates were pear or spindle-shaped, 3.9 to 11.5 µm long and 2.9 to 8.4 µm width. They had a polymorphic oval to globular basophilic, fragmented macronucleus, stained deep blue. They were mostly found inside the digestive tubule epithelia, only few of them were lying in the lumens of digestive tubules. In mussels, where mild infection was noticed, there was only one ciliate in the epithelia of single infected digestive tubule. In moderate infection there were mostly more than one ciliate in the epithelia of single infected digestive tubule and some parasites were also lying freely in the lumens of infected digestive tubules. In all infected digestive tubules there was only one ciliate inside a single epithelial cell.

A slight enlargement of epithelial cells containing ciliates of a large size was observed.

There were no inflammatory response in any of infected mussel and only focal necrosis of digestive glands was detected in few of

infected but also in some of uninfected mussels.

Discussion

Until 2004 the health status of Slovene marine molluscs culture was completely unknown. Taking in consideration the EU legislation brought us to decision to establish two sampling sites in order to monitor all bigger shellfish farms, although the Slovene coast is very short.

Digestive gland ciliates were found in 21.4% of examined cultured mussels from Slovene Sea. The prevalence of infection in two sampling sites was almost the same. It was reported that the prevalence over 40% of digestive gland ciliates was detected in cultured *Mytilus galloprovincialis* in Galician Rias in Spain (Villalba et al., 1997), 4.2 to 8.3% in wild and 0% in cultured blue mussel *Mytilus edulis* from Prince Edward Island in Canada (Weldon, 1999), 4% in wild *Mytilus edulis* on the east coast of the United States (Figueras et al., 1991) and less than 1% on German coast (McGladdery & Bower, 2002).

The difference in percentage of infection between May, with water temperature just below the 20° C, and July, when the water temperature was almost 24° C, was insignificant. Weldon (1999) found 4.2% infected wild mussels in July, with water temperature 16.5° C and salinity 27.3 ppt, and May, when the water temperature was 5.1° C. The prevalence of infection in September, with water temperature 15.2° C and salinity 34.7 ppt, was 8.3%. None digestive gland ciliates were found in November and February, with water temperature 5.8 and -1.5° C (Weldon, 1999).

Establishing our own criteria we determined a mild infection in 94% and moderate infection in 6% mussels. Villalba et al. (1997) reported mostly low infection in *Mytilus galloprovincialis*, not revealing the number of parasites affecting the single mussel. Weldon (1997) counted 67 digestive tubule ciliates in one section from September mussel tissue sample. Ciliates from Slovene Sea were pear or spindle-shaped, 3.9 to 11.5 µm x 2.9 to 8.4 µm with mostly globular basophilic, fragmented macronucleus. Ciliates from Villalba et al. (1997) study were pear-shaped, 7 to 15 µm long, with often fragmented macronucleus. McGladdery & Bower (2002) described spindle-shaped ciliates, 9-16 µm x 4.5-6 µm in size, with polymorphic, oval to globular densely basophilic macronucleus. Our ciliates were mostly inside the digestive tubule epithelia, in a single epithelial cell there was only one parasite. In some tubules, where moderate infection was determined, there were some ciliates also lying freely in the lumens of infected digestive tubules. Ciliates were detected only in epithelial cells of digestive tubules (Figueras et al., 1991; Villalba et al., 1997; Weldon, 1999) and the presence of more than one parasite in the same host cell was noticed in few digestive tubules by Villalba et al. (1997).

No mortality occurred in any of shellfish farms and no abnormalities were detected at macroscopic examination of mussels from The Slovene Sea. Some epithelial cells, that berried ciliates of a larger size, were enlarged. No host response was detected in any of infected mussels. Focal necrosis of digestive glands was noticed in low number of infected mussels but same changes were seen also in

uninfected mussels, so it is quite improbable that those lesions were caused by ciliates. Chollet et al. (2003) and McGladdery & Bower (2002) reported that digestive gland ciliates can cause a disruption of digestive tubule epithelia but no mortalities occurred in infected mussels. Villalba et al. (1997) have also found only enlargement of ciliate-bearing cells with no haemocytic reaction or other lesions in infected mussels. Figueras et al. (1991) reported no specific host response in infected mussels. No data concerning focal necrosis of digestive glands in ciliate's infection were reported in literature.

We can conclude that digestive gland ciliates are quite common parasites in cultured *Mytilus galloprovincialis* in The Slovene Sea and they seem to be harmless. Further investigations are planned in order to study a possible influence of same other epidemiologic factors on infection and also to find out the health status in the wild mussels population.

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