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Protozoan parasites in cultured mussels *Mytilus galloprovincialis* in the Thermaikos Gulf (north Aegean Sea, Greece)

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ABSTRACT: The protozoans *Ancistrum mytili* (Oligohymenophorea: Ancistridae) and *Marteilia refringens/maurini* (Marteiliidea: Marteiliidae) were found parasitizing cultured mussels *Mytilus galloprovincialis* L. in the Thermaikos Gulf (north Aegean Sea, Greece). The former did not affect the condition index of infected mussels, in contrast to the latter, which did so and which also induced hemocyte infiltration in the affected digestive epithelium. The prevalence of both parasites was relatively high in a polluted area.

KEY WORDS: *Ancistrum mytili* · *Marteilia refringens/maurini* · *Mytilus galloprovincialis* · Mussel pathogens

INTRODUCTION

Farming of mussels *Mytilus galloprovincialis* L. constitutes a very important industry in the Thermaikos Gulf (north Aegean Sea, Greece). As a result, the occurrence of a diminished harvest and a weakened mussel condition made it necessary to conduct an investigation into the probable causes (Arsenoudi et al. 2003). The first investigation reported on the impact of *Steinhausia mytilovum* on mussels (Rayyan & Chintiroglou 2003). The present study focuses on 2 additional protistans, *Ancistrum mytili* and *Marteilia refringens/maurini*, which infect the gills and the digestive tubules of the mussels, respectively. The ciliate *A. mytili* has a wide geographical range, affecting mussels from the Atlantic and Pacific coasts of the USA, NW Spain, and Italy (Lauckner 1983, Da-Ros & Massignan 1985, Robledo et al. 1994, Villalba et al. 1997). *M. refringens/maurini*, a combined name suggested to overcome diagnostic challenges (Villalba et al. 1993, Bower et al. 1994), occurs along the Atlantic coast of Europe and in many localities in the Mediterranean Sea (Comps et al. 1982, Ceschia et al. 1992, Villalba et al. 1993, Fuentes et al. 2002). The study herein focuses on the occurrence of both parasites, as well as the prevalence in and effects on the mussel host.

MATERIALS AND METHODS

In total, 294 individuals of *Mytilus galloprovincialis* were collected from 'long-line' mussel cultures in 3 localities in the Thermaikos Gulf (north Aegean Sea, Greece), i.e. 98 individuals from each station: Makrygialos (Stn 1), Halastra (Stn 2), and Nea Michaniona (Stn 3) (Fig. 1). The mussels were 5 to 9 cm in shell length. After collection, the mussels were cleaned of mud and biofouling organisms, measured, and shucked. The condition index (CI) was calculated from total wet weight (TW), wet meat weight (MW), and shell weight (SW) based on the formula:

$$CI = [MW/(TW - SW)] \times 100 \quad \text{(Aguirre 1979)}$$

Pieces of gills and digestive tubule were processed for histology following standard protocols (Martoja &

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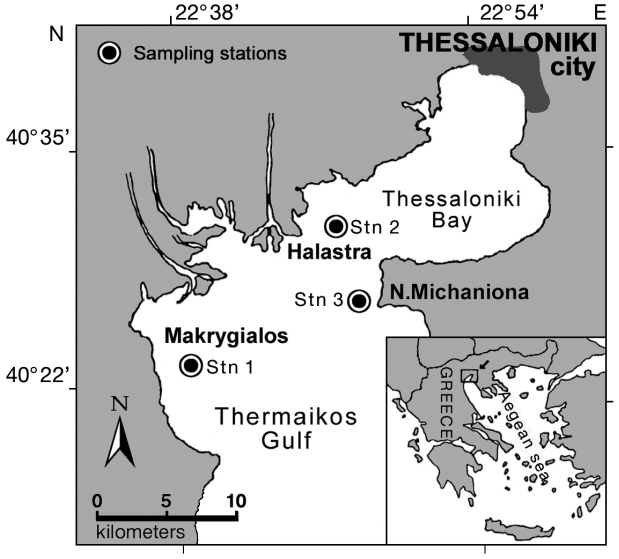

Fig. 1. Sampling stations in the Thermaikos Gulf (north Aegean Sea, Greece)

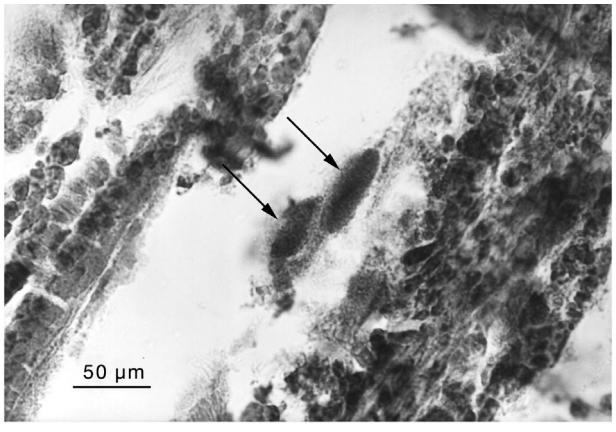

Fig. 2. *Mytilus galloprovincialis*. *Ancistrum mytili* infection (arrows) on the gills

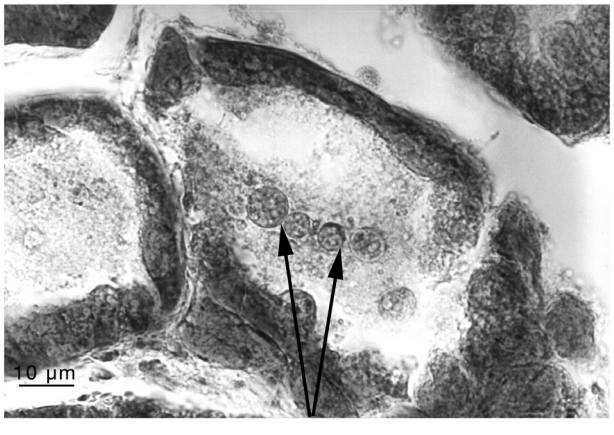

Fig. 3. *Mytilus galloprovincialis*. Various developmental stages (arrows) of *Marteilia refrigens/maurini* in the digestive lumen

Martoja 1967). Mussels were then placed in 10% buffered formalin.

One-way ANOVA was used to compare the CI of infected and non-infected mussels.

RESULTS

Description

Cells of *Ancistrum mytili* (Oligohymenophorea: Ancistridae) were observed moving freely over the gill filaments of the mussels (Fig. 2). The cells were 32 to 57 µm in length, each containing 2 nuclei. Various developmental stages (plasmodia, primary, and secondary cells) of *Marteilia refringens/maurini* (Marteiliidea: Marteiliidae) were detected in the digestive tubules of the host (Fig. 3).

Impact on the host

There were no visible lesions on the tissues in association with the presence of the ciliate *Ancistrum mytili*. There was also no significant difference in the CI of infected and non-infected mussels (Fig. 4, 1-way ANOVA, *F*-ratio = 1.68; p-value = 0.1955).

Marteilia refringens/maurini infected the digestive tubules of the mussels, resulting in a significant difference in the CI of infected and non-infected mussels (Fig. 5, 1-way ANOVA, *F*-ratio = 12.06; p-value < 0.0001).

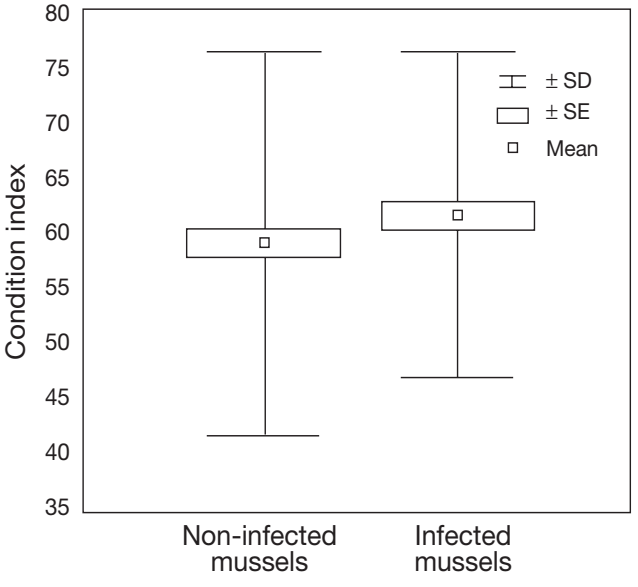

Fig. 4. *Mytilus galloprovincialis*. Condition index of mussels infected by *Ancistrum mytili*, and of non-infected mussels

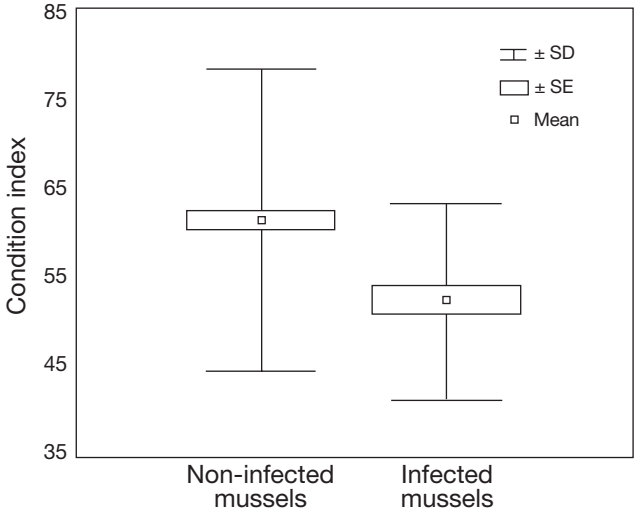

Fig. 5. *Mytilus galloprovincialis*. Condition index of mussels infected by *Marteilia refrigens/maurini*, and of non-infected mussels

Prevalence of the parasites

Among the 294 mussels examined from the 3 stations, 109 (37.1%) were infested with the ciliate *Ancistrum mytili.* The prevalence of 46.9% among the mussels from the polluted area (Halastra) was higher than the prevalence of the parasite at the other 2 stations (31.6% at Stn 1 and 32.6% at Stn 3), which are further from sources of pollution. In contrast, only 43 mussels (14.6%) were infected with *Marteilia refringens/maurini*. The prevalence of this parasite was again higher in mussels from the polluted area (24.5%) than at the other 2 stations (12.2% at Stn 1 and 7.1% at Stn 3).

DISCUSSION

The ciliate *Ancistrum mytili* is common in the gills of bivalves worldwide (Lauckner 1983), and is considered to be more symbiotic than parasitic (Bower et al. 1994, Villalba et al. 1997). Our results support this notion, as the CI was not affected and the tissues of the host were not damaged either. However, the infection could be harmful under unfavorable conditions (Pauley et al. 1966). The high prevalence of *A. mytili* in the Thermaikos Gulf is similar to that found in mussel cultures in NW Spain (Villalba et al. 1997). The prevalence of the infection was high at the polluted site (Halastra, Stn 2). Previous studies (Kim et al. 1998, Powell et al. 1999) recorded similar high rates in carbohydrate-polluted waters. However, Hatzianestis et al. (2001) reported that carbohydrate pollution is more intense at Nea Michaniona (Stn 3). Further experimental surveys are required to examine the relationship between this type of pollution and the prevalence of *A. mytili*.

The effects of *Marteilia refringens/maurini* infection include reduction of nutrient assimilation, gonads, and CI (Villalba et al. 1993, 1997, Fuentes et al. 1995). Infection of the mussels in the present study resulted in significant reduction of the CI, while possible damage (lesions) of the digestive epithelium requires further examination. The prevalence of infection of the parasite *M. refringens/maurini* detected in mussels in the present study was similar to that reported in previous studies on cultured *Mytilus galloprovincialis* from Spain (Fuentes et al. 1995, Villalba et al. 1997), but lower than that previously reported in the Thermaikos Gulf (Virvilis 2003) and in Bretagne (Auffret & Poder 1985). The prevalence of parasite infection was also high at the polluted site (Halastra, ST2), although Bigas et al. (2000) found no relationship between pollution and the prevalence of parasites.

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