

Monitoring of the immune efficiency of *Mytilus galloprovincialis* in Adriatic sea mussel farms in 2005

D Malagoli, L Casarini, E Ottaviani

Department of Animal Biology, University of Modena and Reggio Emilia, Modena, Italy

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Abstract

The monthly evaluation of the cytotoxicity of hemolymph from the mussel *Mytilus galloprovincialis* revealed some variations in the percentage of cytotoxic animals during the year. Cytotoxicity is confirmed to be a dynamic parameter that can be used as an indicator of immune efficiency and, therefore, of the state of health of the animals.

Key words: *Mytilus galloprovincialis*; cytotoxicity assay; cytotoxic activity; Adriatic sea mussel farms

Introduction

The state of health of *Mytilus galloprovincialis* mussels was evaluated in terms of cytotoxic activity in the hemolymph. It is well-known that molluscs, as all invertebrates, possess only innate or natural immunity and recognize and eliminate non-self material mainly through cellular and humoral components. Among the latter, cytotoxicity is one of the most important immune functions (Franceschi *et al.*, 1991). This function has been well conserved during evolution and described both in invertebrates and vertebrates (Ratcliffe *et al.*, 1985; Savary and Lotzová, 1986). Wittke and Renwanz (1984) have demonstrated that circulating cells (immunocytes) from *Mytilus edulis* are able to produce cytotoxic substances, which lyse human erythrocytes. A cytotoxic protein complex has also been found in *M. galloprovincialis* hemolymph (Hubert *et al.*, 1997). Recently, we have designed an easy-to-use and low-cost cytotoxicity test (Malagoli and Ottaviani, 2005) that has been used here to evaluate the cytotoxic activity of *M. galloprovincialis* collected each month during 2005 from Adriatic sea mussel farms.

Materials and Methods

Animals

Specimens of the mussel *Mytilus galloprovincialis* were obtained each month from local fishermen in the Cesenatico area (FC, Italy) immediately after being caught. Forty animals were sacrificed to collect hemolymph on the spot, while the remaining samples were taken to the Department of Animal Biology (Modena, Italy) and maintained in the laboratory aquarium (artificial seawater, temperature 19 ± 1 °C, pH 8.0 ± 0.2 , salinity 35 ± 2 psu) for at least 10 days before control experiments. No animals could be obtained in February because of adverse weather conditions.

Hemolymph preparation and cytotoxicity assay

The detailed procedure for the cytotoxicity assay has been described elsewhere (Malagoli and Ottaviani, 2005). Briefly, the hemolymph was collected from the posterior adductor muscle using a sterile syringe, filtered into sterile tubes with 0.2 µm filters, aliquoted, immediately frozen and maintained at -80 °C for at least 12 h before use. The cytotoxic activity was assessed by checking the cytolysis of human A positive erythrocytes. In order to eliminate damaged erythrocytes, the whole blood sample was washed at least three times in 9 vol. of sterile NaCl 0.9 %, and the erythrocytes were then re-suspended in sterile TBS (50 mM Tris-HCl, 200 mM NaCl, 10 mM CaCl₂, pH 8.5) at a final concentration of 2×10^9 cells/ml. 500 µl of filtered hemolymph were added

Corresponding Author:

Enzo Ottaviani
Department of Animal Biology
via Campi 213/D
41100 Modena, Italy
E-mail: ottaviani.enzo@unimore.it

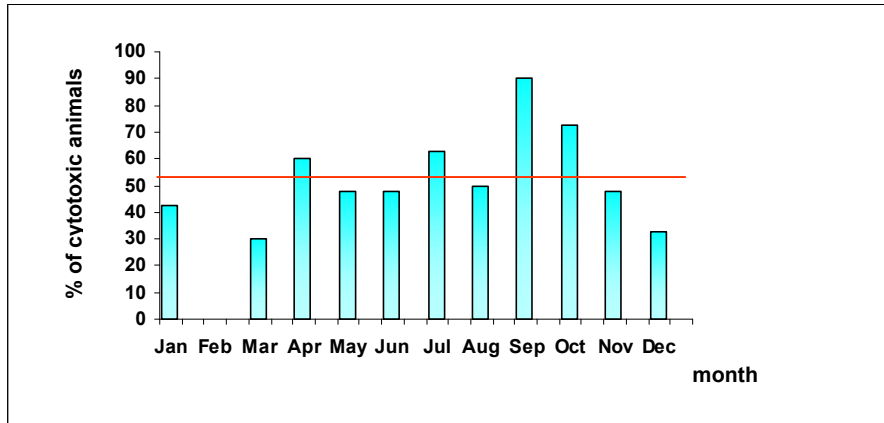


Fig. 1 Mussel cytotoxicity during 2005 in the Cesenatico sea area. The red line represents the mean percentage.

to 500 μ l of erythrocyte suspension and incubated for 1h at 25 $^{\circ}$ C (Hubert *et al.*, 1997; Malagoli and Ottaviani, 2005). After incubation, samples were centrifuged at 3.000xg for 5 min at 4 $^{\circ}$ C, and the optical density (OD) of supernatants was measured by evaluating the absorbance at 541 nm with a Helios β spectrophotometer (Spectronic Unicam, Cambridge, UK). It should be noted here that a threshold OD level of 0,5 was fixed as the minimum for a positive test, while samples that clearly exceeded this value were considered positive without further measurement (Malagoli and Ottaviani, 2005). The experiment was repeated in duplicate two times for each animal.

Statistical analysis

Statistical analysis was performed using the χ^2 test with $p < 0.05$ taken as significant.

Results and Discussion

The periodic evaluation of the cytotoxicity of hemolymph from the mussel *M. galloprovincialis* reveals variations in the percentage of cytotoxic animals during the year (Fig. 1). The mean percentage of mussels positive to the cytotoxicity assay was around 53 %, but there were various peaks. It is interesting to note that the minimum and the maximum values were during the periods in which the water temperature reached its minimum and maximum levels, respectively, as indicated in the annual report on inshore water conditions of the Italian region Emilia-Romagna in 2003. In a previous study, we observed that rapid changes in water temperature, salinity and pH resulted in a significant decrease in the mean cytotoxic activity. A rapid increase in water temperature resulted in a significant drop in the number of animals positive to the cytotoxicity assay (Malagoli and Ottaviani, 2005). Conversely, the observations reported here seem to indicate a positive correlation between water temperature and the percentage of cytotoxic molluscs. However, no sudden

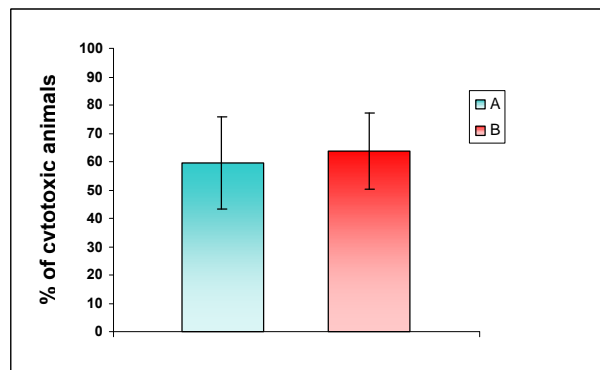


Fig. 2 Comparison between mussel cytotoxicity immediately after being caught (A) or after a period of maintenance in the aquarium (B). The mean values of six independent experiments \pm SD are shown.

modifications in environmental parameters were seen in the present study. It should be underlined that the mean percentages of cytotoxic animals did not differ among mussels maintained in the laboratory aquarium for at least 10 days prior to control experiments (Fig. 2). As far as the peak registered in September is concerned, it should be noted that the samples were collected after a period in which many animals had been died as a result of a loss of byssus. This pathology is still of uncertain origin, but a fungal infection has been observed to be involved in serious damage to the byssus apparatus (Franchini *et al.*, 2005). Even if more detailed studies are needed to clarify the question, the large number of cytotoxic specimens in the survivor population could suggest a link between cytotoxic activity and the animal's ability to overcome epidemics.

Overall, cytotoxicity is confirmed as a dynamic parameter that can be used as an indicator of immune efficiency and, therefore, of the good state of health of the mussels.

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References

- Franceschi C, Cossarizza A, Ortolani C, Monti D, Ottaviani E. Natural cytotoxicity in a freshwater pulmonate mollusc: an unorthodox comparative approach. *Adv. Neuroimmunol.* 1: 99–113, 1991.
- Franchini A, Malagoli D, Ottaviani E. Investigation of the loss of byssus in *Mytilus galloprovincialis* from mussel farms in the Adriatic Sea. *Cell Biol. Int.* 29: 857-860, 2005.
- Hubert F, Cooper EL, Roch Ph. Structure and differential target sensitivity of the stimutable cytotoxic complex from hemolymph of the Mediterranean mussel *Mytilus galloprovincialis*. *Biochim. Biophys. Acta* 1361: 29–41, 1997.
- Malagoli D, Ottaviani E. Cytotoxicity as a marker of mussel health status. *J. Mar. Biol. Ass. UK* 85: 359-362, 2005.
- Ratcliffe NA, Rowley AF, Fitzgerald SW, Rhodes CP. Invertebrate immunity: basic concepts and recent advances. *Int. Rev. Cytol.* 97: 183–350, 1985.
- Savary CA, Lotzová E. Phylogeny and ontogeny of NK cells. In: Lotzová E, Heberman RB (eds), *Immunobiology of natural killer cells*, vol 1, FLA: CRC Press, Boca Raton, pp 45–61, 1986.
- Wittke M, Renwranz L. Quantification of cytotoxic hemocytes of *Mytilus edulis* using a cytotoxicity assay in agar. *J. Invertebr. Pathol.* 43: 248-253, 1984.