Influence of tidal stress on the immunocompetence of hemocytes in soft-shell clam (*Mya arenaria*)

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Introduction

By their large distribution in the intertidal zone and continuous exposure to different biotic and abiotic stress in water and sediments, molluscs, such as *Mya arenaria*, are good sentinel species of environment quality.[1,2] To determine the impact of different stress, an evaluation of the alteration of the main immune function of hemocytes, phagocytosis, can be readily determine in these invertebrates.[3] It has been shown that these stressors could impact on the effectiveness of the immune system in molluscs to fight against diseases.[4]

Indeed hemocytes, which are responsible for phagocytosis, are altered by various environmental factors such as salinity, temperature and pH modification,[5-7] by pathogens,[8-10] and pollution.[5,10-14] Exposure to these multiple stresses affects the immune system of molluscs and could increase their susceptibility to develop infectious diseases and cancers.[15] However, in terms of physical stress, the effect of tides in respect to the position of clams on the shore were rarely been taken into account in immunological research. The objective of the present study is to determine if the shore spatial distribution (upper, middle and lower shore), can also influence the phagocytic competence of soft-shell clams.

Materials and Methods

Animals

Clams were collected, at low tide at three different sites (Anse, Jardins, Motel) and at three altimetric distances from the shore (upper shore, middle shore and lower shore) in Mitis Bay (48°40'N, 68°00'W). Upper shore *Mya arenaria* were exposed to air for *circa* 5 h, the middle shore for 2 h and the lower shore individuals were exposed for 30 min.

Determination of phagocytosis

Hemolymph was extracted from the posterior adductor muscle using 3 mL syringe and 23 G needle and transferred on ice in tubes. Phagocytosis was monitored according to the method developed by our laboratory.[16] Hemocytes were mixed with yellow-green latex FluoSpheres (Molecular Probes Inc., Eugene, OR, USA) at a ratio of 1:100 (hemocytes:beads) in flat-bottom 96 wells plate. The mixtures were incubated at 20°C in the dark. After 18 h, the supernatant was delicately removed by decantation. The cells were fixed with 0.5% formalin. Phagocytosis was measured by flow cytometry (FACScan, Becton Dickinson, San Jose, CA, USA). A total of 3000 events were acquired for each sample and stored in the list mode data format. The data were then analysed, once displayed as two-parameter complexity and cell size, in the process of gating the hemocytes population and as fluorescence (FL1) frequency distribution histogram for phagocytosis. The results of one bead and more represent the phagocytic activity and three beads and more represent the efficacy of phagocytosis. Data collection and analysis were performed with LYSIS-II program.

Statistical treatments

The effect of tides and shore locations were examined by ANOVA followed by Tukey's test for pairwise comparisons. Statistical analyses were performed using SigmaStat (version 3.5). Significance was set at $P<0.05$.

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Results

The phagocytic activity and efficacy of phagocytosis for the site Jardins in Mitis Bay, for three altimetrics levels (top, middle and bottom range) are presented in Figure 1. Note that soft-shell clams located in top and middle ranges have phagocytic activity significantly lower than those from bottom site (18.7% and 12.9% respectively). For the phagocytic efficacy, there is also a significant decrease in top and middle locations when compared to low location. Similar results were obtained for the other two sites.

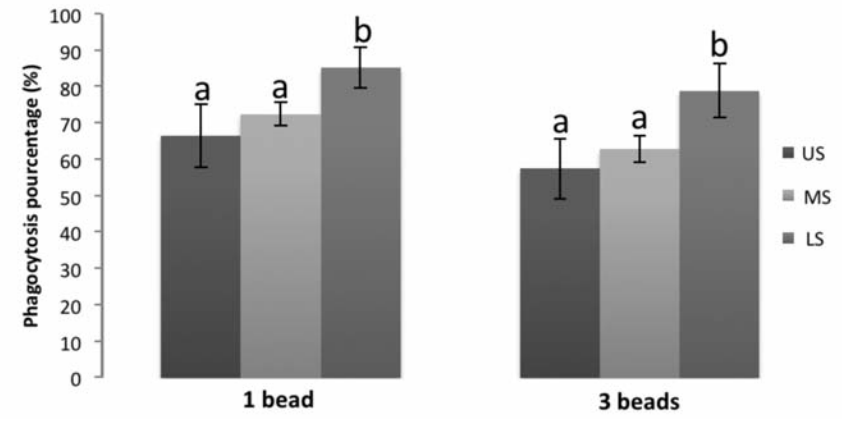

Figure 1. Phagocytosis activity (1 bead and more) and phagocytosis efficacy (3 beads and more) of *Mya arenaria* at upper shore (US), middle shore (MS) and lower shore (LS) from the site Jardins in Mitis Bay on July 31st 2012 (n=15).

Discussion

The distribution of *Mya arenaria* in intertidal zone is highly variable and has an influence on several physiological characteristics of this species. The immersion time, varies with the altimetric position on the shore and has an effect on the allocation of resources in relation to the ingested energy by filtration and the reproductive cycles.[17-21] Among all these physiological changes caused by the positioning in the intertidal area, we can now add the variation of immunocompetence as shown by our results, which tends to decrease with an extension of the period of desiccation. It was also shown that increasing the immersion time changes adaptive strategies[17,22] to optimize the allocation of energy leading to altered growth of soft-shell clams.[17,23] As preliminary results, in our experiment, we have also observed that clams collected lower shore, are at least one and a half fold bigger than those collected in upper shore. Based on our study, in future immunotoxicology studies, it would be important to include the natural variation of the phagocytic activity of soft-shell clams due to their position on the shore with a preference to do the sampling near the low water line to get the best witnesses.

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