Cell tracking and velocimetric parameters analysis as an approach to assess activity of mussel hemocytes *in vitro*

Damien RIOULT[1], Jean-Marc LEBEL[2], Frank LE FOLL[1].

[1] Laboratory of Ecotoxicology EA 3222, University of Le Havre, 76058 Le Havre cedex France.
[2] Physiology and Ecophysiology of Marine Molluscs, UMR 100 IFREMER-UCBN, University of Caen.

Corresponding author: Damien Rioult, damien.rioult@univ-lehavre.fr

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Sessile bivalves belonging to *Mytilidae* are filter feeders characterized by a relatively high tolerance to environmental changes and considered as bioindicator species in many environmental studies. Immune parameters are often proposed as ecotoxicological biomarkers. In invertebrates as in vertebrates, innate immunity relies on humoral responses based on the activity of antimicrobial peptides (such as defensins) and encapsulation of microorganisms by melanization (prophenoloxidase pathway). All these proteins are secreted by the formed elements of hemolymph, collectively named hemocytes. Hemocytes are also responsible for a cell-mediated innate immunity that corresponds to cytotoxic activity (chlorination through ClO-ion production by myeloperoxidase activity) and phagocytosis. Hemocytes are found (and collected) as cells in suspension in circulating hemolymph. Hemocytes are adherent cells as well, infiltrating tissues and migrating to infected areas.

Migration activity of hemocytes remains poorly studied. However, this activity could be considered as a valuable indicator of immunocompetence and, potentially, as a biomarker of immunotoxic damage caused by exposure to environmental contaminants. Motility is related to dynamic cytoskeletal rearrangements and is controlled by external signals from endogenous or bacterial origin.

In order to study potential alterations in the motility of *Mytilus edulis* hemocytes, we have developed a method of long term cell tracking *in vitro*. After staining of nuclei with Hoechst 33342, we are able to monitor cell movemenst over periods encompassing several days and to measuring the instantaneous speeds of more than 20 cells simultaneously. Our results show that velocimetric performances of hemocytes are sensitive to the physicochemical parameters such as temperature and composition of culture media. It seems very likely that the motile activity of hemocytes reflects environmental quality.

Using this method we were able to determine the best culture conditions and thus to maintain hemocyte viability and motility over at least 4-7 days. Exposure to various environmental contaminants and comparisons between a "clean" site (Yport -76) and a site impacted by environmental contaminants (Le Havre -76) are planned to propose this method as a useful biomarker for environmental diagnostics.