

Haemocyte primary-culture from three Mollusc species and its application in ecotoxicology

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Haemocytes play a fundamental role in invertebrate immune system and are responsible for different types of immune responses such as phagocytosis, pathogen hydrolysis or phenoloxydase cascade. These cells could potentially be affected by contaminants such as pesticides and metals. To better understand impacts of contaminants on mollusc immune system, *in vitro* primary-culture of haemocytes could be a useful tool (Mottin *et al*, 2010 ; Latire *et al*, 2012). The aims of this work were to improve culture parameters for haemocytes from three species of marine molluscs (*Crassostrea gigas*, *Sepia officinalis* and *Haliotis tuberculata*) in order to use those cells for ecotoxicity assays.

To assess our cellular culture conditions, MTT reduction assay or Water Solubles Tetrazolium salts (WST-1) assays were performed after different times of culture. After the validation of culture parameters, viability assays and other biomarkers were conducted to assess the effects of different types of contaminants.

Experiments on *Crassostrea gigas* haemocytes showed difficulty to maintain these cells in culture. Different cell concentrations and different kinds of culture media were tested but a high decrease of cell viability was observed on the first 48h of culture.

In *Sepia officinalis*, haemocyte primary-culture is poorly documented and different parameters were thus tested. Combination of modified L-15, Hank's 199 and DMEM media, and two temperatures were assessed. Results showed that the combination of L-15 medium and the temperature of 15°C was the best one for cuttlefish haemocyte primary-culture. First experiments carried out on cuttlefish haemocytes under zinc contamination showed a negative effect on lysosomal system after 48h of exposure.

Parameters for *Haliotis tuberculata* haemocyte primary-culture are well known and those cells could be maintained for 10 days in modified Hank's 199 medium without any decrease of viability. Effects of an herbicide (glyphosate) and a metal (zinc) were tested after 10 days of exposures. Glyphosate appeared to have no effect on haemocyte viability even at very high doses (e.g. 100,000 µg.L⁻¹) whereas an EC₅₀ of 6,300 µg.L⁻¹ could be calculated for zinc.

This approach will allow us to compare haemocyte responses from three species of molluscs which differ phylogenetically and ecologically.

T. Latire, C. Le Pabic, E. Mottin, A. Mottier, K. Costil, Noussithé Koueta, Jean-Marc Lebel, Antoine Serpentine. 2012. Responses of primary cultured haemocytes from the marine gastropod *Haliotis tuberculata* under 10-day exposure to cadmium chloride. *Aquat. Toxicol.*, 109: 213–221.

E. Mottin, C. Caplat, M.-L. Mahaut, K. Costil, D. Barillier, J.-M. Lebel, A. Serpentine. 2010. Effect of *in vitro* exposure to zinc on immunological parameters of haemocytes from the marine gastropod *Haliotis tuberculata*. *Fish Shellfish Immunol.*, 29: 846–853.