

ACADEMIC REGISTRY
Abstract of Thesis Form



Candidate

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| School/PGI: | School of Life Sciences | Degree Sought: | PhD |
| Title of Thesis: (complete in upper and lower case) | <i>Mytilus edulis</i> haemocytes variability: technique, individual and environment | | |
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| Signature: | | Date: | |

Abstract

The widely distributed marine bivalve *Mytilus edulis* is used as a sentinel organism for ecological and toxicological assessments. As a filter feeder, it has the potential to bio-accumulate pollutants. It has been assumed that the cell concentration and cell type ratio of its circulating immune cells, haemocytes, could become complementary sub-lethal indicators of toxicology. These two parameters are respectively referred to as total haemocyte count (THC) and differential cell count (DCC). This study examines these commonly used methods, quantifies their limitations, and develops alternative techniques. The circulating immune cells are investigated to assess their fluctuations. Finally, impacts of environmental challenges on the circulating haemocytes are examined.

Despite its importance in the field of *Mytilus edulis* immunology, THC evaluation is present in only 20% of publications in this field, and DCC in 10%. Ultimately, only 9% of papers consider both THC and DCC before further analyses. The remaining studies disregard THC and DCC, or regard these parameters as being constant and homogenous in *M. edulis* populations.

This study initially quantifies the systematic error induced by sampling, and suggests improvements. For example, a systematic error of 26% is attributed to the use of low precision syringes, and can be reduced with use of higher precision sampling equipment. While the systematic errors in visual count and image analysis of THC and DCC evaluations are equivalent, the computerised methods allow the throughput of larger data sets, reduce workload, and avoid tedious eye counts. Flow cytometry was found to be the most accurate method in THC and DCC evaluation. Furthermore, repeated bleedings influence DCC, triggering the decrease of circulating eosinophils (up to 20%) and the increase of hyaline cells (up to 30%). To mitigate this reaction to sampling, a maximum volume of 50µl using a permanent cannulation is recommended.

However, even with improved methods reducing systematic error by half, this study still reports variations as high as 20-fold in the haemocyte concentration in populations of healthy individuals. In addition, over a 2-hour period in a single cannulated individual, fluctuation of the DCC is observed to be as high as 30% for eosinophils, 10% for hyaline cells, and 20% for basophils. These measured variations are explained by haemocyte reservoirs in the tissues. Eosinophils are found in large numbers in epithelial association in the gills, guts and in the mantle, where their numbers have been evaluated at 3×10^7 cells.

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As a case study, *Mytilus edulis* challenged with barium sulphate smothering, used by the oil industry in drilling muds, shows gill damage and their subsequent infiltration by eosinophils. In bacterial outbreak, basophilic cells are observed to infiltrate the tissue surrounding the stomach and eosinophils are depleted from the epithelium of the digestive tissues. THC is 10-fold lower than in healthy organisms and eosinophils are depleted from the haemolymph.

In conclusion, the THC and DDC methods are shown to be unreliable despite the use of more accurate methods. In addition, *Mytilus edulis* circulating haemocytes present large variations and the assumption of their homogeneity in terms of time, individuals or methods used cannot be made. These results challenge the conclusions of many past publications regarding causalities established between potential stresses and measured effects. Further research is necessary to understand the mechanisms regulating the circulating haemocytes, the inter-individual variability and to improve investigation methods.

Notes

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