I International Symposium on Advances in Marine Mussel Research, 26-27 October 2016, University of Vigo, Spain

Blue mussels mortality outbreak in France is associated with heavy genomic abnormalities detected by flow cytometric analysis of hemolymph

Abdellah BENABDELMOUNA[1]* & Christophe LEDU[1]
[1]Ifremer, RBE-SG2M-LGPMM, Station de La Tremblade, Avenue de Mus de Loup, F-17390 La Tremblade, France
Abdellah.Benabdelmouna@ifremer.fr

INTRODUCTION

In France, blue mussels industry (*Mytilus edulis* and *Mytilus galloprovincialis*) produces more than 77,000 metric tons each year. However, since 2014, the production of French blue mussels decreased dramatically because of sudden and unfamiliar mass mortality outbreaks (90-100%) for which the causes remained uncertain, and both juvenile and adult blue mussels were affected. Several studies have already shown that the exposure of mussels to environmental contaminations gives rise to various DNA damages, including abnormalities in DNA content and distribution as well as progressive development of circulating aneuploid-polyploid cells in the hemolymph. Whatever their origin, the genomic abnormalities of circulating cells in the hemolymph of mussels can be reliably studied by flow cytometry (FCM) which is recognized as a non-tedious, non-subjective, cost-effective and high precision technique to study DNA damages, cell cycle alterations and ploidy changes. In the context of mass mortality outbreaks affecting blue mussels in France since 2014, we hypothesized that the observed mortality outbreaks of blue mussel stocks were probably linked to a greater susceptibility of mussels in relation to their poor cytogenetic quality. To explore this hypothesis, we used FCM to study the DNA content and cell cycle characteristics of circulating cells of hemolymph collected from various wild and cultivated blue mussels stocks of various areas situated in the Pertuis Charentais zone and Bourgneuf Bay.

Results and Conclusion

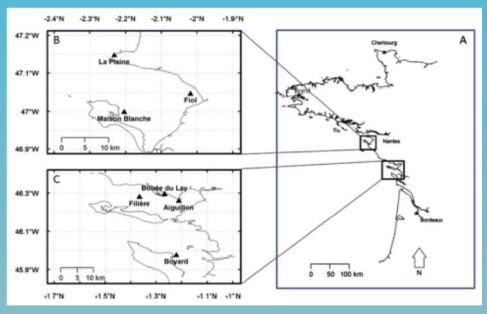

Figure 1. Locations of the sampled blue mussel stocks on Atlantic coast of France (A). Three mussel stocks were sampled in Bourgneuf Bay (B) and four mussel stocks were sampled in Pertuis Charentais zone (C)

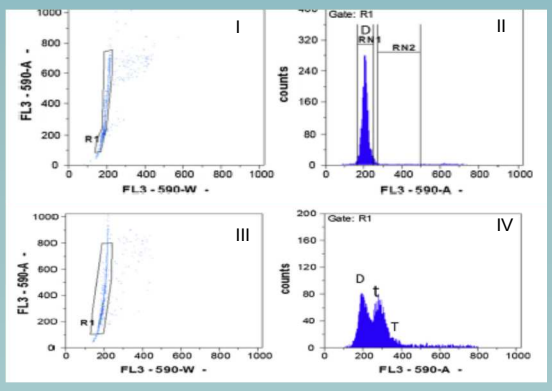

Figure 2. Flow cytometry histograms of propidium iodide stained hemolymph nuclei from normal (I/II) and abnormal (III/IV) blue mussels. Left panels: gating of single nuclei on a width vs area FL3 cytogram. Right panels: histogram of propidium iodide fluorescence of gated single nuclei. Markers RN1 and RN2 were placed to estimate the percentage of nuclei in diploid (D) and non-diploid phases, respectively. t and T : aneuploid nuclei in triploid and tetraploid range, respectively

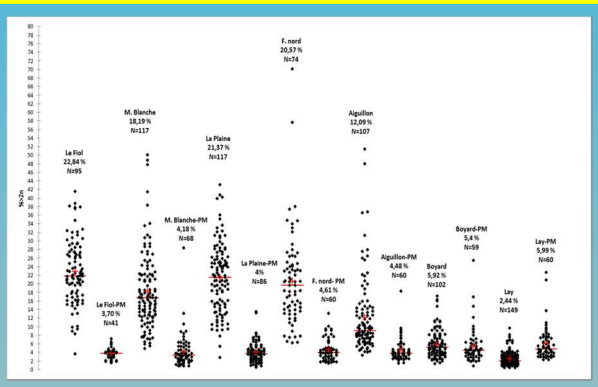

Figure 3. Scattergram representation of the variation of the non-diploid nuclei percentages within and between the different mussel stocks analyzed before and after the mortality event. Mussel stocks analyzed after the mortality event are marked PM (post-mortality). Values under mussel stock names correspond to GA% and indicate the mean percentage of non-diploid nuclei per mussel stock. N is the number of analyzed mussels per stock

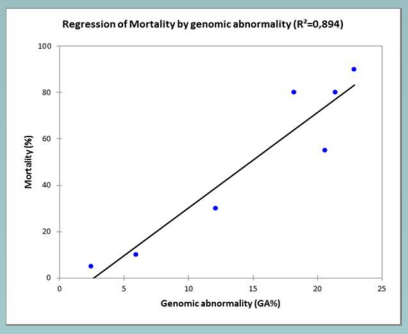

Figure 4. Simple regression between initial genomic abnormality level (GA%) and final level of mortality (mortality %). The equation of the regression is expressed as mortality % = (4.12 x GA%) – 10.93.

- Adult specimens were collected before the mortality of 2015 from 7 different mussel stocks distributed across the Pertuis Charentais zone and the Bourgneuf Bay (Fig. 1). After the mortality event, the same analyses accomplished before the mortality were applied again to surviving mussels collected from the same sites.

- 1300 mussels were individually analyzed and categorized into two groups according to their FCM profiles :
  - Normal mussels with less than 10% of non-diploid nuclei (Fig. 2 I-II). This group is composed of mussels with normal hemocytes presenting multiple pseudopodia and with FCM histograms showing a unique/dominant population of diploid (2n) nuclei in the G0/G1 phase.
  - Abnormal mussels with more than 10% of non-diploid nuclei (Fig. 2 III-IV). This group is composed of mussels with FCM histograms presenting additional populations of nuclei with a non-diploid status that all display aneuploidy patterns from hypodiploid to hypotetraploid. Heavily abnormal mussels exhibited numerous rounded and refractile cells showing a lack of attachment and presenting characteristics of previously described leukemia-like cells present in hemic (disseminated) neoplasia disease.

- Genomic abnormality (GA%) for each mussel stock was estimated by the value of the mean percentage of non-diploid nuclei detected among individual mussels. Thresholds of high/low cytogenetic quality were established at individual and population levels.

- Before the mortality, GA% values for the different stocks showed highly significant differences (fig. 3). For each mussel stock, initial GA% was highly correlated with its final mortality level (Fig. 4).
- After the mortality, no significant differences between the analyzed stocks which all exhibited high cytogenetic quality. the mortality event was accompanied by preferential elimination of all (or the very large majority of) abnormal mussels with heavy genomic abnormalities (Fig. 3).

- FCM is a powerful tool to help manage current mussel mortality in France. This could be performed by combining two complementary approaches. The first one implies extensive determination of cytogenetic quality of wild and cultivated mussel beds with the aim of preserving those with good quality and to eliminate/reduce the ones with poor cytogenetic quality. The second approach implies that only FCM-qualified juveniles that show good cytogenetic quality should be used as seeds in cultured stocks.