MICRONUCLEUS TEST IN MUSSEL AS A TOOL IN BIOMONITORING NETWORKS: RESULTS ALONG THE IBERIAN MEDITERRANEAN COAST

Beatriz Fernández *, Juan Antonio Campillo and José Benedicto
Spanish Institute of Oceanography (IEO). Oceanographic Centre of Murcia. Varadero 1. 30740. San Pedro del Pinatar. Murcia. Spain. - bea.fernandez@mu.ieo.es

Abstract
Micronuclei (MN) frequency was measured in gill tissue of native mussels, *Mytilus galloprovincialis,* along the Iberian Mediterranean Coast. Significant differences ($p<0.05$) on MN frequencies were found between some sampling sites and the reference area. Higher MN frequencies were found in sampling sites located along Murcian Coast and southwest Andalusian Coast which may be indicative of a higher exposition to genotoxic compounds of mussels inhabiting these areas. Results supported the application of the MN test in branchial mussel cells as a sensitive biomarker for applying in biomonitoring networks.
Keywords : Bio-indicators, Monitoring, Western Mediterranean.

Introduction
Micronucleus cell frequency demonstrates objectively the level of genotoxic damage and, among many mutagenesis assays, the MN test has been successfully applied as it is simple, reliable and sensitive. Mussel has proved to be an adequate organism for the study of the genotoxic effect of pollutants due to its capacity to accumulate high levels of contaminants including those with genotoxic properties. In this study, MN frequency was determined on branchial cells of mussels from coastal sites affected by different levels of anthropogenic pollution with the aim of determining the adequacy of this test to detect genotoxic effects of pollutants in a field study performed along the Iberian Mediterranean Coast.

Material and Methods
Native mussels were collected from 18 sampling sites during May-June 2003 (Figure 1). Gills were removed, digested and filtered to obtain a cellular suspension. After centrifugation, cellular pellet was fixed in methanol:acetic acid, dropped onto clean glass slides and then stained with 3% Giemsa. Sampling size (n) ranged from 6 to 8 and at least 1000 cells per mussel were scored in the subpopulation of cells prevailing in gill tissue, the main gill cells [1]. MN identification was based on the criteria described in [2].
Statistical analysis
Results were expressed as mean ± S.E. Significant differences on MN frequencies among sites were tested by applying the independent samples T-test (significance was set at $p<0.05$). Analysis were performed using SPSS 11.0 packet.

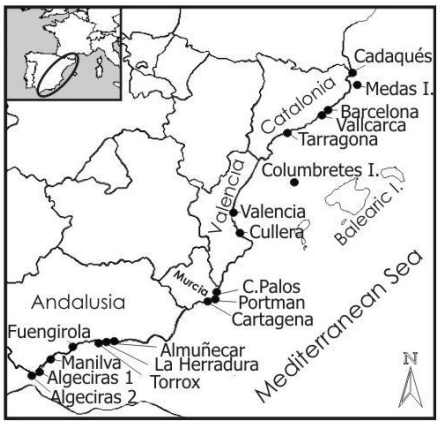

Fig. 1. Map of the Iberian Mediterranean Coast showing the sampling stations.

Results and Discussion
As brachial epithelium represents the primary target for water-borne, gills tend to have higher MN frequencies than haemocytes. According to Brunetti et al [3], for unpolluted areas of the Mediterranean, within the thermal range of 15 to 20 °C (spring), it may be assumed a spontaneous MN gill frequency on *M. galloprovincialis* of $2^{o}/_{oo}$. The lowest MN frequency recorded in Cadaqués ($1.92^{o}/_{oo}$ ±0.50) could be assumed as a spontaneous frequency not related with genotoxic pollution, and therefore, this site could be a realistic reference area for this purpose.

Taking this into account, significantly higher MN frequencies than in this control site, were recorded in two main areas that included Cartagena, Cabo Palos and Portman on Murcia Coast, and Algeciras (1 and 2) and Manilva on the southwest Andalusian Coast ($p<0.01$). Also Tarragona and Vallcarca (Catalonian area), and Torrox and Almuñecar (Andalusian area) showed high MN frequencies ($p<0.05$).
Higher MN frequencies recorded in these areas may be indicating strong exposition of mussels to genotoxic agents. Majority of the areas that showed highest MN levels are well known hot-spot of the Iberian Mediterranean Coast.
This study demonstrated the feasibility of MN test on mussel gill cells to be applied in biomonitoring networks as a tool to detect effect of genotoxic pollution. In further studies, special attention should be paid in areas where mussel seems to be affected by genotoxic damage to identify pollutants which may be causing these observed biological effects.

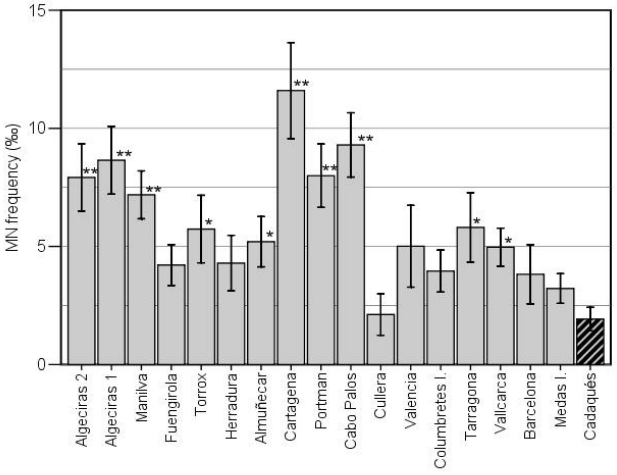

Fig. 2. MN frequencies (per 1000 cells) in the gills of mussels (mean ± S.E.). MN frequency significantly higher than the reference site (darker bar) at $p<0.05$ (*) and $p<0.01$ (**).

References
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