MICRONUCLEUS TEST IN ERYTHROCYTES OF *MULLUS BARBATUS* FROM THE IBERIAN MEDITERRANEAN COAST: A BIOASSAY FOR THE *IN SITU* DETECTION OF GENOTOXIC POLLUTION

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

Abstract
Feral red mullet, (*Mullus barbatus)*, were sampled from four different areas located in the Iberian Mediterranean Coast. Peripheral blood samples were analyzed for erythrocyte micronuclei (MN). The results shown significant differences on MN frequencies among areas ($p<0.05$) but these were only demonstrable for female specimens. Higher MN frequencies were found in Ebro Delta and Cartagena, indicating a higher exposure to genotoxic contaminants in fish from these areas. The results of this study indicate that the MN test in *Mullus barbatus* could be a suitable biomarker for *in situ* monitoring of genotoxic pollution in the marine environment.
Keywords : Fishes, Bio-indicators, Monitoring, Western Mediterranean.

Introduction
In the study of the biological effects of the marine pollution is recommended to apply a battery of test that includes biomarkers of genotoxicity such as the MN test [1]. Micronuclei are small intracytoplasmic pieces of chromatin that result from chromosome breakage during mitosis. The MN test has been adapted to fish species (piscine micronucleus test) where MN can be analysed in peripheral erythrocytes and also in cells from tissues such as gill, kidney, liver and fin. The use of erythrocytes allows a rapid enumeration and blood smears are easily obtained and prepared. In this study we assessed the usefulness of the piscine MN test on erythrocytes of *Mullus barbatus* for the monitoring of genotoxic pollution.

Material and Methods
A survey was carried out in April 2006, to collect specimens of *Mullus barbatus* and superficial sediments from four areas of the Iberian Mediterranean Coast exposed to different degrees of anthropogenic activities (Figure 1.B) in the framework of the MEDPOL Biomonitoring Program [1]. Individual red mullets were sexed and their length measured. Peripheral blood samples were drawn and smeared onto microscope slides and air-dried. After fixation in pure methanol for 10 minutes, slides were left to air-dry and then were stained with 5 % Giemsa solution for 30 min. Due to the high interindividual variability on MN frequency, twelve fishes were analysed in each area and a minimum of 2000 erythrocytes per animal were scored under oil immersion at 1000 x magnification. MN were counted using the criteria of Carrasco et al. [2] and MN frequencies were expressed per 1000 cells.
Statistical analysis
Results were expressed as mean $\pm$ S.E. Data were log10 transformed to achieve normal distribution and homogeneity of variances. Differences between sexes within each area were evaluated by non-parametric Mann-Whitney U-test. One-way ANOVA was used to test differences of MN values among areas, followed the Tukey-b test multiple comparison test (n unequal). Level of significance was set at 0.05. Analysis were performed using SPSS 11.0 packet.

Results and Discussion
Field studies provide most realistic information about the potential genotoxicity of complex contaminant mixtures in aquatic environments than *in vitro* and laboratory assays. None of the four areas selected in this study could be considered as a clean or control site because all of them are influenced by anthropogenic activities. Cartagena and Valencia are highly industrialized cities while Santa Pola Bay and Ebro Delta are under the influence of the Segura and Ebro rivers inputs of contaminants, due to the urban, industrial and agricultural activities developed in their river basins. Although we did not find differences among sexes on MN frequencies within each area (Mann-Whitney U-test, $p>0.05$), one-way ANOVA test applied separately to males and females, shown significant differences in MN levels among areas only in females ($p<0.05$). MN frequencies in females were significantly higher in Ebro Delta than Santa Pola Bay ($p<0.05$). Also higher MN frequencies were recorded in Ebro Delta than Valencia, and in Cartagena than Valencia and Santa Pola Bay, though such differences could not be statistically demonstrable (Figure 1.A).
In this study, we could not assume any area as a control. However, Bolognesi [3] found a spontaneous MN frequency of 0.33 in erythrocytes of *M.barbatus* from a reference area in the Mediterranean Sea. MN frequencies recorded in Ebro Delta and Cartagena indicate a strong exposition to genotoxic compounds in these areas. MN frequencies in Valencia and Santa Pola Bay were lower, though higher than 0.33, so it could be pointing to fishes inhabiting these areas were also exposed to genotoxic contaminants.

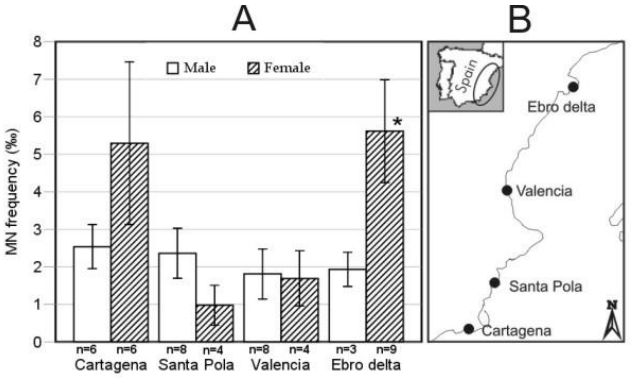

Fig. 1. A) MN frequencies (mean $\pm$ E.S.) in males and females. (*) MN frequency significantly higher than Segura ($p<0.05$); B) Location of the sampling sites.

MN test was able to detect differences among areas affected by different degrees of genotoxic pollution. Only females' results shown such differences, and therefore, further studies should be performed in future to assess the influence of biotic factors on this biomarker. Regardless, the use of erythrocytes from native *M. barbatus* proved to be a sensitive and promising tool for the marine environment genotoxicity monitoring.

References

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