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## BIOLOGICAL EFFECTS OF THE PRESTIGE OIL SPILL IN MUSSELS FROM GALICIA AND THE BAY OF BISCAY USING BIOMARKERS, HISTOPATHOLOGY AND GENOMIC AND PROTEOMIC APPROACHES

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### 1. INTRODUCTION

In November 2002, the tanker *Prestige* sunk in front of the Galician coast (Northwestern Iberian Peninsula). As a result, more than 60 thousand tons of heavy fuel-oil were leaked into the sea affecting more than one thousand kilometres of coastline along the Bay of Biscay. The Galician coast was very severely impacted, but other coastal areas of special high ecological relevance, such as the Biosphere's Reserve of Urdaibai, were also affected at different degrees. After such an enormous environmental disaster, it became necessary to evaluate the effects of the spill over the wildlife and natural resources. Therefore, in the spring of 2003 a monitoring program was started along the Galician and Bay of Biscay coast based on the use of biomarkers in intertidal mussels.

Biomarkers are measurements of body fluids, cells or tissues at cellular, biochemical and molecular levels that indicate the presence of pollutants (exposure biomarkers) or the magnitude of the organism's response (effect biomarkers) (McCarthy & Shugart 1990). These measurements are considered as "early warning signals" since changes at low levels of biological organization (molecule, cell, tissue) can anticipate changes at higher levels such as population, community or ecosystem. The use of individual biomarkers provides partial information about the presence of different types of pollutants and their biological consequences, thus the utilisation of a battery of measurements, including both exposure and effect biomarkers, in selected sentinel species has been strongly recommended to be included in routine monitoring programs. The biomarker approach using mussels or fish as sentinel organisms has been previously employed to assess organism's exposure to and biological effects of accidental oil spills such as those of the Exxon Valdez (1989), Aegean Sea (1992), Sea Empress (1996) and Erika (1999). Studies on the Exxon Valdez have shown a linkage between biomarker responses and long-term effects in populations (Petersen et al., 2003).

In order to assess the biological effects of the Prestige oil spill on coastal ecosystems, mussels (*Mytilus galloprovincialis*) were sampled in 20 locations along the coast of Galicia and the Bay of Biscay. Mussel samples were collected in April, July and September 2003 and in February, April, July and October 2004. Several cell and tissue-level biomarkers were measured: peroxisome proliferation (acyl-CoA oxidase, AOX, activity); lysosomal responses as changes in the structure (volume density, Vv, surface-to-volume ratio, S/V, and numerical density, Nv) and in membrane stability (labilisation period, LP); cell type replacement (volume density of basophilic cells, VvBAS) in digestive gland epithelium; and changes in the morphology of digestive alveoli as mean luminal radius to mean epithelial thickness (MLR/MET). Additionally, flesh condition index and gonad development stages were assessed as supporting parameters. Possible genotoxic effects were assessed by measuring the frequency of micronuclei in isolated gill cells of mussels from selected Basque localities.

## 2. RESULTS AND DISCUSSION

AOX activity was particularly low in Galicia in 2003 and further was markedly induced. LP values were low in all the studied locations, especially in Galicia in 2003. In July 2004, LP values raised slightly, evidencing a certain recovery in mussel health. Concomitantly, lysosomal enlargement was observed in most stations, in 2003 and in 2004 samples, as shown by the significantly low S/V values. VvBAS and MLR/MET values were markedly high in 2003 and showed a decreasing trend throughout 2004. In July and October 2004 no evidence of genotoxic effects were found in the selected Basque locations.

In addition to conventional biomarkers and histopathology, in this study we are using the powerful genomic and proteomic approaches to perform alternative measurements of the same biomarkers and also to develop new biomarkers. We have compared expression profiles of mussel peroxisomal proteins by analyzing the peroxisomal subproteome. We are currently comparing the levels of expression of biomarker genes of interest by semiquantitative RT-PCR. For this purpose, using degenerated primers we have already amplified and cloned portions of the following mussel genes: 2 fragments of nuclear receptors; fragments of the peroxisomal enzymes AOX, catalase, polyamine oxidase and xanthine dehydrogenase; and fragments of the lysosomal enzymes cathepsin-L and hexosaminidase.

In conclusion, employed biomarkers evidenced exposure to toxic chemicals and disturbed health in mussels from Galicia and the Bay of Biscay. Highest degree of disturbance was detected in areas most impacted by the oil spill (Galicia). Biomarker responses were more marked in 2003, a certain recovery trend being envisaged during 2004. Further assessment of mussel health during next years will serve to establish potential long-term effects of the Prestige oil spill by comparison of historical data series. Major drawbacks of the study are the absence of a valid control location along the North Iberian Peninsula coast after the Prestige oil spill and the small amount of previous data on biomarker responses in the study area for comparison purposes. This highlights the urgent need of establishing a regular biomonitoring network along the Iberian coast which should include assessment of the biological effects of pollution based on the use of biomarkers in conjunction with chemical analyses of contaminants (Cajaraville et al. 2000).

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