Effects of the anthropogenic activities on gills of *Pinna nobilis* using oxidative stress biomarkers

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Aquatic organisms are exposed to anthropogenic contaminants that may strongly affect their performance and survival. The periodical oxygen deficiency in coastal areas can induce the production of an excess of reactive oxygen species (ROS). An increase in the formation of ROS and/or a reduced function of the physiological antioxidant defence systems results in oxidative damage to cellular biomolecules. *Pinna nobilis* is the largest endemic bivalve in the Mediterranean Sea, appearing in coastal areas between 0.5 and 60 m depth. *P. nobilis* is under strict protection and all forms of deliberate capture or killing them are prohibited (EEC, 1992). This study aims to estimate the effects of the anthropogenic activities on gills of *Pinna nobilis* through environmental stress biomarkers (nNOS, HIf-1 alfa), determination of antioxidant enzyme response (Cat, Sod, Gpx, Gr) and biomarkers of oxidative damage (MDA). In bivalve molluscs, the gills are an organ highly exposed to environmental factors due to their large surface and their involvement in gas exchange and feeding. The nitrogen monoxide (NO) belongs to the family of free radicals. The production of NO takes place in a reaction catalyzed at the level of several tissues by different isoforms of a monooxygenase known as NOS (nitric oxide synthase). The enzyme has three known isoforms: nNOS, neuronal tissue constitutive enzyme, eNOS, constitutive of vascular endothelial cells and iNOS, an enzyme inducible by cytokines in the liver and activated macrophages. In the nervous system, NO plays multiple roles such as neurotransmitter, is also involved in sensory processes, in synaptogenesis, and in the synaptic plasticity. NO also participates as a molecular mediator of the non-specific immune response against bacterial infections in invertebrates. It is we established that pollution stimulates the gene expression of this enzyme. Furthermore, NO regulates the activity of many transcription factors such as HIF-1α. The neuroepithelial cells, distributed along the axis of the gills and lamellar filaments in the vertebrates, play an important role in oxygen chemoreception and modulate a variety of responses such as oxygen tension in the arteries in response to hypoxia. The HIF-1α is the key gene in hypoxia signalling, being activated under hypoxic stress. In conditions of normoxia, the HIF-1α is not expressed, degraded and kept inactive; on the contrary, when the oxygen tension is reduced HIF-1α is expressed and enters in the cellular nucleus. The hypoxia is characterized by the production of ROS in the cells. The production of ROS and the oxidative stress associated have been recognized as major players in the mediation of a great number of diseases caused by lack or excess of oxygen. An oxidative stress situation occurs when the balance between antioxidants and oxidants is altered and the redox equilibrium is disturbed, resulting in overproduction of free radicals. The relationship between the production of ROS, the several levels of oxygenation and the pathological state of the organism is very complex. The redox homeostasis is subjected to displacement due to an overabundance of factors such as disease states, deficiency or excess of oxygen, infection, inflammation and oxidative damage. The defence systems known to oppose the formation of oxygen radicals are divided in enzymatic antioxidants such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX); and in non-enzymatic antioxidants (such as vitamin E, ascorbic acid and reduced glutathione). The activity of these enzymes is revealed, therefore, as useful biomarkers for assessing the quality status of aquatic environments. For this study, 20 individuals of bivalve *Pinna nobilis* were collected during May-June 2011 in 2 stations with different degree of human impact; the first station located in the Magalluf bay (Mallorca, Esp) along Mallorca Island coasts is considered as a polluted site and the second station in Cabrera Archipelago National Park, (BI, Esp) is considered as a clean non polluted area. The fan mussels were sampled at 8-10 m depth by SCUBA diving, measured and dissected on board in order to remove gills. A portion was frozen in liquid nitrogen for biochemical assays and another portion was stored in paraformaldehyde for histomorphological and immunohistochemical analysis. Histological sections were mounted on glass slides and stained with haematoxylin/eosin to visualize typical morphological features. The morphological staining revealed in individuals of *Pinna nobilis* sampled at Cabrera a regular morphology in the gills with filaments and well organized lamellae, while there were evidenced distinct alterations in the epithelium of gills from the organisms sampled in Magalluf, where it was possible to see thickening and thinning of the lamellar apices close to the filament. The gills of individuals, collected in the waters of Magalluf when analyzed by indirect

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immunofluorescence using antibody anti-nNOS, showed a rather intense immunopositivity in the cells and in the haemocytes of the gill lamellae, compared to those from the reserve of Cabrera. The epithelium of gills from organisms collected to Magalluf showed a greater number of HIF-1α immunopositive haemocytes and cells to respect to what was observed in the gills of the individuals sampled at the control site. All antioxidants CAT, SOD, GPx, GR and GST measured as markers of oxidative stress in gills showed higher activities in Magalluf station respect to the Cabrera. MDA concentration in gills, as a marker of lipid peroxidation, was also increased in the specimens sampled in Magalluf bay. The results suggest that the intense production of vasoactive substances, such as NO found in connective fibbers of gills, may be a local reaction to hypoxia. Due to environmental stress, there is a greater induction of the nNOS, in the nervous elements and in the neuroendocrine-type cells that it can be correlated with the modulation of the chemiosensorial mechanisms for the oxygen and the osmotic control, as it has emerged from the presence of numerous HIF-1α immunopositive cells in the samples from the polluted site. The presences of nNOS and the transcription factor HIF-1α represent adaptive and protective mechanisms. Among the biological assays useful for the assessment of environmental quality, NO is therefore a useful biomarker in providing early information on the presence of environmental changes. We can conclude that human activity induces a biological stress in the fan mussel *Pinna nobilis* which was further highlighted by an increase in the antioxidant enzyme activities and in the lipid peroxidation.