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Histopathology alterations and histochemistry measurements in mussel, *Mytilus edulis* collected offshore from an aluminium smelter industry (Norway)

N. Aarab [a,*], D.M. Pampanin [a], A. Nævdal [a], K.B. Øysæd [a], L. Gastaldi [b], R.K. Bechmann [a]

[a] *International Research Institute of Stavanger (IRIS) – Biomiljø, Mekjarvik 12 N-4070 Randaberg, Norway*
[b] *Dipartimento di Scienze dell'Ambiente e della Vita – DiSAV Università del Piemonte Orientale "A. Avogadro" via Bellini 25/G, 15100 Alessandria, Italy*

Abstract

Histopathological characteristics of specific organs express condition, and represent time-integrated impacts on the organism stemming from alterations at lower levels of biological organisation. As integrative parameters, histochemical investigations have proved to be sensitive tools to detect effects of chemical compounds.

The objective of this study was to determine changes in the tissues of mussels collected at a PAH contaminated site compared to a reference site using histopathological and histochemical parameters: lipofuscin (LF) accumulation in mussel digestive gland, and lysosomal membrane stability (LMS), and using additional information provided by body burden analysis to compare the sensitivity of these parameters.

The histochemical measurements for both LF and LMS gave a clear indication of a high level of stress in animals from the PAH contaminated site. This LF accumulation in lysosomes is the result of peroxidation of autophagocytosed proteins associated with protein aggregates and oxidatively damaged organelles. These measurements were able to detect the effects of PAHs, and showed a strong relationship with the body burden results.

Keywords: *Mytilus edulis*; Immunohistochemistry biomarker; Environmental pollution; Lipofuscin; LMS and PAH

1. Introduction

Mussels are the most widely used sentinel organisms in pollution monitoring programs aimed at studying the health of coastal and estuarine environments, the Mussel Watch (Goldberg, 1975) being the oldest biomonitoring program in progress worldwide. Mussels tolerate pollutant exposure, responding in various ways that can be measured as biological effect parameters. The choice of molluscs in monitoring programs is taken on the basis of their wide geographic distribution and their easy sampling availability.

Histopathological changes are powerful indicators of prior exposure to environmental stressors and are the result of adverse biochemical and physiological changes in an organism. For field assessments, histopathology is often the easiest method of assessing both short- and long-term toxic effects (Hinton and Lauren, 1990). Histopathological alterations in selected organs and tissues are conceived as histopathological or tissue-level biomarkers. By looking at the structure/morphology of digestive glands and histochemistry, it is possible to follow the metabolic activities, especially related to the feeding conditions.

The digestive gland (DG) of molluscs is the main centre for metabolic regulation, participating in the mechanisms of immune defence and homeostatic regulation of the internal medium, as well as in the processes of detoxification and elimination of xenobiotics (Moore and Allen, 2002).

* Corresponding author. Tel.: +47 51875066; fax: +47 51875540.
E-mail address: nadia.aarab@iris.no (N. Aarab).

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Morphology, structure and functionality of the DG in molluscs can be used to study the metabolic activity of the organism. The DG of mussels consists of a series of diverticula connecting with the stomach by a sequence of branching ducts. The epithelium of the digestive diverticula is made of two main cell types, digestive cells and basophilic cells. The digestive cells are primarily involved in intercellular food digestion, whereas basophilic cells (or calcium cells) are thought to be secretory cells that contribute to extracellular digestion and metabolic regulation. DG alterations are a reflection of disturbances at the molecular level and identification of these disturbances can aid in the understanding of whole animal impact due to pollutants and other stress factors. Exposure to pollutants causes severe changes in the relative occurrence of cell types in the DG epithelium (Marigómez et al., 1998). Digestive cells are usually much more abundant than secretory or basophilic cells but, under conditions of exposure to pollutants, an apparent increase in the relative numbers of basophilic cells occurs (Rasmussen et al., 1983; Widdows et al., 1984; Cajaraville et al., 1989; Marigómez et al., 1998).

The histochemical measurements selected for this study were biomarkers of stress. Biomarkers of stress include parameters related to the general physiological stress that the organisms undergo due to a wide range of pollutants, but the biomarkers of this class are not specific. Lysosomal membrane stability (LMS) in cryosections, lysosome membrane stability-neutral red retention time and oxidative stress/ lipofuscin (LF) lysosomal content are three of the main biomarkers of this category.

Lysosomes constitute a group of sub-cellular structures involved in intracellular digestion that compartmentalize and accumulate a wide range of pollutants. Thus pollution causes damage in these organelles, which can be measured by different means, including LMS assays and measuring the extent of vacuolization and LF granules (Etxeberria et al., 1995). The measurement of LMS is used as an integrative biomarker of cellular stress, as membrane integrity is affected by different pollutants (Viarengo et al., 1987). There is evidence of decreased LMS in mussels exposed to pollutants under laboratory conditions and mussels collected from contaminated areas (Widdows et al., 1982). In addition, digestive cells possess a well-developed lysosmal system that is responsive to diverse pollutants, including heavy metals (Moore, 1985, 1988). Exposure to pollutants causes changes in size and number of lysosomes, alterations in membrane permeability and osmotic disruption, which all together lead to vacuolization of digestive cells (Moore, 1985, 1988; Cajaraville et al., 1989; Hole et al., 1995). The relationship between the accumulation of lipid and chemical exposure in the environment has received the greatest amount of attention, and shows the strongest linkage between cause and effect (Feist et al., 2006). In the present study LMS and LF were used, additional information was provided by the body burden analysis.

The objectives of this study were (1) to determine changes in the tissues of mussels sampled at the contaminated site compared to the reference one using a selection of histological parameters and (2) to compare the sensitivity of the different histological techniques.

2. Materials and methods

2.1. Sampling

Sampling was undertaken at 2 sites, Førlandsfjorden and Høgevarde on two dates: March and April 2006. At each station, on both dates, twenty blue mussels, *Mytilus edulis* were collected. This invertebrate has a wide geographical distribution and is available to catch by scraping at 1 m depth.

Two sampling sites were chosen: (1) Førlandsfjorden, located at 59° 19.11N, 5° 27.506E, is an extremely sheltered fjord representing the inner part of a fjord system. There is little activity in this fjord, just some small boat traffic and some small farms that drain into the fjord; (2) Høgevarde, located at N59 19.233, E5 18.952, is a site slightly north of the discharge from an old alumina smelter in Karmsund. The works had a production of about 200,000 tons of aluminium per year, and the discharge of PAH to the Karmsund strait was approximately 450 kg annually (Beyer et al., 1998). A caging study with Atlantic Cod showed that PAHs released from the works were taken up from the water column and metabolised by the caged cod (Beyer et al., 1998). Karmsund is sheltered, with a good water exchange. The site is affected by pyrogenic PAHs.

These sites have been used as reference and polluted sites in BEEP, an EU project (Biological Effects of Environmental Pollution) from 2000 to 2004 (Contract EVK3-2000-00543).

2.2. Histopathology-wax sections

After dissection, DG samples were fixed in calcium-formol (40% formaldehyde, 10% Calcium) and dehydrated in alcohols using ethanol. Tissue is immersed first in 70% ethanol in water progressed through 95% ethanol to 100% ethanol, in this way all the aqueous tissue fluids are removed. Tissue is cleared in methyl benzoate, rinsed in benzene and embedded in paraffin. Histological sections (3 μm thick) were cut on a microtome HM335s (Microm GmbH, Bergman, Germany) mounted on slides, dried at 37 °C for 24 h, and stored at room temperature until staining. Twenty mussels tissue sections per station were stained with hemtoxylin and eosin and examined for histopathological alterations.

2.3. Histochemistry-frozen sections

Small pieces of freshly excised DG tissues from 20 animals from each station were placed on metal cryostat chucks. Each chuck was then placed for 1 min in a small bath of *n*-hexane that had been pre-cooled to −70 °C (using a liquid nitrogen bath). The metal chuck plus the