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Assessing the effects of treated and untreated urban discharges to estuarine and coastal waters applying selected biomarkers on caged mussels

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ABSTRACT

To assess effects of urban discharges, biomarkers were measured in caged mussels in northern Iberian Peninsula. Lysosomal membrane stability and histopathology of gonad and digestive gland were analysed as general effect biomarkers. Exposure to specific pollutants was evaluated by autometallographical detection of metals, peroxisomal acyl-CoA oxidase activity, micronucleus test and transcription levels of vitellogenin and *MT20* genes. Health status of mussels was impaired after 3 days of caging at the untreated outfall discharge and at the waste water treatment plant effluent discharge to the estuary. The most relevant finding was the significant up-regulation of vitellogenin gene transcription in male mussels exposed to the untreated outfall discharge. Metals and xenoestrogenic endocrine disruptors were bioavailable in some discharges and disturbed the health status of mussels. Biomarkers were effective in the assessment of effects of urban discharges and could be implemented in operative controls required to assess the risks associated to effluent discharges.

1. Introduction

In the last decade of the 20th century, the European Union launched a series of Directives with the purpose of managing urban pollution. In 1991, the Council approved the Urban Waste Water Treatment Directive (CEC, 1991) intending to avoid pollution of fresh and marine waters from urban sewerage systems. This Directive concerned the collection, treatment and disposal of urban waste water. In this context, the necessities of urban areas for treatment infrastructures as well as the minimal requirements of depuration were determined. Later, in 1996, the Integrated Pollution, Prevention and Control Directive (CEC, 1996) established a general framework for the integrated prevention and control of pollution, laying down the measures necessary to achieve high level of protection for the environment. The application of this Directive, although focused on industrial activities, entailed the protection of urban areas receiving discharges. In 2000, the European Parliament and the Council published the Water Framework Directive (WFD; CEC, 2000) which purpose was to establish a framework for the protection of inland surface waters, transitional waters, coastal waters and groundwater, preventing them from further deterioration and enhancing their protection in order to achieve by 2015 a "good ecological and chemical status". From then onwards, Member States were required to register river basins and to establish monitoring and, when necessary, improvement programmes including, among others, the control of discharges from sewage treatment plants (Zabel et al., 2001). To assess the "ecological status", biological, hydromorphological and physicochemical parameters, together with some specific pollutants identified in water bodies must be analysed. The assessment of the "chemical status" involves the measurement of priority substances listed within the WFD and amended by the Directive 2008/105/EC (CEC, 2008), where environmental quality standards (EQS) for these and other pollutants were established. Sediment and biota should also be monitored to provide information on the accumulation of those priority substances.

Urban sewage may contain a wide variety of pollutants, derived from the numerous activities carried out in metropolitan areas (Petrović et al., 2003). Pharmaceuticals, surfactants, polyaromatic hydrocarbons and metals, among others, are introduced in sewers

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and wastewater treatment plants (WWTPs) (Aguayo et al., 2004; Bolong et al., 2009; Ellis, 2006; Petrović et al., 2002). Treatment processes have improved over time, but increasing urbanisation has multiplied the concentration of contaminants in wastewater that need to be controlled (Rule et al., 2006). Release of the final effluent in estuarine and coastal areas can produce eutrophication and chemical pollution (Cearreta et al., 2004; Hu et al., 2008; Viguri et al., 2002). Some of those chemicals possess genotoxic, carcinogenic or endocrine disrupting capacities, which entail a risk to health of living organisms (Anderlini, 1992; Chapman et al., 1995; Dagnino et al., 2010; Gagné et al., 2001; Izquierdo et al., 2003; Porter and Janz, 2003). Environmental monitoring of aquatic systems receiving urban discharges has evolved along decades. Traditionally, it consisted of periodic measurements of contaminant levels in the effluent and in the receptor medium (Gasperi et al., 2008). The effects that discharges caused on biota were commonly tackled by analysing assemblages of benthic invertebrates or algae, which offer information of high ecological relevance (Chapman et al., 1995; Echavarri-Erasun et al., 2007). Nevertheless, the spatial and temporal distribution of organisms can be influenced by a variety of natural factors (Johnson et al., 2008) interfering in the establishment of connections between contaminant exposure and biological effects. As time passed, it became recognised that an accurate assessment of the impact on organisms requires the knowledge of the effects existing through a hierarchy of biological organisation, starting at molecular levels (Rice, 2003). Therefore, responses that take place prior to mortality should be assessed to detect prompt responses to contaminant stressors. This is the specific case of biomarkers, defined as measurements at the molecular, biochemical or cellular level, which indicate that the organism has been exposed to pollutants (exposure biomarkers) and/or the magnitude of the response of the organism to the pollutant (effect biomarkers) (McCarthy and Shugart, 1990). In general terms, responses at lower biological organisation levels are more rapid, specific and sensitive than responses at population or assemblage level (Au, 2004). For this reason, biomarkers are utilised as early warning signals of distress (McCarthy and Shugart, 1990) allowing the initiation of remediation strategies before permanent damage to ecosystems has occurred. To obtain data useful for the evaluation of pollution induced stress, a set of biomarkers must be selected, as no individual biomarker can give a concluding evaluation (Cajaraville et al., 2000). The multibiomarker approach brings together different biological responses to make a diagnostic of environmental health condition (Handy et al., 2003; Viarengo et al., 2007).

Besides the selection of precise biomarkers, choosing proper sentinel organisms is critical. Since the 1970s, the importance of molluscs (mainly mussels, *Mytilus* sp.) for pollution monitoring has increased rapidly (see Smolders et al., 2003). Several authors have reported effects on the health of mussels transplanted to areas affected by both treated and untreated urban wastewaters. Immunological responses, effects on the reproductive system, endocrine disruption and histological alterations are among the biological changes often associated with domestic pollution (Akaishi et al., 2007; Bouchard et al., 2009; Gagné et al., 2004). Taking this into account, a variety of biological responses was evaluated on caged mussels, ranging from molecular to organism level. As general stress responses, or effect biomarkers, survival in air, lysosomal membrane stability and histopathological alterations of digestive gland and gonad tissues were evaluated (UNEP/RAMOGE, 1999; Marigómez et al., 2004; Viarengo et al., 2007; Bignell et al., 2008). The presence of bioavailable chemicals in studied scenarios was determined through exposure biomarkers. Exposure to metals was assessed by means of autometallographical detection of metals accumulated in lysosomes and by measuring transcription levels of metallothionein 20 gene (*MT20*) (Dondero et al., 2005; Soto and Marigómez, 1997b). Exposure to organic xenobiotics was assessed through the measurement of acyl-Co A oxidase activity as an indication of peroxisome proliferation (Cancio et al., 1998; Cajaraville and Ortiz-Zarragoitia, 2006). The induction of transcription of the *vtg* gene in male gonad tissues was measured as biomarker of exposure to xenoestrogenic endocrine disruptors (Andrew et al., 2008; Matozzo et al., 2008). Furthermore, data obtained for different biomarkers was integrated in a multibiomarker index (Beliaeff and Burgeot, 2002), to obtain a global picture of pollution occurring at each site.

Thus, the present transplant experiment was performed aiming to validate the usefulness of a selected battery of effect and exposure biomarkers for the detection of biological effects derived from different urban wastewater discharges. With this approach, we aimed to find out the effects that occur at different biological organisation levels in each of the different types of sanitary solutions.

2. Materials and methods

2.1. Study sites

The mussel transplant experiments were performed in October 2009, in estuarine and coastal areas of the northern Iberian Peninsula affected by treated and untreated urban discharges. The study sites are shown in Fig. 1 and their main features as well as geographic coordinates are listed in Table 1. In coastal areas, mussels *Mytilus galloprovincialis* were caged at the mixing zones of two submarine outfalls, Virgen del Mar and Peñarrubia. Virgen del Mar outfall discharges the treated effluent of the sanitation system from Santander city (Cantabria) at 42 m depth. The waste water treatment plant is equipped with biological secondary treatment. Peñarrubia outfall discharges untreated wastewaters from Gijón city (Asturias) at 35 m depth. Raw sewage is discharged through Peñarrubia outfall. Both outfalls discharge their effluents to the Cantabric Sea. To compare results with an area not affected by urban discharges, a common reference site, sharing geomorphological and physicochemical features with both outfall areas was selected at Santoña (Cantabria). One of the cages transplanted to Peñarrubia was lost due to bad weather. Therefore, the transplant was repeated at Peñarrubia and Santoña in April 2010.

In estuarine areas, two urban discharges were monitored, Sestao and Santander. The first discharge is the tertiary treated effluent from Bilbao wastewater treatment plant (WWTP) that discharges to the Nerbioi-Ibaizabal estuary in Sestao (Basque Country). The second discharge is the untreated effluent from a combined sewer overflow of the sanitation system of Santander. Reference sites in nearby estuaries were selected for each effluent. Both sites are industrialised, especially the Nerbioi-Ibaizabal estuary. The reference site for Sestao was established in Plentzia (Basque Country) and the reference site for Santander was established in San Vicente de la Barquera (Cantabria).

2.2. Mussel transplants

Mussels originally from Ría de Arosa (Galicia, Spain) were obtained from a shellfish commercialising company (FRESMAR, Santander). Among all mussels, those from 40 to 50 mm in shell length were utilised for the transplant and the rest were discarded. Before transplants, selected mussels were introduced in plastic cylindrical cages and acclimated in the estuary of Santander for one week, in order to standardise initial conditions. As an exception, mussels destined for caging in Bilbao and Plentzia estuaries were acclimated under laboratory conditions during 5 days to gradual decreasing salinity until 17 PSU, due to the low salinity recorded at those transplant sites.