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Impact assessment of agricultural inputs into a Mediterranean coastal lagoon (Mar Menor, SE Spain) on transplanted clams (*Ruditapes decussatus*) by biochemical and physiological responses

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ABSTRACT

The Mar Menor is a coastal lagoon threatened by the development of intensive agriculture in the surrounding areas. Large amounts of pesticides from these areas are discharged into El Albujón, a permanent watercourse flowing into the lagoon.

We have used a multi-biomarker approach to assess the biological effects of agricultural pollution on a bivalve species. Biomarkers indicative of neurotoxicity (acetylcholinesterase, AChE), oxidative stress (catalase, CAT; glutathione reductase, GR and lipid peroxidation, LPO), phase II biotransformation of xenobiotics (glutathione S-transferase, GST) and physiological stress (scope for growth, SFG) were measured in clams transplanted to four sites of the lagoon (two reference sites and two sites affected by the dispersion of the effluent of the El Albujón), for exposure periods of 7 and 22 days.

The hazards of this effluent were also examined by simultaneously measuring up to 83 contaminants (pesticides, PCBs, PAHs and others) in samples of fresh water from the watercourse mouth and seawater from the deployed sites, as well as the bioaccumulation of organochlorinated compounds and PAHs in the transplanted animals.

Biomarker responses showed marked differences between reference and affected sites after 7 and 22 days. However it was only after 22 days that principal component analysis (PCA) of the biomarker responses distinguished between clams deployed in sites affected by the dispersion of the effluent of the watercourse and those from the reference sites. The chemical analysis of water showed high concentrations of pesticides close to El Albujón watercourse mouth, with the greatest input flux corresponding to the organophosphate chlorpyrifos, followed by pendimethalin and naphthalene, and at lower levels acenaphthene, terbuthylazine-desethyl and chlorpyrifos-methyl. In this regard, PCA analysis showed that the biological effects of the mixture of pesticides in caged clams after 22 days were reduced levels of AchE and SFG and increased levels of GR and phase II GST activity. An integrated biomarker response index was calculated from the combination of these biomarkers, proving useful for the assessment of the impact of agricultural pollution in caged clams.

1. Introduction

Marine bivalves have been used as bioindicators to identify chemical pollutants in coastal environments. However, the concentration of contaminants in tissues alone provides no information on the biological significance and deleterious effects of environmental pollution on biological systems. The need to detect and assess the effects of contaminants has led to the development of markers of their effects (i.e. biomarkers). These biological-effect methods, which range from responses measured at subcellular level (e.g. oxidative stress and DNA adducts) to whole-organism responses (e.g. scope for growth or disease occurrence), can indicate links between contaminants and ecological responses and can be used to indicate the presence of harmful substances in the marine environment (Thain et al., 2008).

Acetyl cholinesterase (AChE) activity measurement is considered a valuable biomarker of exposure to neurotoxic compounds in vertebrate and invertebrate species, such as organophosphorus (OPs) and carbamate compounds used in agriculture as pesticides (Bocquené and Galgani, 1998; Cooper and Bidwell, 2006).

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The toxicity of OPs results from their inhibition of cholinesterase enzymes, which catalyze hydrolysis of the neurotransmitter acetylcholine after it is released at the nerve synapse.

Oxidative stress is a common pathway of toxicity induced by several classes of pollutants (Winston and Di Giulio, 1991) in which production of reactive oxygen species (ROS) is enhanced. ROS can be highly toxic to aquatic organisms as they often cause cellular damage such as lipid peroxidation (LPO) in membranes, altered pyridine nucleotide redox status and DNA damage (Lemaire and Livingstone, 1993). Protection against the toxicity of oxyradicals towards cellular targets is afforded by a complex defence system consisting of both low molecular-weight scavengers and antioxidant enzymes. Measurement of enzymatic activities for the detoxification of ROS and the degree of LPO have been proposed as biomarkers of oxidative stress in bivalves exposed to different types of pollutants (Fernández et al., 2010a; Tsangaris et al., 2007; Vidal-Liñán et al., 2010). Some of the more commonly used antioxidant biomarkers include catalase (CAT), superoxide dismutase (SOD), glutathione transferase (GST), glutathione reductase (GR), and lipid peroxidation (LPO). CAT is responsible for the breakdown of hydrogen peroxide into water and oxygen, which may be produced during basal aerobic metabolism or after a pollution-enhanced oxyradical generation (Winston et al., 1990). Although glutathione reductase (GR) does not play a direct role in the elimination of oxygen radicals it can be regarded as an essential antioxidant enzyme since it reduces oxidized glutathione (GSSG) and maintains the GSSG/GSH balance under oxidative stress, essential for cellular homeostasis and the operation of other enzymes (Winston and Di Giulio, 1991). GST represent a major group of phase II detoxification isoenzymes whose 'natural' substrates range from molecules of foreign origin to by-products of cellular metabolism. GSTs primarily catalyze the conjugation of GSH to various electrophilic compounds, but they can also act as glutathione peroxidase, as isomerases, or simply as binding proteins sequestering hydrophobic molecules, and can therefore be regarded as playing an antioxidant role (Manduzio et al., 2005; Prohaska, 1980).

Scope for growth, SFG, is a biomarker at the individual/whole organism level of biological complexity with a high degree of ecological relevance and for this reason is eminently applicable for biomonitoring programmes (SIME, 2007). This technique involves the calculation of the energy available for growth under standardized laboratory conditions. It consists of evaluating the energy acquired by an organism after absorbing the food it has ingested and that lost in the respiratory and excretory processes, the difference between them being the energy the organism has available for production (growth and reproduction). The presence of contaminants in the marine environment alters this energy balance, making SFG a marker for toxic stress. SFG has been successfully applied in programmes monitoring chronic pollution (Albentosa et al., 2012; Cotou et al., 2002; Halldorsson et al., 2005; Toro et al., 2003a; Widdows et al., 1995, 2002), acute pollution associated with a spill (Fernández et al., 2010b; Larretxea and Pérez Camacho, 1996) and in laboratory contaminant exposure studies (Kraak et al., 1997; Sobral and Widdows, 1997; Wang and Chow, 2002; Widdows and Page, 1993).

Different studies have shown the usefulness of marine clams as sentinel organisms for the detection of the impact of environmental pollution in coastal waters through the application of different biomarkers (Bebianno et al., 2004; Nasci et al., 1999, 2000). These biomonitoring studies have employed native populations of bivalves or organisms that have been transplanted from a reference site to a polluted area (Rank et al., 2007; Tsangaris et al., 2010, 2011). This latter strategy, called active biomonitoring (ABM), is based on comparing chemical and/or biological properties of samples collected from one population that, after randomization and translocation, has been exposed to different environmental conditions at monitoring sites (Roméo et al., 2003). This approach avoids bias related to the age and the reproductive status of the organisms and allows for better control of the accumulation and biological effects of contaminants over a predetermined exposure period. In addition, comparisons of sites are feasible even if natural populations are scarce (Tsangaris et al., 2011).

Overall, coastal lagoon environments are characterized by being isolated from the open sea, which makes them highly vulnerable to impacts. The Mar Menor lagoon is a shallow coastal basin connected with the Mediterranean Sea principally through three sea channels that receives a wide variety of chemical pollutants associated with anthropogenic activities. Its ecological equilibrium is threatened by massive urban growth and intensive agricultural activity (Conesa and Jiménez-Cárceles, 2007). The lagoon receives water run-off from the coastal plain of Campo de Cartagena, where intensive agricultural activity has taken place since 1979. At the present time, El Albujón watercourse constitutes the main collector in the Campo de Cartagena drainage system (García-Pintado et al., 2007), maintaining a regular flux fed by groundwater (drainage of irrigated crops) that is only continuous in the last 3–8 km, depending on the season (Velasco et al., 2006).

In a previous study by our group (Moreno-González et al., 2013a), the seasonal input of all the organic pollutants to the Mar Menor lagoon through El Albujón watercourse (considering both regular and flash flood periods) was characterized. In the aforementioned study, 71 semi-volatile organic pollutants were detected by stir bar sorptive extraction followed by capillary gas chromatography coupled to mass spectrometry (SBSE/GC/MS). Results showed that pesticide concentrations varied significantly along the watercourse and a clear seasonal pattern was detected, with a predominance of insecticides during summer and of herbicides during winter. The most commonly detected analytes were propyzamide, triazinic compounds and chlorpyrifos.

The objective of this study was to assess the effect of the El Albujón watercourse on the water quality of the Mar Menor by means of the biological effects elicited in a characteristic bivalve of this lagoon. For this purpose, a multi-biomarker approach was applied in transplanted *Ruditapes decussatus* clams caged at two sites affected by the dispersion of the watercourse input, according to the main currents, and at a further two sites not affected directly by significant pesticide inputs, which act as reference sites. In previous studies carried out in El Albujón watercourse (Moreno-González et al., 2013a) more pesticides were detected in autumn than in other seasons, and for this reason autumn was the season chosen to evaluate the impact on clam biology. Biomarkers included biochemical measurements which represent important endpoints of particular chemicals or mixtures expected in the study area: AChE, CAT, GST, GR, LPO and bioenergetics such as SFG used to detect general stress effects on the health status of clams. Active biomonitoring is also evaluated for the assessment of the risk of environmental contamination in this coastal lagoon where natural populations of bivalves are scarce due to the deterioration of their ecosystems.

2. Materials and methods

2.1. Study area and experimental design

The Mar Menor (SE Spain) is a hypersaline coastal lagoon located in the Mediterranean Sea with a superficial area of 135 km^2 (María-Cervantes et al., 2009). The salinity of its waters in the period of this study (autumn) ranges from 40 to 43 psu showing a north–south gradient, except in areas close to the principal channels connecting with the Mediterranean Sea or to freshwater inputs. The general circulatory pattern (Pérez-Ruzafa et al.,