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In situ evaluation of wastewater discharges and the bioavailability of contaminants to marine biota

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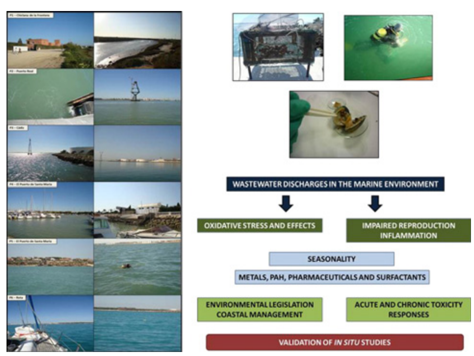
HIGHLIGHTS

- Sediment quality of wastewater discharges evaluated by caged clams *R. philippinarum*
- Exposure was confirmed by measurement of metal and organic compounds in sediments.
- Winter: metabolism by phases I and II, and activation of antioxidant enzymes
- Summer: metabolism by phase I, oxidative effects and neurotoxicity
- Adverse effects related to neuroendocrine disruption: inflammation, spawning delay

GRAPHICAL ABSTRACT



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ABSTRACT

Marine sediment quality of wastewater discharges areas was determined by using in situ caged clams *Ruditapes philippinarum* taking into account the seasonality. Clams were caged in sediment directly affected by wastewater discharges at four sites (P1, P2, P3, P4) at the Bay of Cádiz (SW, Spain), and one reference site (P6). Exposure to contaminated sediments was confirmed by measurement of metals and As, PAH, pharmaceutical products and surfactants (SAS) in bottom sediments. Biological effects were determined by following biomarkers of exposure (activities of 7-ethoxyresorufin O-deethylase — EROD, dibenzylfluorescein dealkylase — DBF, glutathione S-transferase — GST, glutathione peroxidase — GPX, glutathione reductase — GR and acetylcholinesterase — AChE), effects (lysosomal membrane stability — LMS, DNA damage and lipid peroxidation — LPO), energy status (total lipids — TLP and mitochondrial electron transport — MET), and involved in the mode of action of pharmaceutical products (monoamine oxidase activity — MAO, alkali-labile phosphates — ALP levels and cyclooxygenase activity — COX). In winter, urban effluents were detoxified by phase I biotransformation (CYP3A-like activity), phase II (GST), and the activation of antioxidant defence enzymes (GR). Urban effluents lead to the detoxification metabolism (CYP1A-like), oxidative effects (LPO and DNA damage), neurotoxicity (AChE) and neuroendocrine disruption (COX and ALP levels) involved in inflammation (P1 and P2) and changes in reproduction as spawning delay (P3 and P4) in clams exposed in summer. Adverse effects on biota exposed to sediment directly affected by wastewater discharges depend on the chemical contamination level and also on the reproductive cycle according to seasonality.

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1. Introduction

Wastewater treatment plants (WWTPs) is responsible for a wide variety of natural, domestic and industrial products disposal to the environment. Domestic wastewater discharges are frequently observed at the Spanish coast. According to the European Directive 92/271/EEC, Spain should have collecting systems and secondary treatments (or equivalent process) in towns with more than 10,000 inhabitants. The population around the Bay of Cádiz (SW, Spain) comprises approximately 460,000 inhabitants, which increases by 30% during summer season (INE, 2011). The volume of wastewater effluents discharged daily to the Bay of Cádiz does not change along the year, however, discharge composition vary widely. Summer is a dry season in South Spain, and the main driver of contamination comes from the touristic population. Winter is a rainy season characterized by water overflows, re-suspension of sediments and increase of drainage from the vicinity. Hence, municipal effluents from the cities located around the Bay of Cádiz are considered important sources of contamination for the local marine and estuarine environments by industrial, domestic and agriculture residues.

Biomonitoring is an important tool for understanding the linkages between chemical exposures and potential health outcomes in contaminated environments. Sediment compartment is a well-known sink and long-term source of xenobiotics. Previous studies reported concentration of different xenobiotics in sediment sampled at this Bay, including pharmaceutical and personal care products — PPCPs in $ng \cdot g^{-1}$ (Pintado-Herrera et al., 2013), metals in $mg \cdot kg^{-1}$ (Ramos-Gómez et al., 2008, 2011; Morales-Caselles et al., 2008; Martín-Díaz et al., 2008; Riba et al., 2004), surfactants in $\mu g \cdot g^{-1}$ (Lara-Martín et al., 2006), polycyclic aromatic hydrocarbon — PAH in $\mu g \cdot kg^{-1}$ (Ramos-Gómez et al., 2008, 2011; Martín-Díaz et al., 2008; Riba et al., 2004) and polychlorinated biphenyls — PCB in $mg \cdot kg^{-1}$ (Martín-Díaz et al., 2008; Riba et al., 2004). Contaminants might be bioavailable in this bay as reported by previous study about caged polychaetes *Arenicola marina* (Ramos-Gómez et al., 2008). Through the number of studies published on wastewater ecotoxicology, chemical characterization of released discharges and its ecotoxicological effects in situ are generally less understood (Farcy et al., 2011).

In situ exposure approaches, such as clam caging, allow the determination of both exposure and effects in the natural environment (Farcy et al., 2011). Within the Water Framework Directive, there are regulatory arguments in favour of using biota for assessing contamination trends (Gust et al., 2014), highlighting the relevance of caged invertebrates (Besse et al., 2012).

The real consequences of sediment contamination on biota can be determined by the measurement of biomarkers (Ramos-Gómez et al., 2008). Biomarkers are suitable tools recommended for environmental international agencies such as UNEP, OSPAR, OECD, ICES and IOC guidelines applied around the world (Solé et al., 2009; Cajaraville et al., 2000). In addition to the relevance of the studied biological responses, potential biomarkers of environmental stress and the influence of external variables such as temperature and seasonal changes needs to be understood for proper biomonitoring application (Guerlet et al., 2007).

Several endpoints can be assessed in field studies. Gagné and Blaise (2003) proposed two types of biomarkers to measure PPCP effects in field studies. The first group are biomarkers encompassing the effect of drugs with no assumption of their mechanism of action and specificity. This group can be subdivided in biomarkers of exposure and effect. Biomarkers of exposure involve the metabolism of xenobiotics (phases I and II enzymatic activities), antioxidant enzymes and neurotoxicity (AChE activity). Phase I is composed by detoxification enzymatic activities: ethoxyresorufin *O*-deethylase (EROD) and dibenzylfluorescein dealkylase (DBF). Phase II is based on the conjugation of xenobiotics by glutathione S-transferase (GST) activity. Glutathione peroxidase (GPX) and glutathione reductase (GR) are part of the antioxidant system. Acetylcholinesterase activity (AChE) plays an important role in synapses. Biomarkers of effect are related to the oxidative effects, consequence of free radical production, as lysosomal membrane stability (LMS), lipid peroxidation (LPO) and to some extent DNA damage (*strand breaks*). LMS is considered a suitable tool to analyse the health status of populations in different environmental compartments, including the exposure to sediment samples (Buratti et al., 2012). The second group of biochemical responses involves the mode of action (MOA) of different drugs, with possible profound effects on an organism's survival, immune function and reproduction: energy status measured as total lipid content (TLP) and mitochondrial electron transport (MET), monoamine oxidase activity (MAO) (for MAO inhibitor drugs as fluoxetine), cyclooxygenase activity (COX) (for COX inhibitors drugs as ibuprofen) and alkali-labile phosphates (ALP) levels (ALP levels that could be related to estrogenic compounds).

In the present study, a suite of biomarkers were evaluated in clams *Ruditapes philippinarum*, proposed as sentinel species to monitor the environmental quality of the Southern Iberian Peninsula (Cajaraville et al., 2000). *R. philippinarum* has been extensively used in biomonitoring studies (Moschino et al., 2011; Morales-Caselles et al., 2008) and for sublethal measurements (Buratti et al., 2010, 2012; Coughlan et al., 2009; Martín-Díaz et al., 2007, 2008). Clams are at high risk of exposure to such contamination because these benthic bivalves are sedentary, they live at the sediment/water interface and they are filter feeders ingesting suspended materials. Manila clams (*R. philippinarum*) are species of subtropical to boreal low-latitude western Pacific and introduced in the Atlantic, being well distributed in temperate Europe (Goulletquer, 2005). This species has considerable commercial value especially in Ireland, Portugal, Spain and Italy (Goulletquer, 2005).

The main aim of the present study was to evaluate the adverse effects of WWTPs on the benthic biota applying the bioindicator *R. philippinarum*. Sediment quality of the Bay of Cádiz was evaluated, including the traditional approach with chemical analysis and ecotoxicological effects. This purpose has been carried out by means of in situ study of 14-days toxicity test, taking into consideration two distinct seasons: winter and summer.

2. Material and methods

2.1. Test species

Adults of *R. philippinarum* were obtained from an aquaculture farm located at Chiclana de la Frontera (Southern Spain). Specimens were acclimatized for one week under laboratory conditions (pH: 7.9 ± 0.2; salinity: 36 ± 2; temperature: 19 ± 2 °C; dissolved oxygen: 8.5 ± 1.4 mg L^{-1}), before the exposure for 14-days to five stations located at the Bay of Cádiz (SW, Spain) in winter and summer seasons. Spawning period of *R. philippinarum* occurs in summer (Delgado and Pérez-Camacho, 2007) which can interfere in some of the biomarkers responses and influenced by contamination (Blaise et al., 2002).

2.2. Exposure sites

The Bay of Cádiz is characterized by salt-marsh areas and watershed covering about 36.6 km^2 and average depth of 4 m (Forja et al., 1994), which drains a dense urban area in the Southern Spain. The main industries located in this zone are related with ship, offshore, car and aerospace manufacturing. Agriculture and tourism activities are important socio-economic activities. WWTPs receive wastewaters from 460,000 inhabitants, which increase approximately 30% in summer period (INE, 2011). Clams were caged on five locations at the Bay of Cádiz. Four stations (P1–P4) were chosen where the WWTPs effluent discharges occur, comprising the cities of Chiclana de la Frontera — P1, Puerto Real — P2, Cádiz — P3 and El Puerto de Santa María — P4. P6 located in Rota was chosen as reference site. In winter, two cage systems located in P3 and P4 (Figs. 1 and 2) disappeared and it was not possible to recover the animals from the cages. Exposure of clams and sediment