Environment International 35 (2009) 523–531

Contents lists available at ScienceDirect

Environment International

journal homepage: www.elsevier.com/locate/envint

# Pollution biomarkers in two estuarine invertebrates, *Nereis diversicolor* and *Scrobicularia plana*, from a Marsh ecosystem in SW Spain

Montserrat Solé [a,b,*], Justyna Kopecka-Pilarczyk [c,d], Julián Blasco [a]

[a] Instituto de Ciencias Marinas de Andalucía (CSIC), Av. República Saharaui, 11510 Puerto Real, Cádiz, Spain
[b] Institut de Ciencies del Mar (CSIC), Passeig Marítim de la Barceloneta 37-49, 08003 Barcelona, Spain
[c] Polish Academy of Sciences, Institute of Oceanology, ul. Powstańców Warszawy 55, 81-712 Sopot, Poland
[d] Centro Interdisciplinar de Investigação Marinha e Ambiental (CIIMAR), Rua dos Bragas 289, 4050-123 Porto, Portugal

ARTICLE INFO

*Article history:*
Received 18 June 2008
Accepted 24 September 2008
Available online 17 November 2008

*Keywords:*
*Nereis diversicolor*
*Scrobicularia plana*
Biomarkers
Marsh areas

ABSTRACT

The polychaete worm *Nereis diversicolor* and the clam *Scrobicularia plana* were collected from several sites, affected by different types of contamination, in a littoral enclosure in the SW Spain (Caño Sancti-Petri and Rio San Pedro). *N. diversicolor* was present in 6 sampling sites whereas *S. plana* in 4 of them. The aim of our study was to relate several pollution biomarkers to chemical sources (metals and organic pollutants e.g. PCB, PAH) in these species, thereby confirming their adequacy as sentinels for this habitat. The biomarkers surveyed in the two invertebrates were the activities of the antioxidant enzyme catalase (CAT), the phase II detoxifying enzyme glutathione *S*-transferase (GST) and the neurotoxicity marker acetylcholinesterase (AChE). Metallothionein (MT) levels were measured as a biomarker of exposure to metals. The results suggested a different response in the two sediment-dwelling organisms, the sediment-eating polychaete and the water-filtering clam, probably as a consequence of different contamination exposures. The results also suggested that samples from the "Caño Sancti-Petri" were exposed to biologically active compounds that altered some of their biochemical responses. Of all the biomarkers tested, AChE was the most sensitive one and *N. diversicolor* the potentially most robust sentinel in this ecosystem. In this low to moderately polluted environment, the biochemical approach better reflected temporal trends than site-related differences although it was also able to detect punctual chemical insults.



## 1. Introduction

Littoral ecosystems are constantly threatened by pollution due to their proximity to human settlements with their associated agricultural and industrial activities. Our study site, "Caño Sancti-Petri", is a salt marsh situated in the SW of Spain. It is a highly productive area where aquaculture and fishing activities have traditionally taken place. It is also important from an ecological point of view, which justified its qualification as a Natural Park in 1996. Despite this qualification, untreated domestic waste waters from a city with a population of 88,000 discharged directly into its core and, not until February 2002, did most of this load discharge cease thanks to the construction of a Sewage Treatment Plant (STP).

The choice of a suite of biomarkers, rather than the chemical analysis alone, has been proposed as a more appropriate approach to monitor effects of pollutants in aquatic ecosystems (Cajaraville et al., 2000). Several field studies have proved to be successful using a multibiomarker approach in invertebrates (Porte et al., 2001; Kopecka et al., 2006; Durou et al., 2007; Lima et al., 2007; Martín-Díaz et al., 2008) and nowadays this is a recommended tool for pollution monitoring studies within many governmental institutions such as MAFF, IFREMER, UNEP, OECD, EU projects (e.g. BEEP), and it has been adopted by international conventions OSPAR, ICES.

The suite of parameters selected were biomarkers of exposure aiming to detect specific-site contamination, or temporal trends, in the general stress condition of the two sentinels selected for the study. The biomarkers chosen were: the protein yield (PY), metallothionein (MT) levels, catalase (CAT), glutathione *S*-transferase (GST) and the acetylcholinesterase (AChE) activities. PY is a general marker of hepatic/digestive gland protein synthesis and, despite its unspecificity, it is usually enhanced under xenobiotic exposure. MT is a cysteine-rich protein that binds to essential and non-essential metals facilitating either their transport (essentials) or inactivation (non-essential). Demonstration of increased synthesis under metal exposure has been given under field and laboratory studies (Viarengo et al., 2000). CAT is an antioxidant enzyme that eliminates $H_2O_2$ thus preventing cell damage by reactive oxygen species (ROS), although its inducibility under field conditions is more controversial (Livingstone, 2001). GST is a phase II enzyme involved in the metabolism of

* Corresponding author. Instituto de Ciencias Marinas de Andalucía (CSIC), Av. República Saharaui, 11510 Puerto Real, Cádiz, Spain. Tel.: + 34 93 230 95 00; fax: +34 93 230 95 55.
*E-mail address:* msole@icm.csic.es (M. Solé).

0160-4120/$ – see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.envint.2008.09.013

lipophilic organic contaminants (Fitzpatrick et al., 1997), but it has also an antioxidant role, its activity can be modulated by metals (Regoli et al., 1998; Canesi et al., 1999; Geracitano et al., 2004a). AChE is specifically inhibited by organophosphorus pesticides and carbamates in invertebrates (Escartín and Porte, 1997; Fourcy et al., 2002), however, some heavy metals, surfactants and petrogenic compounds can modify its response (Guilhermino et al., 2000; Moreira et al., 2004).

An important factor to consider when using the biomarker approach in invertebrates is the variations on these parameters depending on sex, physiological stage, age and physical characteristics in the environment. These variations have been reported for bivalves (Bodin et al., 2004; Leiniö and Lehtonen, 2005), but also in the ragworm *Nereis diversicolor* (Durou et al., 2007). Thus, in order to minimise these confounding factors, we chose individuals of similar size, caught at the same year period over the four consecutive campaigns. For this study, we selected two benthic, sessile invertebrate species native to this saltmarsh: the clam *Scrobicularia plana* and the polychaete worm *N. diversicolor*. Thus, their respective biochemical responses will be associated to pollutants bound to the particulate fraction of the water (clam) or linked to the sediment (worm).

Bivalves, and in particular mussels, are broadly used as sentinels in pollution monitoring although other invertebrate species are also adequate (Livingstone, 2001). In our studied area as no mussels were available, *S. plana* was the most adequate bivalve alternative. Nevertheless, this species was not present at all the sampled sites due to the characteristics of the sediment. There are only a few recent studies using the set of biomarkers applied in here in *S. plana* (Dauvin, 2008; Coelho et al., 2008; Romero-Ruiz et al., in press). Likewise mussel, *S. plana* possesses the characteristics that make it adequate for pollution monitoring: wide distribution, tolerance to chemical exposure, sedentary lifestyle and low metabolism. Among other invertebrates, polychaete worms are also well adapted to stressful environmental conditions and distributed worldwide, including heavily polluted sites, for which they are also good sentinels. In particular the ragworm, *N.*/*Hediste diversicolor*, has been proved to be a good bioindicator (Díez et al., 2000; Fourcy et al., 2002; Moreira et al., 2006; Ait Alla et al., 2006; Durou et al., 2007, 2008; Sun and Zhou 2008). It is a key-species in soft-bottom communities which behaves as a filter and deposit feeder, scavenging for organic matter and detritus on the sediment surface with a highly significant ecologic role. Therefore, any alterations in their health, feeding behaviour or survival can greatly affect the ecology of their habitat (Moreira et al., 2006).

The aim of the study was to find out the adequacy of the sentinel species selected, using a suite of biomarkers, in identifying pollution sources, as well as, to follow up the recovery of this ecosystem after the cessation of domestic waste discharges over a four year period (2002–2005). Our findings could also be applicable to other ecosystems of similar latitudes under similar challenges.

## 2. Materials and methods

### 2.1. Study area and sample collection

Specimens of ragworm, *N. diversicolor*, and clam, *S. plana*, were collected in the period May–June from the years 2002 to 2005 from 6 and 4 sites, respectively, in the "Río San Pedro" (S0) and "Caño Sancti-Petri" marsh (S1–S5) (SW Spain) (Fig. 1). Due to sediment requirements, *S. plana* was not found in all the selected sites, as muddy sediment is preferred to sandy one, whereas *N. diversicolor* was found in both sediment types. Site characteristics were as follows: (SO) is located in a dead mouth river that does not receive any recognised pollution inputs and was chosen as reference; (S1) is near a military settlement and a shipyard; (S2) used to be the discharge area for untreated domestic water of a 88,000 inhabitants town, the discharges theoretically ceased 4 months previously to the initiation of this study, with the completion of a STP; (S3) is a relatively clean area but under the Chiclana river influence; (S4) is located near a small leisure harbour, and thus influenced by boat traffic and activities; and (S5) located nearer to the sea and under intense water renovation, was also used as reference. Animals were collected during low tides and water salinity and ambient temperature was similar as all sampling were carried out at the same year period. The two invertebrates were transported to the laboratory within 1–2 h of collection, clam's digestive gland and the whole body of *N. diversicolor* were frozen in liquid nitrogen ($N_2$) and stored at −80 °C until biochemical analysis.

### 2.2. Sample procedure for biochemical determinations

Pooled digestive glands of 3 clams or 3 fragments of worm tissue were used for each replicate sample, and from 4 to 6 replicates were prepared per site. The animal's tissue was homogenised in 1:4

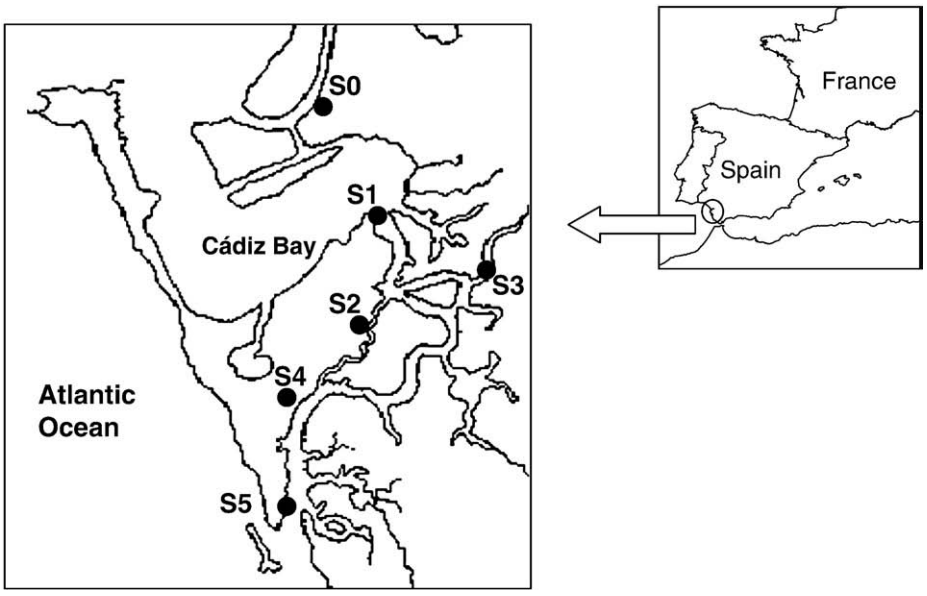


**Fig. 1.** Map of the sampling sites: S0–S3 for *Scrobicularia plana*, S0–S5 for *Nereis diversicolor*. S0 "Río San Pedro" (control). S1–S5 "Caño Sancti-Petri" sites.