Marine Pollution Bulletin 64 (2012) 2681–2691

Contents lists available at SciVerse ScienceDirect

Marine Pollution Bulletin

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

Effects of an experimental *in situ* diesel oil spill on the benthic community of unvegetated tidal flats in a subtropical estuary (Paranaguá Bay, Brazil)

Aline Gonzalez Egres *, César C. Martins, Verônica Maria de Oliveira, Paulo da Cunha Lana

Centro de Estudos do Mar, Universidade Federal do Paraná, Caixa Postal 61, 83255-976 Pontal do Sul, Pontal do Paraná, PR, Brazil

ARTICLE INFO

Keywords:
Diesel
Benthic macrofauna
In situ experiment
Recolonization
M-BACI

ABSTRACT

The effects of diesel oil on benthic associations from unvegetated tidal flats in a subtropical estuary were experimentally evaluated using a Multivariate Before and After/Control and Impact Model (M-BACI). Impacted treatments were contrasted with controls in 14 successive periods before and after the oil spill. An acute effect was recorded just after the impact, but the recovery to pre-disturbance population levels was extremely fast. The increase in the total density of the benthic community after the disturbance was the result of an increase in the densities of *Heleobia australis*, oligochaetes, and ostracods, observed in both impacted and control treatments, as a reflection of background variability and not the presence of the contaminant. The experimental spill had little influence on the biological descriptors of the benthic associations, which were resilient or tolerant to oil disturbance at the temporal (147 days) and spatial (cm^2) scales used in the experiment.

1. Introduction

Oil spills are among the main sources of organic contamination in marine environments and may cause negative effects on the biota in its various levels of organization (ITOPF, 1997; Clark, 1997; Fleeger et al., 2003; NRC, 2003; Ruiz et al., 2005). Under normal conditions, much of the oil is removed by physical forces such as tidal movements, evaporation, and dispersion; however, an important fraction can be introduced into the deep anaerobic layers of the sediment, where it may persist for years (Readman et al., 2002). Unlike exposed rocky or sandy beaches, estuarine habitats are relatively protected from strong winds and currents and tend to gather fine grained sediments, which tend to accumulate and bind toxicants (Sanz-Lázaro and Marín, 2009), as it often happens in tidal flats, salt marshes and mangroves.

Estuaries are habitats to diversified benthic populations that play an important role as transfers of matter and energy between trophic levels. Benthic organisms live in close association with the substrate, which tends to accumulate and retain organic and inorganic contaminants, particularly when poorly oxygenated (Hyland et al., 2005). It is difficult for these organisms to avoid exposure to adverse conditions because of their relatively sedentary nature. Thus, benthic animals have been frequently used on assessments of the intensity and extent of damage caused by oil spills, mainly because they reflect with greater precision and speed the changes in physical and chemical parameters (Dauer, 1993; Poulton et al., 1998; Muniz et al., 2005; Gomez Gesteira and Dauvin, 2005; Borja and Dauer, 2008; Ocon et al., 2008; Dauvin et al., 2010).

Pollutants tend to affect the structure of benthic associations by changing their abundance and composition. Species that are tolerant and opportunistic become relatively more numerous after oil spills, while those considered sensitive become increasingly rare or disappear (Carman et al., 2000; Belan, 2003). Surviving or recolonizing species can form population patches after acute impacts in affected areas, benefiting from the exclusion of those previously dominant. Thus, the composition of benthic communities can be changed temporarily or permanently in comparison with pre-disturbance conditions (Glasby and Underwood, 1996).

Reliable biological indicators of environmental quality need to demonstrate logical connections between their responses and the variables of interest and the idea that a taxonomic group or species is representative of particular environmental conditions should be initially treated as a hypothesis to be tested (Goodsell et al., 2009). Toxicological approaches in laboratories or *in situ* have been widely used for environmental risk assessment, both presenting specific advantages and limitations (Carman et al., 2000; Bhattacharyya et al., 2003; Schratzberger et al., 2003; Chung et al., 2004; Lu and Wu, 2006). However, laboratory bioassays conducted under strictly controlled conditions might not reflect the so-called natural variability (Morales-Caselles et al., 2008a). This creates uncertainty as to the validity of result extrapolations when compared to field studies (Sibley et al., 1999).

In fact, animal associations do not exhibit simple or linear responses to disturbance in their natural habitat. They are more likely to resist or persist until reaching a threshold of disturbance

* Corresponding author. Tel./fax: +55 41 3511862.
E-mail address: egres.aline@gmail.com (A.G. Egres).

0025-326X/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.marpolbul.2012.10.007

(Hyland et al., 2005). In this context, the simulation of small- or meso-scale *in situ* manipulative experiments is often the best way to assess impacts because they best reflect the ecological reality responding to cumulative and synergistic effects (Glasby and Underwood, 1996).

These experimental approaches are very simple from a conceptual and methodological point of view and do not require sophisticated equipment. Additionally, they are reliable tools because they use different lines of evidence and analyze the whole community through key species (Sanz-Lázaro and Marín, 2009), which putatively support ecosystem ecological integrity (structure and productivity).

The subtropical estuarine complex of Paranaguá Bay in southern Brazil is one of the largest and best preserved in the southern hemisphere (Lana et al., 2001). Its economic importance is related to fishing activities, urban and tourist areas and industries, associated with fertilizer plants, fuel terminal and the main South American grain shipping port, being the third most important in loading and unloading operations (Santos et al., 2009; Martins et al., 2010). While not characterized by a large volume of oil operations, the port hosts the Transportation Terminal of Paranaguá (TEPAR) among its retro-port zone components, which operates refining, storing, and transporting oil and derivatives. Intense ship traffic in the bay, together with smaller fishing and leisure boats, must be added as potential impact vectors. The most common fuel in most of these vessels is the marine diesel oil, which regardless of its tendency to persist less time due to its high evaporation rate (FINGAS, 2001), has acute toxic effects on benthic plants and animals (Lytle and Peckarsky, 2001).

Paranaguá Bay has a wide variety of intertidal environments such as mangroves, marshes, and unvegetated tidal flats, which are most exposed to oil impacts. Local unvegetated tidal flats are resting and feeding areas for migratory birds and fishing areas for local fish-folk communities (Ryo et al., 2011). These systems, usually dominant in confined low-energy areas along the southeastern and southern Brazilian coasts, are indeed potential targets of accidental oil spills (Noernberg and Lana, 2002).

In this context, this work aims: (i) to evaluate, through a field experiment, the acute or immediate effects of diesel oil on the benthic macrofauna from unvegetated subtropical tidal flats expressed by the mortality rates and changes in vital signs of the numerically dominant species; (ii) to assess the short-term effects (weeks to months) of diesel oil expressed by variations in total macrofaunal density, species numbers, and densities of the numerically dominant macrobenthic species; (iii) to assess recovery speed to before-impact population levels, e.g. local resilience. We hypothesize that macrofaunal recovery in intertidal areas of a subtropical estuary after a small-scale oil spill will be fast and mostly determined by the mobility strategies of individual species.

2. Materials and methods

2.1. Study area

The Paranaguá Bay (25°30′S; 48°25′W), located in the coastal plain of Paraná State, is an estuarine system bordered by mangroves, marshes, and extensive tidal plains. Unvegetated tidal flats are the most common traits of the bay. They may be covered by banks of macro-algae or diatoms films, which are the main primary producers (Siqueira et al., 2006). The numerical dominance of polychaetes and oligochaetes followed by crustaceans and mollusks are recurrent (Faraco and Lana, 2004).

The *in situ* experiment was performed in unvegetated tidal flats along the Cotinga Channel (Fig. 1), a sub-estuary from Paranaguá Bay, with about 15 km in extension, which receives the freshwater input from the Maciel, Correias, Almeidas, Guaraguaçú, and Itiberê rivers (Lana et al., 2001).

2.2. Experimental design

An *in situ* experimental M-BACI approach was carried out between November 18, 2009 and April 13, 2010, with the simulation of a single acute impact (January 29, 2010) using pre-established temporal scales presented in Table 1. The Multivariate Before and After/Control and Impact (M-BACI) model is a sampling strategy appropriate for analyses over planned impacts (Keough and Mapstone, 1997; Underwood, 2000; Downes et al., 2004).

Three impacted treatments were contrasted with three controls over 14 successive sampling times, seven before and seven after the spill (Table 1). Spatial and temporal sampling were replicated to allow for a proper interpretation of the interactions between treatments (impacted and control) and sampling times (before and after) making the inferences more trustworthy (Underwood, 2000).

The contaminant used in the experiment was the marine diesel oil donated by TRANSPETRO S.A. This fuel is destined to small, mid size ships, and to auxiliary engines in large carriers. The experimental spill was performed in three unvegetated tidal flats located between the mouths of the Maciel and Guaraguaçu rivers: Area 1 (25°33′031″S and 48°25′074″W); Area 2 (25°32′706″S and 48°25′826″W); and Area 3 (25°32′552″S and 48°27′143″W) (Fig. 1).

Impacted treatments with diesel oil spill and the corresponding control treatments were established in each tidal area, 40 m apart and positioned at similar tidal levels, parallel to the coastline. Twelve squares of 1 m^2 arranged in rows and with centralized sampling areas of 0.35×0.35 m were defined for each treatment; walking circulation areas were also pre-established to avoid trampling during sampling (Fig. 1).

The experimental spill was done during the low tide when the unvegetated tidal flats were emersed, thus optimizing the time for the oil to percolate into the sediment. Around 2500 ml of diesel were dumped in each centralized sampling area. Immediately after the discharge, the oil was contained by wooden square artifacts properly allocated to prevent the dispersion of the product and cross-contamination of the control treatments. The amount of oil tested was defined based on pilot experiments to identify threshold effects on the benthic fauna from increasing oil volumes. In lesser oil volumes, no massive mortality rates or loss of vital signs, as indicated by active locomotion in the case of polychaetes and crustaceans and ciliary activity in the mantle of gastropods or bivalves, were observed.

2.3. Faunal sampling and processing

Three sampling units were randomly taken from each treatment (impacted and control), in the three unvegetated tidal flats on each of 14 sampling days (7 before and 7 after the oil spill), using a corer of 10 cm in diameter and 8 cm in length. The samples were sorted in the laboratory through 500 μm mesh sieves. The retained material was fixed in 4% formaldehyde for 48 h and subsequently preserved in 70% alcohol. The benthic macrofauna was counted and identified to the lowest taxonomic level (usually to species).

The samples collected on the first day after the impact were taken to the laboratory and analyzed prior to fixation to assess the immediate (acute) responses of organisms to oil. Organisms were classified as alive and dead and quantified with the aid of a stereoscopic microscope. Alive organisms usually showed active mobility and responses to mechanical or light stimuli.

2.4. Sampling of environmental variables

Sediment samples were collected in each treatment for particle size analysis on the first and last day of the experiment (November 18, 2009 and April 13, 2010, respectively). Additional sediment samples were collected in each treatment to determine the