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DECAPAGE PROJECT: HYDROCARBON DEGRADATION IN COASTAL SEDIMENTS*

Oil spill effects on macrofaunal communities and bioturbation of pristine marine sediments (Caleta Valdés, Patagonia, Argentina): experimental evidence of low resistance capacities of benthic systems without history of pollution

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Abstract The Patagonian coast is characterized by the existence of pristine ecosystems which may be particularly sensitive to oil contamination. In this study, a simulated oil spill at acute and chronic input levels was carried out to assess the effects of contamination on the macrobenthic community structure and the bioturbation activity of sediments sampled in Caleta Valdés creek. Superficial sediments were either noncontaminated or contaminated by Escalante crude oil and incubated in the laboratory for 30 days. Oil contamination induced adverse effects on macrobenthic community at both concentrations with, for the highest concentration, a marked decrease of approximately 40 and 55 % of density and specific richness, respectively. Besides the disappearance of sensitive species, some other species like Oligochaeta sp. 1, *Paranebalia* sp., and Ostracoda sp. 2 species have a higher resistance to oil contamination. Sediment reworking activity was also affected by oil addition. At the highest level of contamination, nearly no activity was observed due to the high mortality of macroorganisms. The results strongly suggest that an oil spill in this protected marine area with no previous history of contamination would have a deep impact on the non-adapted macrobenthic community.

Keywords Macrobenthic communities · Oil pollution · Bioturbation · Resistance capacities · Pristine marine sediments · Patagonia

Introduction

In quantitative terms, crude oil is the most important organic contaminant in the marine environment (Spormann and Widdel 2000; Head et al. 2006). Despite the implementation of active and preventative policies, inputs are still increasing due to the rise of the world consumption and transportation, mainly via maritime routes, of petroleum products (Dargay and Gately 2010). Oil hydrocarbons are either chronically or

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accidentally introduced in many marine areas around the world. They can affect coastal and marine ecosystems in several ways. For instance, toxic effects of some hydrocarbons, particularly polycyclic aromatic hydrocarbons, on feeding, growth, development, and reproduction cascade across trophic levels affecting plankton, fish, marine birds and mammals, as well as benthic organisms (Fleeger et al. 2003; NRC 2003; Wiese and Ryan 2003; Alonso-Alvarez et al. 2007; González et al. 2009; Engraff et al. 2011; Almeda et al. 2013; Bellas et al. 2013; Lee and Lin 2013). The deposition of hydrocarbons or oil/weathered oil at the sediment-water interface can considerably alter the biogeochemistry of benthic systems and impact inhabiting organisms that maintain a close relationship with these substrates (Rivero et al. 2005; Massara Paletto et al. 2008). In particular, reduced diversity, retrogression to opportunistic species, and reduced size of individuals have been reported for macrobenthic organisms in contaminated sediments (Lee and Page 1997; Peso-Aguiar et al. 2000; Blanchard et al. 2002; Belan 2003; Je et al. 2003; Lu 2005; Gomez Gesteira and Dauvin 2005; Lu and Wu 2006; Di Leonardo et al. 2007; Venturini et al. 2008; Gonzalez Egres et al. 2012; Yu et al. 2013; Seo et al. 2014; Zabbey and Uyi 2014). Yet, soft-bottom macrobenthic organisms play a key role in processes such as organic matter degradation and nutrient cycling, dissolved oxygen diffusion, dispersion and burial of sediment particles, and secondary production (Michaud et al. 2006; Gilbert et al. 2003). This is mainly due to excavation or ingestion of mud and particulate organic matter (Rhoads et al. 1978; Aller 1982; Kristensen et al. 2012). Bioturbation was recently redefined by Kristensen et al. (2012) as the displacement of particles (sediment reworking) and solutes (ventilation) due to the infauna activity. These bioturbating organisms, through the construction of tunnels and canals, particularly alter the flow of dissolved oxygen within marine sediments (Pischedda et al. 2008; Pischedda et al. 2012) and thereby positively influence microbial activities (Arndt et al. 2013). This subsequently affects significantly early diagenesis and, more particularly, biodegradation rates and pathways of organic pollutants (Gilbert et al. 1996; Christensen et al. 2002; Banta and Andersen 2003; Granberg et al. 2005; Mermillod-Blondin and Rosenberg 2006; Cuny et al. 2007; Miralles et al. 2007; Cuny et al. 2011; Stauffert et al. 2013). Conversely, bioturbation may be responsible for a large release of contaminant from the sediment into the water column (Thibodeaux and Bierman 2003; Cuny et al. 2011).

In case of contamination, the effects of oil on macrobenthic communities may thus in turn strongly affect the natural attenuation and, overall, the resistance and resilience capacities of the benthic system. Sediments chronically contaminated generally exhibit tolerant or opportunistic species populations, usually polychaetes worms, which can resist to relatively elevated concentrations of hydrocarbons (Gilbert et al. 1996; Peso-Aguiar et al. 2000; Grossi et al. 2002; Dauvin and Ruellet 2007; Miralles et al. 2007; Seo et al. 2014; Gilbert et al. 2015). In contrast, pristine benthic communities without historical exposure to hydrocarbons seem to be particularly sensitive to contamination (Carman et al. 2000). To date, the literature related to oil effects on pristine areas is still scarce, probably because more and more areas around the world are exposed to chronic or massive hydrocarbon spillages. Yet, in this kind of oil-sensitive marine area, a single large spill of crude oil or petroleum products may produce environmental damages that can amount to billions of dollars (Dalton and Jin 2010).

Human activities in coastal areas involve port and industrial activities and maritime transport. Patagonian coast extends over more than 3000 km between 40° and 55° S and oil production is one of the most important economic activities in the region. There are two important oil basins named San Jorge and Austral basins, which produce petroleum in the order of 17.8 million m^3 per year, constituting nearly 50 % of the Argentina total annual production (Nievas and Esteves 2007). The main risk of Patagonian marine ecosystems contamination comes from crude oil loading in buoys and ports and maritime transport from exploitation sites in the south to the refineries in the north of the Country. Although hydrocarbon pollution was found in some punctual zones of the Patagonian coastal sediments (Comendatore et al. 2000; Esteves et al. 2006; Commendatore and Esteves 2007; Commendatore et al. 2012), certain areas such as Caleta Valdés (CV; east of Península Valdés; Chubut province) have been never affected by massive or chronic pollution and remain pristine (unpublished data, Commendatore and Esteves). This ecosystem, established as Humanity Mundial Patrimony (UNESCO 1999) because it is a sensitive zone that constitutes a valuable environment for the reproduction and feeding of birds and marine mammalians, offers a relatively unique opportunity of assessing the response of the macrobenthic communities in case of petroleum contamination.

The objective of this work was to experimentally ex situ study the effects of a simulated oil spill at acute and chronic concentration levels on the macrobenthic community structure and the bioturbation activity of sediments sampled in a pristine site located in Caleta Valdés. Pollution-induced changes in macrobenthic community with a special interest for the determination of indicator species, as well as changes of the reworking activity, were assessed after 30 days of incubation.

Materials and methods

Study area

Caleta Valdés (CV) is a north-south oriented creek located at the eastern side of the Peninsula Valdés and connected to the Atlantic Ocean by a southern mouth. The sampling site for the experimentation was chosen in the muddy north continental zone of CV (42°15′53″ S, 63°40′50″ W) because of the absence of anthropogenic hydrocarbon pollution and it macrofauna richness (Fig. 1).

Bioturbation experimental assay conditions

Sampling

Sediment samples were collected by hand in April 2012 using 10 cm diameter and 25 cm length tubes, in agreement with previous works on bioturbation and reworking studies (Timmermann et al. 2002; Quintana et al. 2007; Hedman et al. 2011). Twelve PVC corers were vertically introduced down to 20 cm sediment depth approximately. Then, they were carefully withdrawn and carried immediately to the laboratory avoiding disturbing the vertical structure of the sediments. Surficial sediment (first centimeter; 4 kg) was also collected for the laboratory experimentation. In addition, 20 L of seawater were also collected in plastic containers.

Laboratory experimental design and incubation conditions

With the aim to assess the oil impact on macrobenthic community structure and activity, three experimental conditions were set up: a control condition without oil addition (E0; 4 sediment cores), a low hydrocarbon concentration condition (E1; 4 sediment cores), and a high hydrocarbon concentration condition (E2; 4 sediment cores). These conditions were carried out by depositing at the surface of the respective cores, a layer of surficial CV sediments (height: 1 cm) either noncontaminated (E0), or contaminated by Escalante crude oil (Chubut) at final concentrations of 1000 ppm (1 g/kg; E1) and 20000 ppm (20 g/kg; E2). The different amounts of added oil were defined to simulate chronic and acute polluted sites, respectively (UNEP/IOC 1992). Then, the three groups of four cores were respectively distributed in three individual 56.1 L tanks. Tanks were filled with seawater, previously collected in CV, until cores were covered. They were then kept aerated (air bubbling) and monitored periodically during experimentation. In order to assess biological reworking activity, in each sediment core, 2 g of luminophores (63–355 μm particulate inert tracers, Duport et al. 2007) were homogeneously spread at the sediment surface at two different experimental times: green particles at initial time (*t*=0 days) and pink particles 15 days after the start of the experiment (*t*=15 days). Luminophores size was selected according to the sediment granulometry. Cores were incubated statically for 30 days at ambient temperature, after which they were manually collected and sliced in order to provide 0.5 cm thick sediment layers from the surface to 2 cm depth and 1 cm thick layers from 2 to 18 cm. Each sediment slice was then separated in four equal parts that were randomly distributed to perform the different further analysis.

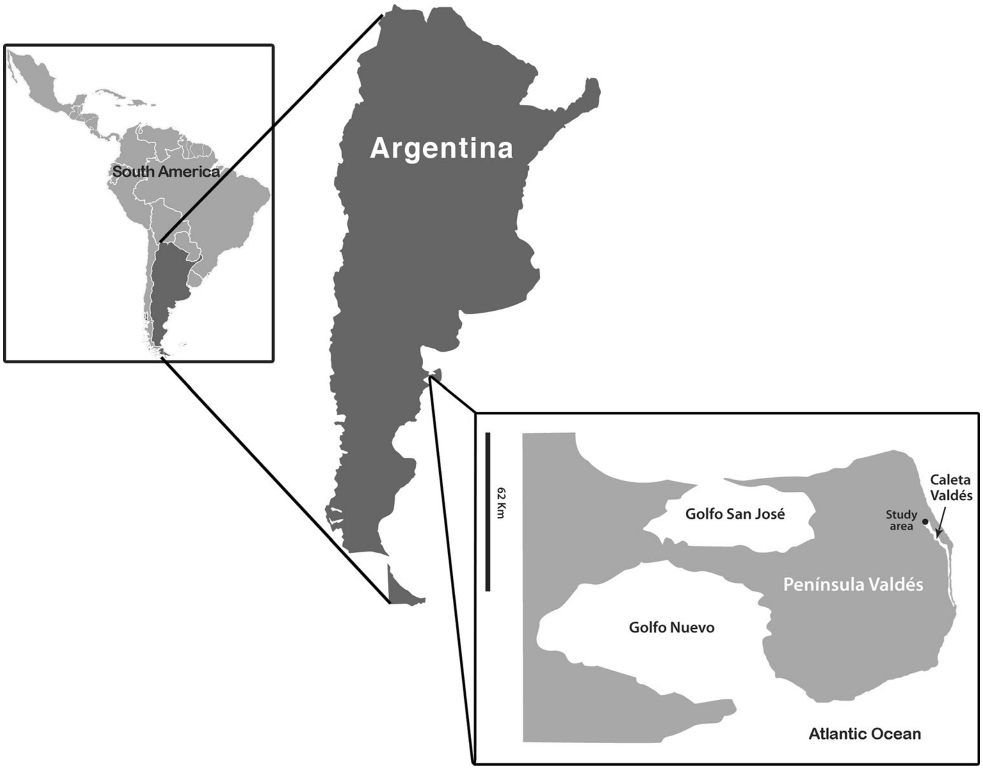

Fig. 1 Sampling site in Caleta Valdés (CV; Península de Valdés, Patagonia Argentina)

Variable assessment

Temperature, dissolved oxygen, and pH were assesses periodically in the microcosm sea water during the experiments using a multiparameter probe YSI-556.

Analytical procedures

Macrobenthic communities

The 75 % of each layer (i.e., three parts) obtained from of each corer was fixed with 4 % formaldehyde to perform macrobenthic community analysis, storing the one resting parts at −20 °C for chemical analysis. The fixed samples were first sieved with a 500 μm mesh to retain macroorganisms and then with a 44 μm mesh to collect the sediment fraction containing the luminophores particles. The sediment retained on the 500 μm mesh was preserved in alcohol 70 % for the counting and sorting of the organisms. The macrofauna was identified to the major taxonomic level possible with stereoscopic and optic microscopes using reference keys (Hartman 1968; 1969; Banse and Hobson 1974; Blake and Ruff 2007; Orensanz et al. inedited, among others). Whole organisms and anterior fragments of each taxon were only registered. In order to provide more information that could support further studies, some classification parameters usually used in taxonomic identification and photographs of the three major undetermined species or group of species are presented in the Online Resource 1.

Bioturbation

The vertical luminophores profile of each sediment column at final time of experimentation (30 days) was obtained from the percentages of the luminophores found in each layer with respect to the total amount in the core. The sediment retained on the 44 μm mesh was homogenized and subsampled to quantify luminophores using a microplate reader (Biotek Synergy Mx) at λex/λem: 565/602 and 460/500 nm for the green and pink luminophores, respectively. According to the respective time of deposition of the tracers at the surface of the cores, data from green particles reflected the reworking activity from the whole experimentation period (30 days), while pink particles indicate activity during the last 15 days.

The quantification of sediment reworking was evaluated from the distributions of luminophores by the gallery-diffusor model (François et al. 2002). This model allows to describe both the biodiffusion-like transport (D_b coefficient) due to the continuous displacement of the tracers and the non-local advective displacement of the tracers (*r* coefficient). The best fit between observed and modeled tracer distribution with depth (i.e., producing the best *Db* and *r* coefficients couple) was estimated by the least squares method (Gilbert et al. 2007).

Statistical data analysis

Data analyses were performed by corer using the software package PRIMER 5.0 (Plymouth Marine Laboratory, UK). Specific richness (*S*), total abundance (expressed as density=number of individuals/m^2), the Shannon diversity index (*H*′), and Pielou's evenness index (*J*′) were calculated for each treatment. Differences in each biological variable were evaluated through one-way ANOVA considering conditions as fixed factors (n=12) (Statistica, version 7). The Tukey test for multiple mean comparisons was used to determine statistically significant differences between conditions.

The ordination and sorting using the total abundance data was carried out with a non-metric multidimensional scaling (nMDS) and CLUSTER analysis (Bray-Curtis index; group average link; square root), respectively. The differences between the macrobenthic communities composition were tested by ANOSIM (Similitude analysis) while SIMPER (Similitude Percentage) was applied to determine the species that most contributed to the observed differences (PRIMER 5.0 program).

Finally, the dominant and subdominant species per treatment were calculated according to Picard (1965). The species were considered dominant or subdominant when the mean dominance index was maximum or ≥5 %, respectively.

A comparison of the biodiffusion (D_b) and bioadvection (*r*) coefficients was performed to assess differences in the sediment transport along the sediment column between the experimental conditions by means of the non parametric Kruskal-Wallis one-way analysis of variance by ranks (Kruskal and Wallis 1952). When significant, it was followed by a Mann-Whitney pairwise comparison test to evaluate the significance of the differences between conditions after a Bonferroni adjustment for multiple comparisons (Siegel and Castellan 1988).

Results

Physico-chemical parameters

The seawater temperature, dissolved oxygen, and pH showed little changes throughout the incubation with mean values of 17.7±0.6 °C, 7.9±0.4 mg/L, and 7.8±0.3, respectively (n=7) for all the experimental conditions. There were no statistically significant differences of means found between conditions (E0, E1, E2) for these three variables.

Macrobenthic communities

Experiments with two levels of oil addition affected macrobenthic community structure in comparison with the control experiment, being these changes mainly evidenced in density and specific richness (Fig. 2a, b, Online Resource 2). The contamination caused a progressive reduction in both parameters with the added oil amount showing a negative tendency. Particularly, the addition of 20 g/kg of oil (E2) resulted in a decrease of approximately 40 and 55 % of density and specific richness, respectively. On the other hand, diversity and evenness showed little changes being diversity only significantly lower for E2 condition in comparison with E1 treatment. The low level contamination condition (E1) did not differ from control for these two parameters (Fig. 2c, d, Online Resource 2).

The density, specific richness, diversity, and evenness profiles of organisms vs. depth layers are shown in Fig. 3. In a general way, the maximum values of these parameters were found in the first 2 cm, and then they gradually decreased with depth. Density was mainly affected by oil addition in the superficial layer of the sediments (0–0.5 cm) where a decrease of approximately 54 and 78 % was observed for the lowly (E1) and highly (E2) contaminated cores, respectively. Specific richness, diversity, and evenness markedly decreased with depth for the highly contaminated cores compared to controls and lowly contaminated cores.

The nMDS and CLUSTER analysis showed that the E1 samples were close to the E0 samples with a 72 % of similarity while the E2 samples, which were located on the opposite side of the diagram, presented a 58 % of similarity with E0 and E1 (Fig. 4, Online Resource 3). The nMDS representation was satisfactory as attested by the low stress value (0.11).

The ANOSIM showed highly significant differences among conditions with a global R of 0.775 ($p<0.02$). The SIMPER results showed which species contributed in greater extent to the differences observed. Higher proportions of Ostracoda sp. 1, Oligochaeta sp. 1, Pseudocumatidae sp. 1, *Leuroleberis poulseni* and Cirolanidae sp. 1 were observed in noncontaminated sediments. Low and high contaminated sediments were characterized by the presence of Ostracoda sp. 2, *Phoxocephalopsis* sp., *Ceratocephale* sp., *Anacalliax argentinensis* and Gammaridae sp. 2, and *Paranebalia* sp., respectively (Table 1).

Lastly, a list of the dominant and subdominant species found in the oil pollution simulation experiments is given in Table 2. Ostracoda sp. 1 was the dominant species in the E0 (uncontaminated) and E2 (highly contaminated) conditions. This species was the most subdominant species in the E1 experience where Ostracoda sp. 2 predominated over there. Finally, Oligochaeta sp. 1 was the most subdominant species in the E2 experience.

Sediment reworking: luminophores vertical distribution

The vertical distribution of the luminophores showed an exponential decrease of the tracers with depth for the three experimental conditions (Fig. 5). Maximal penetration depth for the luminophores was of 14 cm for all conditions (data not shown) with more than 96 % of tracers located in the first 5 cm (Fig. 5). After 30 days of incubation, nearly all the green luminophores remained at surface (98 %) for the highly contaminated cores (E2) while for low contaminated (E1) and uncontaminated (E0) only 54 and 43 % of the initially deposited luminophores were detected in the deposit zone, respectively (Fig. 5a). The effects of oil addition were even more marked during the last 15 days

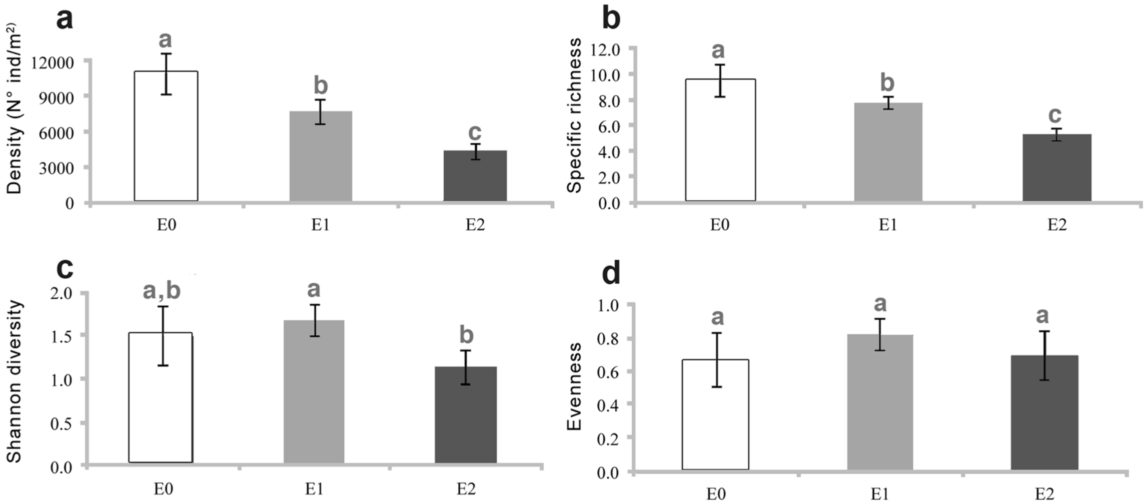

Fig. 2 Global community parameters measured for each experimental condition (mean values for the entire cores±standard deviation; $n=4$). *E0* control (no contamination); *E1* low oil contamination (1 g/kg); *E2* high oil contamination (20 g/kg). *Lowercase letters* indicate significant differences between experimental conditions

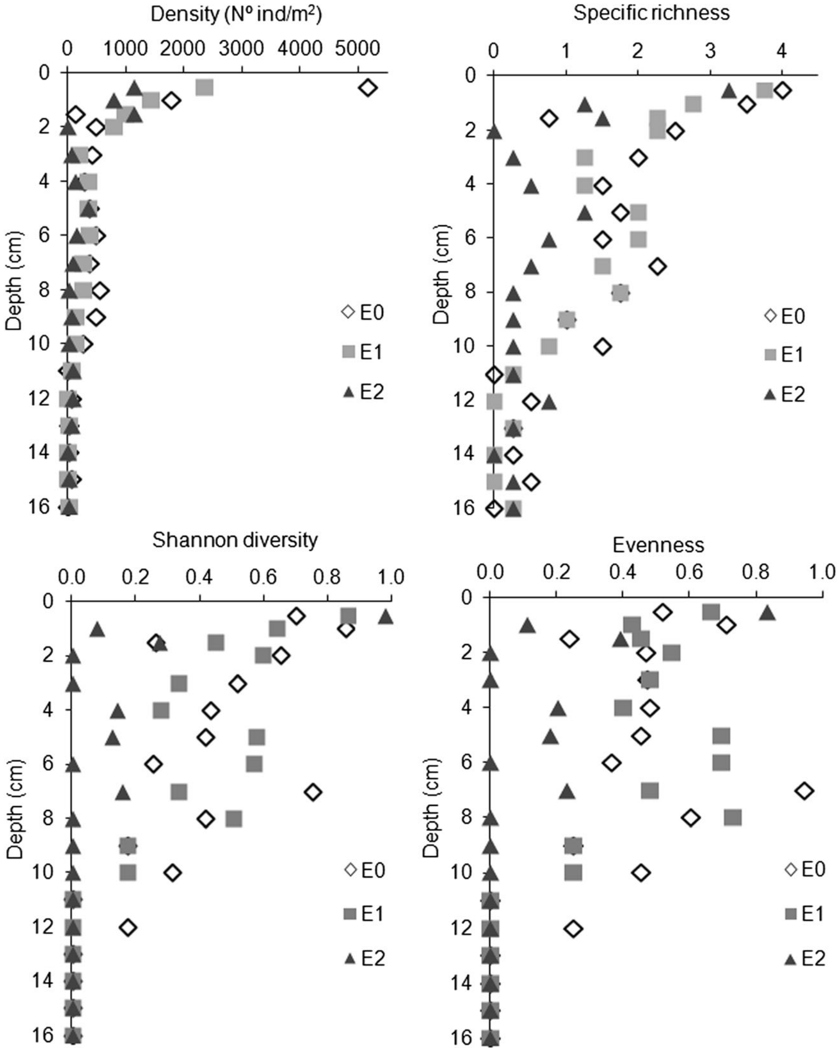

Fig. 3 Community parameters measured at each depth layer for each condition (mean values; $n=4$). *E0* control (no contamination); *E1* low oil contamination (1 g/kg); *E2* high oil contamination (20 g/kg)

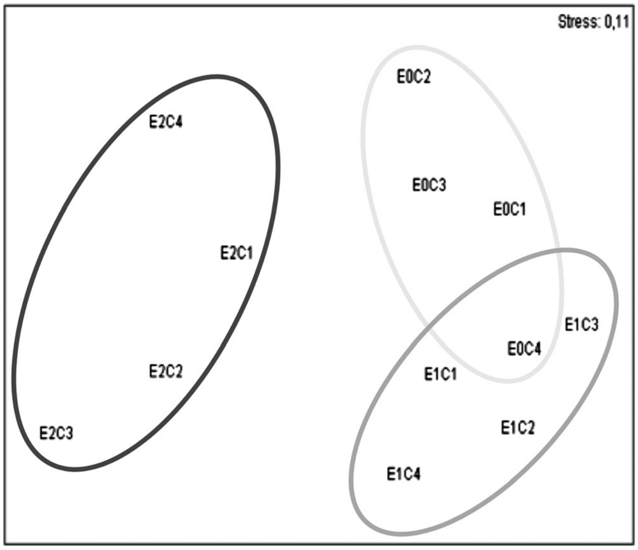

Fig. 4 Non-metric multidimensional scaling diagram (nMDS) for each condition (transformation: square root). *E0* control (no contamination); *E1* low oil contamination (1 g/kg); *E2* high oil contamination (20 g/kg)

Table 1 Similarity percentage analysis (SIMPER) for oil pollution simulation experiments

Species	Mean abundance		
	E0	E1	E2
Ostracoda sp. 1	44.50	16.75	15.25
Oligochaeta sp. 1	14.25	5.50	12.00
Axiothella sp.	10.25	10.25	0.75
Ostracoda sp. 2	5.25	18.75	3.50
Phoxocephalopsis sp.	4.00	4.25	0.00
Pseudocumatidae sp.	3.50	0.75	0.75
Paranebalia sp.	1.00	0.25	1.25
Leuroleberis poulseni	1.00	0.50	0.00
Cirolanidae sp. 1	0.75	0.25	0.00
Ceratocephale sp.	0.50	0.75	0.25
Anacalliax argentinensis	0.25	1.25	0.00
Gammaridae sp. 2	0.25	1.50	0.00

E0 control (no contamination), *E1* low oil contamination (1 g/kg), *E2* high oil contamination (20 g/kg)

Table 2 Dominant and subdominant species in oil pollution simulation experiments

E0			E1			E2		
Species	*P*	*D*	Species	*P*	*D*	Species	*P*	*D*
Ostracoda sp. 1	1112.50	51.74	Ostracoda sp. 2	468.75	30.86	Ostracoda sp. 1	381.25	44.85
Oligochaeta sp. 1	356.25	16.57	Ostracoda sp. 1	418.75	27.57	Oligochaeta sp. 1	300.00	35.29
Axiothella sp.	256.25	11.92	Axiothella sp.	256.25	16.87	Ostracoda sp. 2	87.50	10.29
Ostracoda sp. 2	131.25	6.10	Oligochaeta sp. 1	137.50	9.05			
			Phoxocephalopsis sp.	106.25	7.00			

E0 control (no contamination), *E1* low oil contamination (1 g/kg), *E2* high oil contamination (20 g/kg). *P* mean density, *D* mean dominance index

of the experiments (Fig. 5b). Indeed, the percentages of buried pink luminophores were only 26 and 1 % for E1 and E2, respectively, while 47 % were moved down from the surface deposition layer for the controls.

Quantification of sediment reworking showed significant differences in the biodiffusion coefficient (D_b) between the control and the highly contaminated condition ($p<0.05$) for both the full experience time (30 days) and the last 15 days in comparison with controls (Fig. 6a, b, Online Resource 2). The same pattern was found with the bioadvection coefficient (*r*) (Fig. 6c, d, Online Resource 2). Both coefficients indicated a decrease of sediment reworking intensity with oil concentration increase with almost null values for the highly contaminated sediments. Indeed, in the presence of the high contamination (E2), the 0.1 values given by the model for both *Db* and *r* are the lowest limit values of the model (i.e., they vary from 0 to 0.1).

Discussion

Caleta Valdés is a pristine zone in the Patagonian coast having no oil contamination history. Simulated ex situ oil spills were conducted to study the impact of oil on the structure and activity of the macrobenthic community at two levels of contamination, low and high, simulating a chronic pollution and an acute oil spill situation, respectively. Throughout the whole experimentation, water temperature, dissolved oxygen, and pH within experimental tanks were stable for all the incubation conditions, suggesting that these were not discriminating parameters.

Resistance of macrobenthic community to oil contamination

The results clearly demonstrate the low resistance capacities to oil contamination of the macrobenthic communities of Caleta Valdés sediments. Castège et al. (in press) studied benthic community from Guéthary (Bay of Biscay, France), affected by the Prestige oil spill in 2002. These authors concluded that 5 years after the spill, the macrobenthic community still showed perturbation signs, such as proliferation of grazers, highlighting the complexity and slowness of the recovery process. The severity of oil impacts on the benthic macrofauna depends on many factors, most notably (1) oil amount; (2) chemical composition; (3) form (weathered or not, emulsified

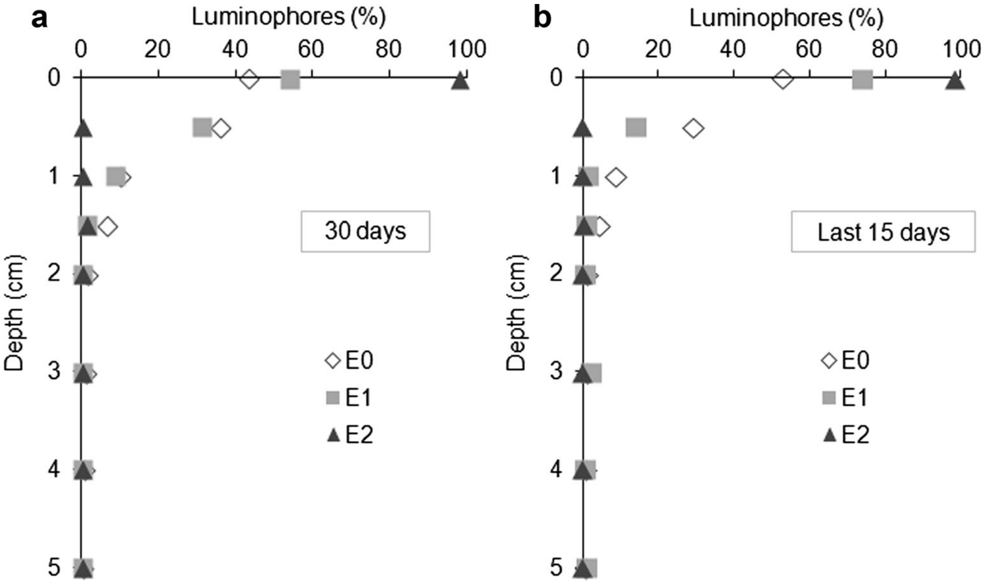

Fig. 5 Vertical profiles of luminophores distribution after 30 days of experimentation and for the last 15 days. *E0* control (no contamination); *E1* low oil contamination (1 g/kg); *E2* high oil contamination (20 g/kg)

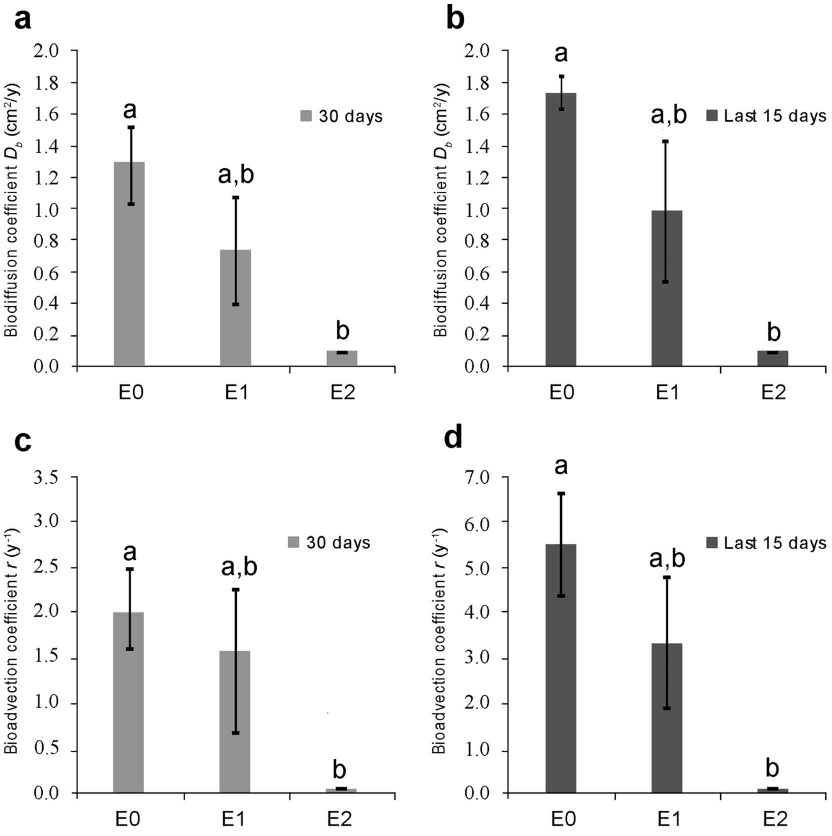

Fig. 6 Sediment reworking biodiffusion (D_b) and bioadvection (r) coefficients after 30 days of incubation and for the last 15 days. *E0* control (no contamination); *E1* low oil contamination (1 g/kg); *E2* high oil contamination (20 g/kg). Note that, in presence of the high contamination (E2), the 0.1 values given by the model for both *Db* and *r* are the lowest limit values of the model (i.e., they vary from 0 to 0.1). *Lowercase letters* indicate significant differences between experimental conditions

or not, type of emulsion); (4) occurrence (i.e. solubilized, in suspension, dispersed, or adsorbed on suspended particulate matter); (5) exposure time; (6) life stage (juvenile or adult forms); (7) season; (8) natural environmental stress associated with fluctuations in temperature, salinity, and other variables; and (9) type of affected environment (Kennish 1997). According to Puente et al. (2009), fine-grained substrates characterized by low permeability and water-saturated sediments, such as the one used in this study, substantially prevents depth penetration of oil. Indeed, the high surface/volume ratio of the sedimentary matrix grains facilitates oil absorption and retention in the superficial layers preventing oil penetration to deep layers. This phenomenon was probably reinforced by our experimental setup, i.e., no simulation of sediment re-suspension due to tidal currents. Such conditions may explain why the density of macrobenthic organisms was mainly affected in the most superficial layer of contaminated sediment (i.e., where the oil was applied). At the same time, no deeper burial of the organisms in order to avoid contamination was detected (Bolam 2011), suggesting a direct toxic impact of the oil compounds on the organisms. According to Baker (1970), low molecular weight compounds present in oil can dissolve the membranes and alter the biota respiration mechanisms. Particularly, the oil water-accommodated fraction (WAF) containing the most polar and low molecular weight compounds from oil, mainly aromatic hydrocarbons (PAHs), have been extensively described to be toxic. For example, LC_{50} on benthic organisms of oil WAF were found to be as low as 1.2–3 ppm of total aromatic hydrocarbon for some amphipods, shrimps, crabs, and snails (Holdway 2002), while Echols et al. (2015) reported >20 % mysid mortality for Gulf of Mexico crude oil WAF in a concentration range of 0.015–0.89 ppm of total PAHs. Escalante crude oil used in our experiments is classified as medium petroleum having low molecular weight compounds aromatic hydrocarbons. A concentration of 1.38 ppm of total BTEXs and 0.23 ppm of total PAHs from naphthalene up to traces of four aromatic ring compounds like pyrene were measured in the Escalante oil WAF in a 90:1 (seawater:oil) ratio volume mixture (Marino et al. 2012). Thus, a top-down gradient of these compounds in the water phase of the oiled experimental cores may be expected due to diffusion processes. This fact may also explain the mortality found in the top layer, in addition to the direct contact of organisms with the organic oil phase.

Regarding oil effect on the structure of macrobenthic community, both the density and specific richness of organisms decreased with regard to the uncontaminated controls with a progressive impact with the increasing oil contamination. Harmful effects of crude oil on different macrobenthic communities have been previously reported. For instance, Hartwick et al. (1982) carried out field and laboratory studies about the effect of Alberta crude oil on behavior and survival of natural littleneck clam (*Protothaca staminea*) populations from uncontaminated sites during 231 and 10 days, respectively. In both studies, superficial sediments were contaminated with different oil concentrations and no mortality was found through the experimental period when the clams were treated with crude oil at 1000 ppm, the same concentration used in our study for the low concentration experiment (E1). Nevertheless, siphon activities were impaired at this contamination level; this functional inhibitory effect would probably render the animals more vulnerable to natural mortality factors (e.g., predation) in their habitat. In another study, carried out in Singapore coasts chronically contaminated by discharges from industry, sewage, and shipping, a significantly negative correlation between the specific richness, abundance, and diversity of macrobenthic organisms and total petroleum hydrocarbons was found (for oil added at concentration>800 ppm) (Lu 2005).

Response of the different macrobenthic species to oil contamination

Benthic organisms with little or no mobility have been widely used in marine environmental impact assessment and monitoring. As a matter of fact, they are sensitive sensors of physical and chemical changes undergone by benthic systems (e. g., 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). Because they are closely associated with the seafloor they can hardly avoid deteriorated conditions of water and/or sediment quality. Furthermore, they have relatively long life cycles and exhibit different stress tolerance (Bilyard 1987; Dauer 1993). According to their response to stress, species can be divided into “sensitive species” (i.e., able to only survive within a narrow range of environment conditions and disappear from polluted areas); “tolerant species” (i.e., being not sensitive to a particular stress and/or pollution); “opportunistic species” (i.e., able to quickly exploit new recourses or ecological niches as they become available, characterized by early reproduction, high reproduction rates, rapid development, small body size, and an uncertain adult survival rate), and “indifferent species” (without real affinity for any particular community and showing no response to pollution) (Dauvin et al. 2010). In Caleta Valdés sediments and with regard to our experimental oil contamination, the three first types of organisms were found. Opportunistic species could be represented by Oligochaeta sp. 1 that reached high densities under the highly contaminated conditions (E2). Different opportunistic species of oligochaetes have been shown to be favored by the organic enrichment of sediments due to discharge of sewage outfall (Gamito 2008), untreated abattoir wastes (Arimoro et al. 2007) and livestock effluents (Solimi et al. 2000). These substrates are mostly covered by bacteria and sewage fungi, which are the main food source for the most part of oligochaetes (Rueda et al. 2003). Species such as *Limnodrilus hoffmeisteri* and *Tubifex tubifex* have been used as indicators of this kind of environment (Brinkhurst and Jamieson 1971; Aston 1973; Miserendino and Pizzolon 2000). Particularly, Gonzalez Egress et al. (2012) found a marked decrease in the density of oligochaetes shortly after the in situ diesel oil spill impact but a recovery trend with a gradual increase from day 2, behaving as tolerant or resilient species to the oil impact (Gomez Gesteira and Dauvin 2000; Ocon et al. 2008). Also, *Paranebalia* sp. (Crustacea: Phyllocarida: Leptostraca) was dominant in the most contaminated sediments 30 days after the oil addition and seems to be a tolerant species. Even if it was known as a primarily tropical-subtropical genus, it was recorded for the first time in the northern Patagonia during the years 2003 to 2007 on sediments and among holdfast of *Macrocystis pyrifera* and *Undaria pinnatifida* (Roccatagliata et al. 2010). Leptostracans are known to occur in a variety of habitats, from hydrothermal vents and marine caves to the intertidal zone showing a wide resistance to adverse environmental conditions (Haney and Martin 2004). Under mild contaminated conditions (E1) Ostracoda sp. 2 was the dominant species at the end of the incubation. Yet, previous field and laboratory studies demonstrated that ostracods seem particularly sensitive to oil (Carman et al. 2000; Ruiz et al. 2005). Nevertheless, after a high mortality event due to the introduction of oil, it has also been observed a high recovery capacity of ostracods communities characterized by the development of high densities, 2 days after the contamination (Gonzalez Egress et al. 2012), or by changes in the trophic structure of the community (Carman et al. 2000). Clearly, the response of the different species of ostracods may vary. Indeed, in the present study, Ostracoda sp. 1 predominated both under uncontaminated and highly contaminated conditions but its abundance decreased with the oil addition. On the other side, some taxa known for their sensibility to contamination such as *Phoxocephalopsis* sp. (Gomez Gesteira and Dauvin 2000) also disappeared when superficial sediments were highly contaminated (E2). The present study constitutes the first characterization of the macrobenthic communities of Caleta Valdés sediments both subject or not to oil contamination. This perturbation led to the selection or disappearance of particular organisms which may serve as indicator species for this kind of environment in case of oil pollution. However, although our results do provide a first indication about potential oil pollution indicators, further studies are needed in order to identify the macrobenthic organisms to species level (e.g., ostracods) but also characterize their functional traits. This information is so far clearly missing for the Patagonian coastal zone where exploitation and sea transportation of oil are rapidly expanding.

Role of the benthic organisms in the polluted sediments natural attenuation

Benthic biota has a marked role in microbial aerobic biodegradation of hydrocarbons in marine sediments (Cuny et al. 2011). Indeed, bioturbation processes such as burrow construction and ventilation carried out mainly by macrofauna are key process to stimulate the availability of oxygen in superficial sediments (Timmermann et al. 2008). In addition, some macrobenthic organisms can affect positively the biodegradation of hydrocarbons through the production of digestive compounds acting like surfactants that modify their dispersion state and improve their bioavailability (Penry and Weston 1998; Weston and Mayer 1998; Gilbert et al. 2001) for hydrocarbon-degrading bacteria (Bertrand et al. 1993; Bonin and Bertrand 1999; Cuny et al. 2007; Cuny et al. 2011). Therefore, in sediments strongly affected by oil spills without active macrobenthic fauna, the microbial bioremediation of hydrocarbons would be markedly reduced (Timmermann et al. 2011).

In the frame of this study, oil affected the structure but also the activity of the Caleta Valdés macrobenthic community. Indeed, the quantification of the sediment reworking clearly showed a negative impact of oil contamination on the transport and mixing of particles by organisms. This impact was highlighted in highly contaminated conditions by the almost null values of the biodiffusion (D_b) and bioadvection (r) coefficients calculated for the full experience and for the last 15 days of incubation. These facts evidenced a general reduction of sediment reworking for both mixing modes with oil addition. This suggests that the activity of the community was inhibited throughout the sedimentary column, even in deeper sediments where community parameters were not strongly affected by oil. Similar observations have been reported for laboratory experiments where *Nereis diversicolor* reworking was found to be reduced in the presence of Arabian Light crude oil (Gilbert et al. 1994). But this is not always the rule, as other authors have found that *Hediste diversicolor* reworking activity may be enhanced in case of oil contamination (Stauffert et al. 2013). These discrepancies underline the complexity of the response of the benthic system to oil contamination, which is controlled by several factors as mentioned above. Overall, the different responses to oil pollution will be given by the resistance capacity of the species according to their biological traits, physiological status, and stage of development.

Finally, the resistance capacity seems to be strongly determined by the different events of contamination that may have led to the selection of an adapted community. As highlighted by the weak resistance capacity to oil of the macrobenthic communities of Caleta Valdés, pristine environments without previous history of contamination would be probably strongly impacted in case of an oil spill (Carman et al. 2000).

In conclusion, this study allowed for the first time to experimentally evaluate the effects of oil exploited in Patagonia on the structure and activity of the benthic macrofauna from a pristine site located in Caleta Valdés. Macrobenthic community structure, and above all, sediment reworking activity were impacted even at the lowest contamination level tested (level corresponding to chronically contaminated sites). On the other hand, in a high level of contamination, similar to those recorded in intertidal areas after an oil spill, results demonstrated an almost complete inhibition of macroorganisms reworking activity. It further causes severe structural changes in the macrobenthic community due to high mortality rates. These results clearly demonstrate the weak resistance capacity of the macrobenthic communities from a pristine environment in case of oil contamination. They also highlight the need to carry out longer-term experiments in order to test the resilience capacities of this type of benthic system. A special attention to the shifts in macrobenthic species functional traits due to oil contamination should also be paid as they can markedly affect biogeochemical cycling with cascading effects on the ecological functioning of the whole ecosystem (Van Colen et al. 2012; Hale et al. 2014; Kristensen et al. 2014). This knowledge is fundamental in developing an efficient environmental management strategy against pollutions particularly for sensitive pristine marine protected areas such as Caleta Valdés.

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