Structure and activity of polychaete assemblages in oil-polluted sediment (Patagonia, Argentina)

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Abstract. One of the tools applied to determine the pollution and stress status of an environment is through macrobenthic bioindicators. This study aimed 1) to validate ex situ experimental conditions by comparing different physical-chemical and biological variables between in situ and ex situ treatments after 13 days; 2) to analyze, through an ex situ experiment, the effect produced by two concentrations of crude oil (1 g/kg and 20 g/kg) on the Caleta Sara polychaete assemblages at different depths of the sediment column (0-8 cm and 8-16 cm), and 3) to evaluate the application of ´taxonomic sufficiency´ concept on crude oil experiment data. The abundance, richness, diversity and bioturbation showed a significant decrease in oil-polluted sediment only at the upper level. *Capitella* (Capitellidae) and *Boccardia* (Spionidae) were the most abundant at the highest oil concentration. The results were similar at both taxonomic levels, indicating that it would be possible to assess the effect of crude oil on the assemblages even at the family level, especially under acute ecotoxicological conditions. However, the ratio of families to genera was 1:1 in all cases, except for Spionidae. Therefore, identification at the family level should only be considered for low-diversity polychaete assemblages. This study is the first to evaluate the assemblages of Caleta Sara under conditions modified by crude oil in the laboratory. Thus, natural conditions are simulated and any negative impact on the study site is avoided.

[Keywords: indicators, hydrocarbons, macrobenthos, reworking, pollution, anthropogenic impact]

Resumen. Estructura y actividad de los ensamblajes de poliquetos en sedimentos contaminados con petróleo (Patagonia, Argentina). Una de las herramientas aplicadas para determinar el estado de contaminación y estrés de un ambiente es a través de bioindicadores macrobentónicos. Los objetivos de este estudio fueron 1) validar condiciones experimentales ex situ comparando después de 13 días, diferentes variables fisicoquímicas y biológicas entre tratamientos in situ y ex situ; 2) analizar, a través de un experimento ex situ, el efecto producido por dos concentraciones de petróleo crudo (1 g/kg y 20 g/kg) sobre los ensamblajes de poliquetos de Caleta Sara a diferentes profundidades de la columna sedimentaria (0-8 cm y 8-16 cm), y 3) evaluar la aplicación del concepto de suficiencia taxonómica a los datos del experimento con petróleo crudo. La riqueza, la diversidad, la abundancia y la bioturbación disminuyeron significativamente en los sedimentos contaminados con petróleo solo en el nivel superior. *Capitella* (Capitellidae) y *Boccardia* (Spionidae) fueron los más abundantes en la concentración más alta de petróleo. Los resultados a ambos niveles taxonómicos fueron similares, indicando que se podría determinar el efecto del petróleo crudo a nivel de familia, sobre todo si las condiciones ecotoxicológicas son agudas. Sin embargo, la relación familias:géneros fue 1:1 en todos los casos, excepto para Spionidae. Por lo tanto, la identificación a nivel de familia debería ser considerada solo para ensamblajes de poliquetos poco diversos. Este estudio es el primero que evalúa en laboratorio los ensamblajes de poliquetos de Caleta Sara en condiciones modificadas por el petróleo crudo. De este modo, se simulan las condiciones naturales y se evita todo impacto negativo en el sitio de estudio.

[Palabras clave: indicadores, hidrocarburos, macrobentos, bioturbación, contaminación, impacto antropogénico]

Introduction

Argentinian Patagonia has ~3000 km of coastline with high marine biodiversity (Helbling and Helbling 2021). This led to the creation of protected areas such as the Interjurisdictional Marine Coastal Park Patagonia Austral in 2007. This coastal park was created to preserve biodiversity, make sustainable use of seashore resources, protect the landscape and the cultural and natural heritage and promote environmental research and monitoring, among other goals (Documental and legislative information 2007). Several socioeconomic activities have also been developed, such as fishing, tourism and oil extraction. Although these activities led to significant economic growth, their development without strict control represents a threat to the conservation of marine ecosystems. For example, on the Chubut coast, there was a low amount of crude oil spill in 1991 (Commendatore and Esteves 2007), while there was a spill of 100 m³ in Caleta Córdova in 2007 (Pucci et al. 2011). In particular, the loading of crude oil at buoys and ports in the south of the country and its subsequent transport by sea in tankers to refineries in the center and north of the country pose a constant threat of pollution to the Patagonian marine ecosystem (Sturla Lompré et al. 2018).

Hydrocarbons are the main component of crude oil. The anthropogenic introduction of hydrocarbons into marine ecosystems can result from large crude oil spills, as well as smaller discharges from fishing activities, urban and industrial effluents, bilge and ballast water from ships (Commendatore and Esteves 2007). In both cases, the effects on the habitat and biota are significant (Lindgren et al. 2014; Ferrando et al. 2015, 2019). Crude oil is considered problematic to organisms due to its toxicity, carcinogenicity and mutagenicity (Christensen et al. 2002) and can negatively affect different marine organisms in their diet, growth and reproduction at different trophic levels (Alonso-Alvarez et al. 2007; Almeda et al. 2013). Changes in the distribution and abundance (Warwick 1988; Muniz et al. 2013), as well as in the activity of benthic communities (Venturini et al. 2011), are usually considered as indicative of pollution.

Many studies have shown that benthic organisms are useful indicators of environmental status because they respond to variable organic loads and pollutant levels (Pearson and Rosenberg 1978; Dauvin et al. 2010 and references therein). Within these communities, there are 'sensitive' species that can only survive in a narrow range of environmental conditions, 'tolerant' species that can resist stress conditions and 'opportunistic' species that can exploit new resources under these conditions due to their characteristics (i.e., high reproductive rates, small body sizes and short life cycles) (Pearson and Rosenberg 1978; Dauvin et al. 2010; Campbell et al. 2019). At polluted sites, these communities produces the continuous mixing of the pollutants adsorbed on the particles from the deep layers of the sedimentary matrix to the surface and vice versa (Christensen et al. 2002). In addition, this activity affects microbial processes and biogeochemical reactions in particular by promoting the infiltration of oxygen into sediments (Kristensen et al. 2012). To quantify sediment redistribution by macrobenthic action and to obtain accurate measurements of bioturbation rates (Gerino et al. 1998), one of the methods used is based on the use of luminophores, which are inert sediment particles marked with fluorescent paint that act as burial and resuspension tracers (Gilbert et al. 1994). Within macrobenthic communities, polychaetes have been widely used as bioindicators in monitoring studies, especially in soft bottoms (Solis-Weiss et al. 2004; Elías et al. 2021). However, a common problem with the use of these organisms as bioindicators is how informative they can be. For example, the classification of these organisms to the species level requires specialists for each taxonomic group (Marrero et al. 2013). In addition, the excessive time required for identification (Kingston and Riddle 1989) increases the economic cost of biomonitoring. In this context, Ellis (1985) defined taxonomic sufficiency as the identification of taxa at the highest possible taxonomic level at which there is no loss of statistical significance in the assessment of pollution effects. This approach assumes that analysis at the specific level is ideal for assessing the effects of these impacts on ecosystems (Ellis 1985; Gesteira and Dauvin 2005). However, as we have already mentioned, the identification of organisms at a lower taxonomic level is a slow and costly process (Muniz et al. 2005; Marrero et al. 2013; Gerwing et al. 2020) while reducing the risk of making mistakes in the classification (Olsgard and Somerfield 2000; Dauvin et al. 2003). In particular, some studies favor the application of this concept in places where the macrobenthic communities were poorly analyzed and

there is little reference bibliography for identification (e.g., Warwick 1988; Dauvin et al. 2003). Furthermore, Warwick (1988) found that natural environmental variation affects macrofaunal populations at a specific level, while anthropogenic disturbance does so at higher taxonomic levels. Therefore, taxonomic sufficiency is an important tool for low-cost monitoring at impacted sites.

The objectives of the present study were 1) to validate ex situ experimental conditions comparing different physical-chemical and biological variables between in situ and ex situ conditions; 2) to analyze, through an ex situ experiment, the effect produced by two concentrations of crude oil (1 g/kg and 20 g/kg) on the Caleta Sara polychaete assemblages at different depths of the sediment column (0-8 cm and 8-16 cm), and 3) to evaluate the application of taxonomic sufficiency concept on crude oil experiment data.

Materials and Methods

Study area

The study site is located in Caleta Sara (44°54'0'' S - 65°34'0'' W) (San Jorge gulf, Patagonia, Argentina) (Figure 1). It is a part
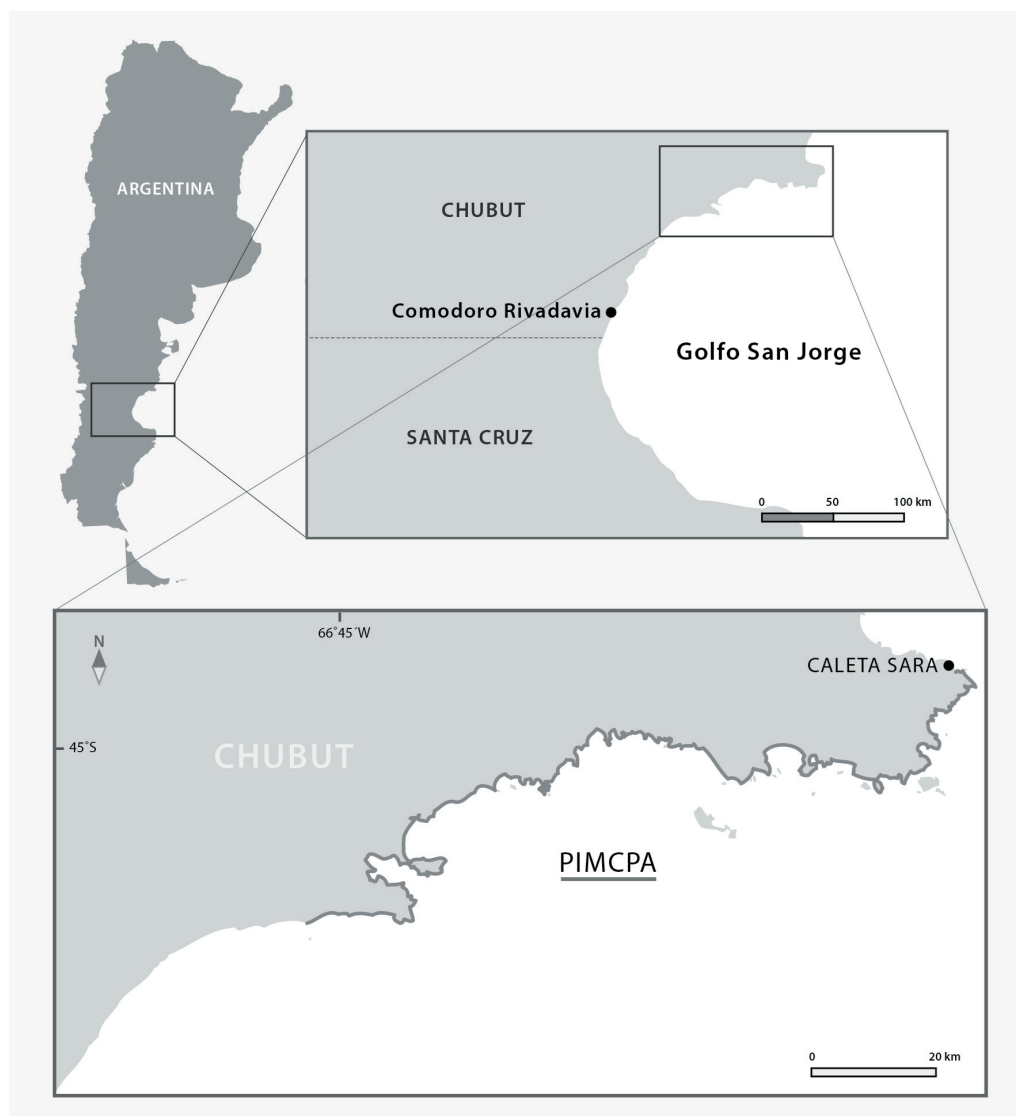
Figure 1. Location of the sampling site in Caleta Sara (Argentine Patagonia). PIMCPA: Interjurisdictional Marine Coastal Park Patagonia Austral.

Figura 1. Ubicación del sitio de muestreo en Caleta Sara (Patagonia argentina). PIMCPA: Parque Interjurisdiccional Marino Costero Patagonia Austral.

of the Interjurisdictional Marine Coastal Park Patagonia Austral, where the climate is temperate and semi-arid, with an average annual temperature of 13 °C. The area includes rocky environments exposed to open sea waves as well as soft substrate environments (mainly mud and fine sand) protected inside small bays, coves and inlets. Caleta Sara was selected for this study due to its predominance of fine sediments, low trace metal content, presence of biogenic hydrocarbons and high biodiversity of macrobenthic assemblages (Ferrando 2015). Although this study describes these assemblages in relation to the environmental characteristics of the Gulf of San Jorge, little is known about assessing the impact of crude oil on these environments (Ferrando et al. 2015, 2019).

Sampling

Validation of ex situ experiment. Eight cores (diameter: 10 cm; length: 25 cm; area:314.15 cm²) were vertically buried up to 20 cm in the lower intertidal of Caleta Sara in November 2011. In addition, a volume of sea water (60 L) was collected for experimentation. The four cores of the in situ conditions remained on the study site for 13 days while the four cores of the ex situ conditions were immediately transported to the laboratory where they were incubated in an aquarium (56 L) with seawater from the site of collection and oxygenated by a double aerator at room temperature (~18 °C) for 13 days (Figure 2a). At the end of the validation experiment, the eight sediment columns (in situ and ex situ conditions) were sliced in layers in the laboratory. The first four layers were 0.5 cm thick while the remaining ones (up to 16 cm) were 1 cm thick (Figure 2b). To assess the percentages of water content and organic matter in them, each sample was homogenized (25% of each layer; 7 layers; with 4 replicates per treatment), in agreement with

previous work on Patagonian coastal sediment characterization (Sturla Lompré et al. 2018; Ferrando and Sturla Lompré 2020; Ferrando et al. 2022). A subsample of 2 g (wet weight) was dried in an oven at 105 °C until constant weight (dry weight). The sample was combusted in muffle at 450 °C for 4 h and weighed again (ash-free dry weight). Percentages of water content and organic matter were calculated using the following formulae (Billen 1978):

Water content (%) = ({wet weight [g] - dry weight [g]} / wet weight [g]) * 100

Organic matter (%) = ({dry weight [g] - calcined weight [g]} / dry weight [g]) * 100

For the analysis of Polychaeta assemblages, we used 75% of each layer, obtaining two composite samples of each sediment column (four replicates per treatment): upper (0-8 cm) and lower (8-16 cm) level (Figure 2b). The samples were screened with a 500 μm mesh and fixed with formalin (10%) and preserved in alcohol (70%) to count and identification of organisms. These samples were analysed at family taxonomic level using a stereoscopic microscope.
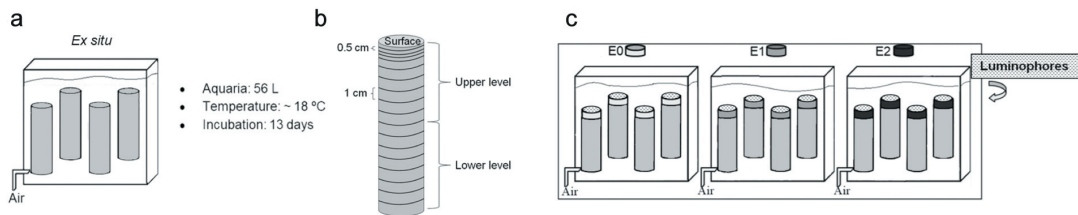
Ex situ experimentation with crude oil. Twelve cores (diameter: 10 cm; length: 25 cm; area: 314.15 cm²) were vertically buried up to 20 cm in the lower intertidal of Caleta Sara in November 2012 and immediately transported to the laboratory. A sample of surface sediment (2 kg) and a volume of seawater (60 L) were collected for experimentation. Ex situ conditions (four replicates per treatment) were: control (without the addition of crude oil, E0), with a moderate concentration of crude oil equivalent to those recorded at a site chronically exposed to this contaminant (1 g/kg, E1) and with a high concentration equivalent to those found at a site affected by a

Table 1. Density (mean±SD) (n=4) of polychaete assemblages recorded in the validation by level of the sediment column (upper level: 0-8 cm; lower level: 8-16 cm).

Tabla 1. Densidad (media±DS) (n=4) de los ensamblajes de poliquetos registrados en la validación por nivel de la columna sedimentaria (nivel superior: 0-8 cm; nivel inferior: 8-16 cm).

Family	Ex situ		In situ	
	Upper level	Lower level	Upper level	Lower level
Maldanidae	891.3±1617.0		38.2±63.7	
Syllidae	891.3±1286.0		38.2±63.7	
Spionidae	865.8±1056.8	38.2±63.7	356.5±318.3	38.2±63.7
Orbiniidae	483.8±127.3		191.0±127.3	
Onuphidae	318.3±216.5		63.7±76.4	38.2±63.7
Nereididae	292.8±241.9			
Capitellidae	38.2±63.7			

Figure 2. Ex situ experimental design with 4 replicates (a), diagram representing the cut of a sediment column in layers (b) and experimental design with crude oil in ex situ conditions (c). Upper level: 0-8 cm. Lower level: 8-16 cm. E0: Control. E1: Moderate concentration of crude oil (1 g/kg). E2: High concentration of crude oil (20 g/kg).

Figura 2. Diseño experimental ex situ con 4 réplicas (a), diagrama representando el corte de una columna sedimentaria en capas (b) y el diseño experimental con petróleo crudo en condiciones ex situ (c). Nivel superior: 0-8 cm. Nivel inferior: 8-16 cm. E0: Control. E1: Concentración moderada de petróleo crudo (1 g/kg). E2: Concentración alta de petróleo crudo (20 g/kg).

spill (20 g/kg, E2) (UNEO / hydrocarbons IOC / IAEA 1992). Escalante crude oil from the San Jorge gulf basin (Chubut province, Argentina) was used to pollute sediments according to experimental conditions. For this, a surficial layer (thick: 1 cm) of Caleta Sara sediments, either uncontaminated (E0) or contaminated with escalante crude oil (E1 and E2), was placed on the surface of corresponding sediment columns (Figure 2c). Each group of cores was placed in three individual tanks (56 L) filled with seawater from the same site and maintained under the same environmental conditions (temperature: 15 °C, photoperiod: 12h/12h) for 30 days. To analyse the bioturbation activity, 2 g of luminophores (fluorescent inert particles of 65-125 μm) were added at the surface of each sediment column (Duport et al. 2007). At the end of the experimentation, the procedure applied to analyze the sediment samples was the same described for the validation experiments, except that the identification of organisms was carried out at the taxonomic level of families and genera. Also, the distribution of buried luminophores in sediment columns was analysed by an optical method using an ad-hoc black box (Black box), where each subsample was illuminated with UV light and photographed. For image analysis, we used the ImageJ 1.46r program.

Statistical data analysis

Validation of ex situ experiment. Differences in physical and chemical parameters (dependent variables) were analyzed by layer while the biological data (dependent variable) were analysed by the level of the sediment column (upper and lower). The differences in water content, organic matter and mean abundance of polychaetes were analyzed using a two-way ANOVA test (Statistica 7.0) considering two fixed factors: conditions (in situ and ex situ) and depth, and an interaction term conditions * depth. Then, a Tukey post hoc test was used to analyze the significant differences between the treatments for each layer and between the layers for each treatment. Previously, the assumptions of normality and homoscedasticity were tested (Statistica 7.0) and when the latter was not met (P<0.01) the data were transformed (natural logarithm) to meet them.

Ex situ experimentation with crude oil. A combined multidimensional scaling (MDS) analysis was made using the absolute abundance data (dependent variable) at family and genera level by treatment (E0, E1 and E2) and level of the sediment column (upper and lower) (PRIMER 6.0). To do that, the Bray-Curtis similarity index (Field et al. 1982) and a fourth root transformation to the data were applied to decrease the effect of very abundant taxa (Field et al. 1982). Moreover, the community parameters (richness, Shannon diversity and total abundance) were also assessed by treatment and level of the sediment column (PRIMER 6.0). Differences in the previous step were evaluated through one-way ANOVA considering the treatments as a fixed factor with three levels (E0, E1 and E2) and the community parameters as dependent variables with four replicates (n=4) in each level of the column (upper and lower) (Statistica 7.0). The assumptions of normality (R Studio) and homoscedasticity (Statistica 7.0) were tested, and when the latter was not met (P<0.01), a Kruskal-Wallis nonparametric test (Statistica 7.0) was performed. Furthermore, the Tukey test or Mann-Whitney test for multiple mean comparisons was used to assess significant differences between

pairs of treatments depending on the case. Spearman correlations were assessed between similarity values recorded at the upper level of sediment columns at family and genera level by treatment (E0, E1 and E2). To do that, the Bray-Curtis similarity index and a fourth root transformation to the data were applied. A permutational multivariate analysis of variance (PERMANOVA) was applied to assess the effect of the treatments (E0, E1 and E2), the depth of the sediment column (upper and lower) (nested to the treatments) and the interaction between both variables (PRIMER v7). A pairwise PERMANOVA was applied to analyze the significant differences between the treatments and the levels of the sediment columns (nested to the treatments). These analyzes were based on Bray-Curtis similarity matrices generated from the transformed absolute abundance data (fourth root) (dependent variable) and 9999 permutations (Anderson et al. 2008). Finally, differences in percentages of buried luminophores for each layer among treatments (E0, E1 and E2) were analysed using one-way ANOVA or Kruskal-Wallis according to the case. The Tukey or Mann-Whitney tests for multiple mean comparisons were used to assess significant differences between pairs of treatments depending on the case (Statistic 7.0).

Results

Validation of ex situ experiment

The percentage of water content and organic matter recorded for the in situ and ex situ sediments showed similar patterns with the highest values in the upper layers, decreasing from the upper to lower layers of the sediment (Figure 3). However, for both parameters in ex situ profiles, this pattern was less evident showing no significant differences among layers (P>0.05). In in situ profiles, organic matter just was significantly higher in layers 1, 2 and 3 than in 4, 5, 6 and 7, while water content showed no differences among layers. Furthermore, although the percentages of water content and organic matter in the first layers were higher in situ than in ex situ, these differences were only significant for organic matter in layers 1 and 2 (P<0.05) (Figure 3).

Two-way ANOVA results showed a significant depth effect (P=0.0008) in the mean abundance of polychaetes. Although, the abundance in upper level was higher in ex situ than in situ (Table 1), the difference

was not significant (P>0.05). Furthermore, the data recorded in lower level not showed significant differences (P>0.05) between conditions. However, for ex situ conditions the mean abundance was significantly higher in upper than in lower level, meanwhile in in situ conditions there were no differences between levels. The families in both conditions were: Maldanidae, Syllidae, Spionidae, Orbiniidae and Onuphidae, while Nereididae and Capitellidae were only recorded ex situ (Table 1).

Ex situ experimentation with crude oil

Density and diversity of taxa decreased in direct proportion to the concentration of crude oil added (Table 2). Similarly to the validation experiments (in situ and ex situ), the mean density at the upper level in the three treatments (E0, E1 and E2) was higher than at the lower level (Table 2). Furthermore, the mean density recorded at the upper level of E1 and E2 was 64% and 80% lower than that recorded in E0, respectively. On the contrary, the density in lower level was similar in all treatments (Table 2). The only families recorded in E2 were Maldanidae, Spionidae and Capitellidae. Within Spionidae, only Boccardia and Rhynchospio were present but the density of the later was very low (Table 2).

The organizational pattern of the MDS analysis showed highest differences in upper level between E0 and E2, with an intermediate group constituted by E1 (Figure 4). This representation could be considered satisfactory given the good stress (0.12). On the contrary, in the lower levels, we observed no tendency because the density of organisms was similar in all treatments (Table 2). Figure 5 shows the community parameters (richness, Shannon diversity and total abundance) calculated at both taxonomic levels for upper level and lower level of the columns E0, E1 and E2. At the upper levels, the highest number of families and genera was recorded in E0. The diversity of organisms decreased more between E0 and E2 than between E0 and E1. Moreover, the total abundance of organisms was also high in E0 and decreased according to the amount of crude oil added to the sediments (Figure 5). Contrary, in the lower levels of the three treatments, and for all the parameters evaluated, the assemblage of Polychaeta showed no differences between them (Figure 5). The test for the values of the
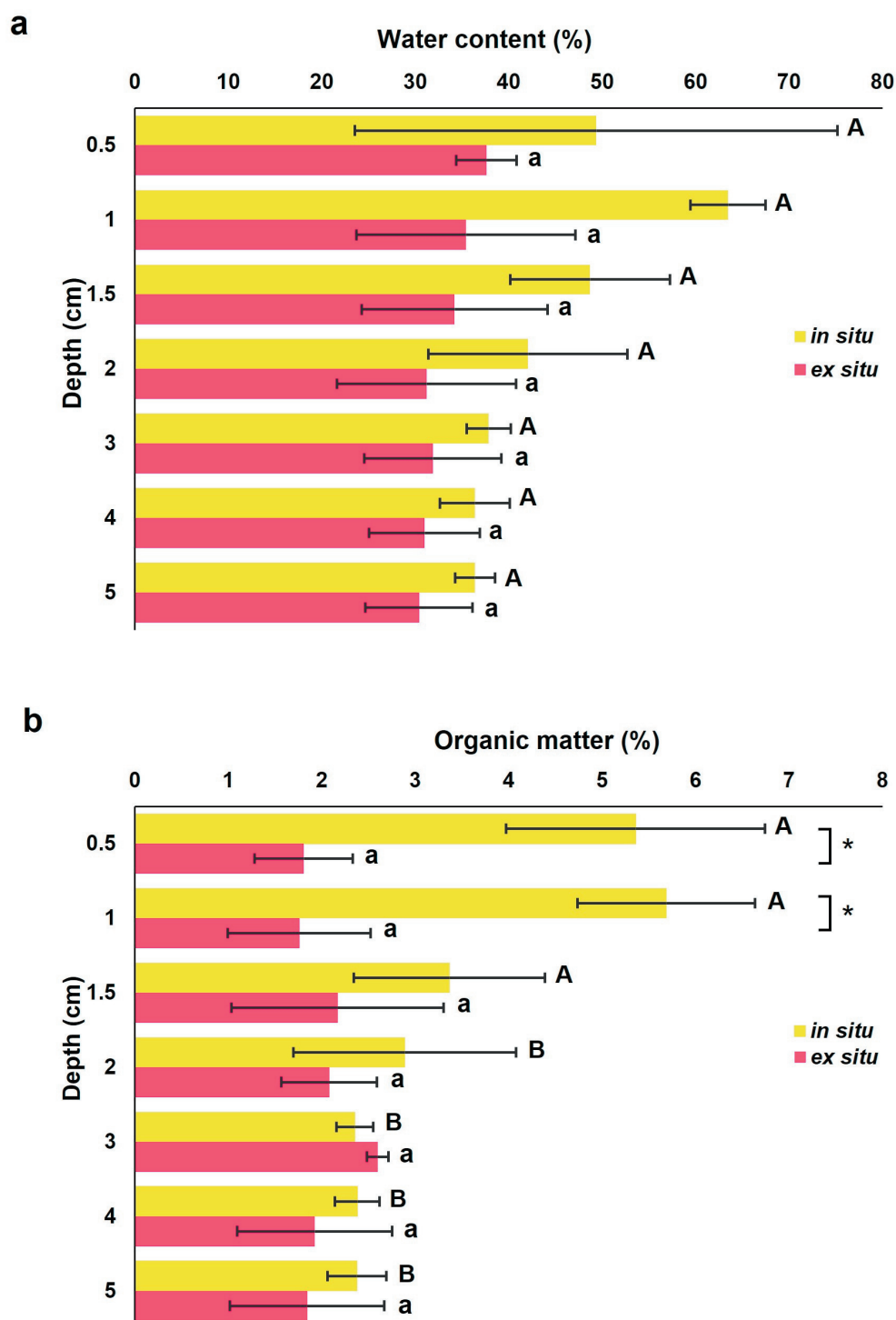
Figure 3. Vertical profiles of water content (a) and organic matter (b) percentages (mean±SD) (n=4) of in situ and ex situ sediments. Significant differences (ANOVA two-way and Tukey test; P<0.05) tested among layers for each condition are represented with different letters (uppercase for in situ and lowercase for ex situ). For each layer, significant differences between in situ and ex situ are represented with an asterisk.

Figura 3. Perfiles verticales de los porcentajes (media±DS) (n=4) del contenido de agua (a) y materia orgánica (b) de los sedimentos in situ y ex situ. Las diferencias significativas (ANOVA de dos vías y prueba de Tukey; P<0.05) testeados entre capas para cada condición experimental son representadas con diferentes letras (mayúsculas para in situ y minúsculas para ex situ). Las diferencias significativas para cada capa entre in situ y ex situ son representadas con un asterisco.

Table 2. Density (mean±SD) (n=4) of polychaete recorded in the three experimental conditions by level of the sediment column. E0: Control. E1: Moderate concentration of crude oil (1 g/kg). E2: High concentration of crude oil (20 g/kg). Upper level (0-8 cm). Lower level (8-16 cm).

Tabla 2. Densidad (media±DS) (n=4) de los poliquetos registrados en las tres condiciones experimentales por nivel de la columna sedimentaria. E0: Control. E1: Concentración moderada de petróleo crudo (1 g/kg). E2: Concentración alta de petróleo crudo (20 g/kg). Nivel superior (0-8 cm). Nivel inferior (8-16 cm).

| Family	Genera	E0 Upper level	E0 Lower level	E1 Upper level	E1 Lower level	E2 Upper level	E2 Lower level
Maldanidae	*Axiothella*	2673.8±802.1		292.8±267.4		165.5±241.9	101.9±190.0
Spionidae	*Boccardia*	1591.5±649.4		891.3±203.7		1056.8±687.5	
	Rhynchospio	891.3±980.4		420.2±267.4	63.7±76.4	38.2±63.7	63.7±76.4
	Boccardiella	229.2±216.5		292.8±127.3			
Capitellidae	*Capitella*	1336.9±522.0		547.5±407.4		292.8±63.7	
Syllidae	*Exogone*	738.5±483.8		101.9±127.3			
Nereididae	*Ceratocephale*	127.3±152.8	63.7±127.3	165.5±241.9			
Onuphidae	*Onuphis*	165.5±165.5					
Hesionidae	*Gyptis*	101.9±63.7	38.2±63.7				
Cirratulidae	*Caulleriella*	63.7±127.3					
Orbiniidae	*Orbinia*			63.7±76.4			
Sabellidae	*Chone*			38.2±63.7			
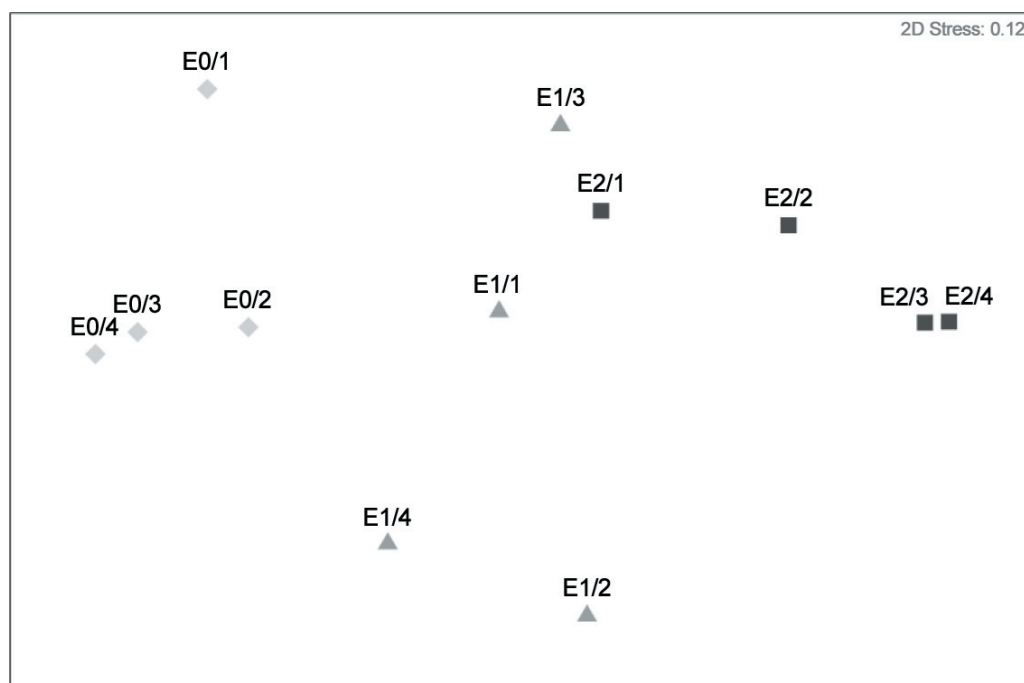
Figure 4. Combined multidimensional scaling (MDS) analysis with the absolute abundance data recorded at family and genera level in the upper level of the sediment column. E0: Control. E1: Moderate concentration of crude oil (1 g/kg). E2: High concentration of crude oil (20 g/kg).

Figura 4. Análisis de escalamiento multidimensional combinado con los datos de abundancia absoluta registrados a nivel de familia y género en el nivel superior de la columna sedimentaria. E0: Control. E1: Concentración moderada de petróleo crudo (1 g/kg). E2: Concentración alta de petróleo crudo (20 g/kg).

community parameters at the upper level, showed significant differences (P<0.05) between E0 and E2 in all cases (Figure 5). For E1, the differences with E0 and E2 were significant (P<0.05) in most of the cases except in richness at the genera level (E0 vs. E1), Shannon diversity at both taxonomic levels (E0 vs. E1) and total abundance (E1 vs. E2) (Figure 5). Conversely, in lower level no significant differences (P>0.05) were found

in the parameters among the treatments (Figure 5). Spearman's correlations between the similarity values recorded at the upper level of the sediment columns at the family and genera level were significant and positive only for E0 and E2 (r=0.82, P<0.05 in both cases). According to the PERMANOVA, only the differences between upper level and lower level (nested in E0, E1 and E2) were highly significant (P<0.01) (Table 3). Furthermore,
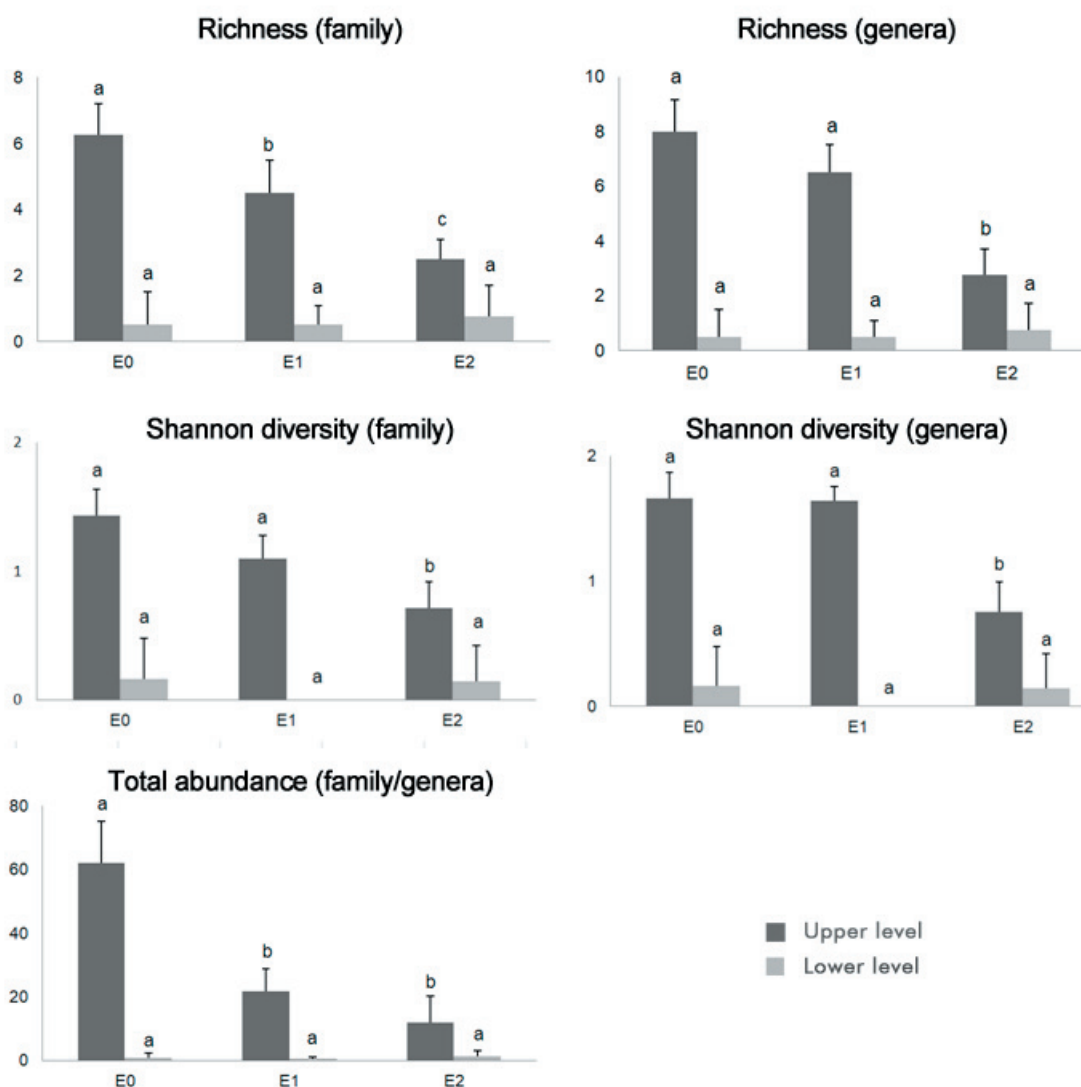
Figure 5. Community parameters calculated at both taxonomic levels (mean+SD) (n=4) in the three treatments. E0: Control. E1: Moderate concentration of crude oil (1 g/kg). E2: High concentration of crude oil (20 g/kg). Upper level: 0-8 cm. Lower level: 8-16 cm. Lowercase indicate significant differences between treatments by level of the sediment column.

Figura 5. Parámetros comunitarios calculados a ambos niveles taxonómicos (media+DS) (n=4) en los tres tratamientos. E0: Control. E1: Concentración moderada de petróleo crudo (1 g/kg). E2: Concentración alta de petróleo crudo (20 g/kg). Nivel superior: 0-8 cm. Nivel inferior: 8-16 cm. Las letras minúsculas indican diferencias significativas entre tratamientos por nivel de la columna sedimentaria

pairwise PERMANOVA showed highly significant differences (P<0.01) at all cases.

Concerning the bioturbation activity in sediments E0, E1 and E2, the retention of luminophores on the surface of the sediment column was 72, 93 and 100%, respectively (Figure 6). In E0, the luminophores were buried up to 4 cm and in E1 up to 1 cm, while in E2, the total luminophores were retained on the surface (Figure 6). The analyses showed significant differences (P<0.05) between E0 versus E1 and E2 in the first 0.5 cm, and between E0 and E2 in the 0.5-1 cm (Figure 6). On the contrary, there were no significant differences (P>0.05) among treatments from the 1 cm to the end of the sediment column.

Table 3. PERMANOVA summary. Res: Residuals. df: Degrees of freedom. SS: Sum of squares. MS: Middle squares. mc: Monte Carlo simulation. **P(mc)<0.01.

Tabla 3. Resumen del PERMANOVA. Res: Residuales. df: Grados de libertad. SS: Suma de cuadrados. MS: Cuadrados medios. mc: simulación de Monte Carlo. **P(mc)<0.01

Source	df	SS	MS	Pseudo-F	P(mc)
Treatments	2	3219.9	1610	0.1699	0.95
Depth (Treatments)	3	28428	9476	22.948	0.00**
Res	18	7433	412.94		
Total	23	39081			
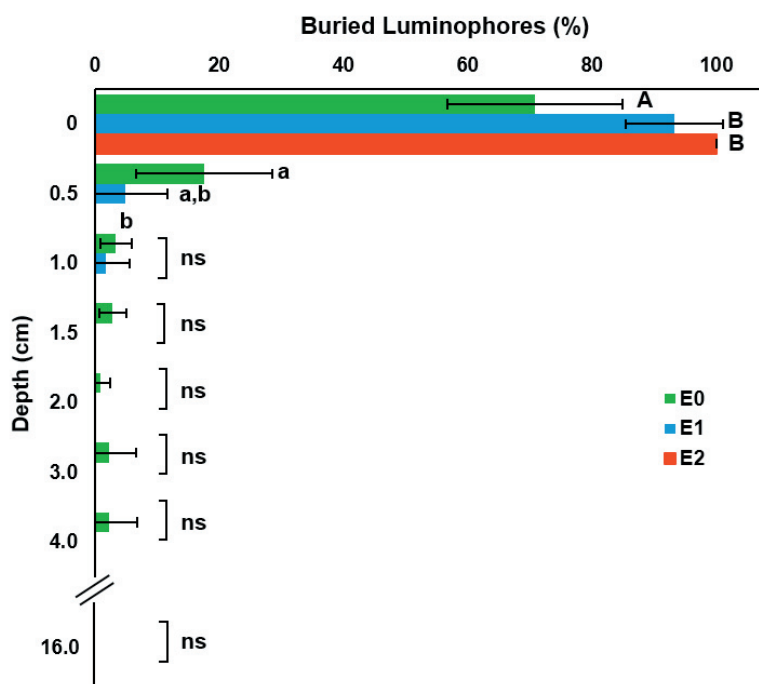
Discussion

In the present study, abundance, richness, diversity and bioturbation activity showed a significant decrease with oil addition only at the upper level. *Capitella* (Capitellidae) and *Boccardia* (Spionidae) were the most abundant at the highest oil concentration. Furthermore, the results were similar at the taxonomic levels of genera and family, indicating that it would be possible to assess the effect of crude oil on the assemblages even at the family level, especially under acute ecotoxicological conditions. However, the ratio of families to genera was 1:1 in all cases except Spionidae. Therefore, identification at the family level should only be considered for low-diversity polychaete assemblages.

In in situ and ex situ conditions, the results showed a higher percentage of water content and organic matter in the superficial layers than in deeper layers. This could be due to the predominance of silt sediments in the first few centimeters of sediments of this type of beach, resulting in greater retention of water and organic matter (Ferrando and Sturla Lompré 2020; Ferrando et al. 2022).

Concerning the in situ and ex situ sediment characteristics, the percentages of organic matter were three times higher in the in situ conditions than in the ex situ conditions,

Figure 6. Distribution of buried luminophores (mean±SD) (n=4) in the three treatments. E0: Control. E1: Moderate concentration of crude oil (1 g/kg). E2: High concentration of crude oil (20 g/kg). Uppercase and lowercase indicate significant differences by layer between treatments.

Figura 6. Distribución de los luminóforos enterrados (media±DS) (n=4) en los tres tratamientos. E0: Control. E1: Concentración moderada de petróleo crudo (1 g/kg). E2: Concentración alta de petróleo crudo (20 g/kg). Las letras mayúsculas y minúsculas indican diferencias significativas por capa entre tratamientos.

which could be related to the presence of phytoplankton blooms that usually occur during the spring months (Guerrero and Piola 1997). In addition, Caleta Sara is a 'low energy' environment with limited water circulation, predominantly fine sediments and high levels of organic matter (Brown and McLachlan 2010). Indeed, the levels of organic matter recorded in the sediments of Caleta Sara were higher than those found in similar Patagonian environments, such as Bahía Nueva (Ferrando et al. 2010; Crespi-Abril et al. 2016) and Caleta Valdés (Ferrando et al. 2022), probably due to the presence of wind-driven coastal downwelling in the study area, which represents an additional source of organic enrichment (Glembocki et al. 2015). However, the community structure recorded under both conditions was similar after 13 days. These results made it possible to carry out experiments with crude oil in the laboratory, simulating natural conditions, without having a negative impact on the study area.

Inputs of nutrients, organic matter and pollutants from anthropogenic activities can significantly alter environmental conditions, although natural variability can be a major source of stress for various organisms (Venturini et al. 2004). In particular, oil deposition at the sediment-water interface can have a negative effect on the biogeochemistry of benthic systems (Rivero et al. 2005; Sturla Lompré et al. 2018). In the present study, a decrease in the density of polychaetes assemblages was observed with the addition of crude oil. This result indicated a direct effect of this contaminant on the organisms living in the sedimentary matrix of Caleta Sara. In particular, the cell membranes of these organisms could be affected because crude oil contains compounds that act as solvents (Baker 2001). Furthermore, oil spills can affect benthic communities by altering habitat characteristics, smothering and/or poisoning flora and fauna (Herkül and Kotta 2012).

Due to their typically high species richness and abundance in benthic environments, polychaetes play a fundamental ecological role (Venturini et al. 2011). Moreover, they exhibit different degrees of stress tolerance (Pearson and Rosenberg 1978; Dauvin et al. 2010; Ferrando et al. 2019; among others). Marrero et al. (2013), working with the assemblage of Polychaeta in the Rio de la Plata estuary, has shown that the family level is the most appropriate compromise between reducing the cost of identification and the efficiency of statistical results. Chainho et al. (2007) suggested that the advantage of using the family level is that most ecologists with taxonomic training are familiar with this level of identification. Other authors suggested that using the family level yields spatial patterns very similar to those obtained for species in areas with strong pollution gradients (Dauvin et al. 2003; Bacci et al. 2009; Soares-Gomes et al. 2012). In addition, the cost of identifying at the family level is half the cost of identifying at the species level, according to estimations made by Ferraro and Cole (1995) and Thompson et al. (2003) with macrobenthic organisms. Our results add to this line of evidence since they were similar at both taxonomic levels, indicating that it would be possible to assess the effect of crude oil on assemblages even at the family level, especially under acute ecotoxicological conditions. However, it should be mentioned that the ratio of families to genera was 1:1 in all cases except Spionidae. Therefore, the identification of the organisms at the family level should only be considered for low-diversity polychaete assemblages.

The only families recorded in the most oil-polluted sediment were Maldanidae, Spionidae and Capitellidae. Although Maldanidae (*Axiothella*) were present in these conditions, their density was much lower than that recorded in the control. Concerning Capitellidae and Spionidae, various species have been widely defined as indicators of organic enrichment (Pearson and Rosenberg 1978; Rivero et al. 2005; Ferrando et al. 2010, 2015, 2019). In this study, the response of the Spionidae was different according to the genera. *Boccardiella* was the only absent in the most polluted sediments. Although *Rhynchospio* was present in these conditions, its density was very low. Finally, *Boccardia* had a similar density under the three experimental conditions. Ferrando et al. (2019) found similar results for *Rhynchospio glutaea* and *Boccardia proboscidea* in an experiment carried out with sediments from Caleta Malaspina (Patagonia, Argentina) applying a similar methodology. Moreover, Ferrando et al. (2010) identified 'disturbed zones' on soft bottom beaches of Puerto Madryn city (Argentina) due to the high abundance of *Boccardia polybranchia* and *Capitella 'capitata'* sp. associated with high concentrations of ammonium, nitrites, nitrates, phosphates and organic matter in sediment. In this sense, although more studies are still necessary to confirm the tendencies observed

in this study, *Boccardiella* and *Rhynchospio* would be behaving as 'sensitive' to the presence of crude oil in sediment, whereas *Boccardia* could tolerate these stress conditions. Particularly, spionids can regenerate different parts of the body and have sexual and asexual reproduction with different types of eggs and larval development (Levin and Creed 1986; Blake and Arnofsky 1999). For example, *B. proboscidea* (Gibson 1997) and *B. polybranchia* (Duchêne 2000) have the ability to produce more than one type of offspring within the same capsules of the egg masses or in the same population (poecilogony). Poecilogony has been proposed as an evolutionary response to the environmental variability (Doherty-Weason et al. 2020) being able to spread to other environments or proliferate where it was formed (Blake and Kudenov 1981). Regarding the complex *Capitella* 'capitata', it presents detritivorous organisms that live buried in sediments, assimilating the organic matter bound in sedimentary particles (García-Garza and De León-González 2011). Similar to *Boccardia*, it has specific reproductive characteristics such as short life cycles, high reproduction rates, small body size, and different types of larvae (planktonic or benthic, planktotrophic or lecithotrophic) that may settle at the same site or disperse to farther environments depending the environmental conditions (Qian and Chia 1991).

The vertical distribution of benthic organisms has attracted the attention of not only marine biologists, but also geologists and geochemists in relation to bioturbation (Muniz et al. 2013). This process modifies the physical, chemical and biological characteristics of the sediment (Soetaert et al. 1996; Quintana et al. 2010). With respect to bioturbation activity, it was null in the most polluted sediment. On the contrary, the tracers were buried up to four centimeters deep in control. The main effect of bioturbation on the sedimentary matrix is the dispersion and mixing of solid particles, including non-living components such as clays, debris, metals and adsorbed contaminants, and living organisms such as microorganisms (Kristensen et al. 2012). Moreover, some macrobenthic organisms can positively affect the biodegradation of hydrocarbons through the production of digestive compounds that act as surfactants, modifying their dispersion state and increasing their bioavailability (Gilbert et al. 2001) for the degrading bacteria present in the sediment (Bonin and Bertrand 1999). Therefore, in sediment heavily affected by oil spill and/or its derivatives and completely devoid of macrofauna, oxygen diffusion to deeper layers could be reduced, affecting the rates and pathways of biogeochemical processes and significantly reducing microbial bioremediation (Timmermann et al. 2011). In the present study, the null bioturbation activity in the most oiled sediments could be due to the direct effect on incapacitated organisms to bury themselves in deeper layers in order to use an evasion mechanism (Bolam 2011). In particular, in all the experiments carried out in this study, it was found that the organisms mostly stayed at the upper level, close to the water-sediment interface. This result coincides with previous findings on this topic (Kristensen and Holmer 2001; Venturini et al. 2011; Muniz et al. 2013) and it could be related to the organisms trend to occupy the first layers, where the concentration of oxygen is higher. In addition, certain genera of Spionidae must remain at the water-sediment interface because their feeding guilds are through ciliated tentacles or palps that they use to collect particles in this zone (Ferrando et al. 2019). Nevertheless, in this study, we considered a large section (0-8 cm) for the upper level that in future studies should consider slice the sediment into shorter layers to make this distinction testable.

The results of this study contribute to general knowledge about the intertidal soft bottom environments of the Patagonian coast. They can also be used as a reference to evaluate the effect of the development of anthropogenic activities in the area or in other environments with similar ecological characteristics. Furthermore, the experimental protocol with crude oil in the laboratory simulating natural conditions proved to be a useful and reliable tool for evaluation on the studied site.

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