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Intertidal macrozoobenthos in sandy beaches of Bahía Nueva (Patagonia, Argentina) and their use as bioindicators of environmental impact

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SUMMARY: Macrozoobenthos were collected from urban sandy beaches of Puerto Madryn and adjacent areas in November 2005. Multivariate analysis techniques (ANOVA, nMDS, ANOSIM, SIMPER and RDA) were used to characterize the area based on the relationships among biological and environmental variables. The areas affected by outfall of fishery factories and pluvial effluent were defined as "perturbed zones" due to the dominance of the pollution indicator polychaetes *Boccardia polybranchia* and *Capitella "capitata"* sp., which are associated with high values of ammonium, phosphates and organic matter. However, sandy beaches located 15 km from the city were considered as "unperturbed zones" because Psammodrilidae and *Puelche orensanzi* dominated, and they had high values of nitrates and dissolved oxygen. These taxa seem to be good candidates as indicators of clean zones for further studies. This is the first record of the family Psammodrilidae in the South Atlantic.

Keywords: Polychaeta, anthropogenic influence, macrobenthic community, *Capitella "capitata"* sp., *Boccardia polybranchia*, Psammodrilidae, sandy beaches.

RESUMEN: Macrozoobentos intermareal en playas arenosas de la Bahía Nueva (Patagonia, Argentina) y su uso como bioindicadores de impacto ambiental. – Se recolectaron muestras de macrozoobentos en playas arenosas de la ciudad de Puerto Madryn y áreas adyacentes, en noviembre de 2005. Los análisis multivariantes (ANOVA, nMDS, ANOSIM, SIMPER y RDA) permitieron caracterizar el área en base a la relación entre las variables biológicas y ambientales. Las áreas afectadas por los desagües procedentes de las fábricas de pescado y los pluviales, fueron definidas como "zonas perturbadas", debido a la dominancia de los poliquetos indicadores de contaminación *Boccardia polybranchia* y *Capitella "capitata"* sp., asociados a altos valores de amonio, fosfatos y materia orgánica. Por el contrario, las playas arenosas localizadas a 15 km de la ciudad fueron consideradas como "zonas no perturbadas" debido a que dominaron Psammodrilidae y *Puelche orensanzi,* con altos valores de nitratos y oxígeno disuelto. Estos taxa podrían ser buenos candidatos como indicadores de zonas limpias para futuros estudios. Se registra por primera vez la familia Psammodrilidae en el Atlántico Sur.

Palabras clave: Polychaeta, influencia antropogénica, comunidad macrobentónica, *Capitella "capitata"* sp., *Boccardia polybranchia*, Psammodrilidae, playas arenosas.

INTRODUCTION

The city of Puerto Madryn, on the Golfo Nuevo (south of Península de Valdés, northern Patagonia), has shown a rapid population increase since the 70s (6183 inhabitants during 1970 and 57614 during 2001; INDEC, 2001). The development of the region has generated environmental pressure on the coastal zone, with decreasing water quality (Esteves *et al*., 1997), toxic phytoplankton blooms (Esteves *et*

al., 1992), and increasing kelp debris on the beaches (Eyras *et al*., 1999). Gil (2001) pointed out that part of these effects could be attributed to industrial effluents from fishery factories as potential sources of organic enrichment. Nevertheless, in some cases, the distinction between changes produced by natural factors, and those produced by anthropogenic activities is not clear (Warwick and Clarke, 1993).

Even when the pollution could be measured from the water column or the sediments (i.e. organic matter, dissolved oxygen, nutrients), these measures cannot clearly identify the impact on the ecosystem. Studies on the biological compartments are needed to assess the environmental impact. Benthic studies, and particularly those related to macrobenthos, are the most useful tool in environmental impact assessment. The effects produced by polluted water or sediments on the environment are determined better through the study of benthic communities than analyses of chemical features only (chemical concentrations higher than background references) (Chapman, 2007).

Polychaetes are one of the most useful organisms in impact studies. The dominance of some species belonging to the families Spionidae and Capitellidae is an indicator of organic pollution (i.e. Tsutsumi, 1990). Polychaetes have been used in bioassays for monitoring toxic compounds and as indicators of perturbed environments at the community, population and specific levels.

Few studies have been carried out on the macrofauna on soft bottoms around Península de Valdés. The only record for the intertidal zone of Golfo Nuevo is an unpublished study carried out at a station located in Puerto Madryn, which comprises macrofaunal aspects, such as biogeography and distribution patterns according to trophic guilds (Escofet, 1983). The relationships with environmental variables were not studied. Other unpublished studies have been performed in the infralittoral zone of the Golfo Nuevo (Carriquiriborde *et al*., 1983; Varela, 1985), and in the infralittoral and intertidal zones of the Golfo San Matías, located in the north of Península de Valdés (Escofet *et al*., 1978); however, only the names of dominant polychaete species were provided (Pastor de Ward, 2000). Recently, Pastor de Ward established polychaete assemblages based on abiotic and biotic factors through depth gradients (0 to 185 m depth). This study was performed in the Golfo San José, located in the north of the Península de Valdés (to the south of the Golfo San Matías), which is a wildlife reserve. None of these studies have focused on environmental impact assessment.

In order to assess the usefulness of the macrobenthic community (polychaetes in particular) in zones subjected to organic enrichment, a study at two tidal levels at 5 sites on sandy beaches around Puerto Madryn city was performed. The macrobenthic (>0.5 mm) community structure and environmental variables were measured in order to characterize zones subjected to different perturbation sources.

MATERIALS AND METHODS

The study area is located in the occidental part of the Golfo Nuevo, between 42°0.63´ and 42°0.76´S and 64°0.01´ and 64°0.98´W (Fig. 1). The semidiurnal tides of 4 m amplitude expose wide sandy intertidal areas, which are sheltered, and have a low wave intensity and slight slope (Monti and Bayarsky, 1996). The water temperature ranges from 10 to 17°C (De Vido and Esteves, 1978). Rain is scarce, with mean annual precipitations of 170 mm (Coronato and Vallejo, 1994).

During November 2005 five sites were sampled, one of them on a beach affected by the effluents of fish factories (*Pesqueras*), two on a beach affected by pluvial effluents and subsurface tap water (*Moreno* and *28 de julio*), and two clean sites (*Doradillo 1* and *2*), which are located on a beach 15 km from the city (Fig. 1). At each level (low and mid tide), 5 cores of 10 cm in diameter and 12 cm depth (78.5 cm^2) were randomly taken in the intertidal zone. One sediment aliquot for each replicate was taken and mixed to conform a single composed sample to determine granulometry, density, porosity and organic matter. The sediments were dried at 80ºC until constant weight to determine porosity, while the organic matter content was measured in dry sediments by weight loss at 450ºC during 4 hours (Billen, 1978). Dried sediments were sieved to obtain percentages of gravel (>2 mm), sand (<2 mm and >0.63 mm) and mud (<0.63 mm) according to Folk (1968).

Salinity, temperature, dissolved oxygen, pH, redox potential discontinuity, ammonium, nitrite, nitrate and phosphate concentrations were measured in the interstitial water. The pH and redox potential discontinuity were determined using Altronix TPA-1 equipment. Salinity was determined with a Plessey-6230N salinometer. Dissolved oxygen and temperature were measured using an YSI model 58

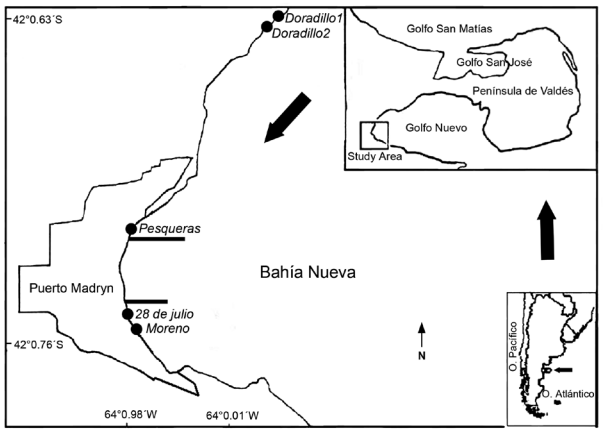

FIG. 1. – Study area showing the collection sites.

oxymeter. Ammonium, nitrite, nitrate and phosphate concentrations in the interstitial water of the lower intertidal level were determined in duplicate following the spectrophotometric techniques described by Strickland and Parsons (1972).

The biological samples were sieved through a 0.5 mm mesh and fixed in a neutral solution of formaldehyde (5%). The specimens were preserved in 70% ethanol for further identification and counting.

Data analyses were performed using the software package PRIMER (Plymouth Marine Laboratory, UK). Species richness (S), abundance (A), the Shannon diversity index (H´), and Pielou's equitativity index (J´) were calculated for each collection site. Differences in each biological variable were evaluated through a two-way ANOVA with levels and collection sites as fixed factors (n=50) (Statistica, version 7). The ordination was carried out with non-metric multidimensional scaling (nMDS) and the Bray-Curtis similarity index, using abundance transformed to the fourth root, which downweights the effect of rare and abundant species, to obtain the minimum stress (Field *et al.,* 1982).

The differences among collection sites due to the presence of benthic organisms were evaluated with a one-way analysis of similarities (ANOSIM), while the analysis of similarity percentage (SIMPER) was used to determine the organisms that contributed most to the differences observed. Abundance data were used throughout. The relationships among the studied environmental variables and macrobenthos abundance were analyzed with an analysis of redundancy (RDA). The dimension of the factors was reduced using the subroutine Biplot of the Excel worksheet. The RDA represented the variables that best explained the high percentage of variation, while the variables that explained redundant information were eliminated (Jongman *et al.*, 1995). Due to the absence of interstitial water at some collection sites of the middle intertidal level, the environmental variables could not be measured. Therefore, in order to compare the sites, only the environmental and biological variables from the lower intertidal level were taken into account for the RDA (since this analysis requires one environmental value for each biological value).

RESULTS

Biological variables

A total of 5317 organisms were counted and identified (Table 1), 90.78% of which were polychaetes, and the rest were oligochaetes, amphipods, copepods, tanaidaceans, insects and nemerteans.

The species richness showed highly significant differences between levels ($p<0.002$), and was higher at the lower level than at the middle one at all the collection sites (Fig. 2B). The mean abundance, equitativity and diversity did not show significant differences between levels. However, significant differences were detected among collection sites in species richness ($p<0.000$), abundance ($p<0.024$) and diversity ($p<0.000$), while equitativity did not show significant differences. Abundance was greater at the lower level from *Pesqueras* (Fig. 2A) and lower at both levels from *Moreno* (Table 1). Species richness and diversity were higher at both levels from *28 de julio* (Fig. 2B and 2C). Finally, an interaction effect was registered (collection sites by level) in abundance ($p<0.036$).

The nMDS showed a good representation (stress= 0.08) and grouped *Pesqueras* with *28 de julio* and the unperturbed sites (*Doradillo 1* and *2*), which were located on the opposite side of the diagram. *Moreno* was in an intermediate position (Fig. 3).

ANOSIM results showed highly significant differences between the two levels ($R= 0.319$, $p<0.2\%$) and between sites ($R= 0.46$, $p<0.1\%$). Differences between pairs of sites are shown in Table 2. Highly significant differences were found between *Pesqueras* and the rest of the sites as well as between *Doradillo 2* compared with *28 de julio* and *Moreno*.

The SIMPER analysis showed that the collection sites were dominated by different species: *Boccardia polybranchia* and *Capitella "capitata"* sp. at *Pesqueras*; *Capitella "capitata"* sp., Oligo-

TABLE 1. – Total abundance (number of individuals) of species (5 replicates) by levels and collection sites (M: middle level; L: lower level).

Species	*Pesqueras*		*28 de julio*		*Moreno*		*Doradillo 1*		*Doradillo 2*	
	M	L	M	L	M	L	M	L	M	L
Polychaeta										
Boccardia polybranchia	192	3538	39	26	1					
Spio quadrisetosa				8						
Scoloplos sp.	4	46	7	18		1				
Capitella "capitata" sp.	20	73	306	36						
Caulleriella sp.1	6	24								
Paraonides sp.1		1	57	123	5	6	2	3	4	
Syllidae indeterminated			4	9	1	1				
Exogone sp.1			2	6						
Axiothella sp.1			6	95						
Nereidae indeterminated			1	1						
Onuphis eremita				1						
Travisia amadoi						1		1		2
Glycera sp.				1						
Psammodrilidae indeterminated								55	3	91
Oligochaeta										
Oligochaeta sp.1			228	9						
Amphipoda										
Puelche orensanzi							1	80		93
Microphoxus cornutus	1		3	33		1				
Monoculopsis valentini				2				1		
Corophiidae indeterminated	2									
Calanoidea										
Calanoidea indeterminated				5						
Tanaidacea										
Tanaidacea indeterminated		10								
Crustacea										
Crustacea indeterminated				1						
Insecta										
Insecta indeterminated								1	2	3
Nemertea										
Nemertea indeterminated			5	1	2	6				

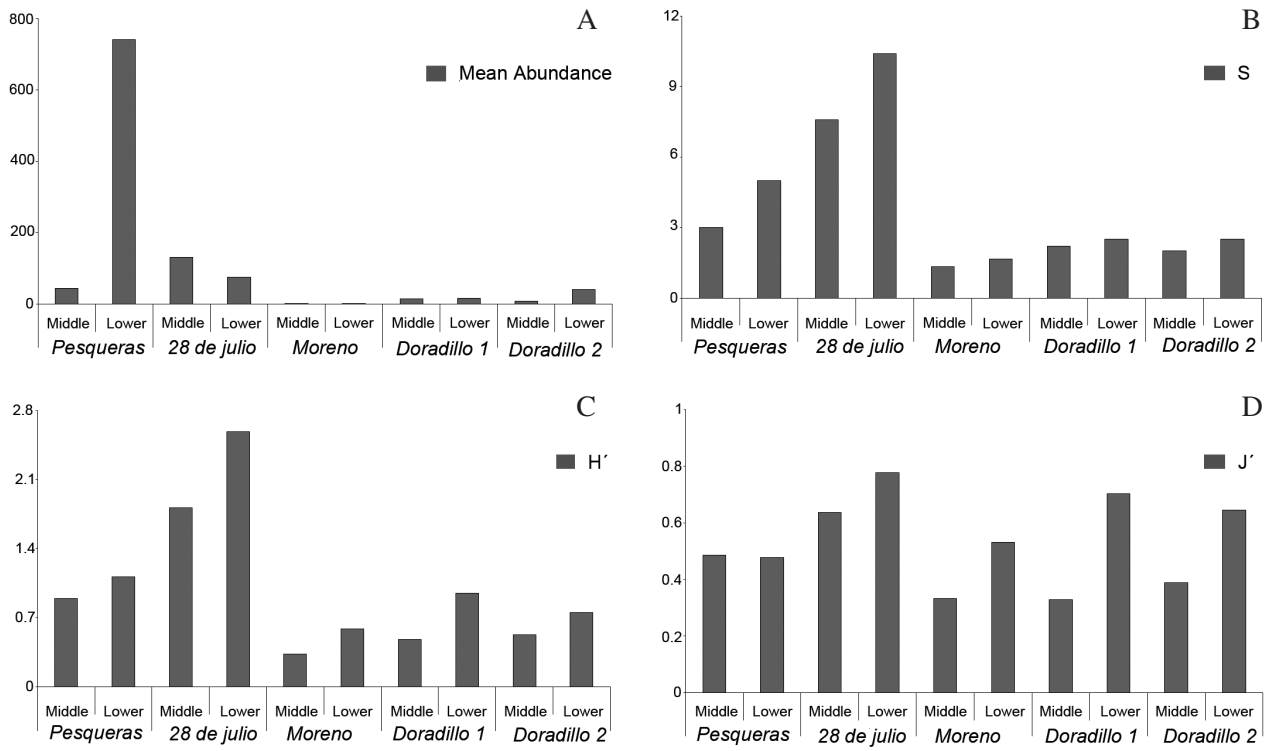

FIG. 2. – Biological variables at the middle and lower levels of the collection sites: A, mean abundance; B, species richness; C, Shannon diversity index and D; equitativity.

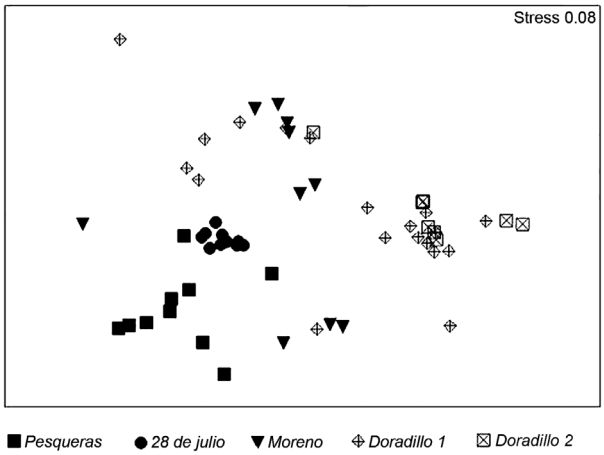

FIG. 3. – Multidimensional scaling diagram (nMDS) for the collection sites on sandy intertidal beaches of Puerto Madryn.

TABLE 2. – R-statistic values and significance levels (between brackets) in pairwaise comparisons among sites using the ANOSIM test. Significant differences in bold characters.

	28 de julio	*Moreno*	*Doradillo 1*	*Doradillo 2*
Pesqueras	**0.836 (0.1)**	**0.504 (0.2)**	**0.57 (0.1)**	**0.938 (0.1)**
28 de julio		0.348 (0.7)	0.444 (0.2)	**0.922 (0.1)**
Moreno			0.388 (0.1)	**0.545 (0.1)**
Doradillo 1				0.018 (40.3)

chaeta sp. 1*, Paraonides* sp.*, Axiothella* sp. and *B. polybranchia* at *28 de julio*; and Psammodrilidae and *Puelche orensanzi* at *Doradillo 1* and these two taxa with higher abundances at *Doradillo 2*. *Moreno* was characterized by *Paraonides* sp., with low mean abundance compared with the other sites (Table 1).

Environmental variables

Table 3 contains the results of the environmental variables measured at the lower level, which include water and sediment analyses. The collection sites showed a predominance of sandy sediments, except for *Pesqueras*, where the gravel fraction (pebbles) dominated. The organic matter contents were below 1% at all the sites. The lowest values were those from the two *Doradillo* sites (0.5%) and the highest values were from *28 de julio* (0.9%). The dissolved oxygen showed high values, except at *28 de julio*. The minimum redox potential discontinuity value was registered at *Pesqueras* (- 311 mV), which suggests that there are reduced chemical species such as hydrogen sulphide, methane and others at this site. The pH was almost neutral in all cases. The ammonium, nitrite and phosphate concentrations were highest at *Pesqueras*. The ammonium and nitrite concentrations were, respectively, one and two orders of magnitude higher than those from the other sites. Nitrate was higher at *Doradillo 1* and *Pesqueras* (9.0 μM and 5.7 μM respectively). The phosphate concentration was higher at *Pesqueras* (21.4 μM) than at the rest of the sites, while the lowest concentration was found at *Doradillo 1* (4.7 μM).

Relationship between biological and environmental variables

Axis I of the RDA performed with the environmental and biological variables from the lower level (Fig. 4) explained 70% of the variance and axis II explained 21.26%, from a total variance of 91.26%. Temperature, pH, Eh, salinity, percentage of gravel, density, porosity, and nitrite concentration, as well as 15 species were eliminated during the analysis because they explained redundant information. A high concentration of ammonium and phosphate were associated with *Pesqueras*, where *B. poly-*

TABLE 3. – Environmental variable values in the lower level.

	Pesqueras	*28 de julio*	*Moreno*	*Doradillo 1*	*Doradillo 2*
Dissolved Oxygen (%)	40	29.3	37.6	67	39
Temperature (ºC)	20.4	19.2	18.5	17	16.8
pH	7.5	7.6	7.2	7.5	7.5
Eh (mV)	- 311	- 38	- 54	- 25	- 34
Salinity	35.13	34.06	32.33	36.60	29.63
Gravel (%)	61.4	2.4	7.6	2.1	2.4
Sand (%)	38.5	95	92	97.7	97.4
Mud (%)	0.1	2.6	0.4	0.2	0.2
Organic matter (%)	0.7	0.9	0.7	0.5	0.5
Density (g/cm^3)	2.0	1.7	1.8	2.0	1.7
Porosity (%)	18	28	24	20	19
Ammonium (μM)	225.2	30.2	23.4	34.2	54.4
Nitrite (μM)	18.6	0.06	0.03	0.07	0.03
Nitrate (μM)	5.7	0.06	3.35	9.0	1.63
Phosphate (μM)	21.4	14.0	13.4	4.7	12.9

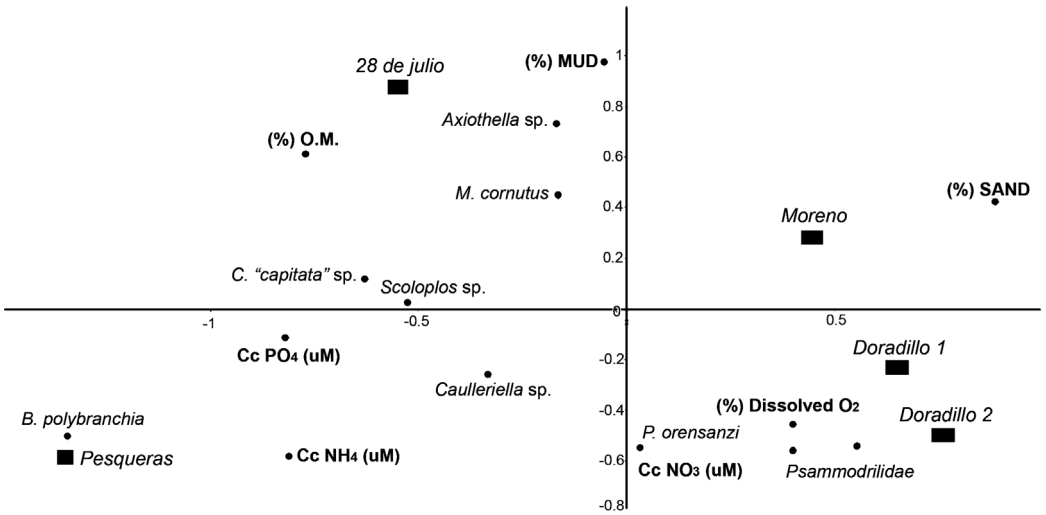

FIG. 4. – Redundancy analysis (RDA) showing collection sites, the main environmental variables and species.

branchia dominated. The site *28 de julio* had high values of organic matter and mud, and *Axiothella* sp. was closely associated with it. *C. "capitata"* sp. was located in an intermediate position between these two sites. High values of nitrate and dissolved oxygen were associated with *Doradillo* beach, with the dominance of Psammodrillid polychaetes and the amphipod *P. orensanzi*. *Moreno* was associated with sand and there were no dominant species.

DISCUSSION

This is the first record of the polychaete family Psammodrilidae Swedmark, 1952 in the South Atlantic. Up to date, five species of this family have been recognized in the North Atlantic and in New Zealand waters, from intertidal to subtidal depths (0-17 m), in clean fine to coarse sand (Worsaae and Sterrer, 2006).

Polychaetes represented the most important taxon of the present study, since they made up 58.33% of the species number and 90.78% of the abundance. Twenty-five years before our study, Escofet (1983) found that this group of organisms represented 70% and 80% of the species number and abundance respectively from the intertidal community of a Puerto Madryn beach (the *28 de julio* site from this study). These percentages are in the same order of magnitude as our data despite the time elapsed between the two sampling periods and the expansive urban development in Puerto Madryn city since the 70s (Esteves *et al*., 1992; Esteves *et al*., 1997; Eyras *et al*., 1999; Gil, 2001). However, polychaetes found in this study are different from those recorded by Pastor de Ward (2000) in the Golfo San José, probably due to the geographic barrier (Fig. 1) and because this zone is not affected by anthropogenic disturbance.

The results from this study suggest that the area has different degrees of pollution based on the analyzed environmental and biological variables. *Pesqueras* showed the highest gravel content (almost 62%) compared with the other collection sites, and had the lowest redox potential value (-311 mV), which indicates the presence of chemical reduced species (Fenchel, 1969). Data analyses indicate that this site is associated with biological and environmental parameters related to pollution, like the dominance of *B. polybranchia* and *C. "capitata"* sp., and high amounts of ammonium and phosphate. This nutrient enrichment can be attributed to the effluents coming from the fishery factory, whose treatment plants do not always function efficiently. Although considerable amounts of organic matter coming from fishery activities are discharged into the environment, at this site the percentage was low (0.7%) due to the coarse nature of the sediments that do not adsorb organic matter.

However, the *28 de julio* site showed the highest diversity as it had the highest species richness, and redox potential discontinuity values as well as the highest organic matter content (0.9%) and percentage of mud (2.6%). This enrichment process was probably the result of a subsurface tap water filtration coming from the black wells and pluvial pipes without any maintenance. These results are similar to those found by Gil (2001), who observed that residual water of Puerto Madryn city could have an

effect on the marine ecosystem. The characteristic species of this site were the detritivorous polychaetes *Axiothella* sp. and *C. "capitata"* sp.

The co-dominance of *B. polybranchia* and *C. "capitata"* sp., as well as the low percentages of organic matter in coarse sediments from *Pesqueras* and *28 de julio* suggest that pollution is not severe enough to consider these areas as "very polluted zones", such as sites with highly organically enriched sediments reported by Reish (1959) in Los Angeles, Bellan (1967a,b) in Marseille, Pearson and Rosenberg (1978) in Loch Eil and Loch Linnhe, Cardell (1996) and Méndez *et al.* (1998) in Barcelona, and Rivero *et al*. (2005) in the Mar del Plata harbour. Therefore, these sites could be defined as "perturbed zones", which are equivalent to the "semi-healthy bottoms" or "polluted zones" as classified by Reish (1959) and Bellan (1967a, b) respectively.

The two sites located at *Doradillo* beach could be considered as "unperturbed zones" like the "healthy bottoms" of Reish (1959) and the "normal zones" of Bellan (1967a, b), due to the high values of dissolved oxygen and nitrates and low values of organic matter, which are not related to polluted conditions. The characteristic species at these two sites were psammodrilid polychaetes and the amphipod *P. orensanzi,* while the pollution indicator species were absent. This beach is located 15 km from the city and no anthropogenic activities are carried out in or around this zone, which explains the results found here.

The nMDS diagram obtained for the *Moreno* site shows that it is located in an intermediate position between the perturbed and unperturbed zones, while the RDA (including the environmental variables) indicates that this site is near the *Doradillo* sites. Although the *Moreno* and *28 de julio* sites are close to pluvial effluents, the differences observed at the two locations may be due to the effect produced by the enriched subsurface tap water filtration in *28 de julio*. Nevertheless, one or more variables that were not measured during the study were probably the main reason for the scarcity of macrobenthos recorded at the *Moreno* site, which could not be characterized based on our results.

Gray and Mirza (1979) pointed out that systems subjected to organic pollution are characterized by the dominance of only a few species. Thus, dominance is a good tool for defining indicator species of different degrees of organic pollution or unperturbed sites in specific areas. The indicator character of *B. polybranchia* and *C. "capitata"* sp. in the perturbed zones was confirmed here. These species have life history patterns that allow their rapid proliferation in available areas (Blake and Ruff, 2007). *B. polybranchia* is widespread in the Southern Hemisphere (Blake, 1983). It was found to be an indicator of intermediate organic pollution in association with an infaunal substrate created by intertidal mussel beds around sewage effluents of Mar del Plata (Elías *et al*., 2003, 2006). Similarly, the *C. "capitata"* species-complex has been considered as a universal pollution indicator when it proliferates in sediments containing high amounts of organic matter (Reish, 1959; Bellan, 1967a, b; Pearson and Rosenberg, 1978; Tsutsumi, 1990; Méndez *et al*., 1998).

The dominance of psammodrilid polychaetes and the amphipod *P. orensanzi* in unperturbed zones suggests that further studies over time are needed to determine whether these taxa are good indicators of clean sediments on Patagonian beaches. This proposal is supported by the fact that several studies performed in North Atlantic and New Zealand waters have found the family Psammodrilidae in clean sediments (Worsaae and Sterrer, 2006).

This study indicates the need for more continuity to better evaluate the health condition of the study zone over time. Thus, we recommend performing multidisciplinary studies using polychaetes to be able to assess the environmental impact produced by anthropogenic activities more easily. The first record of psammodrilids and their dominance observed here shows the importance of this family in the study area; therefore, further taxonomic, molecular and ecological studies should be performed.

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