

ARTICLE

# Spatial changes in the infaunal community of a macrotidal flat in Bahía San Julián, Southern Patagonia, Argentina

Cambios espaciales en la comunidad infaunal de una planicie macromareal en Bahía San Julián, Patagonia Austral, Argentina

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**Resumen.-** El macrobentos intermareal de fondos blandos de Bahía San Julián fue estudiado para identificar las asociaciones infaunales y para analizar su relación con los principales factores ambientales que determinan la distribución de las especies. Se realizaron 4 muestreos estacionales (verano, otoño, invierno y primavera) en los niveles intermareales medio e inferior en 7 estaciones de muestreo establecidas a ambos lados de un sitio de descarga de efluentes urbanos, y se midieron variables del agua y del sedimento. Para explorar las posibles variaciones espaciales en la comunidad, se analizaron los datos ambientales y biológicos utilizando estadística univariada y multivariada. La composición de la comunidad varió con el nivel intermareal, las características del sedimento y la distancia al sitio de descarga de efluentes. En sedimento con alto contenido de fango, la comunidad estuvo caracterizada por *Darina solenoides*, *Mysella patagona*, *Eteone sculpta*, *Scolecopides uncinatus* y *Capitella* sp., en el intermareal medio, y por *M. patagona* y *Ampelisca* sp. en el intermareal inferior. En sedimentos con predominio de arena fina, la comunidad estuvo caracterizada por *Aricidea* sp. en ambos niveles intermareales. Próximo al sitio de descarga de efluentes, la comunidad estuvo ampliamente dominada por *M. patagona*. El nivel intermareal y el tamaño de partícula del sedimento son los principales factores determinantes de la composición de las asociaciones bentónicas en el área de estudio. El vertido de efluentes también afecta la composición de la comunidad infaunal, posiblemente como resultado del aporte de materia orgánica particulada en suspensión, que estimula a las poblaciones de organismos detritívoros.

**Palabras clave:** Comunidades bentónicas, fondos blandos, alteración antrópica, bioindicadores, *Mysella patagona*

**Abstract.-** The soft-bottom macrobenthos of Bahía San Julián intertidal was surveyed to identify infaunal assemblages and analyze their relationships with the main environmental factors determining species distribution. Four seasonal surveys (summer, autumn, winter and spring) were performed in the middle and lower intertidal levels at seven sampling stations established on both sides of an urban sewage discharge point, and water and sediment variables were measured. To explore possible spatial variations in the community, environmental and biological data were analyzed using univariate and multivariate statistics. Community composition varied with intertidal level, sediment characteristics and distance to sewage discharge site. In sediment with high mud content, the community was characterized by *Darina solenoides*, *Mysella patagona*, *Eteone sculpta*, *Scolecopides uncinatus* and *Capitella* sp., in the middle intertidal, and was characterized by *M. patagona* and *Ampelisca* sp. in the lower intertidal. In sediment with dominance of fine sand, the community was characterized by *Aricidea* sp. at both intertidal levels. Near the effluent discharge site, the community was largely dominated by *M. patagona*. Intertidal level and sediment particle size are the main factors determining the composition of benthic assemblages in the study area. Sewage discharge also affects the composition of the infaunal community, possibly as a result of the contribution of particulate organic matter in suspension that stimulates the populations of detritus feeders.

**Key words:** Benthic communities, soft bottoms; anthropogenic disturbance, bioindicators, *Mysella patagona*

## INTRODUCTION

The distribution of benthic communities in tidal flats has been studied both in temperate and tropical regions (*e.g.*, Beukema 1976, 1989; Armonies & Hellwig-Armonies 1987, Reise, 1991, Dittmann 2000, 2002; Dittmann & Vargas 2001). Several studies suggest that tidal height, time of exposure, and substrate characteristics, such as particle size composition of sediment, are the main factors determining composition and distribution of benthic communities in these environments (*e.g.*, Day *et al.* 1989, Brown & McLachlan 1990, Peterson 1991, Dittmann 2000, Glockzin & Zettler

2008). At a small scale, the intertidal faunal assemblages are also structured and regulated by interactions between species (Reise 1985, 1991; Vargas 1996).

Infaunal communities are also structured by the anthropogenic environmental alterations (Grassle & Grassle 1974, Pearson & Rosenberg 1978, Zajac & Whitlatch 1982). Changes in abundance and dominance of benthic species in response to anthropogenic disturbances can be analyzed and used to assess the degree of impact on the marine ecosystem. Accordingly, benthic communities are world-renowned biological indicators of the degree of

impact of human activities on coastal environments (e.g., Anger 1977, Hily *et al.* 1986, Reish 1986). The responses of benthic communities to a certain process of environmental degradation vary between geographical areas, depending on the specific composition of the natural community, the biological and ecological characteristics of the species, and the environmental conditions.

Among macrobenthic organisms, polychaetes are the most frequently used as indicators of environmental disturbance, because they are often the numerically dominant taxon, including both sensitive and tolerant species in a gradient from pristine to heavily disturbed habitats (Grassle & Grassle 1974, Pearson & Rosenberg 1978, Pocklington & Wells 1992). Some species, mainly belonging to the Capitellidae, Cirratulidae and Spionidae families, have become virtually synonymous with bioindicators of organic enrichment, such as *Capitella capitata* (Fabricius, 1780) or *Boccardia polybranchia* (Haswell, 1885), being opportunistic species that commonly reach great abundances in high organic content sediments (Zajac & Whitlatch 1982, Martin & Grémare 1997, Giangrande *et al.* 2005). Other groups of macrobenthos, such as molluscs and crustaceans, have been less frequently used as indicators of organic enrichment, because opportunistic strategies are less common in these groups or have been much less studied. There are, however, some examples of bivalves that profit from organic enrichment and that have been used as bioindicators of moderate or severe anthropogenic disturbances, such as *Mya arenaria* Linnaeus, 1758, *Limecola balthica* (Linnaeus, 1758), *Macoploma tenta* (Say, 1834), *Mulinia lateralis* (Say, 1822) and *Mytilus edulis* Linnaeus, 1758 (Grassle & Grassle 1974, Pearson & Rosenberg 1978).

Benthic communities in tidal flats from southern Patagonia have been scarcely studied. Some works described benthic macroinvertebrate assemblages in Río Gallegos estuary (Argentina) as well as temporal changes in species composition and their relationship with anthropogenic activities (Lizarralde & Pittaluga 2011, Pittaluga 2016, Lizarralde *et al.* 2017). Another work characterized the intertidal macrobenthic community associated with the presence of mytilids in Bahía San Julián (Zaixso *et al.* 2017). In addition, there are reports from the Strait of Magellan (Chile) that contribute to the knowledge of intertidal assemblages in the region (Espoz *et al.* 2008, Cañete *et al.* 2010).

Along the Patagonian coast, fishing and port activities, as well as mining, have increased in recent decades, resulting in a significant urban and industrial development. Therefore, the human impact in the coastal environment has also increased, with three main sources of disturbance having been identified: fishing industry effluents, urban sewage discharges and harbor contamination related to transportation, loading and unloading of goods and fuels

(Commendatore *et al.* 1996, Estévez *et al.* 1997). Despite this, studies on the effect of anthropogenic disturbances on coastal benthic communities are scarce in Patagonia (Cuevas *et al.* 2006, Ferrando *et al.* 2010) and are virtually nonexistent in the southern end of this region (Lizarralde & Pittaluga 2011).

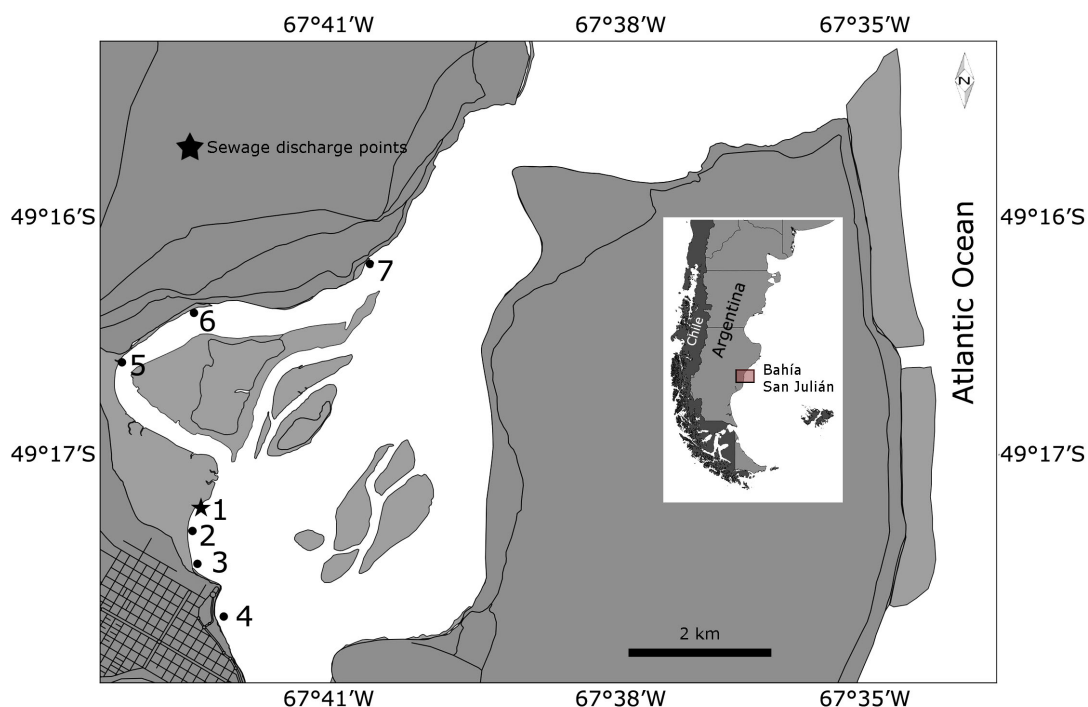
In the present study, spatial variability of soft-bottom macrobenthic community was analyzed in a tidal flat of Southern Patagonia affected by urban sewage discharge, by identifying faunal assemblages and analyzing their relationships with the main environmental factors determining species distribution. This study contributes information about a benthic intertidal community that has been little studied so far, and analyzes the response of macroinfauna to anthropogenic disturbances. This information allows establishing a baseline for future surveys in order to assess the impact generated by wastewater discharge in the coastal ecosystem of the region.

## MATERIALS AND METHODS

### STUDY AREA

Bahía San Julián is located on the central coast of Santa Cruz Province (Southern Patagonia, Argentina) between 67°50'01" and 67°35'50"W and 49°00'12" and 49°47'02"S (Fig. 1). It is a deep inlet of the sea about 20 km long and 8.8 km in maximum width, with a relatively narrow mouth approximately 700 m wide. The bay has a maximum depth of 35 m, and includes a wide shallow terminal area (sac). It receives cold waters of subantarctic origin and others with relatively low salinity from the Patagonian Coastal Current, a branch of the Malvinas (Falkland) Current. Water salinity inside the bay is between 33 and 34 and temperature varies between 5 °C (winter) and 14 °C (summer). The tidal regime is of a semidiurnal macrotidal type, with maximum amplitude of 8.93 m and an average of 6.15 m (Falabella *et al.* 2009, Servicio de Hidrografía Naval 2010)<sup>1</sup>. The coast of the bay is dominated by plains formed mainly by mud (silt+clay) and fine sand, with important extensions of intertidal mudflats. The mudflats are dominated by saltmarshes of *Sarcocornia perennis* in upper intertidal and by infaunal communities dominated by small bivalves and polychaetes in the middle and lower intertidal. Some sectors, with higher proportions of gravel, favor the development of intertidal mussel beds of *Perumytilus purpuratus* (Lamarck, 1819), *Mytilus platensis* d'Orbigny, 1842 and *Aulacomya atra atra* (Molina, 1782) (Martin *et al.* 2015, Zaixso *et al.* 2017).

<sup>1</sup>Servicio de Hidrografía Naval. 2010. Tablas de Marea de Puerto San Julián. <[http://www.hidro.gov.ar/oceanografia/Tmareas/Form\\_Tmareas.asp](http://www.hidro.gov.ar/oceanografia/Tmareas/Form_Tmareas.asp)>



**Figure 1. Study area and location of sampling stations. Distance between stations and the sewage discharge point: station 1 < 3 m, station 2= 500 m, station 3= 900 m, station 4= 1,300 m, station 5= 1,500 m, station 6= 2,500 m, station 7= 3,500 m / Área de estudio y ubicación de las estaciones de muestreo. Distancia entre las estaciones y el punto de descarga del efluente: estación 1 < 3 m, estación 2= 500 m, estación 3= 900 m, estación 4= 1.300 m, estación 5= 1.500 m, estación 6= 2.500 m, estación 7= 3.500 m**

The city of Puerto San Julián, with about 15,000 inhabitants, is located on the northwestern coast of the bay. This city has a plant of sewage treatment that does not work efficiently, so that only the coarser solids are separated. Effluents (about 1,000 to 1,500 m<sup>3</sup> day<sup>-1</sup> between 2009 and 2011) of virtually untreated sewage, with a high load of organic suspended solids, flow through a channel crossing the intertidal flat inside the bay to discharge into the sea water. Moreover, Bahía San Julián is part of a protected area, including Cormorán and Justicia Islands, under a protection regime of limited use; the Peninsula of San Julián was also declared a provincial reserve (Chébez 2005).

#### SAMPLING AND DATA ANALYSIS

Benthic infauna of Bahía San Julián was sampled in lower and middle intertidal levels at seven sampling stations distributed on both sides of the sewage discharge point (Fig. 1). Stations 5 and 6 were located in front of Cormorán Island, on a secondary channel through which water enters and leaves the bay in each tidal cycle.

Four seasonal surveys were conducted in April, August and November 2010, and February 2011, corresponding to autumn, winter, spring and summer in the southern hemisphere. The intertidal flat was divided into three levels according to the tidal height. The lower intertidal level (between 0 and 2 m above low tide line) and middle intertidal level (between 2 and 4 m above low tide line)

were determined using a clinometer and a metric measuring tape, taking the low tide line as a reference (Servicio de Hidrografía Naval 2010)<sup>1</sup>. The position of sampling sites on the shore was fixed, at each station and intertidal level, using a GPS (Garmin GPSMAP 76 CSx). Four replicates were collected per level at each sampling station for each seasonal survey (totaling 224 samples). Samples were taken from the sediment with a corer of 10 cm in diameter (0.00785 m<sup>2</sup>) up to 15 cm in depth. The organisms were separated using a 0.5-mm mesh sieve and fixed in 5% formaldehyde for subsequent identification and quantification. The organisms were identified and counted under a stereoscopic microscope, and then preserved in 70% alcohol. Physical and chemical parameters of water (pH, turbidity, dissolved oxygen, temperature and salinity) were measured using a multiparameter probe (Horiba U-10) during the low tide, and sediment samples were taken to determine particle size and organic matter content.

Sediment particle size was analyzed by the wet sieving method using a metal sieve column after treating the sample with a solution of sodium hexametaphosphate (NaPO<sub>3</sub>)<sub>6</sub> (6.2 g L<sup>-1</sup>) to aid dispersion of clay particles (Bale & Kenny 2005). The total sample was dried in an oven to constant weight at 70 °C, and weighed before sieving. The different sediment fractions were pooled in gravel (4-2 mm), coarse sand (2 to 0.5 mm), medium sand (0.5 to 0.25 mm) and fine sand (0.25 to 0.063 mm). The fractions were dried in an oven at 70 °C to constant weight and weighed, and

were expressed as percentage of the dry weight of the total sample. The fraction of mud (silt+clay) was estimated as the difference between dry weight of the total sample and the sum of dry weight of fractions larger than 0.063 mm. Organic matter content of the sediment was determined by mass loss on ignition in a muffle furnace (450 °C to 6 h) (Bale & Kenny 2005). Weight was obtained using an analytical balance (0.1 mg).

For each macroinfaunal sample we calculated total abundance  $N$ , species richness  $S$ , Shannon-Wiener diversity index  $H' = -\sum p_i \log_2 p_i$ , where  $p_i$  is the proportion of the total number of individuals that belong to species  $i$ ; and Pielou evenness  $J = H' / \log_2 S$ . Ecological and environmental data were compared between intertidal levels (Level) and among sampling stations (Station) via two-way ANOVA and Bonferroni *post-hoc* test, using InfoStat statistical package (Di Rienzo *et al.* 2015). Homogeneity of variances was accomplished using logarithmic transformation, and was verified using Levene test. Normal distribution of the data was checked via Shapiro-Wilks test. When data did not fit the normality and homogeneity of variances, they were subjected to a Kruskal-Wallis non-parametric test and Mann-Whitney U tests. Simple-linear regression analyses were plotted to explore relationship between diversity ( $H'$ ), evenness ( $J$ ), species abundance and distance to the wastewater discharge point, and correlation tests were performed by calculating the Spearman's rank-order coefficient ( $r_s$ ). A significance level of  $P < 0.05$  was used in all tests. The sediment variables were analyzed using principal component analysis (PCA); the data were previously transformed using  $\log_{10}(x+1)$  and normalized (Zar 1996, Dytham 1999).

Abundance data were analyzed via a multivariate analysis using hierarchical agglomerative clustering through the Plymouth Routines in Multivariate Ecological Research (PRIMER) statistical package version 6.1 (Clarke & Warwick 2001). The data were previously transformed

using square root to down-weight the effect of dominant species, and then the Bray-Curtis similarity index was applied. The hypothesis of differences in the composition of the community between intertidal levels and among sampling stations were tested using a two-way crossed analysis of similarities (ANOSIM permutation test). The statistical significance of the groups of samples was tested via similarity profile (SIMPROF), and similarity percentages routine (SIMPER) was then used to identify the species that most contributed to spatial differences in the community (Clarke & Warwick 2001).

A canonical correspondence analysis (CCA) was performed to identify the environmental variables that best explain the variation of the abundance data, using CANOCO 5.0 statistical package. The data were previously transformed using  $\log_{10}(x+1)$ . The relationship of abundance of organisms with intertidal level, percentages of fine sand, mud and organic matter in the sediment, distance to sewage discharge point and water temperature was analyzed. To detect significant environmental variables, a Pearson correlation matrix with Bonferroni adjusted probabilities was previously calculated. The significant variables were selected to perform the CCA analysis. The significance of all primary CCA axes was determined by Monte Carlo permutation test (ter Braak & Smilauer 1998).

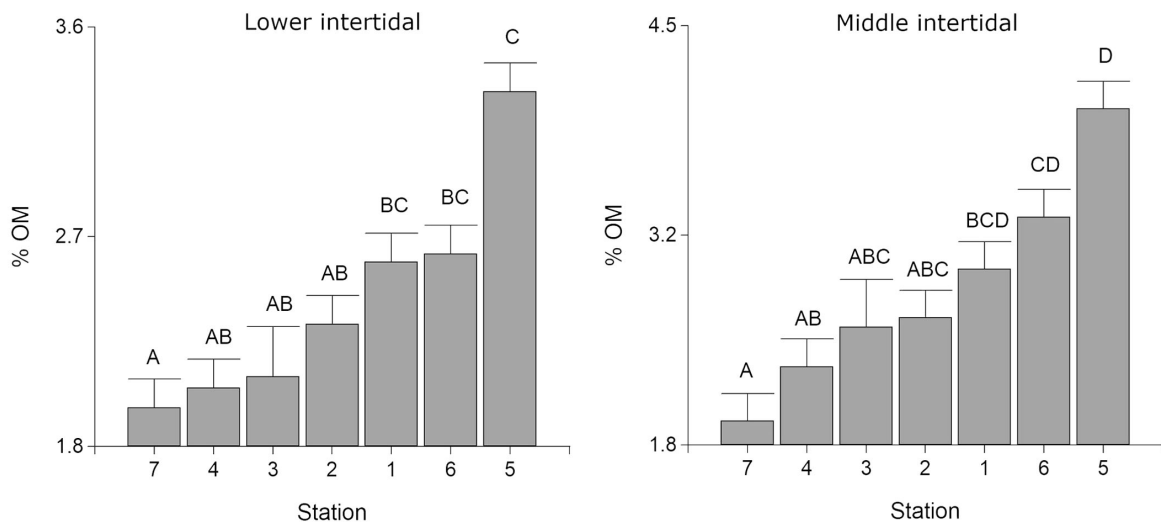
## RESULTS

### ENVIRONMENTAL VARIABLES

The substrate in the intertidal flat was dominated by mud (silt+clay) and fine sand, which together accounted for almost 100% of the sediment at all sampling stations (Table 1). Organic matter content ranged between 2 and 3.27% at the lower intertidal and between 2.10 and 4.23% at the middle intertidal, reaching highest values at station 5 (Fig. 2).

**Table 1. Location of sampling stations and sediment composition / Ubicación de las estaciones de muestreo y composición del sedimento**

Level	Station	Lat. (S)	Long. (W)	Gravel (%)	Coarse sand (%)	Medium sand (%)	Fine sand (%)	Mud (%)
Lower Intertidal	1	49° 17' 31.5''	67° 43' 22.3''	0	0.16	0.11	50.07	49.66
	2	49° 17' 40.6''	67° 43' 27.5''	0.15	0.12	0.12	53.41	46.19
	3	49° 17' 53.7''	67° 43' 24.5''	0	0.06	0.03	54.52	45.40
	4	49° 18' 14.7''	67° 43' 08.4''	0.03	0.15	0.38	60.24	39.21
	5	49° 16' 33.4''	67° 44' 10.6''	0	0.04	0.41	31.83	67.72
	6	49° 16' 13.7''	67° 43' 26.6''	0.08	0.42	0.69	48.71	49.93
	7	49° 15' 54.2''	67° 41' 39.0''	0	0.13	0.09	71.56	28.22
Middle Intertidal	1	49° 17' 31.2''	67° 43' 22.6''	0	0.05	0.13	50.30	49.53
	2	49° 17' 40.5''	67° 43' 28.9''	0	0.03	0.04	31.38	68.55
	3	49° 17' 54.0''	67° 43' 25.4''	0.20	0.24	0.10	39.95	59.52
	4	49° 18' 14.7''	67° 43' 09.4''	0.04	0.04	0.30	51.72	47.91
	5	49° 16' 33.1''	67° 44' 11.1''	0.06	0.27	1.07	27.92	70.68
	6	49° 16' 13.1''	67° 43' 27.0''	0.03	0.03	0.62	31.72	67.61
	7	49° 15' 53.5''	67° 41' 38.1''	0.03	0.03	0.25	62.96	36.73



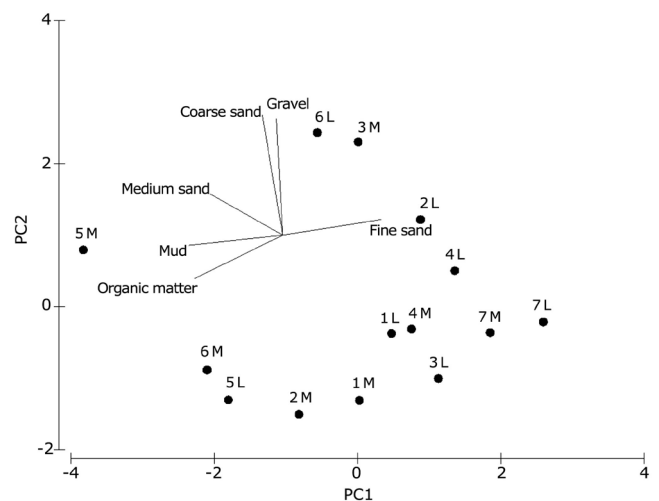
**Figure 2. Organic matter (OM) content in the sediment. Stations with the same letter are not different (Bonferroni *post hoc* test,  $P > 0.05$ ) / Contenido de materia orgánica (OM) en el sedimento. Las estaciones con la misma letra no mostraron diferencias (prueba *post hoc* de Bonferroni,  $P > 0,05$ )**

Two-way ANOVA showed significant differences in organic matter content between intertidal levels (Level) and among sampling stations (Station) (Level:  $F_{(1, 41)} = 20.64$ ,  $P < 0.001$ ; Station:  $F_{(6, 41)} = 28.73$ ,  $P < 0.001$ ; Interaction Level x Station:  $F_{(6, 41)} = 0.89$ ,  $P = 0.52$ ). The highest values were observed at station 5 and the lower values at station 7 in both intertidal levels, although the differences were not always significant among all stations (Fig. 2).

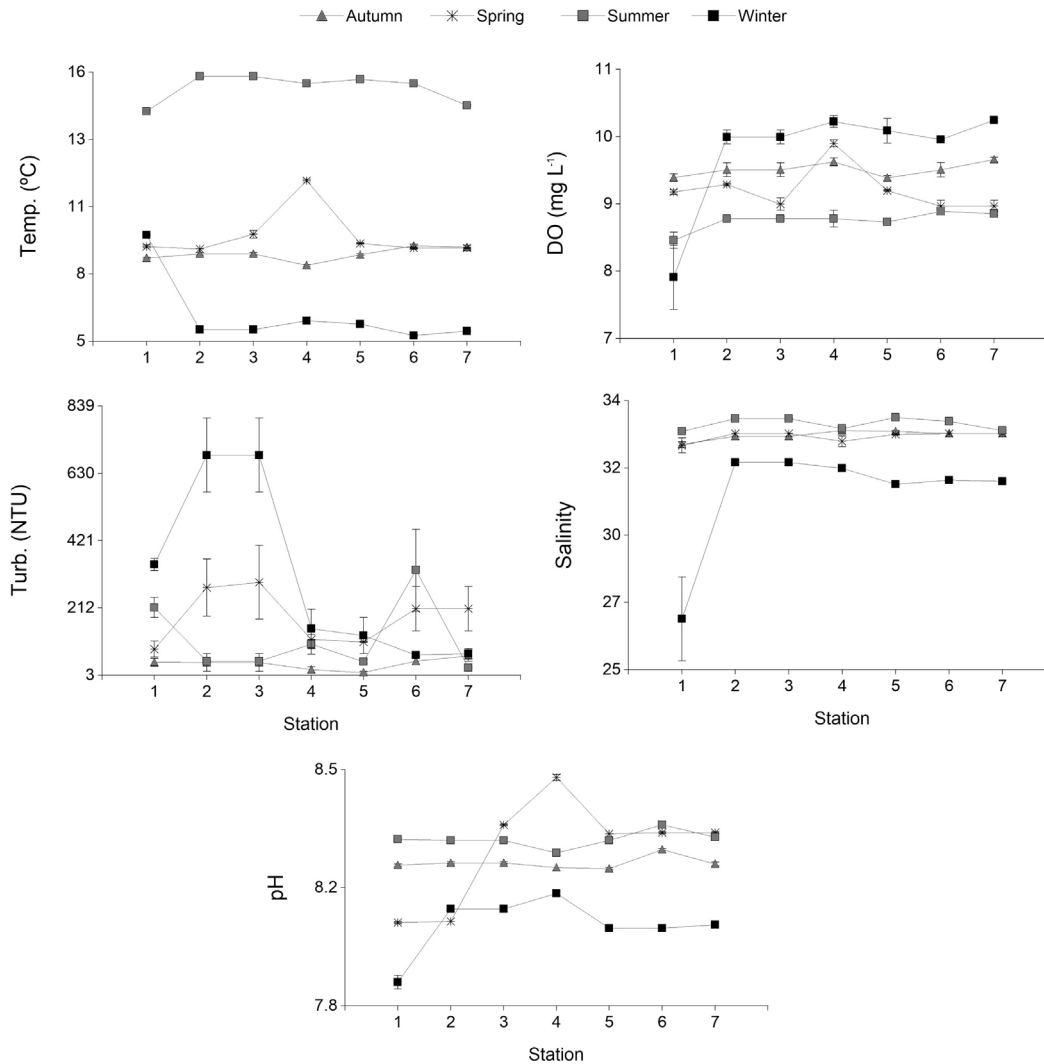
Principal component analysis (PCA) showed spatial variations in sediment composition; PC1 and PC2 axes explained more than 76% of the variance in the data. PC1 axis corresponded to the greatest variations in the substrate, related to the content of mud, fine sand and organic matter. The PCA biplot (Fig. 3) shows an array of sampling stations, with station 7 being located at the end, corresponding to dominance of fine sand, and station 5 of the middle intertidal at the opposite end, where the content of mud and organic matter increases. Stations 1, 2, 3, 4 and 6 were located at intermediate positions. A clearly positive correlation was observed between organic matter content and percentage of mud in the sediment ( $r = 0.78$ ,  $P < 0.001$ ).

Water temperature ranged from 5.4 °C in winter to 16 °C in summer during the study period. At station 1, it was up to 4 °C higher during winter and up to 1.5 °C lower in summer than in the remaining sampling stations.

Turbidity did not show a marked pattern, but proved to be somewhat higher in stations 1, 2 and 3 during the winter. Dissolved oxygen and salinity at station 1 were lower than at the other sampling stations during winter, and water pH at station 1 was lower than in the remaining stations during winter and spring (Fig. 4).



**Figure 3. PCA biplot of sediment variables in sampling stations. M, middle intertidal level; L, lower intertidal level. PC1: eigenvalue= 2.93, explained cumulative variation= 48.8%; PC2: eigenvalue= 1.62, explained cumulative variation= 76.3% / Diagrama de PCA bidimensional de las variables del sedimento en las estaciones de muestreo. M, nivel intermareal medio; L, nivel intermareal inferior. PC1: autovalor= 2,93, variación explicada acumulada= 48,8%; PC2: autovalor= 1,62, variación explicada acumulada= 76,3%**



**Figure 4.** Mean values (±sd) of water variables measured in sampling stations in the different seasons. Temp.: temperature; DO: dissolved oxygen; Turb.: turbidity; NTU: nephelometric turbidity unit / Valores promedio (±ds) de las variables del agua medidas en las estaciones de muestreo en las diferentes épocas del año. Temp.: temperatura; DO: oxígeno disuelto; Turb.: turbidez; NTU: unidades nefelométricas de turbidez

### INEFAUNAL COMMUNITY STRUCTURE

The infaunal community was mainly composed of bivalve molluscs, mostly represented by *Mysella patagona* Ituarte, Martín & Zelaya, 2012 and *Darina solenoides* (King, 1832), gammarid amphipods, with *Ampelisca* sp. and *Monocorophium insidiosum* (Crawford, 1937) being the most abundant, and different polychaetes, with *Scolecoplepides uncinatus* Blake, 1983, *Eteone sculpta* Ehlers, 1897, *Capitella* sp., *Aricidea* sp. and *Gymnonereis fauveli* (Hartmann-Schröder, 1962) being the most abundant (Tables 2 and 3).

Two-way ANOVA showed significant differences for evenness (J), species richness (S) and total abundance (N) between intertidal levels (Level) and among sampling stations (Station). Significant differences were observed for

diversity (H') among sampling stations, but no differences were observed between intertidal levels. The interaction between Level and Station was highly significant in all cases, because changes among sampling stations showed different trends according to intertidal level (Table 4).

The diversity (H') and the evenness (J) at the lower intertidal showed their highest values at stations 4, 5, 6 and 7. Their lowest values were observed at station 1. At the middle intertidal no significant differences were detected in these parameters among sampling stations (Fig. 5, Table 4). The species richness (S) was higher at stations 1 and 6 for both intertidal levels; however, these stations only showed statistically significant differences from station 2, 3 and 4 at the lower level, and from stations 2 and 4 at the middle level. The total abundance (N) at the lower level

**Table 2. Average abundance (individuals per sample) of the species at the lower intertidal level / Abundancia promedio (individuos por muestra) de las especies en el nivel intermareal inferior**

	Lower intertidal						
	1	2	3	4	5	6	7
Nematoda	0.81	0.19	0.25	1.88	4.88	5.08	6.44
Nemertea	0.69	0.19	0	0.19	0.19	0.17	0
Cirratulidae indet. (Pol.)	2.13	0.06	0.25	0.19	0	4.92	0.25
Syllidae indet. (Pol.)	0.5	0.13	0.25	0.06	8.38	13	0.94
<i>Capitella</i> sp. (Pol.)	0.69	0.69	0	0.13	0.19	1	7.25
<i>Mediomastus</i> sp. (Pol.)	5.94	1.69	0.25	0.75	0.69	1.42	1.31
<i>Clymenella minor</i> (Pol.)	0.06	0	0	0	0.13	1.67	0.19
<i>Scolecopides uncinatus</i> (Pol.)	1.69	1.69	0.75	1.5	0.56	5.58	1.5
<i>Lumbrineris magalhaensis</i> (Pol.)	1.63	2.5	0	2.56	0.25	1.08	0.75
Onuphidae indet. (Pol.)	0.56	0.5	0	0.19	0.06	5.67	0.63
<i>Eteone sculpta</i> (Pol.)	0.94	0.38	0.75	0.13	0.5	0.83	0.13
<i>Gymnonereis fauveli</i> (Pol.)	1.13	0.13	1.75	0.38	3.81	22.42	0.44
<i>Ophioglycera eximia</i> (Pol.)	0.06	0	0.75	0.44	0.38	0.25	0.06
<i>Hemipodus patagonicus</i> (Pol.)	0.56	0.13	0.25	0.25	0.38	0.33	0.31
<i>Aglaophamus</i> sp. (Pol.)	0	0	0	0.25	0.06	0	0.19
<i>Aricidea</i> sp. (Pol.)	0.25	0	0	0.19	0.13	0.58	11.38
Oligochaeta	0.88	0.25	0	0.13	3.81	14	0.5
<i>Darina solenoides</i> (Biv.)	10.5	5.25	8.25	2.75	8.81	0.75	2.13
<i>Mysella patagona</i> (Biv.)	232.94	42.69	47.75	11.5	59.13	25.75	2.75
<i>Linucula pisum</i> (Biv.)	0	0	0	0	0	0.42	0
<i>Ampelisca</i> sp. (Amph.)	42.19	66.81	2.75	12.38	17	70.92	3.31
<i>Heterophoxus</i> sp. (Amph.)	1.31	0.13	0	0.13	3.56	15.08	0
<i>Monocorophium insidiosum</i> (Amph.)	0.06	0	0	0.06	69.75	0.17	0.13
Tanaidacea	0	0	0	0	2.38	0.17	0
Cumacea	0	0	0.25	0	0	0.33	0
<i>Priapulus tuberculatoespinosus</i> (Priap.)	0.13	0	0	0	0.25	0	0

Pol., Polychaeta; Biv., Bivalvia; Amph., Amphipoda; Priap., Priapulida

**Table 3. Average abundance (individuals per sample) of the species at the middle intertidal level / Abundancia promedio (individuos por muestra) de las especies en el nivel intermareal medio**

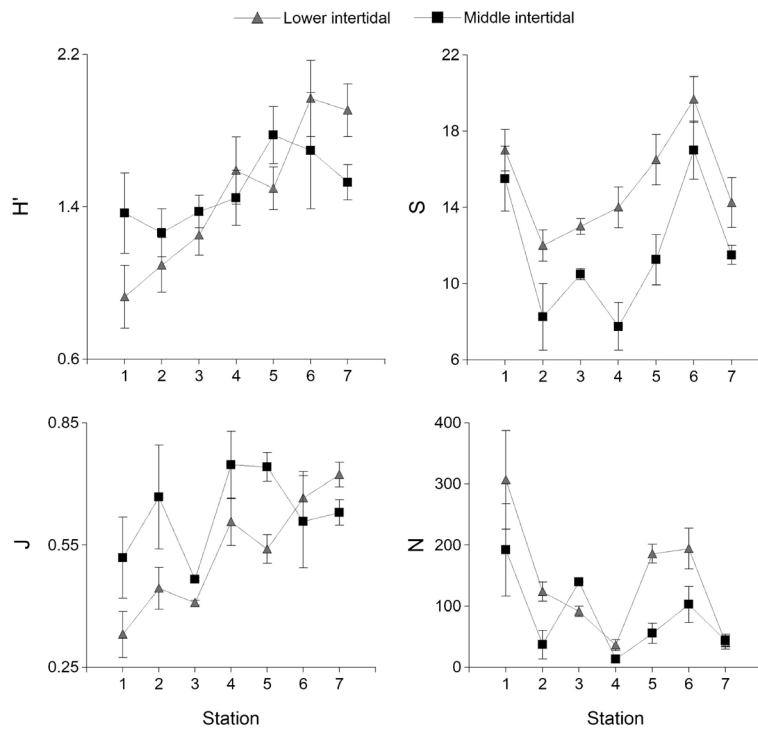
	Middle intertidal						
	1	2	3	4	5	6	7
Nematoda	1.44	0	0	0.44	2.06	0.58	0.69
Nemertea	3.13	0.31	2.5	0.19	3.44	1.58	0.19
Cirratulidae indet. (Pol.)	0.19	0	0	0	0	0.08	0.25
Syllidae indet. (Pol.)	1.5	0.06	0.25	0	0.69	10.33	1.69
<i>Capitella</i> sp. (Pol.)	5.56	5.31	6.75	1.56	5.75	1.58	1.13
<i>Mediomastus</i> sp. (Pol.)	0.56	0	0	0.06	0	0.25	0.25
<i>Clymenella minor</i> (Pol.)	0	0	0	0	0	0.08	0
<i>Scolecopides uncinatus</i> (Pol.)	4	3.06	4	0.38	13.56	2.83	1.75
<i>Lumbrineris magalhaensis</i> (Pol.)	0.19	0.13	0	0.06	0	0	0.94
Onuphidae indet. (Pol.)	0.38	0.25	1.25	0.5	0.25	4.58	2.63
<i>Eteone sculpta</i> (Pol.)	5.44	2.13	17.5	1.13	14.75	6.08	2.31
<i>Gymnonereis fauveli</i> (Pol.)	0.44	0.06	0	0.13	0.06	2.08	0.06
<i>Ophioglycera eximia</i> (Pol.)	0.13	0	0	0	0	0.75	0.06
<i>Hemipodus patagonicus</i> (Pol.)	0.06	0.06	0	0	0	0	0.13
<i>Aglaophamus</i> sp. (Pol.)	0	0	0	0	0.13	0.08	3.44
<i>Aricidea</i> sp. (Pol.)	0.94	0.13	0	0	0.31	1.25	15.31
Oligochaeta	11.69	0	0	0.06	0.06	0.42	0.13
<i>Darina solenoides</i> (Biv.)	23.81	24.31	99.75	6.5	13.31	8.5	12
<i>Mysella patagona</i> (Biv.)	132.06	0.63	6.75	1	0.25	56.42	0.19
<i>Linucula pisum</i> (Biv.)	0	0.31	0	0	0	0.17	0
<i>Ampelisca</i> sp. (Amph.)	0.25	0.13	0	0.19	0	0.5	0
<i>Heterophoxus</i> sp. (Amph.)	0.13	0.13	0	0	0.06	4	0
<i>Monocorophium insidiosum</i> (Amph.)	0	0	0	0	0.19	0	0
Cumacea	0.06	0	0	0	0	0	0
<i>Priapulus tuberculatoespinosus</i> (Priap.)	0.19	0	0.25	0.06	0.19	0.33	0

Pol., Polychaeta; Biv., Bivalvia; Amph., Amphipoda; Priap., Priapulida

**Table 4. Two-way ANOVA and Bonferroni *post-hoc* test of diversity ( $H'$ ), evenness ( $J$ ), species richness ( $S$ ) and total abundance ( $N$ ) values between intertidal levels (Level) and among sampling stations (Station). L, lower level; M, middle level / ANOVA de dos vías y prueba *post-hoc* de Bonferroni de la diversidad ( $H'$ ), la equitatividad ( $J$ ), la riqueza de especies ( $S$ ) y la abundancia total ( $N$ ) entre niveles intermareales (Level) y entre estaciones de muestreo (Station). L, nivel inferior; M, nivel medio**

		F	P	Bonferroni <i>post-hoc</i> test	
				Lower level	Middle level
$H'$	Level	2.61	0.11		
	Station	14.60	< 0.001*	1=2=3<4=5=6=7	1=2=3=4=5=6=7
	Interaction	0.06	< 0.001*		
$J$	Level	16.42	< 0.001*	L<M	
	Station	14.45	< 0.001*	1=2=3<4=5=6; 1<7	1=2=3=4=5=6=7
	Interaction	5.25	< 0.001*		
$S$	Level	66.82	< 0.001*	L>M	
	Station	37.20	< 0.001*	2=3=4<1=5=7<6	2=4<1=3=5=7<6
	Interaction	3.28	0.005*		
$N$	Level	12.73	< 0.001*	L>M	
	Station	21.71	< 0.001*	4=7<2=3=5=6<1	2=4=5=6=7<1=3
	Interaction	3.17	0.006*		

\* significant values  $P < 0.05$



**Figure 5. Mean values ( $\pm$ sd) of diversity ( $H'$ ), evenness ( $J$ ), species number ( $S$ ) and total abundance ( $N$ ) of infaunal community / Valores promedio ( $\pm$ ds) de la diversidad ( $H'$ ), la equitatividad ( $J$ ), el número de especies ( $S$ ) y la abundancia total ( $N$ ) de la comunidad infaunal**

was significantly higher at station 1, 2, 3, 5 and 6 than at stations 4 and 7, whereas at the middle level, stations 1 and 3 showed significantly higher abundance than the remaining sampling stations (Fig. 5, Table 4).

Two-way ANOSIM showed highly significant differences in community composition between intertidal levels (Global  $R = 0.73$ ,  $P = 0.001$ ) and among sampling stations (Global  $R = 0.62$ ,  $P = 0.001$ ). The SIMPER routine identified species

contributing most to differences observed between intertidal levels (Table 5). *Mysella patagona*, *Ampelisca* sp. and *Monocorophium insidiosum* were much more abundant at the lower intertidal than at the middle intertidal. Conversely, *Darina solenoides*, *Eteone sculpta*, *Scolecopides uncinatus* and *Capitella* sp. were more abundant at the middle intertidal than at the lower one.



**Table 5. Results of SIMPER analysis showing the species that most largely contributed to dissimilarity between intertidal levels / Resultados del análisis SIMPER que muestra las especies que más contribuyeron a la disimilitud entre los niveles intermareales**

Species/Intertidal level	Av. Abund.		Cont. %	Cumul. %
	Lower	Middle		
<i>Mysella patagona</i>	6.28	3.01	17.50	17.50
<i>Ampelisca</i> sp.	4.80	0.22	14.68	32.18
<i>Darina solenoides</i>	1.82	3.52	7.82	40.00
<i>Eteone sculpta</i>	0.53	2.03	5.21	45.22
<i>Capitella</i> sp.	0.76	1.71	4.36	49.58
<i>Aricidea</i> sp.	0.80	0.99	4.35	53.94
<i>Scolecopides uncinatus</i>	1.21	1.62	3.84	57.78
<i>Monocorophium insidiosum</i>	1.37	0.04	3.82	61.60
Syllidae	1.21	0.82	3.72	65.32
Nematoda	1.33	0.67	3.65	68.97
Oligochaeta	1.01	0.69	3.48	72.45

Av. Abund., Average abundance (transformed); Cont. %, contribution percentage of each species to dissimilarity; Cumul. %, cumulative contribution percentage to dissimilarity. Cut off for 70% cumulative contribution

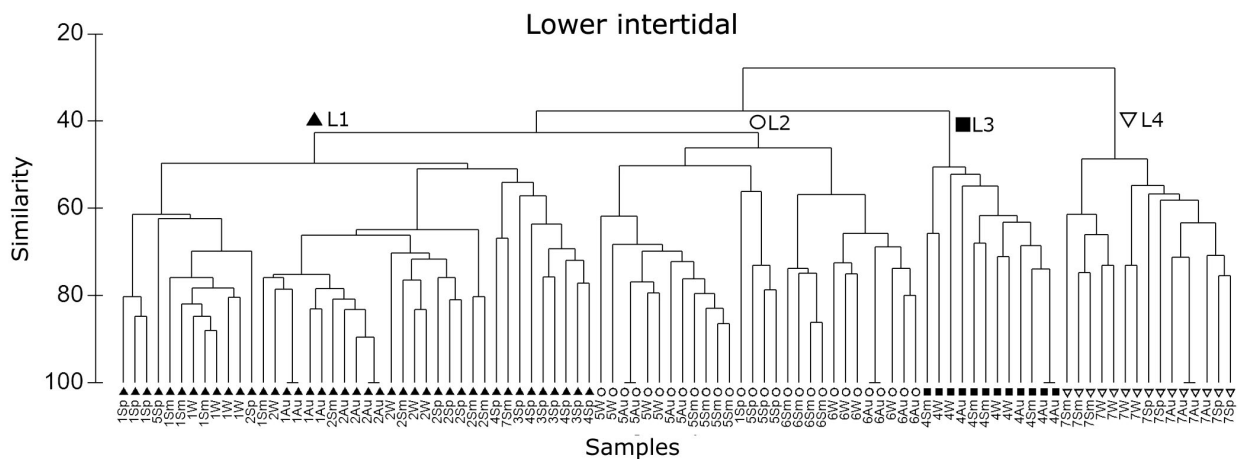
A more detailed analysis was performed for each intertidal level. Community composition varied significantly among sampling stations at the lower intertidal level, and was more significant between station 1 and the stations located at intermediate and the longest distances from the wastewater discharge site (Table 6). Station 7 showed marked differences from the remaining sampling stations at this level. At the middle intertidal, station 1 exhibited the greatest difference from station 7 and the smallest difference from station 6. No significant differences were observed in

community composition among stations 2, 3, 4 and 5 at this intertidal level (Table 6).

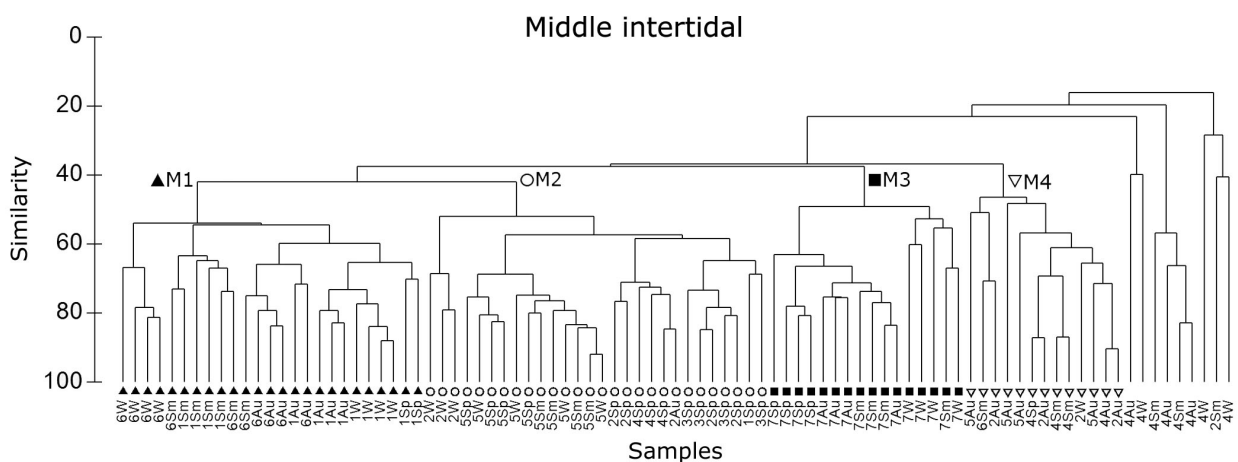
The clustering analysis identified four main sample groups at each intertidal level with similarities higher than 45% between samples (SIMPROF  $P_i = 2.37$ ,  $P = 0.001$  for lower intertidal;  $P_i = 3.18$ ,  $P = 0.001$  for middle intertidal) (Figs. 6 and 7). The species that most contributed to the similarity among samples within these groups for both intertidal levels were identified using the SIMPER routine (Tables 7 and 8).

**Table 6. Results of one-way ANOSIM among sampling stations / Resultados de ANOSIM entre estaciones de muestreo**

Pairwise tests stations	Lower intertidal Global R= 0.775, $P = 0.001$		Middle intertidal Global R= 0.493, $P = 0.001$	
	R	P	R	P
1-2	0.423	0.001	0.576	0.001
1-3	0.717	0.001	0.675	0.001
1-4	0.735	0.001	0.580	0.001
1-5	0.783	0.001	0.670	0.001
1-6	0.765	0.001	0.444	0.001
1-7	0.949	0.001	0.858	0.001
2-3	0.807	0.001	-0.034	48.1
2-4	0.479	0.001	0.076	6.6
2-5	0.927	0.001	0.216	0.2
2-6	0.882	0.001	0.541	0.001
2-7	0.938	0.001	0.511	0.001
3-4	0.503	0.001	0.125	18.2
3-5	0.850	0.001	0.272	9.6
3-6	0.995	0.001	0.773	0.001
3-7	0.941	0.001	0.708	0.001
4-5	0.898	0.001	0.342	0.001
4-6	0.909	0.001	0.478	0.001
4-7	0.811	0.001	0.499	0.001
5-6	0.792	0.001	0.673	0.001
5-7	0.963	0.001	0.589	0.001
6-7	0.923	0.001	0.820	0.001



**Figure 6. Dendrogram of hierarchical agglomerative clustering of lower intertidal samples, based on square root transformation of abundance data, Bray-Curtis similarity index and group average linkage. L1, L2, L3 and L4 are groups of samples with similarities >45%. \_Sm, station number-summer; \_Au, station number-autumn; \_W, station number-winter; \_Sp, station number-spring / Dendrograma de agrupamiento aglomerativo jerárquico de muestras del intermareal inferior, basado en la transformación raíz cuadrada de los datos de abundancia, el índice de similitud Bray-Curtis y ligamiento promedio. L1, L2, L3 y L4 son grupos de muestras con similitudes >45%. \_Sm, número de estación-verano; \_Au, número de estación-otoño; \_W, número de estación-invierno; \_Sp, número de estación-primavera**



**Figure 7. Dendrogram of hierarchical agglomerative clustering of middle intertidal samples, based on square root transformation of abundance data, Bray-Curtis similarity index and group average linkage. M1, M2, M3 and M4 are groups of samples with similarities >45%. \_Sm, station number-summer; \_Au, station number-autumn; \_W, station number-winter; \_Sp, station number-spring / Dendrograma de agrupamiento aglomerativo jerárquico de muestras del intermareal medio, basado en la transformación raíz cuadrada de los datos de abundancia, el índice de similitud Bray-Curtis y ligamiento promedio. L1, L2, L3 y L4 son grupos de muestras con similitudes >45%. \_Sm, número de estación-verano; \_Au, número de estación-otoño; \_W, número de estación-invierno; \_Sp, número de estación-primavera**

**Table 7. Results of SIMPER analysis showing the species that most largely contributed to similarity between samples for lower intertidal / Resultados del análisis SIMPER que muestra las especies que más contribuyeron a la similitud entre las muestras para el intermareal inferior**

	Av. abundance	Cont. %	Cumul. %
Group L1: Average similarity: 56.85			
<i>Mysella patagona</i>	9.22	41.94	41.94
<i>Ampelisca</i> sp.	5.66	26.78	68.72
<i>Darina solenoides</i>	2.00	8.65	77.37
<i>Lumbrineris magalhaensis</i>	1.18	6.07	83.44
<i>Mediomastus</i> sp.	1.45	5.73	89.17
<i>Scolecopides uncinatus</i>	1.01	4.66	93.83
Group L2: Average similarity: 54.47			
<i>Mysella patagona</i>	5.75	20.55	20.55
<i>Ampelisca</i> sp.	5.32	17.32	37.87
Syllidae	2.78	9.85	47.72
<i>Heterophoxus</i> sp.	2.52	8.76	56.48
<i>Gymnoreris fauveli</i>	2.68	8.61	65.10
<i>Monocorophium insidiosum</i>	4.20	8.58	73.68
Nematoda	2.03	7.72	81.40
Oligochaeta	2.02	5.20	86.60
<i>Darina solenoides</i>	1.72	3.01	89.61
<i>Scolecopides uncinatus</i>	1.11	2.29	91.90
Group L3: Average similarity: 57.96			
<i>Ampelisca</i> sp.	3.67	44.58	44.58
<i>Lumbrineris magalhaensis</i>	1.64	20.96	65.54
<i>Mysella patagona</i>	1.28	10.79	76.33
<i>Scolecopides uncinatus</i>	0.94	8.14	84.47
<i>Darina solenoides</i>	0.74	4.53	89.00
Nematoda	0.67	3.14	92.13
Group L4: Average similarity: 55.42			
<i>Aricidea</i> sp.	3.30	36.70	36.70
<i>Capitella</i> sp.	2.41	18.28	54.99
<i>Darina solenoides</i>	1.23	9.71	64.70
<i>Scolecopides uncinatus</i>	1.03	7.82	72.52
Nematoda	1.72	6.30	78.82
<i>Mediomastus</i> sp.	0.89	5.72	84.54
<i>Ampelisca</i> sp.	1.21	4.68	89.21
<i>Mysella patagona</i>	0.89	3.11	92.33

Average abundance (transformed); Cont. %, contribution percentage of each species to similarity; Cumul. %, cumulative contribution percentage to similarity. Cut off for 95% cumulative contribution

At lower intertidal level, Group L1, composed of all samples from stations 1, 2 and 3 and by spring samples from station 4, and Group L2, composed of all samples from stations 5 and 6, were characterized by *Mysella patagona* and *Ampelisca* sp. Group L3, composed of samples collected in autumn, summer and winter from station 4, was characterized by *Ampelisca* sp., *Lumbrineris magalhaensis* and *Mysella patagona*. Group L4, comprising only the samples from station 7, was characterized by *Aricidea* sp. and *Capitella* sp.

At the middle intertidal level, the four main sample groups shared *Darina solenoides*, *Eteone sculpta*, *Scolecopides uncinatus* and *Capitella* sp., as abundant and constant species. However, Group M1, composed of samples from stations 1 and 6, was characterized by *Mysella patagona*, whereas Group M3, composed only of samples from station 7, was characterized by *Aricidea* sp.

**Table 8. Results of SIMPER analysis showing the species that most largely contributed to similarity between samples for middle intertidal / Resultados del análisis SIMPER que muestra las especies que más contribuyeron a la similitud entre las muestras para el intermareal medio**

	Av. abundance	Cont. %	Cumul. %
Group M1: Average similarity: 59.20			
<i>Mysella patagona</i>	8.03	37.54	37.54
<i>Darina solenoides</i>	3.56	16.78	54.33
<i>Eteone sculpta</i>	2.23	11.52	65.85
<i>Capitella</i> sp.	1.79	8.54	74.39
<i>Scolecopides uncinatus</i>	1.43	5.58	79.97
Syllidae	1.78	5.04	85.01
Oligochaeta	1.89	4.89	89.90
<i>Aricidea</i> sp.	0.75	2.34	92.24
Group M2: Average similarity: 60.83			
<i>Darina solenoides</i>	5.39	35.38	35.38
<i>Scolecopides uncinatus</i>	2.92	20.78	56.16
<i>Capitella</i> sp.	2.56	20.46	76.61
<i>Eteone sculpta</i>	3.02	19.74	96.35
Group M3: Average similarity: 58.90			
<i>Aricidea</i> sp.	4.21	46.09	46.09
<i>Darina solenoides</i>	2.98	21.31	67.40
<i>Capitella</i> sp.	1.32	9.30	76.70
<i>Scolecopides uncinatus</i>	1.18	7.10	83.79
Onuphidae	1.23	6.50	90.29
Group M4: Average similarity: 36.74			
<i>Darina solenoides</i>	1.53	50.07	50.07
<i>Eteone sculpta</i>	0.93	19.51	69.58
<i>Capitella</i> sp.	0.75	14.94	84.52
Onuphidae	0.54	8.45	92.97

Average abundance (transformed); Cont. %, contribution percentage of each species to similarity; Cumul. %, cumulative contribution percentage to similarity. Cut off for 95% cumulative contribution

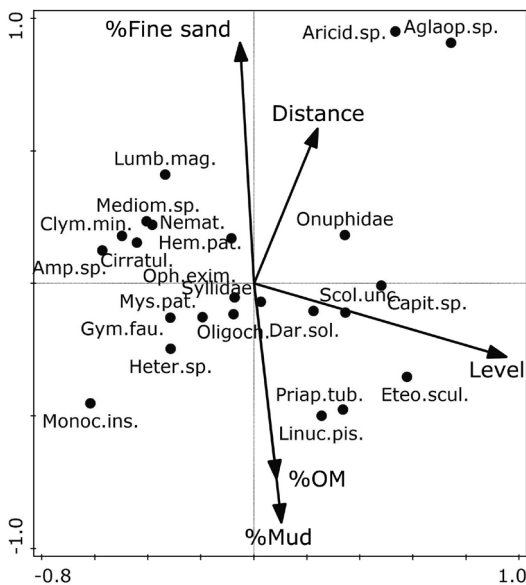
#### RELATIONSHIP BETWEEN INFAUNAL COMMUNITY AND ENVIRONMENTAL FACTORS

The relationship between species abundance and environmental variables was explored using a canonical correspondence analysis (CCA). The variables selected to perform the CCA analysis were intertidal level ( $P < 0.001$ ), percentage of fine sand ( $P < 0.001$ ), percentage of mud (silt+clay) ( $P < 0.001$ ), distance to sewage discharge point ( $P < 0.001$ ) and percentage of organic matter in sediment ( $P = 0.006$ ). Temperature was not a significant environmental factor ( $P = 0.8$ ).

The results of CCA analysis showed that 43.44% of the cumulative variance in species abundance was accounted for by the first four ordination axes (Table 9). The CCA biplot (Fig. 8) summarized the variation in species distribution explained by environmental variables. The first ordination axis indicates that intertidal level had the greatest effects on the distribution of species. The second ordination axis reflected a gradient mostly related to sediment characteristics, such as percentage of fine sand, mud and organic matter content. The distance to sewage discharge point also showed an effect, although less important, on species occurrence.

**Table 9. Summary of canonical correspondence analysis (CCA) between species and environmental variables**  
/ Resumen del análisis canónico de correspondencia (CCA) entre especies y variables ambientales

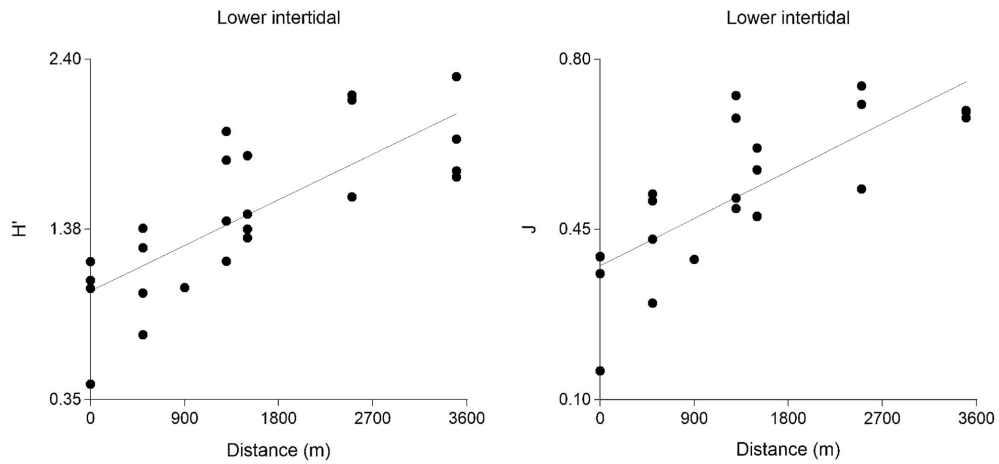
Axes	1	2	3	4	Total inertia
Eigenvalues	0.24	0.12	0.10	0.06	1.20
Explained cumulative variation	19.64	29.94	38.32	43.44	
Species-environment correlation	0.87	0.89	0.85	0.82	
Test of significance					
Axis 1: $F= 10.3, P < 0.001$					
All canonical axes: $F= 6.9, P < 0.001$					



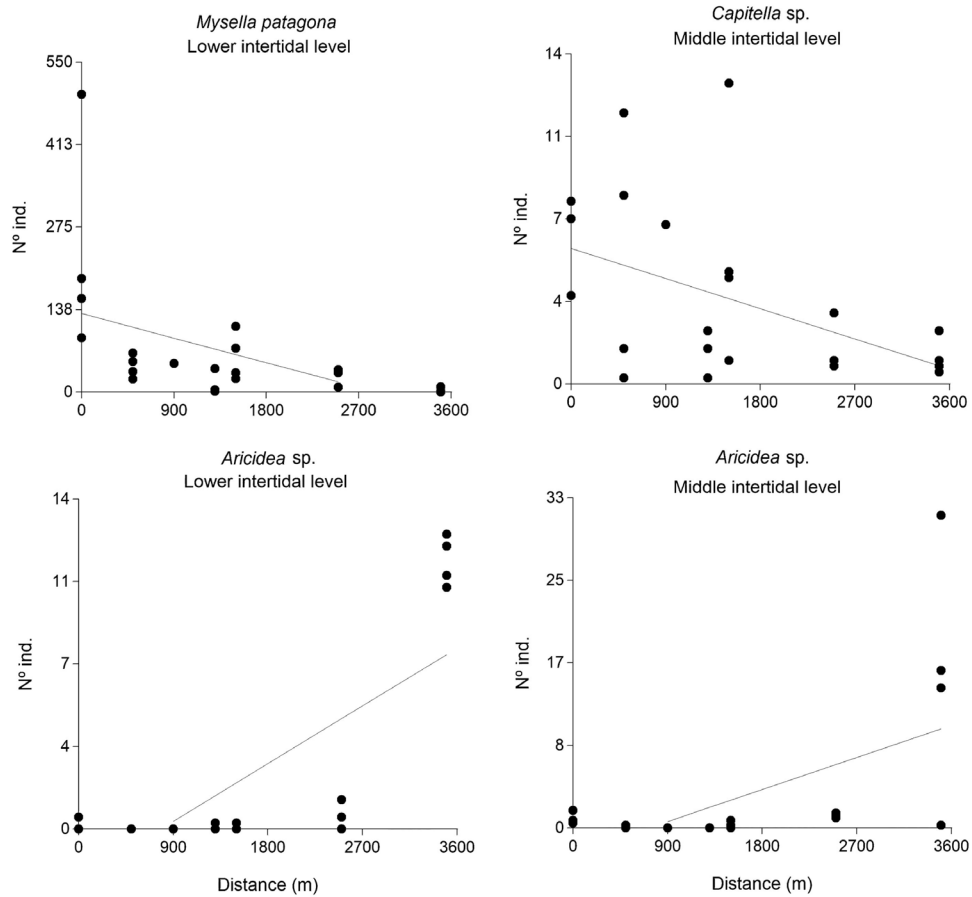
**Figure 8. CCA biplot performed on species abundance and environmental variables. Level: intertidal level; Distance: distance to sewage discharge point; %OM: organic matter percentage. Aricid.sp.: *Aricidea* sp.; Aglaop.sp.: *Aglaophamus* sp.; Lumb.mag.: *Lumbrineris magalhaensis*; Mediom.sp.: *Mediomastus* sp.; Clym.min.: *Clymenella minor*; Nemat.: Nematoda; Hem.pat.: *Hemipodus patagonicus*; Cirratul.: Cirratulidae; Amp.sp.: *Ampelisca* sp.; Oph.exim.: *Ophioglycera eximia*; Mys.pat.: *Mysella patagona*; Oligoch.: *Oligochaeta*; Gym.fau.: *Gymnonereis fauveli*; Heter.sp.: *Heterophoxus* sp.; Monoc.ins.: *Monocorophium insidiosum*; Scol.unc.: *Scolecopides uncinatus*; Capit.sp.: *Capitella* sp.; Dar.sol.: *Darina solenoides*; Priap.tub.: *Priapulus tuberculatoespinosus*; Eteo.scul.: *Eteone sculpta*; Linuc.pis.: *Linucula pisum* / Diagrama bidimensional de CCA de abundancia de especies y variables ambientales. Level: nivel intermareal; Distance: distancia al punto de descarga del efluente; %OM: porcentaje de materia orgánica**

Species mainly associated with sediment with higher content of mud and organic matter of the lower intertidal level, were located in the lower left quadrant of the CCA biplot. Species most linked to sediment with higher content of mud and organic matter of the middle intertidal level were located in the lower right quadrant, and species linked to sediment with higher proportion of fine sand of the lower intertidal level were in the upper left quadrant. Species in the upper right quadrant were associated with sediments with a higher content of fine sand and that were farther from the wastewater discharge point (Fig. 8).

Simple-linear regression analyses showed that diversity ( $H'$ ) and evenness ( $J$ ) had a significant positive correlation with the distance to sewage discharge site at the lower intertidal level ( $r_s = 0.80, n = 28, P < 0.001$  for  $H'$ ;  $r_s = 0.80, n = 28, P < 0.001$  for  $J$ ) (Fig. 9), but did not show correlation at the middle intertidal level. *Mysella patagona*, *Capitella* sp. and *Aricidea* sp. were the only species whose abundance was related to distance to wastewater discharge site (Fig. 10). *M. patagona* abundance showed a significant negative correlation with distance to discharge site only at the lower intertidal level ( $r_s = -0.69, n = 28, P < 0.001$ ) and *Capitella* sp. abundance showed a significant negative correlation only at the middle intertidal level ( $r_s = -0.49, n = 28, P = 0.014$ ). *Aricidea* sp. abundance was positively correlated with distance to discharge site at the lower intertidal ( $r_s = 0.62, n = 28, P = 0.001$ ), and at the middle intertidal level ( $r_s = 0.42, n = 28, P = 0.038$ ).



**Figure 9. Regression of diversity ( $H'$ ) and evenness ( $J$ ) against distance to wastewater discharge site in the lower intertidal level / Regresión entre la diversidad ( $H'$ ) y la equitatividad ( $J$ ) y la distancia al sitio de descarga del efluente en el nivel intermareal inferior**



**Figure 10. Regression of species abundance (number of individuals per sample, average among replicates) against distance to wastewater discharge site / Regresión entre la abundancia de las especies (promedio del número de individuos por muestra entre réplicas) y la distancia al sitio de descarga del efluente**

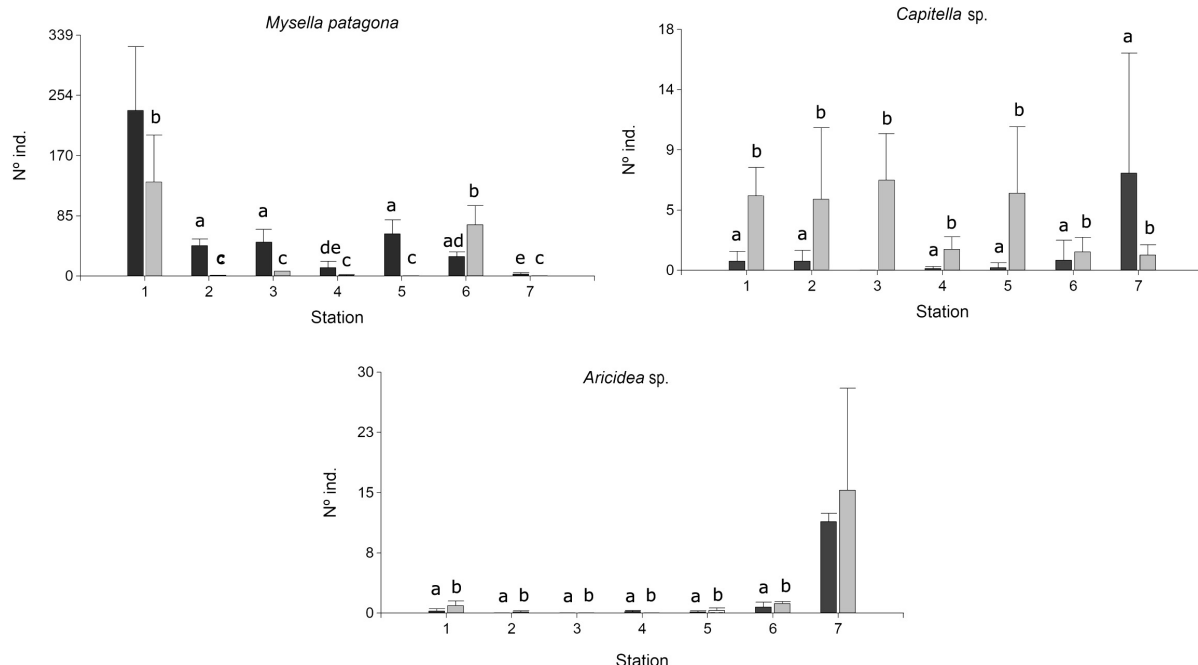
*Mysella patagona* reached its greatest abundance near the effluent discharge site in both intertidal levels. Its abundance at station 1 in the lower intertidal was significantly higher than that observed in the other sampling stations (Kruskal-Wallis  $H= 17.98$ ,  $P= 0.006$ ). In the middle intertidal, *M. patagona* abundance at station 1 showed significant differences from stations 2, 3, 4, 5 and 7 (Kruskal-Wallis  $H= 17.20$ ,  $P= 0.007$ ) (Fig. 11). *Capitella* sp. had greatest abundance at stations 1, 2, 3 and 5 of the middle intertidal; however, no significant differences were observed among sampling stations in either intertidal level (Kruskal-Wallis  $H= 10.56$ ,  $P= 0.07$  for lower intertidal;  $H= 10.3$ ,  $P= 0.11$  for middle intertidal), and *Aricidea* sp. was significantly more abundant at station 7, both in the lower and middle intertidal levels (Kruskal-Wallis  $H= 14.26$ ,  $P= 0.015$  for lower intertidal;  $H= 17.86$ ,  $P= 0.004$  for middle intertidal) (Fig. 11).

## DISCUSSION

The infaunal community in the intertidal flat of Bahía San Julián showed spatial changes in composition, mainly related to intertidal level, sediment characteristics and distance to sewage discharge site. At the lower intertidal, the community was characterized by the assemblage of *Mysella patagona* and *Ampelisca* sp. in substrates with high proportion of mud. Other species present in this assemblage are *Darina solenoides*, *Lumbrineris magalhaensis*, Syllidae,

*Gymnonereis fauveli* and *Heterophoxus* sp. In sediment with a dominance of fine sand of the lower intertidal level, the community was characterized by the assemblage of *Aricidea* sp. and *Capitella* sp. At the middle intertidal, the infaunal community was characterized by *D. solenoides*, *Eteone sculpta*, *Scolecopides uncinatus* and *Capitella* sp., among the most abundant and constantly present species in this level. This assemblage was dominated by *M. patagona* in sediment with high proportion of mud and by *Aricidea* sp. in sediment with a dominance of fine sand.

These results partially agree with previous observations in Río Gallegos estuary, in the south of Santa Cruz (Argentina) by Lizarralde & Pittaluga (2011), and with records reported by Espoz *et al.* (2008) for sand-mud intertidal flats of the Strait of Magellan (Chile), which exhibits very similar sediment characteristics to those in our study area and where a macrobenthic community dominated by *Darina solenoides* was described. Among the most abundant companion species, these authors mentioned the polychaetes *Eteone sculpta* and *Scolecopides uncinatus*, which were also recorded in the present work, but did not mention the presence of *Mysella patagona* and *Capitella* sp. Espoz *et al.* (2008) also indicate a high abundance of *Aricidea* sp. for the sand-mud intertidal dominated by fine sand of Bahía Lomas in the Strait of Magellan. *Capitella* sp. has been recorded for other locations in the Strait of Magellan, in marshes affected by sewage discharges (Cañete *et al.* 2010).



**Figure 11.** Abundance of species that presented a relationship with the distance to wastewater discharge site (average number of individuals per sample  $\pm$ sd). Comparison between stations for each intertidal level. Stations with the same letter are not different (Mann-Whitney U test,  $P > 0.05$ ) / Abundancia de las especies que presentaron correlación con la distancia al sitio de descarga del efluente (número promedio de individuos por muestra  $\pm$ ds). Comparación entre estaciones para cada nivel intermareal. Las estaciones con la misma letra no mostraron diferencias (Test U de Mann-Whitney,  $P > 0,05$ )

*Darina solenoides-Scolecoplepides uncinatus-Eteone sculpta* assemblage is a characteristic in the sand-mud intertidal bottoms in the southernmost region of Patagonia; however, the dominance of *Mysella patagona* observed in benthic assemblages of Bahía San Julián has not been recorded in other intertidal flats of the region to date. *Mysella patagona* Ituarte, Martin & Zelaya, 2012 was first described in the last years, with its type locality being Bahía San Julián. The species was first found in Bahía San Julián on sand-mud bottoms of the lower intertidal (Ituarte *et al.* 2012) and later on the tidal flat of the Río Gallegos estuary (Pittaluga 2016), but it has not still been recorded farther north of Bahía San Julián or in southern Patagonia in Chile.

Spatial changes in the infaunal community of Bahía San Julián related to distance to the wastewater discharge site were also observed, being more pronounced in the lower intertidal level. At the station closest to the discharge point, the community was largely dominated by *Mysella patagona*, mainly at the lower intertidal level. At this sampling station, the community also had the lowest diversity and evenness values, which were significantly lower than those of the most remote stations at the lower intertidal. In addition, the clear positive correlation between the diversity and evenness values of the community and the distance to the wastewater discharge point suggest that there could be environmental alterations related to the sewage discharge that affect the benthic community at the lower intertidal.

The measured water parameters suggest that near the effluent discharge site, the infaunal community of Bahía San Julián could be exposed to changes in environmental conditions, since discharge of waste waters would seem to affect temperature, dissolved oxygen concentration, turbidity and salinity. The effect of the effluent seems to be limited to a short distance from the point of discharge, since the parameters measured are normalized at the stations closest to station 1. However, the measurements made in this study are not conclusive and further measurements of the water parameters should be made at different tidal levels to confirm these observations.

No clear gradient was observed in the organic matter content of the sediment, which correlated directly with the percentage of mud. Near the dumping site, organic matter in the sediment was higher than that found in the most distant station (station 7); however, it was lower than organic matter content at station 5, located approximately 1,500 m away from the discharge site. Station 5 also presented a great proportion of mud; hence, the high organic matter content detected would be related to the high proportion of fine sediment. These results would evidence attenuated hydrodynamic energy in the secondary channel formed between Cormorán Island and the coast of the bay that favors deposition of fine particles and organic detritus at this site.

Although there are no other important anthropogenic sources of organic matter in Bahía San Julián, it should be noted that the upper intertidal level of the bay is occupied by a well-developed saltmarsh dominated by *Sarcocornia perennis* that covers almost the entire substrate. The saltmarshes have long been recognized as highly productive plant communities in the world, which make an important contribution of nutrients to tidal flats through detritus export (Little 2000, McLusky & Elliott 2004). Then, an important contribution of organic matter from the saltmarsh to the macrotidal flat of the bay can be expected. Given this important natural source, the contribution of organic detritus from Puerto San Julián sewage, of moderate flow rate, could be masked.

On the other hand, the macrotidal regime of Bahía San Julián generates strong tidal currents that reduce deposition of detritus contributed by the effluent, scattering them all across and outside the bay as suspended particulate matter. Measurements made after this study showed that particulate organic matter content in the water near the discharge site was 6 times higher than that found in the most distant stations towards the mouth of the bay (station 1=  $37.1 \pm 5.73$  mg L<sup>-1</sup>; station 6=  $6.01 \pm 3.14$  mg L<sup>-1</sup>, Martin unpublished data), indicating an important contribution of suspended organic detritus at station 1. This fact could explain the observed relationship between *Mysella patagona* abundance and the distance to the wastewater discharge point. This species significantly increased its abundance near the effluent discharge point and reached its highest value (up to 63,184 ind. m<sup>-2</sup>) at station 1 in the lower intertidal. This fact suggests that this species could benefit from suspended particulate organic matter contributed by the wastewater discharge, using it as an extra source of food to sustain the observed high abundance.

The geographical distribution of *Mysella patagona* is still not fully known; however, its link to anthropogenic sources of organic enrichment has been indicated for other intertidal flats of the region, such as the Río Gallegos estuary (Pittaluga *et al.* 2013, Pittaluga 2016). The genus *Mysella* (Galeommatoidae) comprises a group of small bivalves of only a few millimeters in size, many of which live buried in soft bottoms. Many of these bivalves feed on organic detritus deposited in the sediment and are species highly specialized in brooding (Reid *et al.* 1992, Domaneschi *et al.* 2002, Passos *et al.* 2005, Passos & Domaneschi 2006, 2009).

*Mysella charcoti* (Lamy, 1906) from Antarctica, for example, is an infaunal species that inhabits the upper millimeters of muddy sediments enriched with organic matter (Passos *et al.* 2005). According to Reid *et al.* (1992), *M. bidentata* (Montagu, 1803) feeds on detritus in the sediment by a 'sweep pedal-feeding', which would allow it to capture food particles through anterior inhalant-pedal

opening. Although this behavior would not have been observed in *M. charcoti*, Passos *et al.* (2005) indicate that this species could benefit from living buried in areas where there is a diatom film on the sediment surface and flocculent ooze deposited on the water-sediment interface. In these areas, *M. charcoti* may profit from this source of potential food. Although no study of gut contents has been performed, *M. charcoti* likely derives part of its food from such labile settled organic material, with the anterior inhalant current driving this deposited material into the mantle cavity.

*Mysella narchii* Passos & Domaneschi, 2006, also from Antarctica, shows a similar behavior to that described by Passos *et al.* (2005) for *M. charcoti*. The activity of the foot and its respective ciliature is involved in the burrowing process only, while the anterior-posterior inhalant current drives water and fine sediments into the mantle cavity. Deposit-feeding, however, may switch to a suspension-feeding behavior whenever the specimens hang attached by byssal threads to firm substrata and immersed in water (Passos & Domaneschi 2006). Likewise, *M. patagona* in Bahía San Julián might feed on organic detritus and associated microfauna near the sewage discharge point, consuming both the deposited detritus and organic particles suspended on the water-sediment interface.

The ability to incubate embryos, also observed in *Mysella patagona* (Ituarte *et al.* 2012), gives advantages for reproduction and development in a changing environment, where physical and chemical characteristics of disturbed sediment could be fatal for free larvae. On the other hand, the holobenthic life cycles (with absence of planktonic stages) allow species to keep the larvae in the adult population area and, through benthic larvae, this species could achieve rapid recruitment and accelerated population growth in the disturbed area, at the expense of extra organic matter. This strategy has been shown for other opportunistic species (Wilson 1986, Méndez 1995, Bhaud & Duchêne 1996, Méndez *et al.* 2000, Martin & Bastida 2006).

*Mysella patagona* is also present in the tidal flats of estuarine areas of the region, such as the estuaries of the Río Gallegos (Lizarralde & Pittaluga 2011, Pittaluga *et al.* 2013) and Río Santa Cruz (Martin unpublished data), indicating the relatively large degree of tolerance of this species to changes in environmental parameters such as salinity and temperature. This euryoic feature confers the species with a greater advantage to colonize areas of unpredictable characteristics and changing conditions. It suggests a certain capacity of this species to settle and colonize disturbed environments, taking advantage of the increased supply of food available in organic-enriched areas.

Based on the characteristics of *Mysella* and the observations made in this study, we suggest that *M. patagona* could be a relatively opportunistic species, as proposed by Pearson & Rosenberg (1978) to define those

species able to colonize rapidly and become dominant in areas subject to disturbance. It could also be classified as a tolerant species, based on criteria to order the soft-bottom macrofauna according to the sensitivity to increasing stress gradient, summarized by Grall & Glémarec (1987). Tolerant species may occur under normal conditions, but their populations are stimulated by organic enrichment. Further studies are necessary to understand aspects related to the feeding mode and life history, such as population growth rate, mortality rate, life span, reproduction mode and dispersal strategies of this species.

In the areas of the bay more distant from the point of effluent discharge, where attenuated hydrodynamic energy allows increased mud deposition, *Mysella patagona* was second in abundance after other organisms that can exploit the organic detritus, such as *Monocorophium insidiosum* at station 5. This gammarid amphipod is a cosmopolitan species usually present in high densities in biofouling of harbors, and often associated with organic enrichment. *M. insidiosum* builds tubes of mud and detritus that fastens together using mucus threads; therefore, its higher abundance at station 5 could be related to the higher proportion of mud in the sediment. The conditions of low energy and high hydrodynamic stability, facilitating the deposition of sludge and organic detritus, could also generate favorable conditions for this amphipod that feeds on sediment particulate matter.

The abundance of *Capitella* sp., a widely recognized opportunistic polychaete, also showed a significant negative correlation with the distance to the sewage discharge site in this study. However, this polychaete was found in relatively low abundance in the infaunal community of Bahía San Julián, even at the effluent discharge site and at those sites with higher organic matter content. It is important to note that not all species of the *Capitella* 'capitata' complex exhibit the same degree of opportunistic behavior. In addition, other studies have indicated that *C. capitata* can be present at low abundances or absent at sites where the community is relatively diverse, presumably as a result of competition or predation (Grassle & Grassle 1974, 1976; Pearson & Pearson 1991). In Argentina, this species can also be found in low and relatively stable abundance throughout the year at intertidal flats subject to a moderate impact by organic enrichment in the Río de la Plata estuary (Martin *et al.* 2004, Martin & Bastida 2006). This could also be the case for Bahía San Julián, where the community at the dumping site was composed of a relatively high number of species, some of which, as in the case of *Mysella patagona*, could compete for food with *Capitella* sp. The poor competitive ability of this polychaete may result from shortage of the most labile food components. A species such as *C. capitata* may digest only the parts of the sediment that may be most readily converted to tissue to sustain the high growth rate that is observed in opportunistic species. Thus, this species



would be more severely limited by competition than others (Grassle & Grassle 1976, Tsutsumi & Kikuchi 1984).

The general environmental conditions near the wastewater discharge site into the Bahía San Julián were sufficiently favorable for most species of the community, whereas the extra supply of organic detritus from the effluent could stimulate the community by increasing the number of species and overall abundance. This effect was noted by several authors and is due to the increased supply of food in relatively good environmental conditions, which reduces interspecific competition and allows the coexistence of more species in the community (Pearson & Rosenberg 1978, McLusky & Elliott 2004). At this site, a relatively high number of infaunal species coexist, some of which, detritus feeders particularly benefited from the excessive food supply, become very abundant. As a consequence, the sharp increase in *Mysella patagona* abundance produces a decrease in the values of diversity and evenness observed at a short distance from the Puerto San Julián dumping site.

Intertidal level height and sediment particle size, determined by the hydrological characteristics of the system, are the main factors driving the composition and distribution of benthic assemblages in the study area. Environmental alterations, product of urban effluents discharge, have also an effect on the composition of the infaunal community. This effect is mainly observed in the lower intertidal near the point of discharge of the wastewater, possibly as a result of the contribution of particulate organic matter in suspension that stimulates the populations of detritus feeders.

This work allows establishing a baseline for future studies in order to assess the impact generated by Puerto San Julián wastewater discharge in the ecosystem of the bay. More studies on the biology and ecology of *Mysella patagona* should be conducted to confirm its opportunistic or tolerant characteristics. If the usefulness of this species as indicator of organic enrichment is confirmed, the evolution of environmental quality and degree of disturbance in Bahía San Julián and other intertidal flats of the region could be monitored through the abundance of this species along with the structure of the infaunal community and its relationship with environmental variables.

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