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Baseline

Assessing sewage impact in a South-West Atlantic rocky shore intertidal algal community

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ABSTRACT

The spatial and seasonal variation of the specific composition and community parameters (abundance, diversity, richness and evenness) of the intertidal algal assemblages was studied at four coastal sampling sites, distributed along an environmental gradient from the sewage water outfall of Mar del Plata, Buenos Aires, Argentina. Two of them were located close to the sewage outfall (<800 m) (impacted area) and the two other were 8 and 9 km distant (non-impacted area). The algal abundance was monthly analyzed from October 2008 to May 2009. The algal assemblages varied according to the pollution gradient in spring, summer and autumn, being autumn the season when the highest difference was observed. *Ceramium uruguayense* was recognized as an indicator species for the non-impacted areas, while *Berkeleya* sp. represented an indicator species for the sewage outfall impact. *Ulva* spp. did not reflect the typical pattern observed for other sewage pollution areas.

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The structure of intertidal macroalgal communities changes along the coastline in relation to environmental salinity gradients, wave action, and the slope and texture of substrates (Lobban et al., 1985). Human activity impacts on benthic macroalgal communities through waste waters, urban runoff or chemicals spilled in coastal areas, which can lead to a decrease in macroalgal species richness and abundance; with a consequent simplification of the community structure (Borowitzka, 1972; Littler and Murray, 1975; Díez et al., 1999). Inversely, the abundance of opportunistic species with high reproductive capacity and a wide tolerance range to pollution is expected to increase (Murray and Littler, 1978; Fairweather, 1990; Gorostiaga and Díez, 1996; Soltan et al., 2001; Dongyan et al., 2007). Contamination by sewage outfall is the main anthropogenic stressor in many intertidal macroalgal communities of rocky shores around the globe (Borowitzka, 1972; Littler and Murray, 1975; López Gappa et al., 1990, 1993; Díaz et al., 2002; Arévalo et al., 2007; Dongyan et al., 2007). High nitrogen input from sewage outfalls was observed to promote the development of early successional stages of macrophytes in the surrounding communities (Soltan et al., 2001; Bokn et al., 2003; O′ Shanahan Roca et al., 2003) because of their high nutrient requirement (Karez et al., 2004; Kraufvelin, 2007).

European countries adopted the Water Framework Directive (WFD) in 2000 in order to protect and manage European water bodies. The WFD identified macroalgae as a suitable biological indicator for the water quality and suggested that they provide an appropriate statistical tool for the assessment of the ecological status of coasts (Soltan et al., 2001; Pinedo et al., 2007; Patrício et al., 2007; Juanes et al., 2008). The use of macroalgae as bio-indicators in water bodies was based on their prolonged exposure to adverse conditions, altering the structure of communities. Several pollution indexes were used in the Northern Hemisphere, mainly based on the relative abundance of some macroalgal indicator species (Orfanidis et al., 2001; Orfanidis et al., 2003; Ballesteros et al., 2007; Wells et al., 2007; Neto et al., 2012). A systematic assessment of macroalgal communities is required to properly evaluate the level of pollution of any waterbody, and to understand the relationship between abundance, diversity and richness of macroalgal communities and environmental factors, as was previously observed for several areas of the European coast (Krause-Jensen et al., 2007; Ballesteros et al., 2007; Wells et al., 2007; Puente and Juanes, 2008).

Several studies were conducted in the Southwestern Atlantic in order to evaluate different types of coastal impacts, mainly the sewage impact on littoral communities (López Gappa et al., 1990; Díaz et al., 2002; Vallarino et al., 2002; Elías et al., 2006; Torres and Caille, 2009; Muniz et al., 2011; Jaubet et al., 2011; Sánchez et al., 2013). Most of them were focused on the intertidal macrofauna, rejecting the macroalgae species response to that impact.

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In this study, we compared the algal assemblages in the rocky shore intertidal communities nearby the sewage outfall of Mar del Plata city with two non-impacted control areas; with respect to their specific composition and community parameters (abundance, diversity, richness and evenness). The aim of the study was to provide a valuable tool for the assessment of the wastewater pollution impact on the mid-littoral communities in the area by means of the intertidal algal assemblage composition.

Mar del Plata city is placed at the Southwest Atlantic coast of Argentina (38° 00′S; 57° 32′W) (Fig. 1).The shoreline is characterized by many sandy open beaches alternating with abrasion platforms of consolidated loess, forming cemented sandstones (Amor et al., 1991). The coastline is influenced by a littoral current, predominantly flowing from South, and undergoes severe wind storms (from the SSE sector) mainly during autumn and winter. Tides have a semidiurnal regime, with a tidal amplitude range around 0.8 m; and 1.6 m during exceptional tides. Sea surface temperature ranges between 9.3 °C in winter and 20 °C in summer (Guerrero and Piola, 1997), while seawater pH stays between 7 and 8.5 (Isla et al., 1998).

Mar del Plata has a sewage pre-treatment plant since 1989. The sewage is first screened to remove large particulates (>0.5 mm) and finally delivered to the intertidal sector of the coast (Scagliola et al., 2006). Mar del Plata is one of the major seaside resorts of Argentina, being visited by over 2,000,000 people during the summer season (December to February) (Bouvet et al., 2005). Consequently, the sewage average discharge increases from 2.8 m^3 seg^{-1} in winter to 3.5 m^3 seg^{-1} in summer (Scagliola et al., 2006). The environmental features in the study area were previously analyzed by Sánchez et al. (2013). They observed that both sediment organic matter and water turbidity in impacted areas were 1% and 50% respectively higher than in non-impacted areas.

Four sampling sites were distributed at the intertidal loess platforms with different distances from the sewage outfall (Fig. 1). Two of them were located 125 m (site E) and 800 m (site I1) south from the outfall, and the two other were located 8 km (site C1) and 9 km (site C2) north from the outfall. Sampling sites C1 and C2 were considered as non-impacted areas according to previous studies (Elías et al., 2009; Vallarino et al., 2014). Only rocky substrates with similar slope, orientation and wave exposure were compared. Two transects perpendicular to the coastline and ca. 50 m far from each other, were sampled at each site. All transects comprised the mid-littoral level of the local eulittoral zone (about 6 m long) (Raffaelli and Hawkins, 1999). The relative abundance of algae was assessed within each transect using a 0.5 × 0.5 m sampling unit, placed at regular intervals of 1.5 m, where the cover (%) of individual species was visually estimated. A total of 5 sampling units were sampled at each transect. The species were identified to the lowest possible taxonomical level. A preliminary identification was made in situ in order to estimate the different species coverage. The species were collected and latter identified at the laboratory. Even though the study was focused on the macroalgal community, the diatom *Berkeleya* sp. was considered in the data analysis as it was present with very high abundance near the sewage outfall, forming an evident biofilm on the substratum. Although the species was present at the non impacted area, biofilm was never observed. Sampling was performed during low tides in spring (October, November and December 2008), summer (January and February 2009) and autumn (April and May 2009).

The total abundance of algae (N), species richness (S), Shannon–Wiener diversity index (H′) (Shannon and Wiener, 1963) and evenness index (J′) (Pielou, 1969) were calculated for each sampling unit. The variation of these indexes between sampling sites was tested using

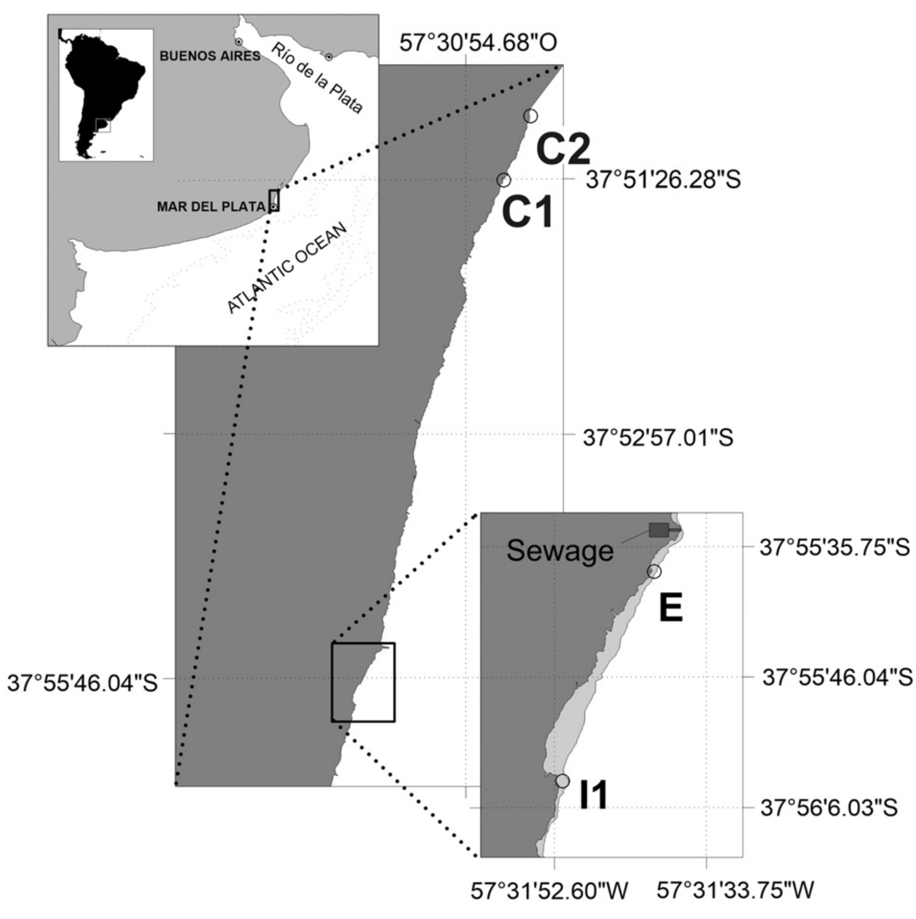

Fig. 1. Distribution of sampling sites and sewage outfall location in the study area.

Table 1
Mean density (%coverage.m^{-2}) of algal taxa in sites E, I1, C1 and C2.

ESPECIES	E	I1	C1	C2
Berkeleya sp.	107.26	99.2	96.8	60.51
Petalonia sp.	–	–	1.66	–
Porphyra sp.	0.13	2.23	5.77	1.08
Ralfsia sp.	3.53	4.57	5.66	1.37
Callithamnion sp.	–	–	1.77	–
Ceramium uruguayense (W.R. Taylor)	0.33	0.97	34.8	22.57
Chondria sp.	–	–	0.4	0.4
Polysiphonia fucoides (Hudson) Greville	0.13	1.37	2.8	2.06
Polysiphonia sp.	0.13	0.34	11.08	6.28
Gelidium sp.	–	–	–	0.28
Gymnogongrus torulosus (J.D. Hooker & Harvey) F. Shmitz	–	–	–	1.26
Ulva spp	130.46	119.37	118.34	192.4
Ulva lactuca (Linnaeus)	0.2	0.06	3.94	25.66
Cladophora sp.	–	–	0.17	0.57
Bryopsis plumosa (Hudson) C. Agardh	1.46	13.08	19.88	14.11

one-way ANOVA. Comparisons among means were performed using a posteriori Tukey test (Zar, 1999). In cases that assumptions of ANOVA test were not accomplished, the Kruskal–Wallis non-parametric analysis was performed. Statistical analysis of the data was achieved using R software, Version 2.5.1 (R Development Core Team, 2004). Changes in the algal assemblages between sampling sites were analyzed by combining a hierarchical agglomerative clustering using group-average linking, and a non-metric multidimensional scaling (NMDS) with a one-way analysis (ANOSIM) on a Bray–Curtis similarity matrix after a 4th-root transformation (Clarke and Warwick, 2001). In order to favor the understanding of generated plots (CLUSTER and NMDS), the sampling units from the same intertidal level in each sampling site were averaged for each season, resulting in a total of 5 averaged samples for each sites in each season. The SIMPER routine was used to determine the species accounting for the greatest contributions to dissimilarity between assemblages. A k-dominance curve was used to compare the diversity patterns between sampling sites (Lambshead et al., 1983). The non- parametric multivariate analyses were performed using the PRIMER v 5.0 software package (Clarke and Warwick, 2001).

Fifteen algal taxa were recorded during this study. 8 of these species were common to all sampling sites (Table 1). Moreover, several species of *Ulva* were recorded in all sampling sites. A high coverage of the diatom *Berkeleya* sp. was observed in both impacted and non-impacted areas. *Berkeleya* sp. is a pennate diatom living in mucilage tubes up to 2 cm height attached to substratum.

The ANOVA showed highly significant differences between sampling sites in total algal abundance ($F_{3,\ 346} = 9$, $P << 0.01$). Tukey *post hoc* comparisons showed a reduction of the mean abundance in the impacted sites (E and I1) (all $P << 0.001$). A similar total algal abundance was observed between sites E and I1 ($P = 0.889$) as well as between both non-impacted sites (C1 and C2) ($P = 0.995$) (Fig. 2). However, no significant differences in diversity, evenness and richness (Kruskal–Wallis $X^2 = 1.341$ $P = 0.71$; $X^2 = 1.137$ $P = 0.76$; $X^2 = 2.851$ $P = 0.415$ respectively) among sampling sites were found (Fig. 2).

The impacted sites were grouped separately from the non-impacted sites in the dendrogram clustering plot according to a 70% similarity (Fig. 3). A similar pattern was distinguished in the NMDS ordination plot (Fig. 3). However, some exceptions of this general pattern were observed. In spring, a C1 site was placed within the group of impacted sites, and one E site and another I1 site were placed in the group of non-impacted sites. In summer, one I1 site was mixed with the non-impacted sites group, and in autumn, one I1 site was placed within the group of non-impacted sites and one C1 and two C2 sites among the impacted sites group. Analysis of similarity (ANOSIM) showed that macroalgal composition of impacted sites (E and I1) differed significantly from that observed in non-impacted sites (C1 and C2) in spring, summer and autumn (Global probability $= 0.01$, Paired contrasts all $P = 0.001$; Global probability $= 0.001$, Paired contrasts all $P < 0.005$; Global probability $= 0.003$, Paired contrasts all $P < 0.081$ respectively). Contrast

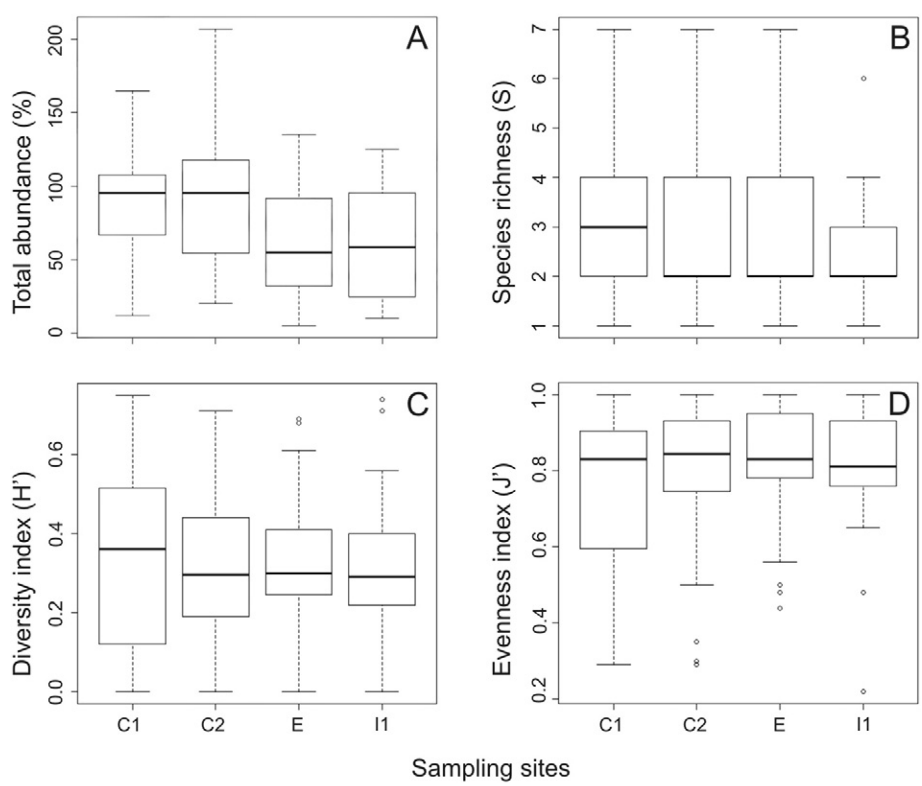

Fig. 2. Box-plots of (A) total abundance, (B) species richness, (C) Shannon–Wiener diversity index and (D) evenness index in the sampling sites. Solid line represents the median. The top and bottom limits of the rectangle coincide with the third and the first quartile of data respectively. Circles represent the outliers and whiskers the non-outliers range.

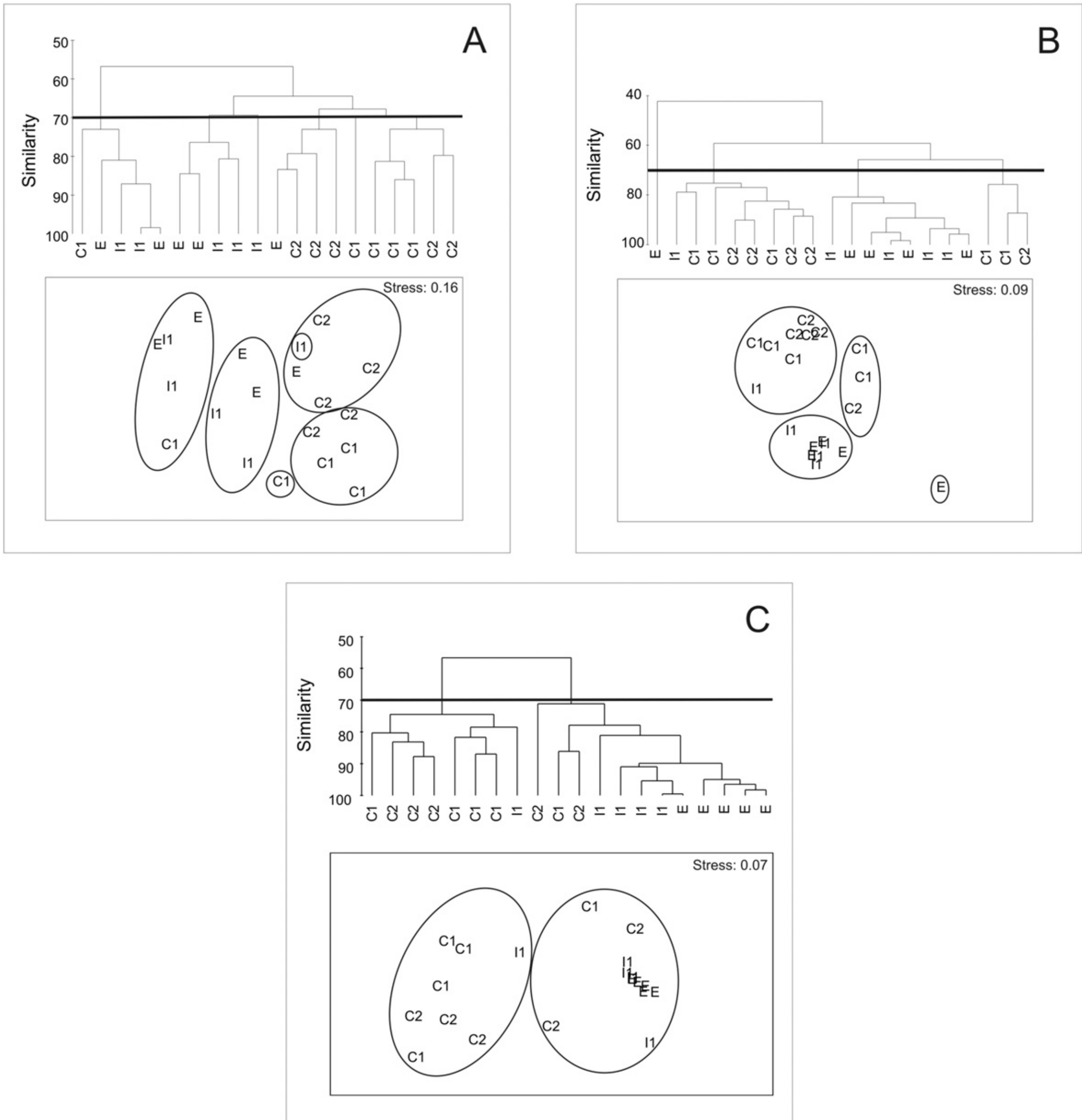

Fig. 3. Cluster. Clusters formed at 70% of similarity are superimposed on the 2-dimensional NMDS obtained from the same similarity matrixes. Composition of algal assemblages in spring (A), summer (B) and autumn (C).

between both non-impacted sites (C1 and C2) reached significance in spring (Paired contrast $P = 0.001$) and autumn (Paired contrast $P = 0.025$). While contrast between both impacted sites (E and I1) never reached significance (Paired contrasts all $P > 0.191$). SIMPER analyses showed that species responsible for the difference in composition between impacted sites and non-impacted sites, where the cumulative percentage did not exceed 60%, were *Berkeleya* sp., *Ulva* spp., *Ceramium uruguayense* and *Bryopsis plumosa* (Table 2). *Berkeleya* sp. was more abundant in impacted sites with exception of site C1 during spring when its abundance was higher than in both site E and site I1. *Ulva* spp. was abundant in all sites, regardless of the impacted and non-impacted areas, and showed no consistent seasonal preferences. Although high values of *Ulva* spp. were recorded in site C2 during spring. *B. plumosa* had higher abundance values in non-impacted sites during summer and autumn and *C. uruguayense* was almost the only species registered in non-impacted sites (Table 2). The k-dominance curves showed that algal communities were increasingly dominated by a few species in impacted sites, where the curve described a quadrangular shape; while a higher diversity of algae was observed in non-impacted sites, and thus the curve described a diagonal shape (Fig. 4).

This study shows that algal assemblages vary according to the distance from the sewage outfall of Mar del Plata. The previous studies performed in this area for the last 10 years indicated that the zoobenthic intertidal community showed an increasing sewage impact, characterized by a decrease in *Brachidontes rodriguezii* abundance and a lower diversity and richness in the resident community; and also an increased abundance of the polychaete *Boccardia proboscidea*, an indicator of organic enrichment (Elías et al., 2001, 2006; Vallarino et al., 2002, 2014; Jaubet et al., 2011; Sánchez et al., 2013).

The total abundance of algae decreased and the specific composition of community changed according to the distance from the sewage outfall. This is coincident to some extent with previous studies conducted in the Atlantic coast of some European countries (Gorostiaga and Díez, 1996; Díez et al., 1999, 2003; Soltan et al., 2001; Arévalo et al., 2007), in the Pacific coast of USA (Littler and Murray, 1975; Murray and Littler, 1978), Australia (Fairweather, 1990), China (Dongyan et al., 2007) and also in the southwest Atlantic Ocean (Díaz et al., 2002;

Torres and Caille, 2009). In all these studies, phytobenthic communities responded to sedimentation, turbidity, high nutrient concentration or water toxicity derived from waste waters, by simplifying their specific composition and community structure (decreasing species richness, diversity and algal abundance), with a high or almost complete dominance of ephemeral green algae. The algal specific composition changed between impacted and non impacted areas, but diversity, richness and evenness of macroalgal assemblages showed no variation between all sampling sites; and the dominance of ephemeral green algae associated to the sewage outfall was not observed. Díez et al. (2003) found that macroalgal coverage; species richness and diversity remained fairly constant from unpolluted to slightly polluted sites, but decreased sharply under moderately polluted conditions in the western Basque coast. However, the difference in the pollution conditions between areas in the present study is not enough to produce statistical differences in the algae community parameters.

The multivariate analyses indicated that the algal community differed between impacted and non-impacted areas with respect to their specific composition. The non-impacted area (sites C1 and C2) were characterized by more homogeneous groups according to their specific composition in summer and autumn. This probably owed to the fact that algal populations generally present an explosive growth during early spring, and a decline in abundance during late autumn and winter (May to July) (Sar et al., 1984). According to their specific composition, the sites were similar during the low richness months (autumn and summer) because *Ulva* spp. dominated the community during all seasons, showing the highest coverage percentages.

The abundance of *Ulva* spp. was not necessarily related to an enrichment impact in this study. The high abundance of *Ulva* spp. in all sampling sites could result from other disturbance processes, as waves and storms, which commonly produce high levels of sand deposition on the intertidal rocky shore of the area. The sand is deposited on the local community, forcing the beginning of a new successional process every time it is washed away (author, pers. Obs.).

The diatom *Berkeleya* sp. was the main species that allowed to distinguish between the pollution status of the area (impacted and non-impacted). Some species of *Berkeleya* were related with polluted areas and high metal concentration in a recent study on the microphytobenthic community of the hyper-saline lagoon of Mar Menor (Spain) (Belando Torrentes, pers. Comm.). *Berkeleya rutinalis* response positively to nutrient increase in the absence of grazers in laboratory conditions (Hillebrand et al., 2000). However, *Berkeleya* sp. response to nutrient concentration in our sampling sites deserves a more detailed study.

C. uruguayense was almost the only registered species in non-impacted sites. However, *Ceramium* sp. was considered as an opportunistic species in some polluted areas of Spain (Díez et al., 1999; Juanes et al., 2008). Karez et al. (2004) showed that the composition of the ephemeral assemblages changes according to nutrient availability. They also observed that corticated filamentous algae (i.e. Ceramiales) were more abundant at low nutrient concentration, while thin foliose algae (i.e. Ulvales) become more abundant with higher nutrient availability in most of the surveyed assemblages.

The impacted area was characterized by a lower abundance of *B. plumosa*, a common species in wave-sheltered areas of the mid-rocky shore of Mar del Plata (Sar et al., 1984; Becherucci et al., 2014). The species was frequently found in lower intertidal tide-pools of the sewage outfall impacted area at Quequén (López Gappa et al., 1990), but no relation between *B. plumosa* and the sewage impact area was established.

Our results of K-curves dominance were consistent with previous studies performed in typically impacted sites (Warwick, 1986; Arévalo et al., 2007). We found lower species richness, with maximum values of cumulative dominance in the impacted areas. However, there were not significant differences in richness or in the diversity index between both impacted and non-impacted areas.

Pollution by organic matter in the coastal waters of Mar del Plata represents a serious environmental issue affecting the local economy and decreasing both the health and quality of the beaches (Isla et al., 1998). Recently, the sewage pre-treatment plant of Mar del Plata celebrated the setting up of a submarine outfall that moved away the discharge 3.5 km far from coast. Futures studies focusing in the restoration of the resident communities in the area will improve the knowledge on the algae as a suitable biological indicator for marine pollution in the coast of Mar del Plata.

In conclusion, the sewage outfall of Mar del Plata impacts on the intertidal benthic algal community by altering the specific composition. The algal assemblages varied according to the pollution gradient in spring, summer and autumn, being autumn the season when the highest difference was observed. *C. uruguayense* emerged as an indicator species for the non impacted area, and *Berkeleya* sp. as an indicator species for the sewage impacted area.

Ulva spp. did not reflect the typical pattern observed for other sewage polluted areas.

Table 2
SIMPER analyses. Seasonal contribution of algal taxa to sampling sites dissimilarities.

Species	Av. abundance	Av. abundance	Contribution (%)	Cum. contribution (%)
Spring	I1	C1		
Berkeleya sp.	29.77	32.27	21.61	21.61
Ulva spp.	21.70	20.87	17.54	39.15
C. uruguayense	0.40	8.47	14.92	54.08
	E	C1		
Berkeleya sp.	13.30	32.27	22.03	22.03
Ulva spp.	27.53	20.87	18.13	40.16
C. uruguayense	0.17	8.47	15.48	55.63
	I1	C2		
Ulva spp.	21.70	51.43	21.96	21.96
Berkeleya sp.	29.77	11.80	20.24	42.20
C. uruguayense	0.40	8.17	13.82	56.02
	E	C2		
Ulva spp.	27.53	51.43	22.22	22.22
Berkeleya sp.	13.30	11.80	19.27	41.49
C. uruguayense	0.17	8.17	15.20	56.69
	C1	C2		
Berkeleya sp.	32.27	11.80	17.54	17.54
Ulva spp.	20.87	51.43	16.40	33.94
C. uruguayense	8.47	8.17	14.38	48.31
Summer	I1	C1		
Berkeleya sp.	19.90	19.45	23.52	23.52
C. uruguayense	0.00	10.00	23.08	46.60
	E	C1		
Berkeleya sp.	26.75	19.45	26.13	26.13
C. uruguayense	0.00	10.00	21.96	48.10
	I1	C2		
Berkeleya sp.	19.90	22.00	25.87	25.87
Ulva spp.	24.05	33.05	16.69	42.56
B. plumosa	1.90	3.40	14.75	57.31
	E	C2		
Berkeleya sp.	26.75	22.00	26.68	26.68
Ulva spp.	35.40	33.05	18.62	45.29
C. uruguayense	0.00	6.00	13.98	59.27
Autumn	I1	C1		
Berkeleya sp	22.25	16.85	26.54	26.54
B. plumosa	2.00	8.95	18.38	44.92
C. uruguayense	0.25	7.75	14.56	59.48
	I1	C2		
Berkeleya sp.	22.25	13.25	38.78	38.78
Ulva spp.	47.85	58.15	19.25	58.03
	C1	C2		
Berkeleya sp.	16.85	13.25	25.46	25.46
B. plumosa	8.95	2.95	17.07	42.53
C. uruguayense	7.75	1.50	14.26	56.79
	C1	E		
Berkeleya sp.	16.85	67.50	34.69	34.69
B. plumosa	8.95	0.00	17.03	51.72
	C2	E		
Berkeleya sp.	13.25	67.50	54.03	54.03

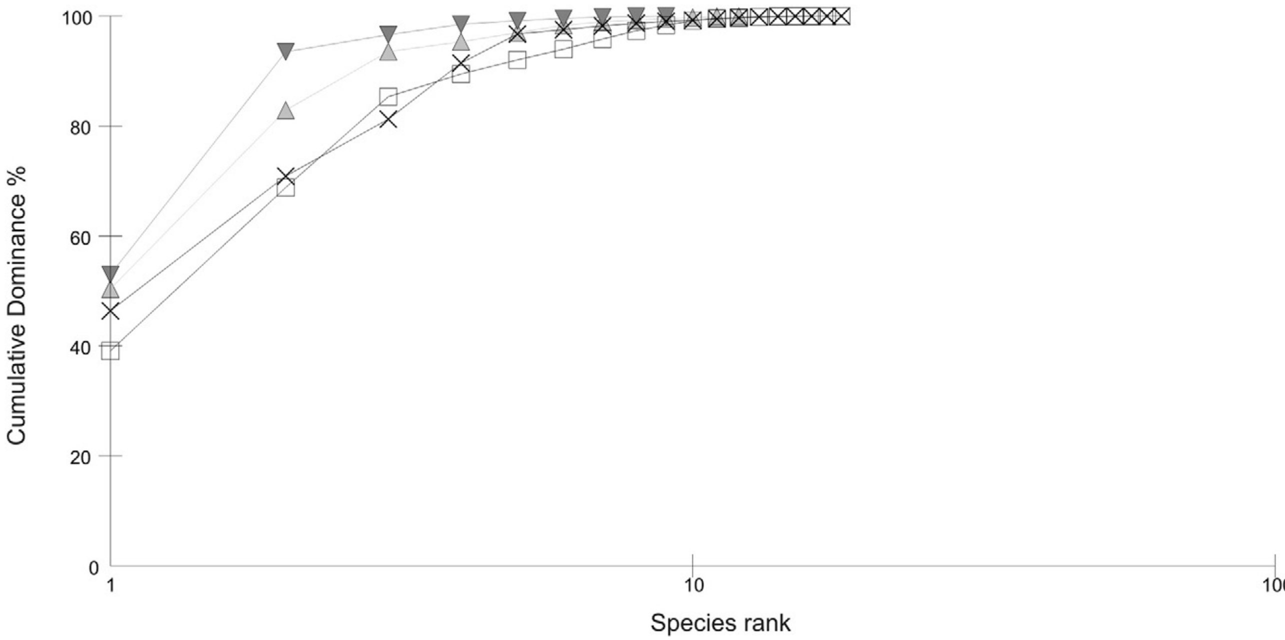

Fig. 4. K-dominance curve for mean coverage at each sampling sites. △I1, ▽E, □C1 and ×C2.

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