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Rapid sewage pollution assessment by means of the coverage of epilithic taxa in a coastal area in the SW Atlantic

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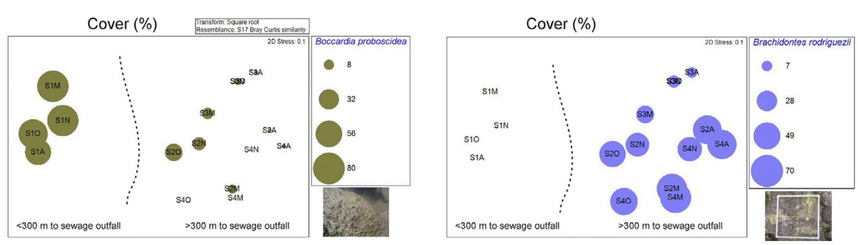
HIGHLIGHTS

- Sewage pollution impact over the benthic community was studied in a SW Atlantic area.
- High content of organic matter and *Enterococcus* characterized the outfall area.
- Diversity of the benthic community decreased in polluted sites.
- *Boccardia proboscidea* dominated the polluted site decreasing community diversity.
- *Brachidontes rodriguezii* and several algae species dominated unpolluted sites.

GRAPHICAL ABSTRACT



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ABSTRACT

The sewage pollution impact over coastal environment represents one of the main reasons explaining the deterioration of marine coastal ecosystems around the globe. This paper aims to detect promptly a putative sewage pollution impact in a Southwestern Atlantic coastal area of Argentina as well as to identify a straightforward way for monitoring, based on the relative abundance coverage of the intertidal epilithic taxa. Four sampling sites were distributed at increased distances from the sewage outfall where the cover of individual epilithic species was visually estimated. The surrounded outfall area (i.e. outfall site) resulted polluted with high percentages of organic matter in sediment and *Enterococcus* concentration in seawater. The structure of the community showed a remarkable difference between the polluted site (outfall site) and the unpolluted sites. The polychaete *Boccardia proboscidea* dominated the outfall site with variable abundances of the green algae *Ulva* sp. during the period of study, decreasing the diversity of the community, while the mussel *Brachidontes rodriguezii* and variable abundances of several algae species dominated the unpolluted sites. The monitoring of the benthic community represents an effective, non-destructive, relative inexpensive and rapid method to assess the health of the coastal environment in the study area. The large abundance of *B. proboscidea* along with the absence of *B. rodriguezii* individuals at <300 m to the sewage outfall discharge allowed the success of this classical monitoring method in a temperate marine-coastal ecosystem with certain gradient of pollution.

1. Introduction

Nowadays an increasing concern regarding conservation of aquatic systems, particularly marine coastal system, has acquired a greater

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public attention. For example, organic and nutrient enrichment due to domestic waste (i.e. sewage discharge) represent one of the main reasons explaining the deterioration of marine coastal ecosystems around the globe (Cloern, 2001). The ecosystem deterioration by sewage discharge is enhanced by the lack of the appropriate treatments to waste waters, which, in general terms, include the screened of the waters, the oxidation of the dissolved organics and the remove of the nutrients such as N and P (Lobban et al., 1985). Although in many countries the waste waters are dumped further out to sea, in many cases these are discharged directly onto coastal areas. The bulk of the studies dealing with the coastal impact by sewage outfall have been concerned with the response of benthic communities to the supply of organic matter to the ecosystem (i.e. eutrophication) and the consequent nutrient enrichment that suffer the eutrophicated systems. Generally, these studies confirm a decrease in species richness and abundance; with a consequent simplification of the community structure. Inversely, the abundance of opportunistic species with high reproductive capacity and a wide tolerance range to pollution is expected to increase (e.g. *Ulva* spp. *Cladophora* spp. among the macroalgaes and Spionidae species like *Boccardia proboscidea* among the polychaetes) (Soltan et al., 2001; Díaz et al., 2002; Díez et al., 2003; O'Shanahan Roca et al., 2003; Borja et al., 2006; Arévalo et al., 2007; Wells et al., 2007; Jaubet et al., 2011; Kotta and Moller, 2014; Cabrita et al., 2015).

In this context, benthic communities have been widely used as biological elements to monitor coastal waters and to evaluate their ecological and conservation status (Orfanidis et al., 2003; Ballesteros et al., 2007; Wells et al., 2007; Pinedo et al., 2007; Dauvin et al., 2007; Borja et al., 2008; Neto et al., 2012; Cecchi et al., 2014; Le Gal and Derrien-Courtel, 2015). However, the difficulties inherent to the assessment of benthic communities in coastal waters are well known, mainly due to the natural variability of the benthos and the high sampling and laboratory processing effort (e.g. taxonomical determination, biomass data or the abundance determination of higher number of organisms by counting them), producing a long-time consuming method of monitoring (Patrício et al., 2007; Puente and Juanes, 2008; Jaubet et al., 2011). These monitoring methods are somehow against the political and management requirements which demand quick responses in pollution tasks. Consequently, considerable attention has been paid to the establishment of straightforward and cost-effective ways to assess the pollution level of a particular area, without neglecting the ability for detection of benthic communities' changes and the precision of data acquired (Wells et al., 2007; Puente and Juanes, 2008).

In the Southern Hemisphere, 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; Elías et al., 2014; Becherucci et al., 2016a; Garaffo et al., 2017). The majority of these studies were focused on the intertidal benthic communities, considering the responses of both infaunal and epilithic species to sewage impact.

Taking into account the above mention, this paper aims (1) to detect promptly a putative sewage pollution impact in a Southwestern Atlantic coastal area of Argentina and (2) to identify a straightforward way for monitoring, based on the relative abundance coverage of the intertidal epilithic taxa.

2. Materials and method

2.1. Study area

The study area was located surrounding the sewage outfall in Punta Carballido (38° 34′ 07.68″ S; 58° 39′ 06.28″W) 4 km east of Quequén Harbour, between Quequén city and the summer resort of Costa Bonita, SW Atlantic coast of Argentina (Fig. 1). The shoreline is characterized by sandy open beaches alternating with rocky substrate composed of loess platform. The coastline is influenced by a mixed predominantly semidiurnal regime tides, with a tidal amplitude range around 1 m with c.a. 1.7 m of maxima during exceptional tides, and undergoes severe wind storm (from the SSE sector) mainly during autumn and winter (Servicio de Hidrografía Naval, 2016). Untreated effluents produced by c.a. 83,883 inhabitants of Necochea and Quequén cities (National data, CENSO 2010) are discharge onto the intertidal zone. The volume discharged was estimated to be c.a. 14,000 m^3 day^{-1}, although this quantity is probably doubled during the tourist season (López Gappa et al., 1990). The intertidal zone in the study area is directly exposed

Fig. 1. Study area in relation to the location of Quequén and Necochea cities, southeastern Buenos Aires province, Argentina.

to sewage discharge and it represents the only source of exposure. The access to the sampled beach is available for both residents and tourists. Given that a significant response of the benthic community to the discharge was registered almost two decades ago (López Gappa et al., 1990; López Gappa et al., 1993), it is strongly desirable to know the current ecological status of the area.

2.2. Sampling design

Four sampling sites were distributed at the intertidal loess platforms with different distances from the sewage outfall: Site 1 located in the point of discharge, Site 2 and Site 3 were located 330 m and 700 m respectively northeastern from the outfall, and Site 4 located 700 m southwestern from the outfall (Fig. 1). The relative abundance of individual epilithic species was visually estimated (measured as % coverage) from an overall of 9 randomly sampled quadrates (i.e. sampling units) (50 cm side) in each site. The sampling units were distributed over the horizontal platforms at the mid-intertidal and both vertical substrates and rock pools were excluded from the sampling. The species were identified in situ to the lowest possible taxonomical level. Sampling was performed during low tides in 4 times: October and November 2015, March and April 2016. Thus, the sampling size was $n = 144$ (9 replicates × 4 sites × 4 times). Representative samples of environmental variables of the seawater: temperature (°C), salinity (ups) and pH were measured in each sampling site and time, as well as the organic matter (MO) contented in sediment (%) by calcinations method. Moreover, 200 mL of seawater was collected in each sampling site and time to determinate the *Enterococcus* concentration (NMP/100 mL) by Standard Methods for the examination of water and wastewater, (AWWA, APHA, and WEF. 22nd Edition 2012).

2.3. Data analysis

Species richness (S), Shannon–Wiener diversity index (H′) (Shannon and Wiener, 1963) and evenness index (J′) (Pielou, 1969) of the community (i.e. diversity parameters) were calculated for each sampling unit. The spatial and temporal variability of diversity parameters was evaluated using two-way ANOVA. The factors were as follows: site (fixed) with four levels and time (fixed) with four levels, with nine replicates each. The assumption of normality and homogeneity of variance were previously tested, being all variable homogenous and normal.

The resemblance of the epilithic assemblages according to site and time of sampling was analyzed by combining a hierarchical agglomerative clustering using group-average linking, and a non-metric multidimensional scaling (NMDS) with a PERMANOVA analysis 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 sampling site were averaged for each sampling time (i.e. October, November, March and April), resulting in a total of 4 average samples for each sampling site (one for each time). The SIMPROF routine was used to know if the groups found in the cluster were significant or they were obtained by chance. The SIMPER routine was used to determine the species accounting for the greatest contributions to dissimilarity between assemblages. Taxa and sites were assessed by a direct gradient analysis of ordination technique (i.e. canonical correspondence analysis, CCA). Ordination helps to identify relationships between species composition at a site and the underlying environmental variables (i.e. explanatory variables). To determine associations between the data and the main explanatory variables, a biplot from the CCA was obtained by overlaying a vector diagram, based on coefficients from the canonical functions describing each canonical axis, on the ordination graph. The CCA analysis was done using only species or taxa with constancy (i.e. frequency of occurrence) >10%. Species or taxa with lower constancy are considered occasional.

3. Results

A total of 20 taxa were recorded during the sampling period, being *Brachidontes rodriguezii* and *Boccardia proboscidea* the most common species among the zoobenthos and *Ulva lactuca* and *Ulva* sp. between the algae.

3.1. Diversity parameters

Species richness reached a maximum of 10 taxa. The Shannon-Wiener diversity and evenness index ranged from 0 to 2 and 0.2–1

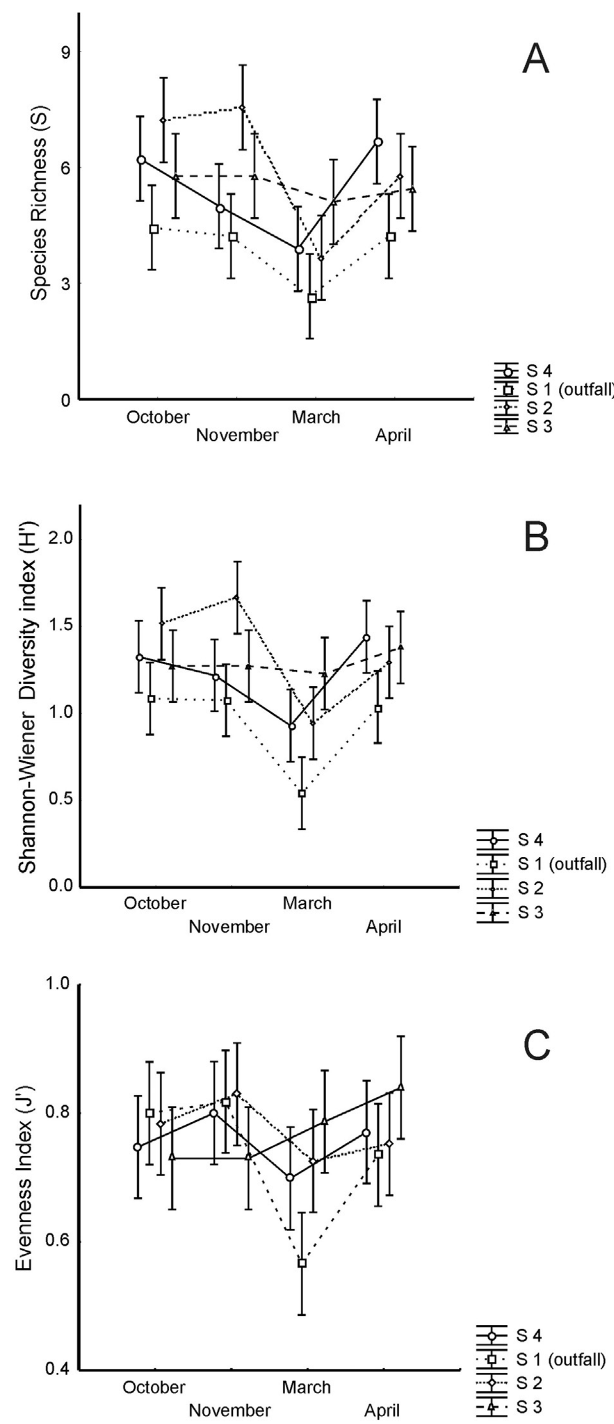

Fig. 2. Mean value (±SE) of species richness (A), Shannon-Wiener diversity index (B) and evenness index (C) in each sampling sites and times. S1 = site 1, S2 = site 2, S3 = site 3, S4 = site 4.

respectively. The interaction site x time had a significant effect on species richness (S), Shannon-Wiener diversity index (H′) and evenness index (J') ($F_{S,\ \text{site x time}\ 144,\ 9} = 2.087$, $p = 0.035$; $F_{H',\ \text{site x time}\ 144,\ 9} = 2.103$, $p = 0.034$; $F_{J',\ \text{site x time}\ 144,\ 9} = 2.516$, $p = 0.011$). Overall, the three parameters decrease at the end of summer (i.e. March). Evenness decrease more deeply in site 1 ($F_{36,\ 9} = 4.962$, $p = 0.006$,) when compared with the rest of the sites ($F_{S2\ 36,\ 9} = 1.628$, $p = 0.202$; $F_{S3\ 36,\ 9} = 2.173$, $p = 0.110$; $F_{S4\ 36,\ 9} = 1.394$, $p = 0.262$). Diversity decrease in site 1 ($F_{36,\ 9} = 4.966$, $p = 0.006$) and site 4 ($F_{36,\ 9} = 4.482$, $p = 0.009$), while richness decrease in site 3 ($F_{36,\ 9} = 8.900$, $p < 0.0001$) and site 4 ($F_{36,\ 9} = 4.493$, $p = 0.007$) (Fig. 2).

3.2. Benthic assemblages and environmental condition

The site 1 was grouped separately from the rest of the sites in the dendrogram clustering plot according to >60% dissimilarity (SIMPROF, Pi = 8.77, $p = 0.001$) (Fig. 3). A similar pattern was distinguished in the NMDS ordination plot (Fig. 3). PERMANOVA showed that the benthic community from Site 1 differs significantly from the rest of the sites in the 4 sampling times (Pseudo $F_{S\ x\ T} = 2.676$, $p = 0.001$, see Table 1). Although it is not so clear in the plots (cluster and NMDS) the rest of the sites differ between their self in all sampling times, with the exception of Site 2 and Site 4 in March, where those community not be different. SIMPER analyses showed that the species and taxa responsible, up to 50% of cumulative percentage, of the difference between composition assemblages of site 1 and the rest of sites were *Boccardia proboscidea*, *Ulva* sp., *Brachidontes rodriguezii* and *Ulva lactuca*, being in all sampling times the first two more abundant (>50% and >15% average of cover respectively) in the site 1, and the last two more abundant (10–50% and >15% average of cover respectively) in sites away for the discharge (i.e. sites 2, 3 and 4). The rest of the species are shown in the Table 1.

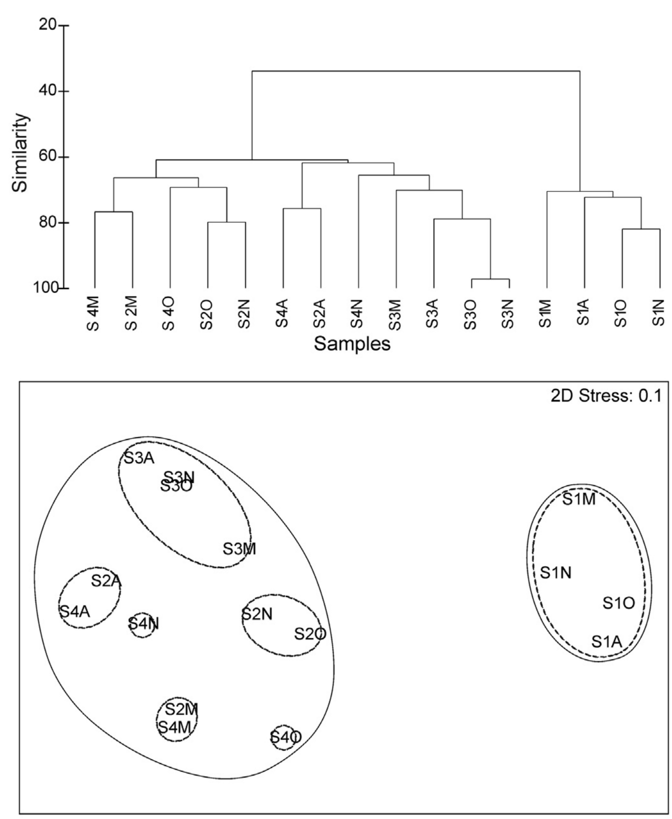

Fig. 3. Cluster formed at 60% (full line) and 70% (dotted line) of similarity are superimposed on the 2-dimensional NMDS obtained from the similarity matrixes. S1 = site 1, S2 = site 2, S3 = site 3, S4 = site 4, M = March, A = April, O = October and N = November.

The CCA showed the main environmental variables associated with the occurrence of benthic species and sites. Species or taxa were sorted in relation to the two main axes, showing a total inertia of 1.3456 and explaining 48.5% of the total variance (eigenvalues for axis 1 and 2 were 0.505 and 0.148 respectively). Axis 1 separated the species *Ulva* sp., *Cladophora* sp., *Gelidum crinale*, *Boccardia proboscidea*, and the group of biofilm and diatoms associated with site 1 from *Brachidontes rodriguezii*, *Siphonaria lessoni*, *Balanus glandula*, *Ulva lactuca*, *Schizymenia dubyi*, *Jania rubens*, *Porphyra* sp. *Ralfsia* sp. *Ceramium uruguayense* and *Chaetomorpha* sp. witch were associated with sites 2, 3 and 4. Greater organic matter (MO) in sediment (c.a. 2.5%) and the concentration of *Enterococcus* (c.a. 1.5×10^6 NMP/100 mL) in seawater were related to the first assemblages in site 1, and greater temperature (range between 16 and 25 °C according to sampling month) and salinity (>31 ups) with the second assemblages (Fig. 4). The pH was not correlated with axis 1 (c.a. 8.5 pH units). The occasional species (with frequency of occurrence lower than 10%) not included in this analysis comprised the red macroalgae *Gastroclonium trichodes*, *Corallina officinalis* and *Chondria* sp.

4. Discussion

Benthic community structure is a good pollution indicator and it has been usefully applied in coastal marine areas worldwide (Orfanidis et al., 2001; Niemi and Mc Donalds, 2004; Simboura et al., 2005; Salas et al., 2006; Devlin et al., 2007; Ballesteros et al., 2007; Wells et al., 2007; Juanes et al., 2008; Borja et al., 2008; Dauvin et al., 2010; Cecchi et al., 2014; Le Gal and Derrien-Courtel, 2015). The untreated discharge of sewage in the intertidal area produce direct consequences in the abundance of the species, since it respond, in general, negatively to sedimentation, turbidity, high nutrient concentration, water toxicity, freshwater and organic matter content derived from waste water. The assay of benthic community structure is usually better than the assay of seawater variables, since the former can be considered as reflecting an average of varying environmental conditions. Thus, covering benthos samples result a rapid and straightforward way for monitoring pollution.

According to our results, diversity and evenness indexes responded to sewage discharge decreasing significantly their values near the sewage outfall (i.e. site 1) during March. Those parameters decreased in site 1 especially during March given that the dominance of *B. proboscidea*. Also the community composition of sites 2 and 4 were similar during this month (see Table 1). It could be explained by an accumulative effect of the summer where higher volumes of discharge, given that the tourism season (i.e. December–March), and the desiccation stress, that suffer specially the macroalgae intertidal species, have a negative effect over the intertidal benthic organism. Part of these results are in line with López Gappa et al. (1993) who concluded that the desiccation stress is the main factor which control the temporal variability in the benthic community composition in the study area. The combination of such factors (desiccation and sewage outfall stress) reduces the diversity of the community, thus further highlighting the dominating species (i.e. *B. proboscidea* in impacted site), while the stress desiccation effect alone reduce the macroalgae abundance denoting the dominance of *B. rodriguezii* in sites located away from the outfall (e.g. decreased richness in sites 2 and 4). In many studies around the world, differences in diversity, total abundance and species composition between impacted and non-impacted areas was observed where species richness and diversity decreased under polluted conditions (Gorostiaga and Díez, 1996; Díez et al., 2003; Arévalo et al., 2007; Wells et al., 2007; Elías et al., 2014). In general those patterns are consistent with the typical dominance of ephermeral green algae association in enriched condition (e.g. green tides) and the consequent hypoxia process affecting the environment (Menesguen, 1992; Sfriso et al., 1993; Valiela et al., 1997; Liu et al., 2007; Teichberg et al., 2010; Shi et al., 2015). In the present study there was not observed a clear dominance of the ephemeral green

Table 1
Pair-wise test and SIMPER result showing the taxa contribution to sampling sites dissimilarity according to sampling month (October, November, March and April). Cumulative percentages that exceed 50% are not show.

	Pair-wise test				SIMPER		
	t	P	Taxon	Av. Abund.	Av. Abund.	Contrib. (%)	Cum. (%)
October							
S4 ≠ S1	4.271	0.001		S4	S1		
			Boccardia proboscidea	0	7.83	21.92	21.92
			Brachidontes rodriguezii	6.64	0	17.97	39.89
			Diatoms	0	3.88	10.14	50
S4 ≠ S2	2.203	0.01		S4	S2		
			Boccardia proboscidea	0	4.8	18.02	18.02
			Ulva lactuca	2.54	3.65	12.37	30.39
			Ulva sp.	2.97	3.04	11	41.38
S1 ≠ S2	3.206	0.001		S1	S2		
			Brachidontes rodriguezii	0	6.46	20.19	20.19
			Boccardia proboscidea	7.83	4.8	11.84	32.03
			Diatoms	3.88	0.42	11.74	43.78
S4 ≠ S3	3.128	0.001		S4	S3		
			Ulva lactuca	2.54	7.18	18.78	18.78
			Brachidontes rodriguezii	6.64	2.43	17.25	36.02
			Ulva sp.	2.97	0	10.99	47.01
S1 ≠ S3	5.275	0.001		S1	S3		
			Ulva lactuca	0	7.18	18.68	18.68
			Boccardia proboscidea	7.83	1.38	17.45	36.12
S2 ≠ S3	3.277	0.001		S2	S3		
			Brachidontes rodriguezii	6.46	2.43	15.41	15.41
			Ulva lactuca	3.65	7.18	15.39	30.8
			Boccardia proboscidea	4.8	1.38	12.01	42.81
November							
S4 ≠ S1	5.082	0.001		S4	S1		
			Boccardia proboscidea	0	8.68	22.7	22.7
			Brachidontes rodriguezii	5.71	0	14.57	37.27
			Ulva sp.	0	4.56	12.37	49.63
S4 ≠ S2	2.05	0.001		S4	S2		
			Brachidontes rodriguezii	5.71	5.75	10.41	10.41
			Cladophora sp.	0.5	2.89	10.1	20.51
			Ulva lactuca	4.76	5.83	9.25	29.76
			Porphyra sp.	2.39	0	9.04	38.8
			Boccardia proboscidea	0	2.5	8.47	47.27
S1 ≠ S2	3.97	0.001		S1	S2		
			Boccardia proboscidea	8.68	2.5	19.29	19.29
			Brachidontes rodriguezii	0	5.75	16.56	35.85
S4 ≠ S3	2.522	0.001		S4	S3		
			Brachidontes rodriguezii	5.71	2.43	16.21	16.21
			Ulva lactuca	4.76	7.18	15.58	31.79
			Porphyra sp.	2.39	0	10.2	41.99
S1 ≠ S3	5.485	0.001		S1	S3		
			Boccardia proboscidea	8.68	1.38	20.9	20.9
			Ulva lactuca	1.49	7.18	17.05	37.95
S2 ≠ S3	2.505	0.002		S2	S3		
			Brachidontes rodriguezii	5.75	2.43	13.89	13.89
			Ulva lactuca	5.83	7.18	11.02	24.92
			Cladophora sp.	2.89	0.38	10.99	35.91
			Boccardia proboscidea	2.5	1.38	8.93	44.84
March							
S4 ≠ S1	5.595	0.001		S4	S1		
			Boccardia proboscidea	0	8.37	27.75	27.75
			Brachidontes rodriguezii	7.91	0	27.42	55.17
S4 = S2	1.246	0.192					
S1 ≠ S2	5.24	0.001		S1	S2		
			Brachidontes rodriguezii	0	7.79	28.99	28.99
			Boccardia proboscidea	8.37	1.35	26.46	55.45
S4 ≠ S3	3.132	0.001		S4	S3		
			Brachidontes rodriguezii	7.91	3.04	24.49	24.49
			Corallina officinalis	0.68	4.77	19.33	43.82
S1 ≠ S3	3.819	0.001		S1	S3		
			Boccardia proboscidea	8.37	2.84	22.39	22.39
			Ulva lactuca	0.35	5.75	20.05	42.44
S2 ≠ S3	2.45	0.002		S2	S3		
			Brachidontes rodriguezii	7.79	3.04	27.55	27.55
			Corallina officinalis	2.4	4.77	17.23	44.78
April							
S4 ≠ S1	6.097	0.001		S4	S1		
			Brachidontes rodriguezii	7.33	0	19.19	19.19
			Boccardia proboscidea	0.5	7.08	17.06	36.25

Table 1 (*continued*)

	Pair-wise test				SIMPER		
	t	P	Taxon	Av. Abund.	Av. Abund.	Contrib. (%)	Cum. (%)
			Ulva sp.	0	4.74	12.16	48.41
S4 ≠ S2	1.929	0.004		S4	S2		
			Ceramium uruguayense	3.76	0.25	16.81	16.81
			Corallina officinalis	0.5	3.04	12.28	29.1
			Brachidontes rodriguezii	7.33	7.24	9.96	39.06
			Ulva lactuca	3.77	3.61	7.81	46.87
S1 ≠ S2	6.611	0.001		S1	S2		
			Brachidontes rodriguezii	0	7.24	19.9	19.9
			Boccardia proboscidea	7.08	0.5	18.1	38
S4 ≠ S3	3.336	0.001		S4	S3		
			Brachidontes rodriguezii	7.33	1.52	22.06	22.06
			Ceramium uruguayense	3.76	0	14.14	36.2
			Corallina officinalis	0.5	3.27	11.5	47.69
S1 ≠ S3	6.046	0.001		S1	S3		
			Boccardia proboscidea	7.08	0.54	19.44	19.44
			Ulva lactuca	0	5.52	16.43	35.88
			Ulva sp.	4.74	0	13.87	49.75
S2 ≠ S3	2.912	0.001		S2	S3		
			Brachidontes rodriguezii	7.24	1.52	25.48	25.48
			Corallina officinalis	3.04	3.27	14.39	39.87

algae, although higher abundance of *Ulva* sp. were registered in the sewage outfall rather than away the of it.

Several exceptions, in which the diversity of the community in eutrophicated coastal area increases, were registered (López Gappa et al., 1990; Vallarino and Elías, 2006; Martinetto et al., 2010; Fricke et al., 2015). In some of those studies, large amount of water (e.g. tidal cycle) flushing enough to prevent the hypoxia process, making the environment suitable for consumers and thus, the high nutrient

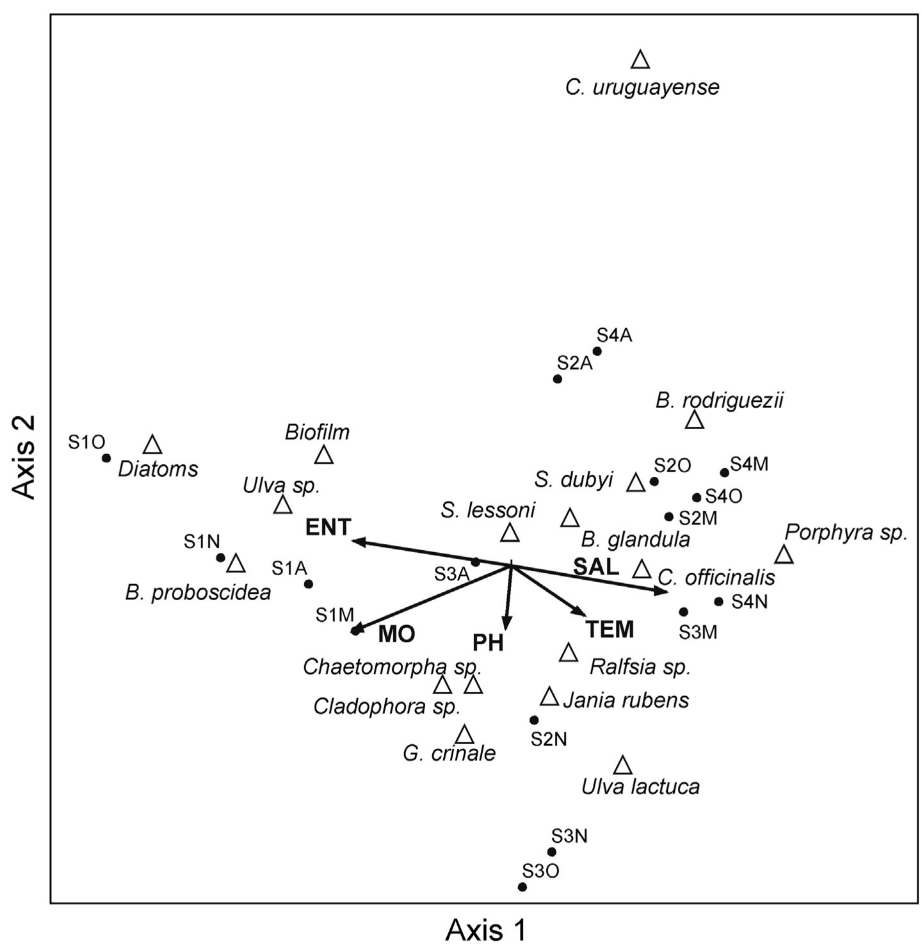

Fig. 4. Ordination diagram of species and sites (sites code: "S" = site, "number" = number of the site, "letter" = initial season) in the two principal axes of the CCA. The relative lengths of the arrows indicate the importance of a variable. *B. glandula* = *Balanus glandula*, *B. proboscidea* = *Boccardia proboscidea*, *B. rodriguezii* = *Brachidontes rodriguezii*, *C. officinalis* = *Corallina officinalis*, *C. uruguayense* = *Ceramium uruguayense*, *G. crinale* = *Gelidium crinale*, *S. dubyi* = *Schizymenia dubyi*, *S. lessoni* = *Siphonaria lessoni*. ENT = Enterococcus, MO = organic matter, PH = pH, TEM = temperature and SAL = salinity.

concentration supports high densities of primary producers and consumers by increasing food availability (Martinetto et al., 2010, Fricke et al., 2015). Otherwise, the abundance of the benthic community dominant specie (e.g. the mussel *Brachidontes rodriguezii* in northern SW Atlantic coast, Adami et al., 2004) decreased because the sewage impact, and thus, the development of other epilithic or infaunal species are allowed, increasing the species richness and avoiding the monopolization of the dominant specie (i.e. intermediated disturbance hypothesis, Connell, 1978) (López Gappa et al., 1990; Vallarino and Elías, 2006). In the present study, the abundance of the dominant *B. rodriguezii* was almost zero near the discharge; however, it was replaced by *Boccardia proboscidea* who dominated the impacted site avoiding the settlement of other species and decreasing drastically the diversity parameters. Although it was not registered here, a decrease in the dominant species (*B. rodriguezii*) and the consequent increase in community diversity were mentioned for intermediate levels of pollution (i.e. 100–150 m) in the study area (López Gappa et al., 1990).

The sewage impact in this case has a short range (i.e. <330 m from the outfall), but it was high enough to observe the drastic decrease in the diversity parameters near the sewage outfall. Another similar study was carried out in the sewage outfall of Mar del Plata city (a seaside resort of Argentina located at 120 km from the present site of study) 9 years ago (Elías et al., 2009). Likewise, the intertidal abrasion platforms, inhabited (still are) by the mussel *Brachidontes rodriguezii* and affected by sewage discharge, were tested using both coverage and quantitative methods in order to assess the effect of a non-functioning period of the pre-treatment plant. Analysis with either quantitative and coverage data showed significant differences between a sewage-impacted point and reference locations and between before and after a period of no function of the sewage plant. The reference locations were characterized by high coverage of *B. rodriguezii* and *Ulva* spp., while the impacted location was characterized by >80% of bare space and sand. Although the scale of sewage discharge of Mar del Plata city is quite different when compared to the sampled city (Mar del Plata dumps >3 $m^3 \cdot sec^{-1}$ raw sewage while Quequén discharges 0.16 $m^3 \cdot sec^{-1}$), the response of the benthic community was similar to the present study. Also the coverage method showed similar results respect to quantitative method (Elías et al., 2009).

Important explanatory variables of benthic species assemblages in impacted sites were organic matter and *Enterococcus* concentration. Earlier studies has already indicated that *Boccardia proboscidea*, *Ulva* sp., diatoms and biofilm abundance (this two latter related with bare substrata) and the bacteriological burden were related to high organic matter concentration discharged by the sewage outfall of Mar del Plata city (Pérez Guzzi, 2006, Garaffo et al., 2012, Jaubet et al., 2013, Sánchez et al., 2013, Elías et al., 2014, Becherucci et al., 2016a and 2016b). The absence of *B. rodriguezii* was also an indicator of sewage impact in the present study and its abundance was related with the opposite environmental pattern (i.e. low organic matter and *Enterococcus* concentration) as was previously registered by López Gappa et al. (1990). As those authors discuss, the absence of *B. rodriguezii* from the most heavily polluted area may be attributed to the high proportion of suspended particles contained in sewage, which could negatively affect the filtering mechanism. As well as given that the insufficient oxygen supply during low tide, since microorganisms would consume high proportion of initially available oxygen at 0 to 50 m from the outfall. The high concentration of *Enterococcus* near the outfall was in accordance with this lasted hypothesis. The absence of *Brachidontes rodriguezii* and associated species is not compensated by the increase in other epilithic species populations, so that extensive areas remain unoccupied by macrobenthic organisms or occupied by *Boccardia proboscidea*. In the study of López Gappa et al. (1990) the presence of dense infaunal populations of *Boccardia* sp. is mentioned in the highly polluted area.

Considering the algae fraction, contrary to the literature, an increase of *U. lactuca* with higher pollution levels was not observed in the present study. Although *U. lactuca* is the most abundant macroalgae species from 330 m, it reaches peak cover values in sites located away from the outfall (i.e. site 3) and it was replaced by *Ulva* sp. near the outfall. This result is in accordance with it registered by López Gappa et al., 1990 in the same study area and by Becherucci et al., 2016a in Mar del Plata sewage outfall impacted coastal area in which *U. lactuca* dominate the algae fraction in unpolluted sites and *Ulva* sp. in sewage polluted sites.

As was above mentioned, there was a local (i.e. short range) sewage outfall effect in the study area since in site 2 (i.e. 330 NW away from the discharge) the respond of benthic community (in terms of diversity and the species composition) there was not registered. This is in accordance with López Gappa et al. (1990) who registered polluted condition in 50 to 100 m away the outfall and unpolluted condition at 200 m of it in the same study area. In Mar del Plata coast, the impact of the sewage outfall comprise until 800 m of the coast with a clear differences in the algae (with dominance of the diatom *Berkeleya* sp., Becherucci et al., 2016a) and faunal composition (with dominance of *Boccardia proboscidea*, Sánchez et al., 2013, Jaubet et al., 2011), however, the sewage average discharge in Mar del Plata city is c.a 20 times higher than average discharge of Quequén city, reaching 2.8 m^3 seg−1 in winter and 3.5 m^3 seg−1 in summer (Scagliola et al., 2006). Although it was possible to conclude that >330 m away from the discharge point there was no evident sewage impact over the benthic community, the precise information and the recommendation of the unsafe use beaches in close proximity to the discharge should be highlighted by the council authority, mostly given that the concentration of *Enterococcus* registered in this study (i.e. around 1.5×10^6 NMP/100 mL in the outfall site) is c.a. 42,000 times higher than the maximum recommended value (i.e. 35NMP/100 mL) by the United States Environmental Protection Agency (USEPA) for recreational coastal waters.

The benthic community studied integrate environmental conditions and changed in a very effective manner, allowing therefore account the disturbances produced by organic enrichment. The response of macrobenthic communities to several types of stress was well studied, based on multivariate analyses that consider variations in species diversity and their relative abundance between perturbed and control sites (Pearson and Rosenberg, 1978; Warwick and Clarke, 1993; Gray et al., 2002; Orfanidis et al., 2003; Ballesteros et al., 2007). In that sense, the use of the cover values of the macrobenthic species according to the polluted impact generated by the sewage outfall of Quequén city resulted in a usefully, rapid and not cost way to evaluate the environmental health of the coastal area.

5. Conclusions

Boccardia proboscidea dominated around the outfall with variable abundance of *Ulva* sp. during the period of study. Consequently, the structure of the community (referring to as specific composition and diversity parameters) showed a remarkable difference between the polluted area (outfall site) and the rest of sites (from 330 up to 700 m away the outfall). The high proportions of both organic matter in the sediment and *Enterococcus* concentration in seawater led to such changes in the community of the polluted area. The combinations of both increasing discharge volume and desiccation stress during the summer season reduces the specific richness of the community further highlighting the dominating species (i.e. *B. proboscidea* in impacted site and *B. rodriguezii* in the rest of the sites). The structure of the epilithic benthic community responded significantly to the sewage outfall and, thus, its monitoring represent an effective, non-destructive and semi-quantitative method to assess the health of the coastal environment in the study area in a relative inexpensive and rapid way.

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