Massive polychaete reefs as indicator of both increase sewage-contamination and chlorination process: Mar del Plata (Argentina) as a case not of study

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Introduction

Mar del Plata city, located 400 km south of Buenos Aires, was in the earlier XX century the recreational seaside place where the people of the high society enjoy sand and sun. At the beginning all houses has cesspit or septic tank, and when they need maintenance (frequent emptying) the wastes where dumped 9 km north to the city. Stockholders choose the same place for sewage discharge. Popular politics transform the city into a popular place. Today Mar del Plata (c.a. 600,000 inhabitants in 2010 census) has the worst scenario for a sea side resort: 2-3 million people spend their summer time (January-March) in the city ([1]), and the sewage effluent discharge untreated on intertidal zone affecting 10km of bathing beaches ([2]).
Sewage discharge is one of the oldest forms of contamination. To assess the effect of contaminants on the environments the use of biological indicators is recommended, because the organisms could show the healthy of the environment ([3]). In particular, benthos (organisms closely related to bottom) is particularly useful. These organisms live in the water-sediment interface, that is biologically active and chemically reactive ([4]), and due to the null or very limited motility they reflect the situation at the time of the sampling but also the precedent situation.
Benthic studies on Mar del Plata's intertidal community were conducted in both natural areas and around sewage effluent since 1997. The intertidal community in natural habitats is dominated by mussel beds of *Brachidontes rodriguezii,* forming a secondary infaunal habitat for several invertebrate fauna, including several species of polychaetes. Studies for monitoring purposes in coastal, especially in soft-bottom, habitats have used extensively polychaetes ([5]). Among benthic groups, polychaetes are, in fact, one of the best indicators of environmental disturbance, since this taxon contains both sensitive and tolerant species in a gradient from pristine to heavily disturbed habitats ([6]). In Mar del Plata both the polychaetes and community-level were used to assess the sewage impact to the environment ([7, 8, 9, 10]). This work describes the polychaetes composition and distribution associated to the intertidal community in both, natural and sewage-affected community along more than 10 years of study, till the development of unique, massive reef of organic contamination indicator polychaete.

Materials and Methods

The quantitative samples were collected from November 1997 to November 2008 in the intertidal community developed in both the Impacted and the Control site. Most samples were performed in November and March, but also some monthly or seasonal samples were taken in some years. Due to climate problem like tide, some samples didn't be taken in some monthly or seasonal samples.

Samplings in November-March (before/after summer) were taken in order to evaluate the effect of summer and increasing organic pollution on benthic community. This effect was partially analyzed in other studies ([7]) but is not an objective of this contribution.

Four sampling stations were sampled along the putative gradient, at 50, 200, 800 and 1200 m south to the effluent. A Control site was 9,000 m to the north to the effluent. Sampling was carried out at each area at upper or media intertidal level on the intertidal benthic community developed in abrasion platforms, exposed during regular tide. At each sample point four random sampling units were taken in independent rocks with a 78 cm^2 and 20 cm long corer. For the present analysis three areas were considered by pooling data from the Impacted site (between 50-200 m south to the sewage effluent), the site called Control 2 (between 800 to 1,200 m south to the effluent), and the Control site (an area 9,000 m north to the effluent) (Fig. 1).

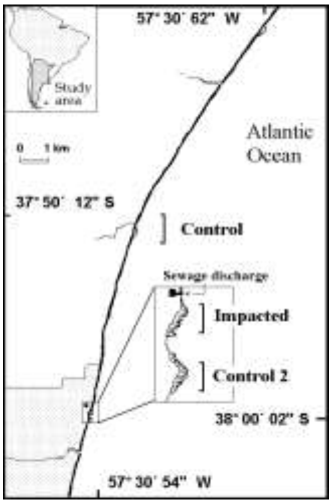

Figure 1. Sampling area and the three sampling sites analysed from 1997 to 2008: Impacted, Control and Control 2 sites along the putative gradient.

The samples were fixed in a 10 % formaldehyde solution with sea water until laboratory analysis. In the laboratory these samples were sieved through a 1 mm screen mesh and the number of polychaetes retained was quantified and preserved in 70% alcohol.

The environmental variables of seawater (pH, turbidity, salinity and temperature) were also measured *in situ* with a U-10 Horiba. The total organic Carbon content (%) was determined by titration from samples of the sediment retained among mussel beds.

The non-metric multi-dimensional scaling (MDS) technique was employed to show the distance among samples along the years in the same way to show the distance among the years used the environmental variables. The Bray-Curtis index and the double root transformation were used. To assess the differences between the three groups previously outlined an Analysis of Similarities (ANOSIM) was carried out, followed by a Similarity Percentage analysis (SIMPER) to assess the species that most contributed to the differences. In order to relate environmental data to biological data (species abundance matrix) and explain the biological pattern observed, the BIO-ENV procedure was applied. The best matches of abiotic and biotic similarity matrices were measured using the weighted Spearman rank correlation coefficient (qw). All this analyses were carried out using the PRIMER package.

The quantitative analysis was limited to the graphical display of each species according to areas and years, because assumptions for parametric analysis were not achieved (normality, homogeneity of variance, and number of observations in each cell). Proportion and densities of main polychaete species were plotted to show the main relationships of polychaetes. The first 10 species according to their abundance were selected and plotted year by year in the three areas.

Additionally a cover method was used to assess the percent of occupation of the dominant fauna in two control and two impacted sites (Fig. 2). In each site the percent cover of main species were measured and averaged from the ten squares of 50x50 cm. Samplings were carried out from June 2008 to February 2009.

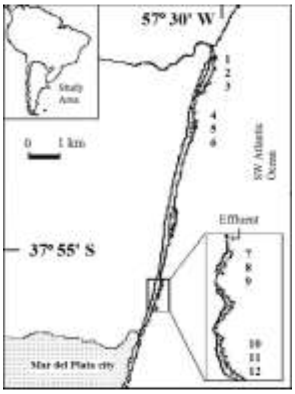

Figure 2. Complementary cover method in two Control (1-3, 4-6) and two Impacted (7-9, 10-12) sites, carried out from June 2008 to February 2009.

RESULTS

A total of 27 polychaete species belonging to 11 families (64,723 individuals) were recorded from 1997 to 2008 in the intertidal reference areas and those affected by sewage discharges. The first 10 species in order of abundance accomplish the 99.5 % of the total individuals (Table 1).

Table 1. Ranking of polychaetes taxa in mussels' bed. Dominance is the percentage of the abundance of each species in relation to the total abundance of polychaetes.

Taxa	Dominance
Boccardia proboscidea	**40,19**
Boccardia spp.	**22,96**
Syllis prolixa	**12,86**
Leodamas uncinata	**7,74**
Rhynchospio glutea	**6,88**
Syllis gracilis	**3,96**
Capitella "capitata" sp.	**3,73**
Alitta succinea	**0,60**
Protocirrineris angelicolliato	**0,35**
Caulleriella bremecae	**0,24**
Syllis indet. 2	0,13
Polychaete indet.2	0,11
Polychaete indet.1	0,10
Lumbrineris tetraura	0,04
Polydora sp.	0,02
Syllidae indet.	0,03
Phyllodoce sp.	0,03
Dodecaceria meridiana	0,02
Polychatete indet. 3	0,01
Hesionidae	<0,01
Spionidae 1	<0,01
Glycera americana	<0,01
Heteromastus similis	<0,01
Terebellidae	<0,01
Syllis indet. 1	<0,01
Paleanotus intermedius	<0,01
Halosydnella sp.	<0,01
Lumbrineriopsis mucronata	<0,01

The spatial analysis

The non-metric multi-dimensional scaling (MDS) with the 75 sampling units show samples of year 2008 far from main cluster (Fig. 2). This was due to the great dominance of *Boccardia proboscidea* in this year (see tables 2, 3). The rest of sampling units without *B. proboscidea* (above the dotted line) show a separation among the different areas from Control site (black squares, below) to Impacted site (open squares, above). The Control 2 site constitutes an intermediate zone, and sampling units are placed roughly between the Impacted and the Control sites (squares with a cross).

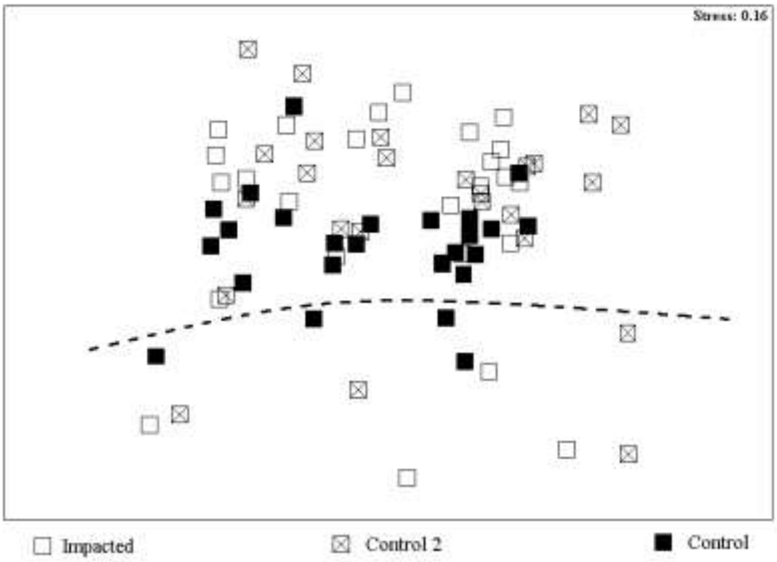

Figure 2. MDS with all sampling units discriminate by areas. Note the 2008 sampling units clustered in the bottom (separated by a dotted line) due to the dominance of *Boccardia proboscidea*.

The Analysis of Similarity (ANOSIM) show significant differences (Global R= 0.116, p 0.2%), been all groups (areas) different from each other ($R_{Impacted,\ Control\ 2}$= 0.059, p 4.3%; $R_{Impacted,\ Control}$= 0.171, p 0.2%; $R_{Control\ 2,\ Control}$= 0.123, p 0.4%). The Similarity Percentage (SIMPER) analysis show the species that most contributed to the differences among sites by pooling the years analyzed (Table 2). The sewage-impacted areas are characterized by the capitellid *Capitella "capitata"* sp. and the spionids *Boccardia* spp. and *B. proboscidea*. The later reached so high abundance in year 2008 that has a significant contribution in average abundances of the ten years. The Control 2 area is characterized by high abundances of the spionids but also by *Leodamas uncinata* and *Syllis prolixa*. The Control site have the same polychaetes species, but in lower abundances.

Table 2. SIMPER showing the species that characterize the sites affected by sewage discharges.

Average dissimilarity = 73.31						
	Impacted	Control 2				
Species	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum%
Boccardia spp.	40.48	38.81	18.74	0.96	25.57	25.57
B. proboscidea	54.70	101.39	14.52	0.51	19.80	45.37
S. prolixa	13.69	37.55	13.30	1.00	18.15	63.52
L. uncinata	9.66	19.79	7.38	0.76	10.07	73.58
C."capitata" sp.	15.07	1.67	6.92	0.75	9.44	83.02
R. glutea	2.60	29.33	6.15	0.39	8.39	91.41

Average dissimilarity = 76.68						
	Impacted	Control				
Species	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum%
Boccardia spp.	40.48	28.24	26.05	1.19	33.97	33.97
C. "capitata" sp.	15.07	0.25	11.92	0.83	15.55	49.52
B. proboscidea	54.70	2.86	11.81	0.45	15.41	64.93
S. prolixa	13.69	7.60	10.27	0.81	13.39	78.32
L. uncinata	9.66	6.78	7.80	0.68	10.17	88.48
S. gracilis	3.80	3.92	2.98	0.89	3.89	92.37

Average dissimilarity = 75.48						
	Control 2	Control				
Species	Av.Abund	Av.Abund	Av.Diss	Diss/SD	Contrib%	Cum%
Boccardia spp.	38.81	28.24	23.90	1.08	31.66	31.66
S. prolixa	37.55	7.60	18.25	1.12	24.18	55.84
B. proboscidea	101.39	2.86	10.76	0.44	14.25	70.09
P. uncinata	19.79	6.78	8.18	0.98	10.84	80.93
R. glutea	29.33	0.30	5.90	0.35	7.82	88.75
S. gracilis	10.55	3.92	5.55	0.91	7.36	96.10

The species that most contributed to the differences among years is shown in another SIMPER analysis (Table 3). It is clear for the average abundance why the year 2008 is far from other sampling year due to *Boccardia proboscidea*. The year 2003 is also different due to the high dominance of *Boccardia* spp., while 2005 has a great dominance of *Rhynchospio glutea*.

Table 3. Average abundances of species in the different years, and discriminated by the SIMPER analysis as important in pair by pair comparisons. The sub index represents the order within the year, not the comparison between years.

	1997	1998	1999	2000	2001	2002	2003	2004	2005	2008
S. prolixa	68.2_1	9.6_3	6.5_2	17.7_2	25.9_2	8.3_2	1.90_4	34.1_1	9.1_4	17.8_2
Boccardia spp.	8.9_6	36.0_1	42.9_1	22.8_1	38.3_1	38.3_1	220.8_1	24.3_2	34.1_1	0
L. uncinata	11.9_3	16.6_4	2.5_6	5.19_3	6.4_5	0	13.9_2	23.1_4	18.3_2	7.2_3
S. gracilis	24.4_2	8.3_2	2.9_3	4.06_4	6.2_3	3.1_3	2.9_3	7.4_3	3.6_3	0
C. "capitata" sp.	6.4_{13}	6.7_5	3.8_4	11.4_5	7.4_4	3.2_4	0.8_6	6.7_5	6.3_6	0
A. succinea	0.5_{11}	1.4_7	1.14_5	1.9_6	0.9_6	0.2_5	0.04	0.8_7	0.2_7	0
R. glutea	1.4_5	0.0	0	3.1_7	0.07_9	0	1.3_5	17.8_6	83.3_5	0
C. bremecae	5.6_4	0.6_6	0.2_6	0	0.03_{10}	0	0	0.02_9	0	0
P. angelicolliatio	0.6_{10}	1.7_8	0.2_7	0.6	0.2_7	0	0	0.11_7	0	0
B. proboscidea	---	---	---	---	---	---	---	---	---	406.2_1

In the other hand, the analysis of the percent covers of the community show significant changes from June, when *Boccardia* spp. are present but only in Control sites, to November-December 2008 and January 2009 when polychaete reef of *Boccardia proboscidea* develops (Fig. 3). Thereafter, the reef dominates all surfaces of rocks in sewage-impacted sites, showing a cycle of storm-destruction/fast rebuild. The cycle continues until local water authority start chlorinate the untreated sewage effluent, resulting in complete depletion of flora and fauna from February 2009.

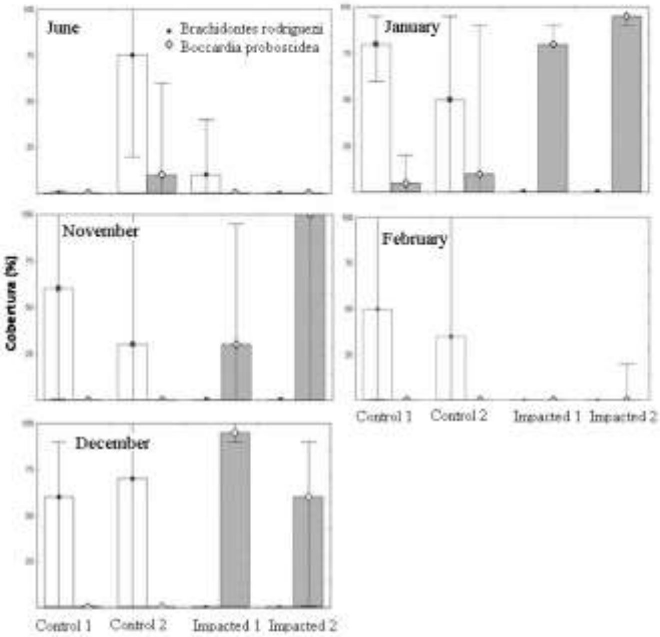

Figure 3. Percent cover of the mussel *Brachidontes rodriguezii* and the invasive polychaete *Boccardia proboscidea* in Control and sewage-Impacted sites close to Mar del Plata seaside resort. The invasive polychaete dominates sewage-impacted sites during 2008 but disappeared after the chlorination process starting in January 21/28th 2009.

Environmental variables

Organic matter (Fig. 4) show regular values along the time, with a peak in the impacted area during 2003 and other greater in 2008 in the three areas. Turbidity (Fig. 7b) show peaks in different areas in different times and an increasing pattern in the last years, except during 2002. Temperature of water show erratic values, perhaps due to years when sampling occur all along the year (including winter months). The pH is very variable, while Salinity is relatively constant, except for the low values in the impacted area (due to freshwater running). Salinity drops in 2008 in all areas. The results of the BIO-ENV procedure showed that the maximum correlation (r= 0.394) among environmental variables and intertidal benthic community was reached when all the 5 variables were used.

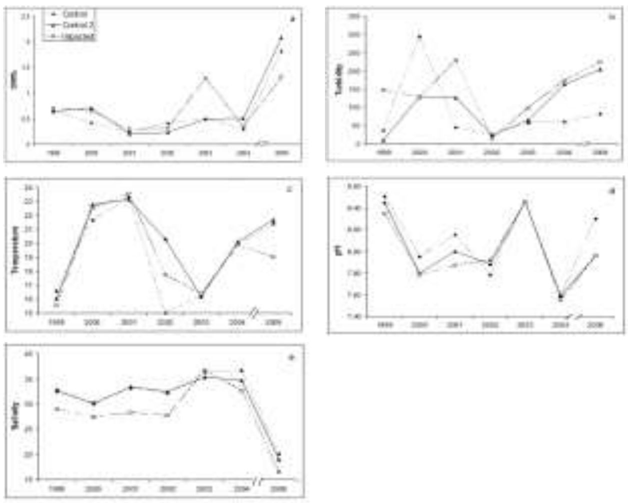

Figure 7: Environmental variables in the three sampling sites a long the years.

Discussion

The site herein named Control 2 behaves in the past as a Control or reference site ([8, 9, 10]). Long shore currents and dominant winds (from east or south) push the sewage flume to the northward far from the sampling area and the city. During summer, predominant winds came from the North, then the flume set down to the South, i.e. to the sampling area and to the city (where are placing the popular beaches). During summer massive tourism produces a significant increase in sewage volume up to 3.5 $m^3.sec^{-1}$ (about 60 % greater). These two factors produced a significant increasing pollution effect on intertidal benthic organisms, and the site call Control 2 was not longer a reference site ([7, 8, 9, 10]). The present study shows the area named Control 2 is now permanently sewage-impacted. The change was confirmed from 2005 by the presence of a community structure and indicator species characteristic of a sewage-impacted site. The same tolerant polychaete species of Impacted site can be seen in the Control 2 site. Further more, from year 2008 a new, massive pollution indicator, a spionid polychaete develops around the sewage discharge. Spionids are classical indicator of disturbance, responding to organic enrichment and pollution ([6, 11] among others). *Boccardia* spp. are also frequently mentioned as being in or around sewage outfalls in the Southern Hemisphere, like B. *polybranchia* or *B. proboscidea* in Australia ([12]) and also in California ([13]). In organically enriched areas around Mar del Plata city *Boccardia* spp. showed a peak very high during 2003 in relation to an increase of the organic pollution, producing a decrease on the other species. These species proliferate in both the Impacted and Control 2 sites due to the continuous flow of organic matter from the sewage outfall. In another year the spionid that produce a peak was *Rhynchospio glutea* in Control 2 site (2005).

B. proboscidea is an invasive species and beginning to constitute part of the landscape in the Impacted and Control 2 site during spring of 2008, showing high densities (140,000 $ind.m^{-2}$ average densities). In their natural habitat, the Pacific area, the species could reach densities no greater than 20,000 $ind.m^{-2}$ ([13]). In this locality of the SW Atlantic, this species have beginning to build biogenic reefs, massive sand tubes that could support a person walking. The reef covers almost completely the entire impacted area, from the outfall to more than 2,000 m south to the sewage effluent (including the Control 2 site). Due to their own population dynamics (highly effective reproduction plus high growing rate), and the massive organic matter flow the worms cover the entire surface of rocks and excluded any other intertidal flora or fauna. Some sampling units reach densities as high as 656,250 $ind.m^{-2}$ ([14]). The data show polychaete reefs in rapid cycles of creation/destruction by storms, however, soon after the chlorination process started ([15]) the reef decline until disappear. Although no Environmental Impact Assessment was performed (compelled by law) the chlorination started (40 hours of 7-10 ppm of sodium hypochlorite in 4 days) between January-February 2009 ([15]), resulting in depletion of all flora and fauna from the area.

No other fauna or flora could colonize the area until several moths later. Chlorine can react with naturally occurring organic compounds found in the water to produce compounds known as disinfection by products (DBPs), causing environmental harm at low levels. Chlorine is especially harmful to organisms living in water and in soil ([16]).

The data strongly suggest that the explosive development of polychaete reefs was trigged by the progressive increasing of sewage-contamination. The disappearance of the reefs in February 2009 is also highly correlated to the chlorination process, and the release of chlorinate water to the environment. Mitigation process kills both the invasive polychaete reef and natural fauna.

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