



The impact of sewage on environmental quality and the mesozooplankton community in a highly eutrophic estuary in Argentina

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Dutto, M. S., López Abbate, M. C., Biancalana, F., Berasategui, A. A., and Hoffmeyer, M. S. The impact of sewage on environmental quality and the mesozooplankton community in a highly eutrophic estuary in Argentina. – ICES Journal of Marine Science, doi:10.1093/icesjms/fsr204.

Received 1 April 2011; accepted 1 December 2011.

Eutrophication caused by human sewage is a growing phenomenon along coasts. Mesozooplankton abundance, community structure, and environmental quality were compared at two sites in the inner Bahía Blanca Estuary (BBE), Argentina, one receiving regional sewage discharge (Canal Vieja), and the other a non-impacted area (Bahía del Medio). Comparisons of mean abundance and multivariate analyses were performed to detect spatial and temporal variations in both environmental and biotic variables. Significantly higher amounts of nitrogen and phosphorus and lower oxygen and pH were recorded at Canal Vieja. Larvae of the detritivorous crab *Neohelice granulata* were more abundant at Canal Vieja, whereas the common estuarine copepod species *Acartia tonsa* was more abundant at Bahía del Medio, where the mesozooplankton community was typical for the estuary. Although mesozooplankton is likely to be adapted to the high organic matter content in the BBE, the environmental conditions at Canal Vieja appear to favour *Neohelice granulata*, and this species should be considered as a potential indicator of the impact of sewage in future monitoring programmes. Particulate organic carbon and chlorophyll *a* were not influenced by organic loading in this naturally eutrophic estuary.

Keywords: environmental quality, estuary, mesozooplankton, multivariate analyses, nutrient enrichment, sewage.

Introduction

Understanding how coastal ecosystems respond to anthropogenic disturbance is critical for resource management. Recent ecological studies evaluating the impact of anthropogenic activities on the functioning of ecosystems in coastal environments have identified plankton as a useful indicator for assessing ecological status (Conley, 2000; Beaugrand, 2005). Coastal eutrophication, i.e. water enrichment with nutrients and organic matter (Nixon, 1995), has been driven by human activities, and it has affected pelagic habitats directly (Nixon and Fulweiler, 2009). Enhancement of hypoxic conditions, water turbidity, and primary production have been the most common changes associated with eutrophication (Cloern, 2001). Shifts at the base of the food chain may modify trophic interactions and hence carbon transfer towards upper trophic levels (deYoung *et al.*, 2008). Monitoring coastal environments provides information valuable for assessing ecosystem vulnerability to aquatic habitat loss as a direct and an indirect effect of human activity (Halpern *et al.*, 2007).

Eutrophication of estuarine environments is a key subject in ecosystem dynamics because it often leads to habitat deterioration

(Pinckney *et al.*, 2001) and it has implications at different levels of biological organization, from individuals to communities. For instance, limited population growth and changes in community structure in response to pollutants and eutrophication have been well documented in some estuarine systems in the northern hemisphere (Park and Marshall, 2000; Gordina *et al.*, 2001; Uriarte and Villate, 2004). In warmer waters affected by domestic and industrial waste, a greater abundance of zooplankton and a shift from a natural to an opportunistic-dominated community have been documented (Siokou-Frangou and Papathanassiou, 1991). Given the central role zooplankton has in foodwebs, i.e. linking environmental and ecological variability, it emerges as a suitable indicator of changes in the ecosystem status, including climate modifications and eutrophication-related processes.

The Bahía Blanca Estuary (BBE), Argentina, is a eutrophic system in the Southwest Atlantic whose drainage area is mostly occupied by urban and industrial settlements and farms. The deposition of human-derived wastes into this productive system has increased in the past decade (Marcovecchio *et al.*, 2008). In particular, the considerable quantity of nutrient and organic matter

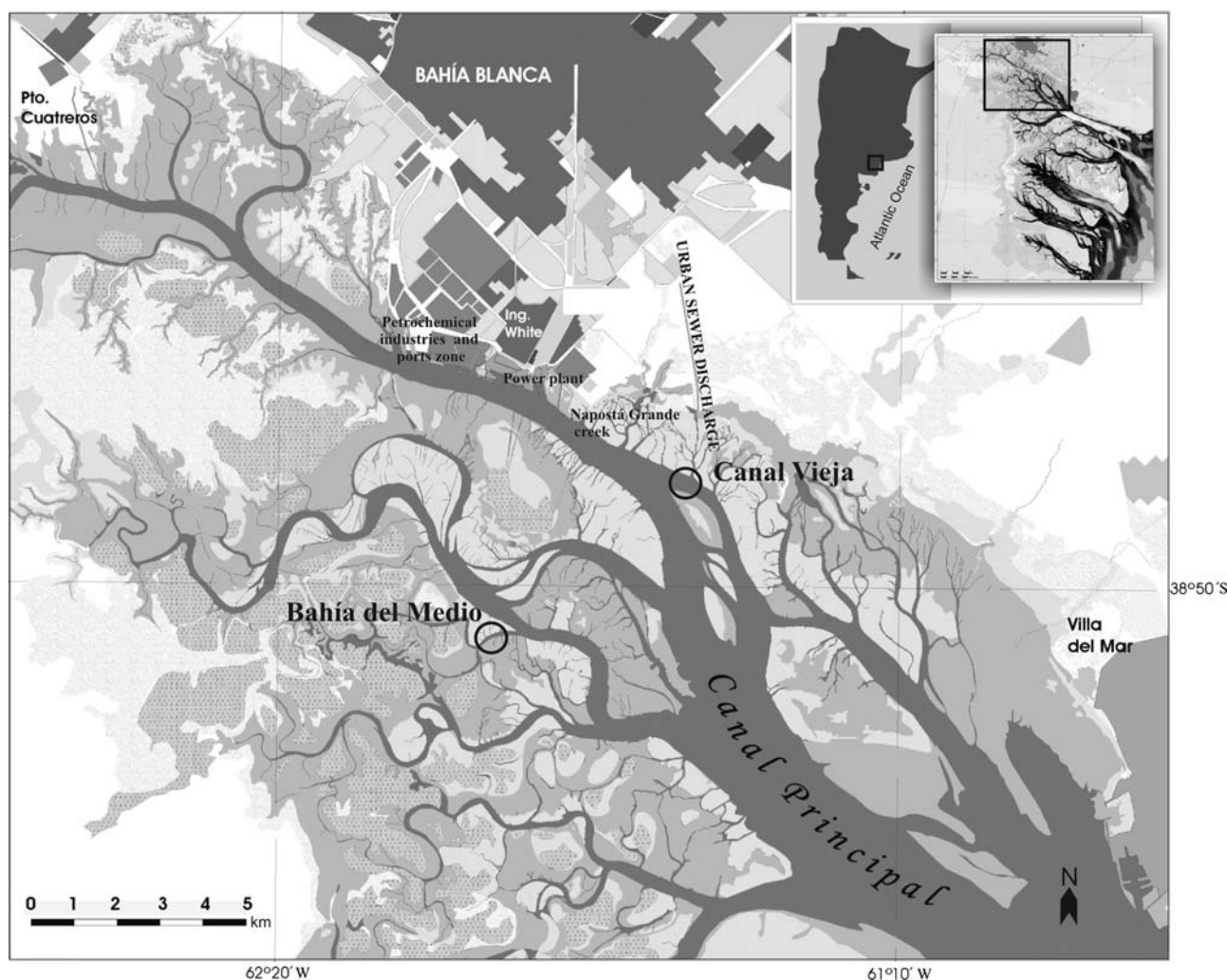


Figure 1. Map of the BBE showing the sampling sites: Canal Vieja (impacted site, sewage discharge) and Bahía del Medio (non-impacted site, tidal channel).

from urban sewage is of growing concern in its management (Limbozzi and Leitão, 2008). Some of the environmental and ecological shifts documented in the discharge-affected area were oxygen depletion, elevated organic matter, nutrients, and bacteria, and deleterious effects on the microzooplanktonic community (Lara *et al.*, 1985; Cabezali and Burgos, 1988; Barria de Cao *et al.*, 2003). Here, we compared environmental quality and the mesozooplankton community in an effluent-affected area and a non-impacted area within the BBE during contrasting seasons. Allochthonous organic matter and nutrient input alter the natural biochemical conditions and planktonic assemblages in the affected zone, and these modifications are detectable across seasons. The aims were to (i) analyse the differences in environmental quality between the sewage-affected and the non-impacted site, and (ii) detect variations in the mesozooplankton community structure at the two sampling sites.

Material and methods

Study area

The BBE is a mesotidal coastal plain environment (Perillo *et al.*, 2001) located in a temperate, semiarid zone on the southern

Atlantic coast (38°45′–39°40′S 61°45′–62°30′W) of Argentina (Figure 1). It covers ~2300 km² and is formed by a series of NW–SE channels separated by many interconnected tidal channels, islands, extensive tidal flats, and low marshes. The main navigation channel is funnel-shaped, with a total length of ca. 68 km and a width that varies from 200 m at the head to 3–4 km at the mouth (Perillo and Piccolo, 1991).

The estuary is turbid and shallow (mean depth 10 m), with a homogeneous water column vertically mixed by the effect of tides and winds (Perillo *et al.*, 2001). Freshwater inflow is low and mainly provided by two tributaries in its inner area, with a mean annual run-off of, respectively, 1.9 and 0.8 m³ s^{−1} (Perillo and Piccolo, 1991). Water circulation is driven by a standing semi-diurnal tidal wave, which constitutes the principal energy input into the system. Circulation is strongly affected by the prevailing winds that modify the tidal wave and the particular geomorphology of the estuary that displays a progressive meandriform pattern (Perillo *et al.*, 2001). The mean tidal amplitude varies between 3.5 m at the head and 2.2 m at the mouth, and the tidal range and tidal current amplitude increase headwards (Perillo and Piccolo, 1991). Residence time for the inner area was calculated as ca. 28 d (G. Perillo, pers. comm.). The residual circulation

shows a significant difference in the direction of the mass transport, causing salt concentrations in the inner portion of the estuary often to exceed those of the inner continental shelf. However, negative circulation has not been documented (Perillo *et al.*, 2001).

Salinity ranges from 17.3 to 41.9, depending on seasonal rainfall, winds, and temperature (Freije *et al.*, 2008), and it increases exponentially in the first 6 km from the head (Perillo *et al.*, 2001). Hypersaline conditions are usually recorded during hot dry summers, when elevated evaporation rates produce high concentrations of salt in the adjacent tidal marshes (Freije *et al.*, 2008). Mean surface-water temperature is about 23°C in summer and 7.1°C in winter, and it is always slightly higher at the head of the estuary (Freije *et al.*, 2008). The elevated nutrient content observed in the estuary year-round can be detected on the adjacent shelf, several miles off the estuary (Marcovecchio and Freije, 2004), making it one of the most productive coastal ecosystems in the region (Freije *et al.*, 2008). The typical weather pattern of the region is characterized by a broad temperature gradient, strong N–NW winds (mean velocity 6.7 m s⁻¹ with events of >28 m s⁻¹) and low rainfall (498 mm year⁻¹), influenced by marked seasonality with most precipitation during spring and summer and least during winter (Piccolo, 2008).

The estuary, especially its north coast, is subject to high anthropogenic impact (Arias *et al.*, 2010) as a result of human settlement, commercial ports, and petrochemical and chemical industries (Figure 1). The sampling points were chosen based on previous investigations and technical reports (e.g. Baldini *et al.*, 1999; Tombesi *et al.*, 2000; Marcovecchio *et al.*, 2008). Both sampling sites are located in the inner part of the estuary (Figure 1): Canal Vieja, which lies on the northern shore of the estuary, is a channel 300-m wide with a mean depth of 6.5 m that receives untreated sewage from more than 300 000 inhabitants (Tombesi *et al.*, 2000), whereas Bahía del Medio is a small tidal channel located in the southeast of the estuary which has a mean depth of 4.5 m and mean width of 70 m. The latter is associated with a particular looping channel system surrounded by a great expanse of tidal flats, covered by the perennial cordgrass *Spartina alterniflora* (Hoffmeyer and Barriá de Cao, 2007). Its location, geomorphological features and bacteriological condition make it suitable for aquaculture activity (Baldini *et al.*, 1999; Hoffmeyer and Barriá de Cao, 2007, and references therein).

Sampling and laboratory procedures

To detect the differences in the mesozooplankton assemblages that dominate contrasting seasons, five sampling campaigns were carried out during summer (December 2008 and February and March 2009) and winter (July and August 2009) at both study sites. At each site, four horizontal tows were conducted in the upper layer (0–2 m) during mid-ebb tide at ca. 2 knots, using a plankton net of 200 µm mesh. A General Oceanics® digital flowmeter was used to estimate the filtered volume of seawater. Environmental variables including temperature, salinity, pH, turbidity, and dissolved oxygen (DO) were measured at the time of each tow using a HORIBA® U-10 multiparameter probe. Additionally, surface-water samples were collected to determine inorganic dissolved nutrient concentration (nitrite, nitrate, phosphate, silicate, and ammonium), chlorophyll *a* (Chl *a*), phaeopigments, and particulate organic carbon (POC). Sampling was performed during daylight.

Chemical determinations were carried out by the Marine Chemistry Laboratory at IADO following internationally validated methods (Strickland and Parsons, 1968; Technicon®, 1973; Tréguer and Le Corre, 1975; Hansen and Grasshoff, 1983; Eberlein and Kattner, 1987; APHA-AWWA-WEF, 1998). Mesozooplankton samples were preserved in 4% buffered formaldehyde–seawater solution until analysis. All were qualitatively and quantitatively analysed under a Wild M5 stereoscopic microscope. Samples with high abundance, collected in December and February, were diluted to 250 ml and after homogenization, five aliquots of 5 ml were placed into counting chambers (subsampling volume: 1/10), and all mesozooplankton taxa in each aliquot were counted. The remaining samples (collected in March, July, and August) were counted in total. Identification was to the lowest possible taxon using appropriate identification keys and descriptions (Boltovskoy, 1981), and abundance was expressed as ind. m⁻³.

Data analysis

Univariate analyses

To ensure thorough comparative analysis, different indices were used to estimate the diversity at the two sites during both seasons: the Shannon–Wiener index ($H' = -\sum p_i \log p_i$, where p_i is the proportion of each taxon in the sample), the Margalef richness index [$d = (S - 1)/\log N$, where S is the total number of taxa and N the total number of individuals], and the Pielou evenness index ($J' = H'/\log S$). The PRIMER® 5 package (Clarke and Warwick, 1994) was used to determine these indices. Environmental and biotic variables at the two sites were compared for each season and between seasons to seek significant differences. Owing to the rejection of normality and homoscedasticity assumptions, a Mann–Whitney test was used to compare the variables between sites and seasons, and the mean abundances of species with relative abundance >1% were compared between sites and seasons also using a Mann–Whitney test. Remaining taxa with <1% relative abundance were grouped under “other taxa”. All statistical analyses were conducted at a significance level of 0.05 using NCSS® 2007 software.

Multivariate analyses

Principal component analysis (PCA) by Spearman's rank correlation matrix was used to arrange and visualize the data and to detect relationships among environmental variables and their association with the study sites. Sampling sites were included as a supplementary variable to determine their spatial arrangement according to data input. Diversity indices, total mesozooplankton abundance, and the abundances of the dominant species (*Acartia tonsa*, *Neohelice granulata*, and *Balanus glandula*) were examined in terms of their correlation with the environmental variables. The first two principal components (PC1 and PC2) were retained because they explained a meaningful part of the total variation. Plotted variables showed a reconstruction percentage >50% in the two-dimensional plot. Chlorophyll *a* was excluded from the analysis because of its very low contribution to the first two axes.

Hierarchical agglomerative clustering coupled with group average linkage was used to represent the community under study and to determine mesozooplankton assemblages. Groups were then spatially displayed by non-metric multidimensional scaling (MDS) ordination according to sample similarities. Both techniques were based on triangular matrices using the Bray–Curtis similarity index on fourth-root transformed

Table 1. Environmental and biotic variables (mean \pm s.d.) recorded at the two sampling sites during summer and winter 2009.

Variable	Between seasons						Between sites (<i>p</i> -values)	
	Canal Vieja			Bahía del Medio			Summer	Winter
	Summer	Winter	<i>p</i> -value	Summer	Winter	<i>p</i> -value		
pH	8.08 \pm 0.11	8.32 \pm 0.09	0.000	8.17 \pm 0.13	8.43 \pm 0.06	0.001	0.047	0.004
Dissolved oxygen (mg l ⁻¹)	5.77 \pm 0.62	5.86 \pm 0.20	0.349	6.55 \pm 0.39	6.41 \pm 0.57	0.295	0.010	0.018
Salinity	38.57 \pm 0.65	38.78 \pm 0.44	0.266	38.57 \pm 0.44	39.2 \pm 0.21	0.000	0.500	0.005
Turbidity (NTU)	87 \pm 36.13	40.5 \pm 5.86	0.001	82.98 \pm 44.05	77.97 \pm 33.44	0.268	0.595	0.004
Temperature (°C)	23.33 \pm 2.03	12.20 \pm 1.92	0.000	23.71 \pm 0.66	9.53 \pm 2.73	0.000	0.274	0.029
Nitrite (μM)	3.27 \pm 0.71	0.24 \pm 0.08	0.000	0.74 \pm 0.83	0.06 \pm 0.04	0.071	0.000	0.001
Nitrate (μM)	9.28 \pm 30.4	1.52 \pm 0.92	0.000	2.21 \pm 2.07	0.23 \pm 0.27	0.032	0.000	0.002
Phosphate (μM)	2.45 \pm 0.76	3.48 \pm 4.19	0.198	1.77 \pm 0.76	1.18 \pm 0.30	0.015	0.030	0.016
Silicate (μM)	71.43 \pm 3.20	42.76 \pm 12.20	0.001	66.42 \pm 4.00	37.03 \pm 4.42	0.000	0.116	0.186
Ammonium (μM)	25.05 \pm 18.25	110.15 \pm 153.33	0.001	14.39 \pm 10.67	30.23 \pm 8.80	0.001	0.028	0.002
Chl <i>a</i> (μg l ⁻¹)	7.49 \pm 2.81	8.25 \pm 4.70	0.327	8.09 \pm 3.88	5.36 \pm 2.49	0.045	0.333	0.073
Phaeopigment (μg l ⁻¹)	4.45 \pm 4.19	5.24 \pm 3.66	0.335	7.32 \pm 3.46	8.85 \pm 8.07	0.311	0.040	0.134
POC (μg l ⁻¹)	3 956.72 \pm 1 110.15	3 330.25 \pm 449.20	0.049	4 360.02 \pm 1 491.38	2 870.40 \pm 1 160.56	0.019	0.282	0.162
<i>d</i>	1.43 \pm 0.41	3.09 \pm 0.69	0.000	2.08 \pm 0.76	3.43 \pm 0.83	0.001	0.009	0.193
<i>J'</i>	0.32 \pm 0.10	0.46 \pm 0.12	0.005	0.23 \pm 0.13	0.59 \pm 0.23	0.002	0.049	0.200
<i>H'</i>	0.77 \pm 0.29	1.24 \pm 0.29	0.001	0.70 \pm 0.47	1.65 \pm 0.67	0.004	0.345	0.231
Mesozooplankton abundance (ind. m ⁻³)	1 540.09 \pm 837.71	111.15 \pm 56.29	0.000	3 914.93 \pm 4 824.66	153.88 \pm 113.64	0.001	0.059	0.263

d, Margalef index; *J'*, Pielou index; *H'*, Shannon–Wiener index. Comparison between sites and seasons (Mann–Whitney test; *n* = 12 for all variables during summer, and *n* = 8 for all variables during winter). *p*-values of <0.05 are shown emboldened.

abundance data to enhance the contribution of the less abundant taxa (Clarke and Warwick, 1994). A matrix was constructed based on taxa with >0.01% of the total abundance found at each sampling site. Species that were most different in abundance between sites were identified in the MDS ordination with superimposed circles of increasing size, representing increasing abundance. Similarity percentage analysis (SIMPER), using the Bray–Curtis similarity index, was used to examine the contribution of each taxon to the similarity within a group, and to the dissimilarity between groups (on a temporal scale). To determine environmental variables that best explain the community pattern, a BIOENV analysis was performed using a Euclidean-distance-based similarity matrix of the environmental data with the taxa-similarity matrix. Environmental data were transformed using log₁₀ (*x* + 1; Clarke and Warwick, 1994). ANOSIM was applied to detect significant differences between the discriminated groups. The PRIMER[®] 5 package was used to carry out these analyses.

Results

Environmental conditions and biotic parameters

Chemical, physical, and biotic differences between sampling sites during the seasons studied are shown in Table 1. Canal Vieja, the sewage discharge area, consistently had significantly higher levels of all nutrients (nitrate, nitrite, phosphate, and ammonium) except silicate, whereas the non-impacted site, Bahía del Medio, had significantly higher pH and DO in both winter and summer. Salinity and turbidity were significantly higher at Bahía del Medio during winter, whereas temperature was significantly higher at Canal Vieja during the same season. Species richness (*d*) was significantly higher at Bahía del Medio during summer, but evenness (*J'*) was significantly lower than at Canal Vieja. No differences in these indices were found in winter. There were no significant differences between sampling sites during the same

season in silicate, Chl *a*, POC, *H'*, or mesozooplankton abundance. Phaeopigments were consistently higher at Bahía del Medio, but significantly higher only in summer.

Seasonal differences between sampling sites were also observed. Both sites showed significant differences in pH, temperature, nitrate, silicate, ammonium, POC, mesozooplankton abundance, and diversity (*d*, *H'*, *J'*) between summer and winter. Diversity indices, ammonium, and pH were higher during winter, and the other variables were higher during summer. At Canal Vieja, nitrite and turbidity values were significantly higher in summer. Bahía del Medio showed significantly higher values of phosphate and Chl *a* in summer and of salinity in winter. There were no significant differences between summer and winter at Canal Vieja in DO, salinity, phosphate, Chl *a*, or phaeopigment, or in DO, turbidity, nitrite and phaeopigment at Bahía del Medio.

Mesozooplankton abundance and composition

In all, 68 taxa belonging to ten phyla (Arthropoda, Mollusca, Chaetognatha, Annelida, Bryozoa, Cnidaria, Priapulida, Nematoda, Echinodermata, and Chordata) were identified during the study at the two sampling sites. Crustaceans dominated (99% of total abundance), and among the crustaceans, copepods and decapods were the most diverse groups (34 and 7 taxa, respectively). The 2 sites shared 26 taxa in common, 13 (19%) were exclusive to Canal Vieja, and 19 (28%) were found only at Bahía del Medio.

Of the taxa observed, only a few at either site had relative abundances >1% during summer or winter (Table 2). In summer, the copepod *A. tonsa* dominated at both sampling sites, but its abundance was significantly greater at Bahía del Medio (Table 2), where it accounted for 92% of total abundance at its maximum abundance during the study (10 869 ind. m⁻³; Table 2). Its relative abundance at Canal Vieja was 55%. The abundance of larvae of the crab *N. granulata* was significantly greater in summer at

Table 2. Main mesozooplankton species found during the study period at the two sampling sites (Canal Vieja and Bahía del Medio) in summer (top panel) and winter (bottom panel).

Taxon	Canal Vieja			Bahía del Medio			p-value
	Mean \pm s.d.	Minimum	Maximum	Mean \pm s.d.	Minimum	Maximum	
<i>A. tonsa</i>	854 \pm 785	165	2 649	3 589 \pm 4 533	41	10 869	0.031
<i>N. granulata</i>	502 \pm 753	11	2 309	108 \pm 117	4	340	0.049
<i>B. amphitrite</i>	26 \pm 31	0	98	120 \pm 150	0	363	0.028
<i>E. acutifrons</i>	107 \pm 202	0	695	39 \pm 55	0	179	0.312
<i>P. parvus</i>	20 \pm 17	0	51	–	–	–	–
Other taxa	36 \pm 35	9	138	42 \pm 36	8	124	–
<i>B. glandula</i>	80 \pm 43	22	146	82 \pm 98	11	311	0.231
<i>A. tonsa</i>	6 \pm 5	1	16	16 \pm 16	3	49	0.172
<i>P. parvus</i>	4 \pm 3	2	12	12 \pm 7	5	28	0.006
<i>C. carinatus</i>	6 \pm 4	1	11	12 \pm 6	7	22	0.002
<i>E. acutifrons</i>	4 \pm 5	0	15	6 \pm 5	1	15	0.200
<i>E. americana</i>	2 \pm 3	0	8	–	–	–	–
<i>N. granulata</i>	3 \pm 3	0	7	–	–	–	–
<i>H. aff. crassica</i>	–	–	–	4 \pm 4	0	12	–
<i>M. aff. littorale</i>	–	–	–	7 \pm 8	1	25	–
<i>Longipedia</i> sp.	–	–	–	4 \pm 5	0	13	–
<i>N. aff. palustris</i>	–	–	–	3 \pm 4	0	13	–
Acari	4 \pm 3	0	9	–	–	–	–
Foraminifera	–	–	–	2 \pm 5	0	14	–
Other taxa	5 \pm 2	3	9	7 \pm 5	1	17	–

Mean abundances \pm s.d. (ind. m⁻³), minimum, and maximum values are shown. Comparisons between sites were made by the Mann–Whitney test, and significant values ($p < 0.05$) are shown emboldened. Species that made up $< 1\%$ of total abundance at each sampling site and each studied season are included in the grouping “Other taxa”.

Canal Vieja, where it accounted for 33% of total abundance, with a maximum of 2309 ind. m⁻³ (Table 2). The cirripede *Balanus amphitrite* and the copepod *Euterpina acutifrons* were also present in summer at both sites (Table 2). *Balanus amphitrite* was significantly more abundant at Bahía del Medio in summer, but the abundance of *E. acutifrons* did not differ significantly between sites in summer (Table 2).

In winter, there tended to be a pattern of a single dominant species; in all, 13 taxa of relative abundance $> 1\%$ were recorded in winter (Table 2). The cirripede *B. glandula* was dominant at both sampling sites, but its abundance did not differ significantly between sites in winter (Table 2). Its relative abundance was 72% at Canal Vieja and 53% at Bahía del Medio. Other species detected at both sites during winter were the copepods *E. acutifrons*, *Paracalanus parvus*, and *Calanoides carinatus*, the last two of which were significantly more abundant at Bahía del Medio (Table 2). There was no significant difference in the abundance of *E. acutifrons* between sites (Table 2). Other taxa with relative abundance $> 1\%$ were *Eurytemora americana*, *N. granulata*, and Acari at Canal Vieja, and *Halicyclops* aff. *crassicornis*, *Microarthridion* aff. *littoralis*, *Longipedia* sp., *Nannopus* aff. *palustris*, and Foraminifera at Bahía del Medio (Table 2). Harpacticoid and cyclopoid copepods, among other taxa included in the grouping “other taxa”, were found during summer and winter at both sampling sites, their relative abundance peaking at 5% in winter at Bahía del Medio.

There were differences between seasons at the sampling sites: *A. tonsa*, *N. granulata*, and *E. acutifrons* consistently showed significantly higher values of abundance in summer at the two sites (Table 3). In contrast, the abundance of *P. parvus* did not differ significantly between summer and winter at Canal Vieja (Table 3).

Table 3. Comparison (Mann–Whitney) of the abundance of species found in common at both sampling sites (Canal Vieja and Bahía del Medio) in summer and winter (Table 2).

Shared species	Between seasons	
	Canal Vieja	Bahía del Medio
<i>A. tonsa</i>	0.000	0.000
<i>N. granulata</i>	0.000	–
<i>E. acutifrons</i>	0.029	0.010
<i>P. parvus</i>	0.123	–

Significant values ($p < 0.05$) are shown emboldened.

Multivariate environmental and biotic analysis

The first two principal components of the environmental variables and main species abundances explained 61.37% of the total variation (Figure 2) and revealed significant correlations among the analysed variables (Table 4). Mesozooplankton and *A. tonsa* abundances, *d* (Margalef richness index), *N. granulata* abundance (H' ; Shannon–Wiener index), *B. glandula* abundance, pH, and temperature had the highest percentage contribution to the first axis (PC1 47.70%).

Variables with strong seasonal variability formed two clearly arranged groups along the PC1 axis (Figure 2). As suggested by the distribution and position of these variables, there were highly significant positive correlations among variables within each group, and highly negative correlations among variables between groups (Figure 2, Table 4). Mesozooplankton abundance was significantly positively associated with turbidity, temperature, nitrate, nitrite, silicate, POC, and *N. granulata* and *A. tonsa* abundance (Table 4), and significantly negatively correlated with pH, ammonium, diversity (H' , d , J'), and *B. glandula* abundance (Table 4). *Acartia tonsa* showed the same trend as total

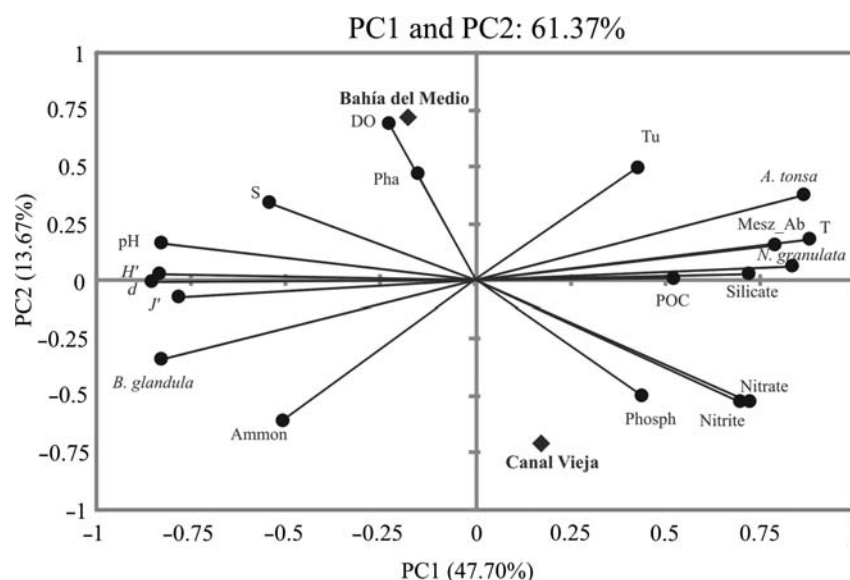


Figure 2. PCA plot of environmental variables and main species (*A. tonsa*, *N. granulata*, and *B. glandula*). Sampling sites are represented as a supplementary variable (T, temperature; Tu, turbidity; DO, dissolved oxygen; Phosph, phosphate; Ammon, ammonium; Pha, phaeopigment; Mesz_Ab, mesozooplankton abundance; S, salinity; POC, particulate organic carbon; H' , Shannon–Wiener index; J' , Pielou evenness index; d , Margalef richness index).

mesozooplankton abundance plus a significant negative correlation with salinity (Table 4). The pattern for *N. granulata* was similar, but with a positive significant correlation with phosphate and no association with turbidity and POC. Positive associations were found between *B. glandula* and pH, salinity, ammonium, and diversity (H' , d , J') and negative correlations with turbidity, temperature, nitrate, nitrite, silicate, and POC (Table 4). DO and ammonium were the variables that best explained the second axis (PC2 13.67%). These variables were spatially linked with the sampling sites and arranged in opposite quadrants, showing spatial ordination on PC2 (Figure 2). Canal Vieja was positively correlated with most of the nutrients (except silicate) in the bottom-right quadrant (Figure 2, Table 4). Bahía del Medio, located in the upper left quadrant (Figure 2), was closely associated with DO and highly significantly positively correlated with both DO and turbidity (Table 4).

Two groups of taxa were determined by cluster analysis at a similarity level of 22% (Figure 3), corresponding to seasonal distribution (summer and winter groups; Figure 3). *Acartia tonsa* and *B. glandula* were the species with the greatest contribution to average similarity in summer and winter, respectively. The average dissimilarity between groups was determined by *A. tonsa*, *B. glandula*, and *N. granulata* (Figure 3). Summer and winter groups were significantly different from each other (ANOSIM, Global $R = 0.993$, $p = 0.000$). The two sampling sites were visually discriminated using the MDS method with a stress of 0.08 (Figure 3). *Acartia tonsa* and *N. granulata* showed the greatest discrimination between sites as a consequence of their differential contribution in terms of abundance. *Acartia tonsa* was more abundant at Bahía del Medio and *N. granulata* at Canal Vieja. DO, pH, and temperature best explained the observed spatial pattern (BIOENV; $\sigma = 0.755$; Figure 3). The study sites differed significantly when taking environmental data into account (ANOSIM; Global $R = 0.299$, $p = 0.001$).

Discussion

Environmental variation

The sewage disposal site, Canal Vieja, was clearly differentiated by chemical and physical characteristics. It consistently showed higher levels of nutrient, and lower DO and pH, in accord with the findings of Lara *et al.* (1985) and Barría de Cao *et al.* (2003), and others related to other coastal environments receiving sewage (Mateus *et al.*, 2008). Corroborated by the multivariate analyses, allochthonous organic matter, nutrients, and polluting compounds from sewage discharge lead to enhanced bacterial productivity and the uncoupling of natural seawater buffer mechanisms (Thompson and Bonnar, 1931; Waksman and Renn, 1936). Temperature contributed to the differentiation between study sites mostly in winter, possibly indicating a short-term localized impact. The higher temperature at Canal Vieja in winter seems to be directly associated with both the slightly higher temperature of wastewater and the impact of overheated water discharge from a thermoelectric power plant located upstream (Hoffmeyer *et al.*, 2005). Although there is artificial overheating also in summer, it is likely that a naturally driven process at Bahía del Medio causes the temperature there to rise, matching the thermal fluctuation between sites then. The effect of thermal variation and bottom-wall friction is probably intensified by the shallowness and narrowness of Bahía del Medio, generating greater interaction with the surrounding saltmarshes and producing a high rate of soil–seawater heat exchange, i.e. seawater heat gain during summer (Beigt *et al.*, 2003).

Contrary to *a priori* assumptions, wastewater discharge in the BBE appears not to be associated with local increases in turbidity and POC. Although those variables were consistently high throughout, reflecting the turbid nature of this eutrophic estuary (Perillo and Piccolo, 1991; Freije *et al.*, 2008; Popovich and Marcovecchio, 2008; Guinder *et al.*, 2010), there was a clear decrease in both variables in winter. The decline in turbidity at

Table 4. Spearman's correlation matrix corresponding to the PCA.

Variable	pH	Turbidity	DO	Temperature	Salinity	Amm onium	Phaeopigment	Nitrite	Nitrate	Phosphate	Silicate	Particulate organic carbon	H'	d	J'	Mesozooplankton abundance	A. tonsa	B. glandula	N. granulata
pH																			
Turbidity	−0.144																		
DO	0.169	−0.027																	
Temperature	−0.795	0.088	0.119																
Salinity	0.511	0.023	0.339	−0.395															
Ammonium	0.263	−0.472	−0.281	−0.493	0.092														
Phaeopigment	0.106	0.207	0.333	−0.128	0.068	−0.088													
Nitrite	−0.663	0.137	−0.458	0.382	−0.381	−0.116	−0.226												
Nitrate	−0.642	0.115	−0.451	0.440	−0.393	−0.100	−0.252	0.944											
Phosphate	−0.379	0.048	−0.329	0.161	−0.382	0.100	−0.109	0.587	0.626										
Silicate	−0.433	0.191	−0.120	0.545	−0.341	−0.510	−0.212	0.441	0.490	0.404									
Particulate organic carbon	−0.398	0.147	0.021	0.401	−0.320	−0.320	−0.037	0.444	0.350	0.240	0.351								
H'	0.635	−0.495	0.411	−0.585	0.487	0.317	0.208	−0.490	−0.520	−0.243	−0.537	−0.422							
d	0.621	−0.506	0.256	−0.570	0.287	0.345	0.151	−0.582	−0.614	−0.393	−0.519	−0.309	0.753						
J'	0.554	−0.449	0.335	−0.569	0.498	0.367	0.153	−0.353	−0.408	−0.181	−0.580	−0.442	0.955	0.616					
Mesozooplankton abundance	−0.687	0.513	−0.162	0.674	−0.271	−0.419	−0.093	0.479	0.498	0.239	0.544	0.393	−0.774	−0.838	−0.738				
A. tonsa	−0.711	0.592	0.077	0.732	−0.367	−0.572	0.108	0.411	0.431	0.231	0.486	0.432	−0.695	−0.761	−0.673	0.897			
B. glandula	0.669	−0.433	−0.209	−0.762	0.246	0.680	−0.083	−0.481	−0.519	−0.285	−0.694	−0.411	0.503	0.669	0.475	−0.689	−0.822		
N. granulata	−0.601	0.308	−0.227	0.676	−0.263	−0.424	−0.182	0.473	0.519	0.377	0.762	0.244	−0.616	−0.750	−0.604	0.843	0.711	−0.732	
Canal Vieja	−0.295	−0.328	−0.576	0.033	−0.227	0.368	−0.295	0.654	0.628	0.472	0.061	0.022	−0.004	−0.230	0.147	0.030	−0.126	0.034	0.208
Bahía del Medio	0.295	0.328	0.576	−0.033	0.227	−0.368	0.295	−0.654	−0.628	−0.472	−0.061	−0.022	0.004	0.230	−0.147	−0.030	0.126	−0.034	−0.208

The supplementary variable was the sampling sites (Canal Vieja and Bahía del Medio).

Significant correlations ($p < 0.05$, $n = 40$) are shown emboldened. H' , Shannon–Wiener index; d , Margalef index; J' , Pielou index.

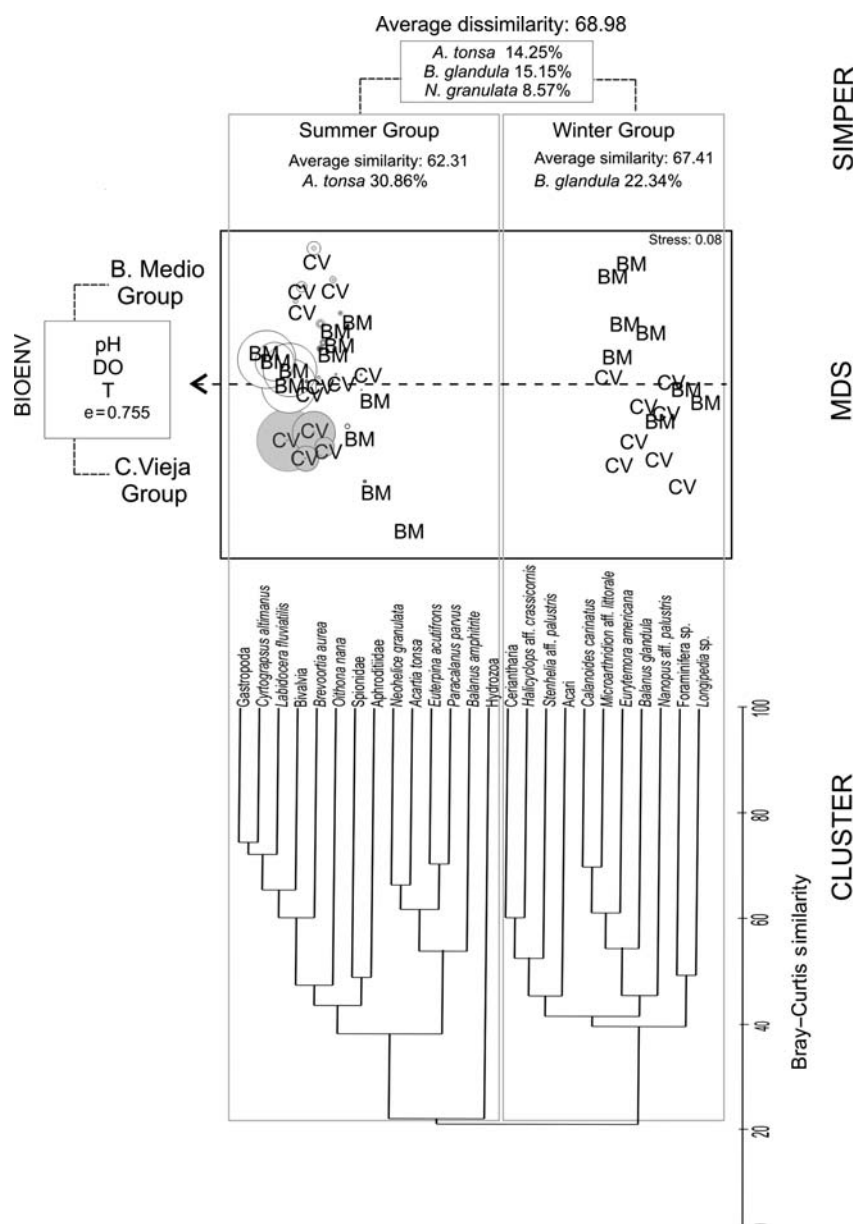


Figure 3. Relationships between groups of taxa found by cluster analysis and sampling site in both study seasons (CV, Canal Vieja; BM, Bahía del Medio), displayed by non-metric MDS using a taxon composition matrix (fourth-root transformed, ind. m⁻³). The abundance of the two most pertinent species on the spatial scale is distinguished by superimposed circles (*A. tonsa*, white circles; *N. granulata*, grey circles). Variables that best explain the spatial pattern were determined by BIOENV (Euclidean distance), using both taxon composition and environmental matrices (log-transformed). Summer and winter groups are shown at 22% of the Bray–Curtis similarity index. Similarity and dissimilarity percentages and species contributing most to group discrimination are shown by SIMPER. Significant differences between spatial (CV and BM) and temporal groups (summer and winter) were found (ANOSIM, $p < 0.05$).

Canal Vieja reflects greater responsiveness to small-scale local changes such as continental run-off. The cold dry winters of the region result in less freshwater input, which minimizes sediment resuspension and detritus contribution to the water column (Piccolo, 2008), specifically at Canal Vieja, which receives the direct influence of continental run-off. Climatic seasonality is also reflected in the salinity rise during winter, especially at Bahía del Medio which is not affected by continental discharge. Salinity is a key factor in controlling the spatial distribution of plankton in estuarine systems (Laprise and Dodson, 1994;

Cervetto et al., 1999; Marques et al., 2007). However, it is unlikely to have a great effect on zooplankton distribution in the BBE, given the limited variation in the inner zone (Popovich and Marcovecchio, 2008).

Although chlorophyll is widely used as an indicator of nutrient enrichment (Valiela, 1995; Nixon, 1995), chlorophyll *a* was not associated with the allochthonous organic matter or nutrient source in the BBE. As in other shallow turbid systems (Cloern, 1987; Kocum et al., 2002; Araujo et al., 2008; Domingues et al., 2011), phytoplankton seems not to be nutrient-limited in the

BBE, because light availability in the water column is the main factor regulating primary productivity (Popovich and Marcovecchio, 2008; Guinder *et al.*, 2009). Changes in phytoplankton composition can be inferred through variation in the N:Si ratio, which usually rises during eutrophication, promoting the growth of flagellates over diatoms (Sommer, 1994; Roberts *et al.*, 2003). Given that nitrogen values were significantly higher at the sewage-affected site and the silicate levels were similar between the two sites, a higher N:Si ratio at Canal Vieja could promote shifts in the phytoplankton assemblages from a community dominated by diatoms to one dominated by microbia. Chlorophyll *a*, POC, and silicate, on the other hand, varied strongly between seasons, but not between sites, showing a neutral association with the sewage effect.

Compared with other estuaries, the BBE is an organically enriched system (Guinder *et al.*, 2009) in which autochthonous sources provide a notable input of organic matter throughout (Popovich and Marcovecchio, 2008; Guinder *et al.*, 2009; Negrin *et al.*, 2011). The POC content followed the same pattern as that of total mesozooplankton, both peaking in summer and reflecting the crucial role of plankton in the energy flux of the system.

In terms of spatial distribution, dissolved inorganic nitrogen and phosphate, DO, and pH were the variables with the clearest association with the sewage contribution. In addition, phaeopigments, chlorophyll degradation products, and indicators of zooplankton grazing activity (Lorenzen, 1967; Helling and Baars, 1985; Head and Harris, 1992) were lower at Canal Vieja, yet another environmental difference between the sites. Further studies on zooplankton grazing and food quality are being carried out currently to evaluate the effect of the sewage contribution to the pelagic trophic web of the estuary.

Mesozooplankton variation

The present findings are consistent with those of previous studies that have reported that the mesozooplankton of the BBE seems to be effectively tolerant of various types of stress (Fernández-Severini *et al.*, 2009; Biancalana *et al.*, 2011). However, there were differences in specific composition, evenness, and richness between sampling sites, perhaps indicative of the modulating effect of the source of disturbance on mesozooplankton community structure. Although total mesozooplankton abundance was not statistically different between areas, it was consistently higher at the non-impacted site, especially in summer. This would suggest that nutrient-loading from Bahía Blanca City's sewage may involve non-favourable compounds for mesozooplankton development, because the growth rates of zooplankton taxa would be expected to show a positive response to nutrient enrichment (Gismervik *et al.*, 2002). In fact, the sewage of Bahía Blanca City has been identified as one of the most pertinent sources of heavy metal contribution to the system (Marcovecchio *et al.*, 2008), and such pollutants affect the reproduction of copepods and subsequent egg viability (Jiang *et al.*, 2007; Mohammed *et al.*, 2010). In addition, sewage-related changes in food quality could restrict the development of the common species at Canal Vieja (Pinckney *et al.*, 2001). Food diversity and availability influence the reproductive success of *Acartia* spp. (Keppel and Hazzard, 2000; Calliari and Tiselius, 2005), so the presence of small-size planktonic groups at Canal Vieja (Barría de Cao *et al.*, 2003) as food may be one sewage-related factor that could limit *A. tonsa* development.

Continuous input of allochthonous organic matter would sustain the development of detritivorous populations such as *N. granulata*, whose larvae were significantly more abundant at the disturbed site. Favourable conditions at Canal Vieja would allow the development of a sustainable population of *N. granulata*, because this species has expanded its distribution at this site beyond its seasonal occurrence (MSD and AAB, pers. obs.). The presence of mudflats at Canal Vieja, which enhance the conditions for development of this species compared with saltmarshes (Silva *et al.*, 2009), also appears to favour the reproductive traits of this ecologically relevant estuarine crab. Given its pollution resistance, *N. granulata* has been postulated as a useful bio-indicator/biomonitor (Ferrer *et al.*, 2006; Simonetti *et al.*, 2011). Although the selection of effective system-level indicators is restricted by their ability to quantify the magnitude of stress and to represent processes across a range of ecological scales (Dale and Beyeler, 2001), the range of a biomonitor at a species level may be a warning signal of changes in natural environmental conditions (Dale and Beyeler, 2001). Further studies on specific physiological responses to allochthonous organic loading are clearly needed. Nevertheless, *N. granulata* is suggested as a potential indicator of sewage pollution in the BBE, so ideally should be considered in future ecosystem management programmes.

However, as mentioned above, most of the environmental variation in the study area is driven by the pronounced seasonality of the region, meaning that short-term climatic variation plays a relevant role in modulating plankton dynamics within this estuary (Hoffmeyer *et al.*, 2009; Berasategui *et al.*, 2009).

Given that the BBE is subject to cumulative regional sewage input, long-term environmental and biological monitoring programmes are needed to assess ecosystem resistance to such input. Wider planktonic spectrum studies, and research on zooplankton physiological responses, by bioassays and *in situ* experiments on sewage pollutants are therefore required.

Acknowledgements

We thank Luis Kaufman and IADO boat staff Camilo Bernárdez, Enio Redondo, and Alberto Conte for their technical support and help during sampling. We also thank Walter Melo for drawing the map of the estuary, C. Javier Chazarreta and Facundo Barrera for their assistance in the laboratory, and Valeria Guinder, M. Clara Menéndez, and J. Carlos Molinero for valued comments on an earlier version of the manuscript. Suggestions made by Carina Marón, Andrés Arias, Hugo Freije, and Carla Spetter were also much appreciated, as were the comments of two anonymous reviewers. The study was supported by the Instituto Argentino de Oceanografía, IADO (CONICET-UNS) and the Agencia Nacional de Promoción Científica y Tecnológica (FONCYT-PICT 1713).

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