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Assessing the ecological quality status of a temperate urban estuary by means of benthic biotic indices

M. Hutton [a,*], N. Venturini [b], F. García-Rodríguez [a], E. Brugnoli [a], P. Muniz [a]

[a] Oceanografía & Ecología Marina, IECA, Facultad de Ciencias, Universidad de la República, Iguá 4225, Montevideo 11400, Uruguay
[b] Laboratorio de Biogeoquímica Marina (LABIM), IECA, Facultad de Ciencias, Universidad de la República, Montevideo, Uruguay

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ABSTRACT

Benthic indices are commonly used tools for assessing the environmental quality, because they represent a simple source of scientific information. However, their performance could vary depending on the application area and perturbation types, thus they should be tested before used in other remote geographic regions. This study aims to test the use of some of the most widely common benthic biotic indices for assessing the environmental quality of Montevideo's coastal zone at a seasonal scale against many physicochemical variables. From all the evaluated indices, AMBI appears to be the most suitable one to assess the environmental quality. The study also allowed us to infer the most relevant physicochemical variables: protein, lipid and heavy metal sediment concentration. Additionally, site-specific threshold effect levels for heavy metals and biopolymers were established, which appear to be useful to determine tolerable levels of such stressors in future assessments or monitoring programs for the study area.

1. Introduction

The ability to quantitatively assess the ecological health status is of great interest for ecosystem monitoring and conservation. In this sense, ecological indices are particularly useful to communicate scientific concepts in a simple format to the public opinion and managers, because they integrate numerous environmental factors of the system (Salas et al., 2006). According to Borja et al. (2009) the use of ecological indices is widely accepted for these ends. In the last few years, benthic biotic indices have become a frequent tool to evaluate the environmental quality of marine and estuarine coastal zones (Forchino et al., 2011; Borja et al., 2009; Carvalho et al., 2006). That is because the benthic communities are good indicators to assess the whole ecosystem quality due to their limited mobility, which makes them depend on local environmental conditions. Nevertheless the worldwide use of benthic biotic indices presents some shortcomings, such as the fact that their performance could vary depending on the specific geographic area of application and the specific perturbation types (Salas et al., 2006). More recent benthic indices were developed at certain coastal and estuarine areas of the United States and Europe (Borja et al., 2009). Similarly, most of them are based on the organic matter enrichment model of Pearson-Rosenberg (Pearson and Rosenberg, 1978), and thus, they should be validated for other type of perturbations as well (Quintino et al., 2006). For this reason, benthic biotic indices may not be universally applicable, and they should be tested in different geographic areas and perturbations (i.e., natural or anthropogenic). Some indices were tested mainly in Europe, where a variable performance according to the environmental type was observed. For example, in muddy sediment or estuaries some indices underestimated the environmental quality of the study area (Marín-Guirao et al., 2005; Muxika et al., 2005; Dauvin et al., 2007; Blanchet et al., 2007; Zettler et al., 2007; Simboura and Reizopoulou, 2008).

Since there is not a perfect index for evaluating the environmental quality, it is widely recommended to use several indices based on different approaches. According to Ruellet and Dauvin (2007) and de Paz et al. (2008) the Shannon–Wiener diversity, ITI (Infaunal Trophic Index), BENTIX and AMBI (*AZTI Marine Biotic Index*) have been the most used indices for their categories (diversity, trophic groups and ecological groups, respectively), and the M-AMBI is being widely used as multivariate index (Borja et al., 2012). In South America there are few assessments for such indices. Albano et al. (2013), Omena et al. (2012) and Muniz et al. (2005, 2011, 2012) have tested the efficiency of AMBI on estuaries and marine coastal areas of Argentina, Brazil and Uruguay, where different degrees of anthropogenic pressure are well documented.

* Corresponding author.
E-mail address: mhutton@fcien.edu.uy (M. Hutton).

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The area of study in placed within the Río de la Plata estuary, which is the second largest estuary in South America, and is characterized as a highly variable ecosystem both biologically and environmentally (Guerrero et al., 1997; Calliari et al., 2003; García-Rodríguez et al., 2014). It displays high micro- and meso-scale variability, depending on the freshwater input, tidal oscillations, wind patterns, and other episodic events such as 'El Niño' and 'La Niña'. The Río de la Plata Estuary is a highly productive and variable environment characterized by a well-developed turbidity front and mostly by strong vertical salinity stratification (Framiñan and Brown, 1996). Strong and unpredictable wind events can generate alternative pulses of stratified and partially mixed conditions in few hours (Nagy et al., 1997). Specifically, the study area corresponded to the metropolitan coastal zone of Montevideo, which is located on the middle portion of this estuary, and it is affected by several anthropogenic activities. According to previous studies, based on environmental and biotic characteristics of the benthos, it was possible to distinguish zones with different degree of anthropogenic influence along the coast of Montevideo (Muniz et al., 2004a,b, 2002; Burone et al., 2006). At a seasonal scale, the worst environmental quality was found in summer according to the assessments of several physicochemical variables (Danulat et al., 2002; Muniz et al., 2004a). However, there are only few studies assessing the temporal changes in the environmental quality in the study area. Recently, Venturini et al. (2012) evaluated the benthic trophic status in the study area using the biochemical composition of sedimentary organic matter as a synthetic descriptor and showed that spatio-temporal patterns in this property were related both to natural and human pressures.

In this context, the aim of this study was to test the performance on some of the most widely utilized benthic biotic indices (ITI, BENTIX, AMBI and M-AMBI) to correctly evaluate the ecological quality of the coastal/estuarine system of Montevideo. As a reference we compared the ecological classification obtained from the indices against physicochemical variables on a seasonal scale during one year. Two main questions are addressed in this work: (i) Are the different benthic biotic indices reliable to assess the degree of anthropogenic impact measured by the physicochemical variables in this stressed system? (ii) Are there any significant spatial and/or temporal variations within the anthropogenic impact gradient of the metropolitan area?

2. Material and methods

2.1. Study area

The study was performed at the Montevideo's coastal zone, which is in the intermediate zone of the Río de la Plata estuary (34.75–34.90° S, 56.73–57.25° W), and it is characterized by a variation in salinity between 0.5 and 30. This is a large coastal plain microtidal estuary (tidal amplitude <1 m), partially mixed with a distinct stratification located in the southwestern Atlantic Ocean (Guerrero et al., 1997; Nagy et al., 2002).

According to previous research in the area (Muniz et al., 2002, 2005), Montevideo's coastal zone can be spatially divided into three main zones due to anthropogenic activities: (1) The area of *Punta Carretas*, containing a sewage pipe that concentrates the sanitation system of the east area of the city. (2) The Montevideo's Bay, which contains the main commercial harbor of the country, a steam water plant and an oil refinery plant. It also has three streams flowing into it (*Miguelete*, *Pantanoso* and *Seco* Streams) all of which carrying wastes from many different industries, the urban center and a large number of sewage pipes. (3) *Punta Yeguas* zone considered as the least anthropogenically impacted, although it does not hold an urban sanitation system (Fig. 1).

2.2. Sampling design, field and laboratory methods

Seasonal sampling was carried out at 18 stations (Fig. 1). Sampling stations were located in order to represent the spatial gradient of environmental impact previously established for the study area. Four sampling surveys were carried out in July and October 2007, and also in January and May 2008, during austral winter, spring, summer and autumn, respectively. In the summer survey station 'M' could not be sampled due to adverse weather conditions.

Surface sediment samples (the first two centimeter) were taken (in triplicate) with a van Veen grab (0.05 m^2, three independent grabs) for the analysis of grain size, photosynthetic pigment content, organic biopolymers, total organic matter content and heavy metals concentration (Cd, Cu, Cr, Pb and Zn). Five benthic macrofaunal replicates were collected at each station. We used only those grabs that presented well-preserved surface sediment. Macrobenthic samples were sieved through 0.5 mm mesh, and the retained material was preserved in 70% ethanol. Macrofauna was sorted, identified to the lowest possible taxonomic level, usually species level, and counted.

Grain size analysis was performed using the "Low-angle laser light scattering" method, with a "Droplet and Particle Analyser – Malvern" series 2600. Frequency and size classes were calculated according to Suguio (1973). Sub-samples of ~1.5–2.0 g were analyzed for total organic matter (TOM) by weight loss on ignition at 550 °C for 4 h following Heiri et al. (2001). Photosynthetic pigment content was determined according to Lorenzen (1967) modified by Sunbäck (1983). Total protein (PRT) analysis was conducted following extraction with NaOH (0.5 M, 4 h) and determined according to Hartree (1972) modified by Rice (1982) to compensate for phenol interference. Total carbohydrates (CHO) were analyzed according to Gerchacov and Hatcher (1972). Blanks for each analysis were performed with precombusted sediments at 450–480 °C for 4 h. PRT and CHO concentrations were expressed as BSA and glucose equivalents, respectively. Total lipids (LPD) were extracted from 1 g of freeze-dried homogenized sediment by ultrasonication (20 min) in 10 ml chloroform:methanol (2:1 v/v), analyzed following the protocol described in Marsh and Weinstein (1966) and expressed as tripalmitine equivalents.

Trace metal analyses were carried out according to the method no. 3051 of the Environmental Protection Agency (Anonymous, 1990). The sediment was dried at 85 °C to constant weight, prior to homogenization in an agate mortar and pestle. In order to avoid organic matter interference in the measurements, and to convert the metals to their free form, duplicate sub-samples of 0.5–1 g were mixed with 10 ml concentrated nitric acid and digested by microwave (CEM, MDS 2100) in a closed fluorocarbon vessel. Quantification was performed by ASS (Shimadzu AA-680) with graphite furnace atomization (Shimadzu GFA-4B). Quality control included procedural blanks, measurement of standards from the National Institute of Standards and Technology (NIST), and spiked samples. Results are reported in g^{-1} dry sediment as mean values of duplicate analyses. Coefficient of variation between duplicates was always lower than 5%.

2.3. Data analysis and benthic indices

A Principal Component Analysis (PCA) was performed for the physicochemical variables of each sampling survey, in order to established groups of stations with similar characteristics (Primer v. 6.1.6). Variables were previously standardized by subtracting the mean value and dividing them by the standard deviation.

Each station was classified according to their trophic status, their degree of heavy metal contamination and their ecological status. The trophic state was evaluated following Dell'Anno et al.

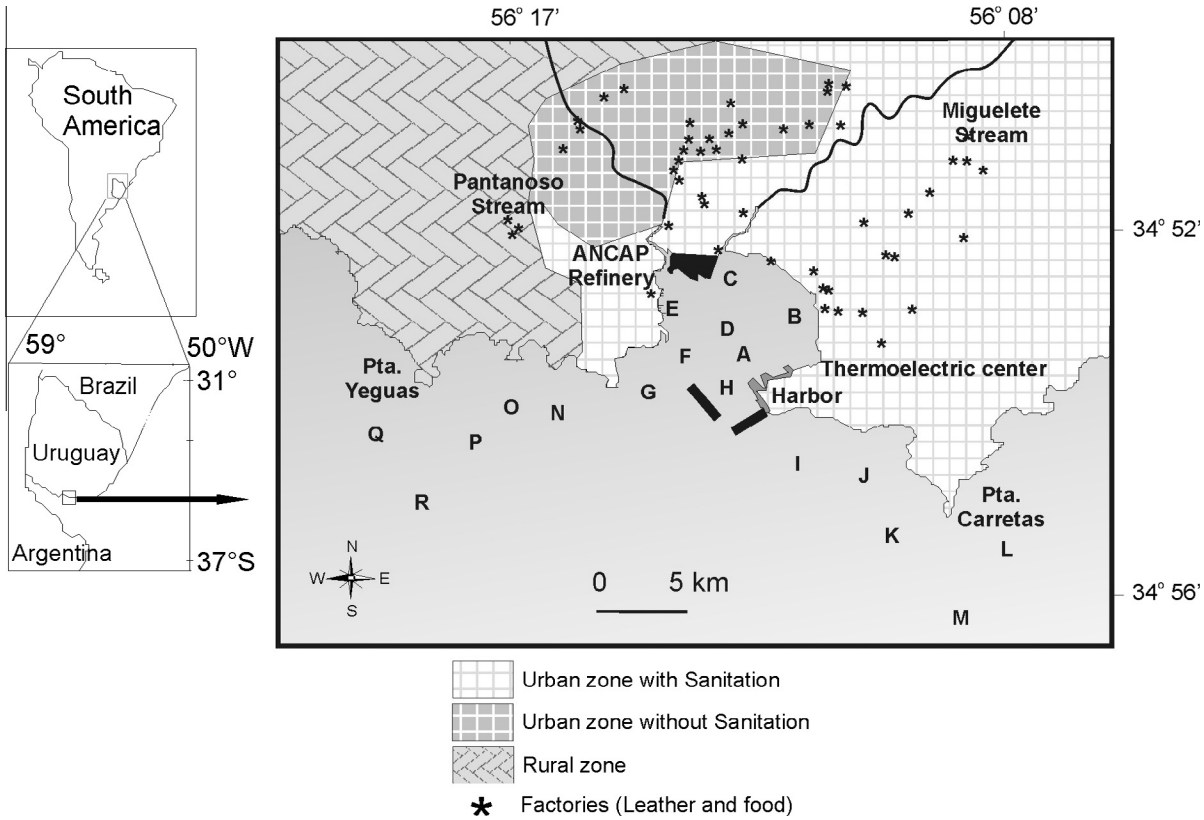

Fig. 1. Study area in Río de la Plata estuary showing the sampled stations (A–R).

(2002) classification, on the basis of both protein and carbohydrate trophic thresholds: hypertrophic (PRT > 4.0 mg g^{-1}; CHO > 7.0 mg g^{-1}; PRT/CHO > 1), eutrophic (PRT = 1.5–4.0 mg g^{-1}; CHO = 5.0–7.0 mg g^{-1}, PRT/CHO > 1) and meso-oligotrophic (PRT < 1.5 mg g^{-1}; CHO < 5.0 mg g^{-1}, PRT/CHO < 1). The degree of heavy metal contamination was evaluated according to the biological effect levels determined by MacDonald et al. (1996): Threshold Effect Level (TEL) and Probable Effect Level (PEL). According to these, three possible conditions are possible: (1) at concentrations below TEL, effects are expected to occur only rarely, (2) at concentrations between TEL and PEL, effects are expected to occur occasionally and (3) at concentrations above PEL, effects are expected to occur frequently.

The ecological quality status was determined according to several biotic indices based on different approaches: the Shannon–Wiener diversity index (H′) (Shannon and Weawer, 1963), Azti Marine Biotic Index (AMBI) (Borja et al., 2000) and BENTIX (Simboura and Zenetos, 2002) based on the classification of ecological group of soft-bottom species according to their sensitivity to an increasing stress gradient, the Infaunal Trophic Index (ITI) based on trophic groups (Maurer et al., 1999) and Multivariate-AMBI (M-AMBI) which is based on a factorial analyses of AMBI, H′ and species richness (S) (Muxika et al., 2007). The M-AMBI index is the only one that requires references conditions of a high and a bad ecological status. The bad status was established with the lowest values of the three components (AMBI = 6, H′ = 0, S = 0). The high ecological status refers to a totally or nearly totally undisturbed condition, however there are no available data prior to the human impact in the study area, neither an area with similar characteristic without human impact. Consequently, we decided to use the following reference conditions: AMBI was calculated considering the abundance of all species that were observed at all surveys and removing the opportunistic species (ecological groups IV and V) from the list (AMBI = 0.196), following the arguments given by Muxika et al. (2007). For species richness, the maximum number of species recorded among the four sampling periods was taken (S = 10) and the diversity was calculated as the logarithm (Ln) of this richness, since it is the maximum possible diversity for this richness value (H = 2.3) (Gray and Elliot, 2009).

Table 1 shows the computational details for all indices. At each station the mean value of the five replicates was utilized for classifying the ecological status (High, Good, Moderated, Poor or Bad) according to Ruellet and Dauvin (2007), Borja et al. (2000, 2003), Simboura and Zenetos (2002), Afli et al. (2008) and Borja and Tunberg (2011) for H′, AMBI, BENTIX, ITI and M-AMBI respectively.

In order to evaluate the relationship between the indices a Spearman correlation matrix was performed ($p = 0.05$) among them, and also between each of them and the macrobenthic abundance. Such analysis evaluates the entire value of the indices, but not the relationship between their ecological status classifications. Hence, the degree of similarity in the classification of the ecological status was calculated for each index, as the percentage of stations with the same ecological status between each possible combination, as stated by Afli et al. (2008).

To evaluate the relationship between the classification of the indices and the physicochemical variables, three kind of methodologies were used: (1) a Spearman correlation matrix (non parametric linear model). (2) Radar plots, which represent the average concentration of each physicochemical variable for groups of stations with the same classification of ecological status by a benthic biotic index, and are normalized by the average concentration at all sites (Benyi et al., 2009). (3) Conditional Probability Analyses (CPA, non linear model). The CPA was performed to evaluate the associations between the variables and the ecological status, in cases where differences were found at radar plots. The conditional

Table 1
Algorithms of the calculated indices and the threshold values of each ecological status.

		Ecological status				
		High	Good	Moderate	Poor	Bad
H′	$\sum_{i=1}^{s} p_i Ln(p_i)$	5.0–4.25	4.25–2.27	2.27–0.92	0.92–0.3	0.3–0
AMBI	$[(0 \times \%GI) + (1,5 \times \%GII) + (3 \times \%GIII) + (4,5 \times \%GIV) + (6 \times \%GV)]/100$	0–1.2	1.2–3.3	3.3–4.3	4.3–5.5	5.5–7
BENTIX	$[6 \times \%(GI + GII) + 2 \times \%(GIII + GIV + GV)]/100$	6.0–4.5	4.5–3.5	3.5–2.5	2.5–2.0	0
ITI	$100 - [33 \times 1/3(0n_1 + 1n_2 + 2n_3 + 3n_4/n_1 + n_2 + n_3 + n_4)]$	100–80	80–60	60–30	30–0	
M-AMBI		>0.77	0.53–0.77	0.38–0.53	0.2–0.38	<0.2

probability is the probability of an event if another event has occurred. Thus, it was calculated the probability of occurrence of a poor and/or bad ecological status if a given concentration of a physic-chemical variable (pollutant) was exceeded. In this way, it is assumed that the probability of occurrence of impairment on ecological quality will increase, with an increase of the concentration of the pollutant (Paul and MacDonald, 2005). The probability of an impairment on the ecological quality is observed when a certain concentration of a pollutant is exceeded, over the entire range of the concentrations, thus providing an empirical curve of conditional probability. Confidence intervals for this empirical curve were estimated by bootstrap resampling ($n = 100$) with a confidence level of 95%. The first point of the curve is the unconditional probability of impact, which measures the probability of an impair in the ecological quality independently of the pollutant concentration. The relationship will be statistically significant when the confidence interval range of the unconditional probability do not overlap the confidence interval range of the conditional probability, thus suggesting that the behavior of the ecological quality is responding to the increase of the pollutant concentration. These analyses were performed with R program, using the commands provided by CProb (Hollister et al., 2008).

3. Results

3.1. Environmental variables

The water column salinity showed a seasonal variation, and generally it was constant in a spatial scale (data not shown). In May and October a low and spatially constant salinity of ~5 was found, while in January it was also spatially constant but with higher concentration (~25). In July, a low salinity (~5) was found in the bay stations while higher values (~25) were present outside the bay. The sediment was seasonal and spatially homogeneous, being silt the dominant fraction (greater than 50%), except at station H where sand was the dominant one (data not shown). For more details of these natural variables see Venturini et al. (2012).

The PCA detected in all surveys similar groups of variables explaining the data variation (see Fig. 2). The axis I was mostly correlated with heavy metals, protein and lipid, while axis II with mud percentage and phytopigments. The percentage of the explained variance of the first two axes was always higher than 65%. Usually, stations of the inner bay (A, B, C and D) were grouped on the left-side of axis I, and they were characterized by high concentrations of heavy metals, lipids and proteins (Fig. 2).

3.2. Trophic status

According to the CHO concentration the studied area was mostly classified as meso-oligotrophic status (67% of the total stations), although all stations showed an eutrophic or hypertrophic status in at least one survey (Fig. 3). However, according to PRT levels, 71% of the stations were classified as hypertrophic, 26% as eutrophic and only 3% as meso-oligitrophic.

Protein and lipid concentrations showed a clear pattern, with highest values in the inner portion of the bay, which was classified as hypertrophic according to the protein levels of all surveys. The trophic status of the remaining stations of the bay showed temporal differences from eutrophic to hypertrophic levels. In addition, the PRT/CHO ratio indicated eutrophic or hypertrophic status in all surveys (Fig. 3).

3.3. Degree of heavy metal contamination

In all surveys, higher concentrations of heavy metals (Cd, Cu, Cr, Pb and Zn) were recorded at the inner portion of the bay than in adjacent coastal zones. The Cd values were higher than TEL in the inner portion of the bay and lower in the remaining stations (Fig. 4). A similar pattern was observed for Cr, Pb and Zn, but with concentrations higher than PEL in some stations of the inner bay (mainly in July, October and May). Nevertheless, Cu concentrations were higher than TEL in almost the entire study area and higher than PEL in the station B of the inner bay.

3.4. Ecological status

A total of 152,358 individuals from 31 species were identified and counted. They corresponded to four phyla: Nemertina (1 species), Annelida (19 species), Arthropoda (6 species) and Mollusca (5 species). Polychaeta was the most diverse group with 16 species, while the gastropod *Heleobia* cf. *australis* was the dominant species ($n = 142{,}988$ individuals), accounting for 90% of the total abundance. Hence the general pattern of the total macrobenthic abundance was shaped by this gastropod abundance. No station was azoic.

3.4.1. Shannon–Wiener diversity index

According to the diversity, the moderate status was the best reached condition throughout the study period. The stations of the inner portion of the bay and the western coastal zone exhibited a bad ecological status, in almost all surveys. The remaining stations ranged either between bad and poor or poor and moderate. On a temporal scale, only four stations did not present any changes (Fig. 5).

3.4.2. AMBI

The species not assigned to an ecological group were always lower than 5%, although station K (in July), B and C (in October) and L (in January) showed three or less species, and consequently, these stations should deserve special consideration according to what was established by Borja and Muxika (2005). After assignment, the highest percentage of organisms in almost all stations throughout the study corresponded to ecological group IV (second-order opportunistic species) and it was dominated by *H.* cf. *australis*. In term of temporal variability, 13 of the 18 sampled stations showed changes in their ecological status over the study period, according to this index classification. Most of the changes occurred among adjacent categories, mainly moderate and poor status (stations A, D, E, F, G, H, I, K, M, N and Q), and only one

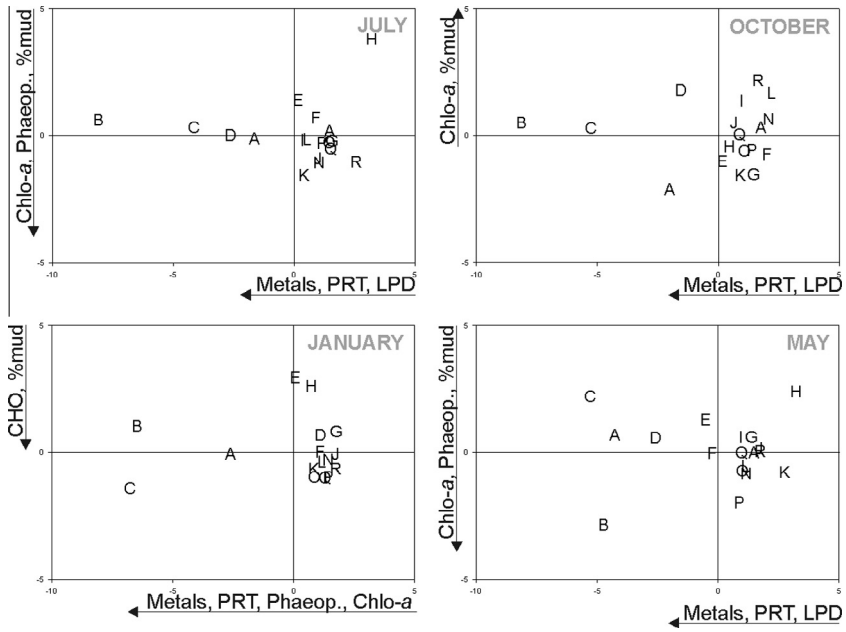

Fig. 2. Principal Components Analysis (PCA) ordination diagram of the sampling stations (letters) according to environmental data for each sampling season. For July survey, the first two axes accounted for 71.6% of the total variance, axis I (60.1%) correlated negatively with heavy metals (Pb, Zn, Cd, Cu and Cr), protein (PRT) and lipid (LPD) concentration, while axis II correlated negatively with phaeopigment (pheop.), chlorophyll-a (chlo-a) concentration and percentage of mud (%mud). For October survey, the first two axes accounted for 75.2%, axis I (62.8%) correlated negatively with Pb, Cu, Zn, Cr, Cd, PRT and LPD, while axis II correlated positively with chlo-a and %mud. For January survey, the first two axes accounted for 73.6% of the total variance, axis I (60.7%) correlated negatively with Zn, Pb, Cu, Cd, Cr, pheop., chlo-a and PRT, while axis II correlated negatively with carbohydrate concentration (CHO) and %mud. For May survey, the first two axes accounted for 67.5% of the total variance, axis I (53.8%) correlated negatively with Cd, Cu, Pb, Cr, Zn, PRT and LPD, while axis II correlated negatively with pheop. and chlo-a and %mud.

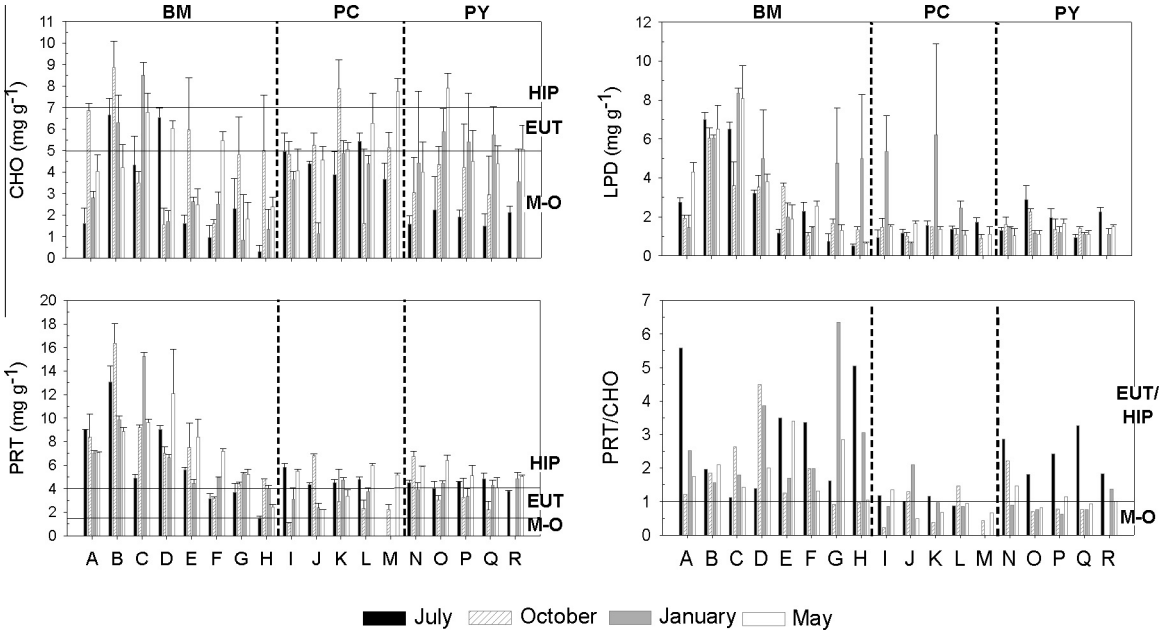

Fig. 3. Concentration of organic biopolymers in sediments and PRT/CHO ratio from Montevideo coastal zone in the four sampling surveys. The thresholds level of the trophic status, stated by Dell'Anno et al. (2002) are indicated. Hipertrophic (HIP), eutrophic (EUT) and meso-oligotropich (M-O).

station (J) changed among poor, moderate and good status. The stations with no temporal changes were classified as poor ecological status (B, C, L, P and R) (Fig. 5).

3.4.3. BENTIX

Twelve stations showed a poor status with no temporal change. The remaining stations showed temporal changes among adjacent status (moderate and poor), except for two stations (J and O) that showed an improvement in their ecological quality, i.e., from moderate to good status. Station F showed deterioration in the May survey (from moderate to poor) owing to a high percentage of species from the ecological group II (tolerant and opportunistic species), which were mainly represented by the gastropod *H.* cf. *australis*. Stations G and H improved their ecological quality in January to a moderate status. Similarly, stations J and O improved their quality from moderate to good status, in all cases due to a greater percentage of species from the ecological group I (sensitive), represented by *Erodona mactroides*. The station I showed a

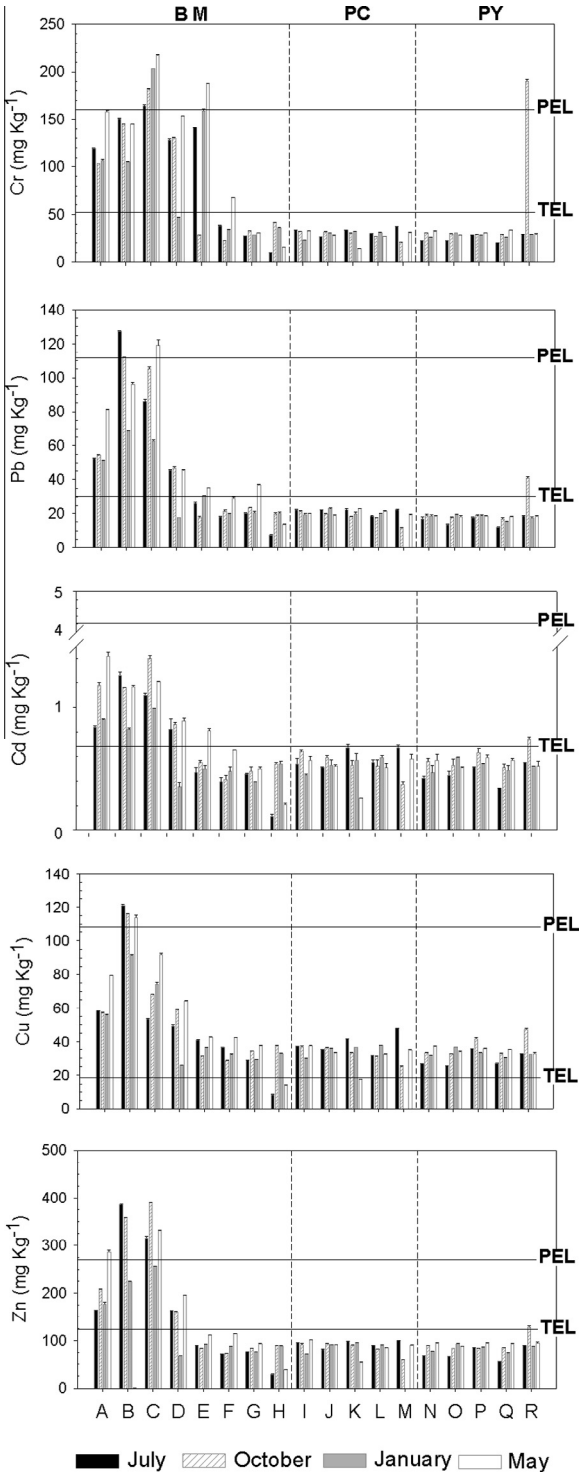

Fig. 4. Concentration of heavy metals (Pb, Cr, Cd, Cu and Zn) in sediments from Montevideo's coastal zone in the four sampling surveys. The Threshold Effect Level (TEL) and Probably Effect Level (PEL) determined by MacDonald et al. (1996) are indicated.

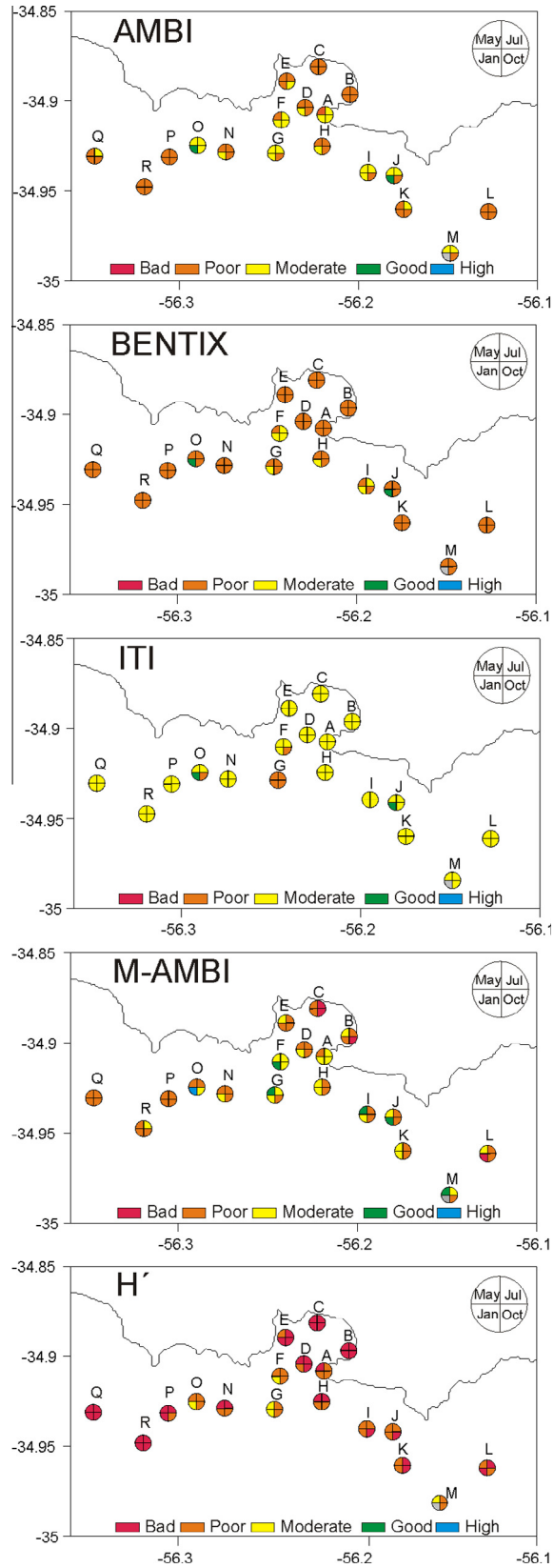

Fig. 5. Spatial distribution of the ecological quality classification obtained for each index in the four sampling surveys.

better quality in July, due to a high percentage of the ecological group I (sensitive species), represented mainly by *Nephtys fluviatilis* and *E. mactroides* (Fig. 5).

3.4.4. ITI

Most of the stations were classified with a moderate status during the whole study. However, the station G remained with a poor status without changes over the time, while stations F, J and O showed changes in their quality. Station F presented a moderate status in October while stations J and O presented good status in

January. The stations classified as moderated status, presented high percentage of species in the trophic group III (surface deposit-feeders), represented mainly by *H.* cf. *australis*. However, those stations that reached poor status were represented by species of the trophic group IV (sub-surface deposit-feeders), mainly by the polychaete *H. filiformis*.

3.4.5. M-AMBI

Only three stations (P, Q and R) presented a poor status over the four surveys (Fig. 5). Among the stations that showed temporal changes, nine stations (A, C, D, E, F, H, K, L and N) ranged between adjacent status (moderate/poor, good/moderate or poor/bad), and the remaining stations alternated through several status (i.e. station O changed from poor to high). Stations B and C reached bad status and only one station reached high status (station O).

3.5. Relationship between biotic indices

The degree of similarity of the ecological status obtained with the indices, showed a high correlation between AMBI/BENTIX (74%), followed by AMBI/M-AMBI (66.5%). ITI and H′ displayed the lowest degrees of similarity with other indices; their highest percentages were 33.8% (ITI/AMBI) and 38% (H′/BENTIX). Moreover, Spearmańs correlation among indices (considering all samples, $n = 70$) only showed a non-significant correlation ($p > 0.05$) between ITI and M-AMBI, and between them and macrobenthos abundance. The remaining correlations were all significant (Table 2).

3.6. Relationship between indices and environmental variables

AMBI, BENTIX and Shannon diversity (H′) correlated negatively with CHO, Cd, Cu and Zn (Table 3). The H′ also showed a negative correlation with LPD and Cr concentrations. Moreover, ITI was the only index that correlated with non-polluting variables (salinity and sand percentage) and M-AMBI was not correlated with any of the analyzed variables.

Conversely, in the radar plot, AMBI showed an association with CHO, PRT, LPD, Cu, Cr, Pb and Zn, since these physicochemical variables showed clear differences between sites with poor or moderate status. In this case, generally the group of stations with better ecological status showed lower concentration of these variables than the group of stations with worse ecological status. Nevertheless, BENTIX, ITI and M-AMBI showed some incongruence, because those sites with better ecological status showed higher percentage of heavy metals or organic matter biopolymers. However, it is worth to notice that the best ecological status observed in January corresponded only to stations J and O (Figs. 5 and 6).

Table 2
Spearman correlation matrix among indices and abundance of macrobenthos ($n = 70$).

	Abundance	AMBI	BENTIX	ITI	H′
AMBI	0.32*				
BENTIX	−0.36*	−0.92*			
ITI	0.02	−0.54*	0.51*		
H′	−0.32*	−0.90*	0.81*	0.26*	
M−AMBI	0.02	−0.71*	0.56*	0.22	0.79*

* $p < 0.05$

3.7. Conditional probability

The Conditional Probability Analysis was only performed for those environmental variables that showed an association with the AMBI classification in the radar plot (CHO, PRT, LPD, Cr, Pb, Cu and Zn). The associations between physicochemical variables and BENTIX, ITI and M-AMBI were not analyzed because they did not show appreciable differences between sites of different ecological status. Figs. 7 and 8 show the probability to obtain an impairment in the biota, equivalent to a poor or bad ecological status (AMBI > 4.3), with the increasing concentration in the corresponding environmental variables.

The unconditional probability of impact (i.e., the probability to obtain "poor" or "bad" ecological status regardless the concentration of the environmental variable) was 62.8%. The unconditional confidence intervals are represented in the graphs with a grey band (Fig. 7 and 8). The probability that the biotic impact (AMBI > 4.3) was caused by the "pollutant", is statistically significant if the confidence interval (solid lines) does not overlap the unconditional confidence interval (grey band).

A statistically significant probability of finding an AMBI value higher than 4.3 was detected for concentrations of CHO between 3.88 and 5.4 mg g^{-1} (Fig. 7, grey box). The probability due to the PRT concentrations was significant for concentrations higher than 6.73 mg g^{-1} (probability = 75.7%), and for concentrations higher than 9 mg g^{-1} (probability = 100%). For LPD concentrations higher than 5.35 mg g^{-1} the significant probability was 77.1%, and for concentrations greater than 6.03 mg g^{-1} the probability was 100%.

For Cu the significant probability of finding impairment on the biota (AMBI > 4.3) for concentrations higher than 38.03 mg kg^{-1} was 75.7%, and for a concentration of 58.95 mg kg^{-1} the probability increased to 100%. For Cr the probability of impairment was 71.4% for concentrations higher than 37.21 mg kg^{-1}. For Pb concentrations higher than 22.32 mg kg^{-1} was 72.9%, while for Zn concentrations higher than 101.3 mg kg^{-1} the probability was 75.8%.

4. Discussion

The present results showed that ecological quality of the study temperate urban estuary (Montevideo coastal zone) varied between poor and moderate conditions, where the inner bay portion exhibited the worst quality. The concentration of lipids, proteins and heavy metals, together with the AMBI index, represented the set of variables with higher resolution to assess the environmental quality of the study area.

4.1. Environmental variables

The high values of the detected organic matter were into the thresholds normally established for estuarine systems under

Table 3
Spearman correlation matrix between biotic indices and environment variables. Only are presented the Rho values of the statistically significant correlations ($p < 0.05$). Neither clo-a, % TOM nor M-AMBI showed significant correlations.

	Phaeopig.	CHO	PRT	PRT/CHO	LPD	%sand	%clay	Cd	Cr	Cu	Pb	Zn
Abundance	0.5		0.5	0.3	0.3				0.4	0.3	0.3	0.3
H′		−0.3			−0.2			−0.3	−0.2	−0.3		−0.3
AMBI		0.4						0.4		0.3		0.3
BENTIX	−0.3	−0.5	−0.2					−0.3		−0.3		−0.3
ITI						−0.2	0.4					

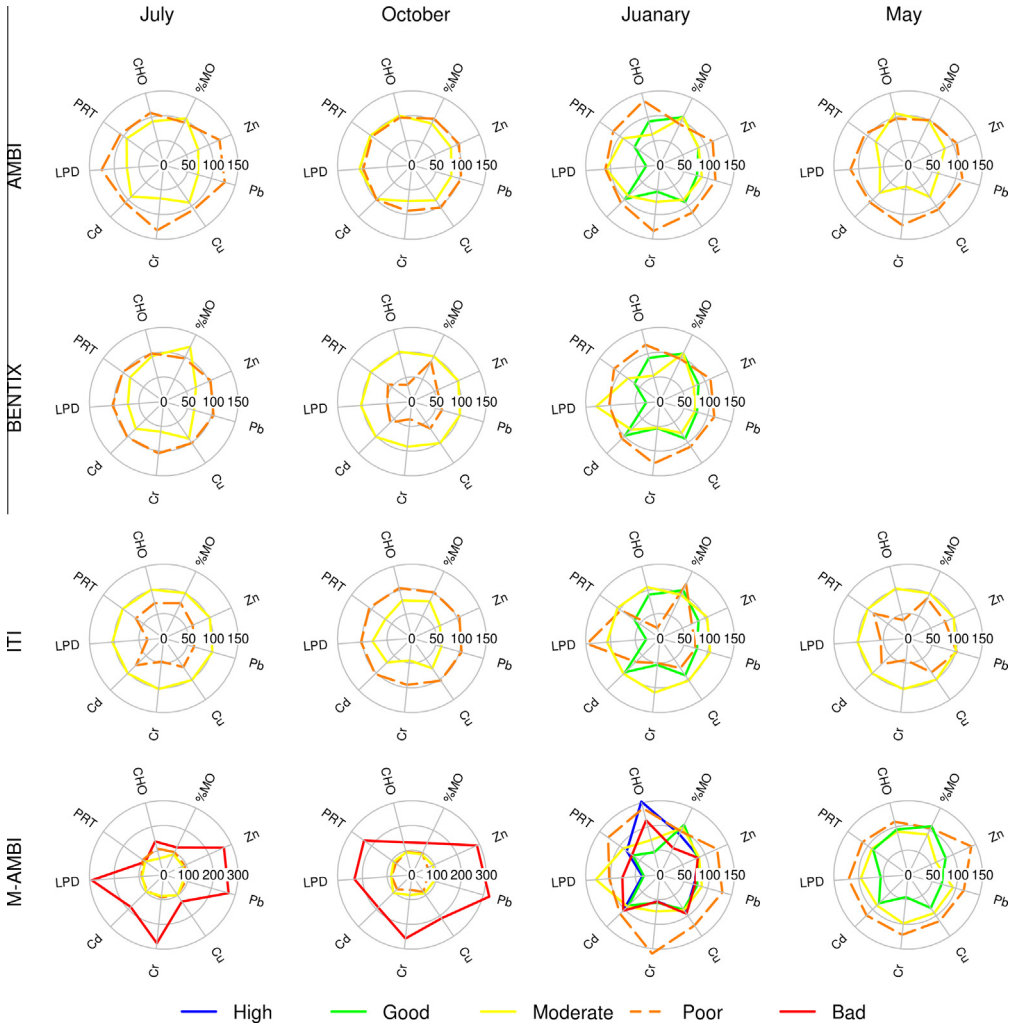

Fig. 6. Radar plots showing mean concentration of environmental variables for each group of stations with equal ecological quality, expressed as the percentage of the average for all stations. One plot per index and per sampling surveys is presented. The radar plot for BENTIX in May survey is not showed because all stations were classified as moderate.

anthropogenic impacts (Cotano and Villate, 2006). However, in this study not regular spatial/temporal pattern was detected, so is it not appropriate to establish an association with trophic state. Eutrophic systems (such as estuaries) accumulate large amounts of organic carbon, a conservative variable with a low degradation rate, due to the associated refractory compounds (Danovaro et al., 1999). Neither CHO concentrations displayed any clear pattern of distribution. This component of the organic matter could be related to the natural high productivity of the estuary, therefore, it could be rapidly degraded (Venturini et al., 2012). This high degradation ratio could be the reason of the low trophic level detected by this biopolymer.

However, PRT and LPD concentration are more related to anthropogenic sources (Venturini et al., 2012). These two variables could be considered important components of the environmental structure in the study area, due to their weight in the principal component analysis (PCA). High PRT concentrations in the inner bay stations could be associated with the refractory organic matter, from both phytodetritus and anthropogenic wastes. These facts together with the high degradation ratio of the CHO could explain the differences in the trophic classifications provided by CHO and PRT (meso-oligotrophic vs. hypertrophic, respectively). In addition, the high ratio of PRT:CHO (always >1) in this zone suggests intense detritus mineralization and an increment in their protein content due to bacterial activity (Venturini et al., 2012). High values of LPD in the inner bay could be associated to the domestic wastes discharged into the bay (through the streams), and to the presence of hydrocarbons related to the oil-refinery and vessels of the harbor (Muniz et al., 2002; Pinturier-Geiss et al., 2002).

The observed spatial distribution of heavy metal contamination in the present work was in agreement with previous studies (Muniz et al., 2004b, 2011). A clear pattern of heavy metal contamination was found, where stations B and C in the inner bay presented a severe contamination, with concentrations of Cr, Pb, Cu and Zn above the probable adverse biological effect level (PEL), and above of the threshold effect level (TEL) for Cd. In the outer bay, the concentrations of heavy metals were lower, but such levels, occasionally might cause some adverse effect on the biota. Pb and Cr concentrations were lower than previously reported (Muniz et al., 2002), but they are still considered within the problematic range of TEL levels.

This group of metals (Cd, Cr, Pb, Cu and Zn) appear to be important for the environmental quality of the study area, since it showed high correlations with the fist axis of the PCA. In this sense, is important to keep in mind that only one variable above the

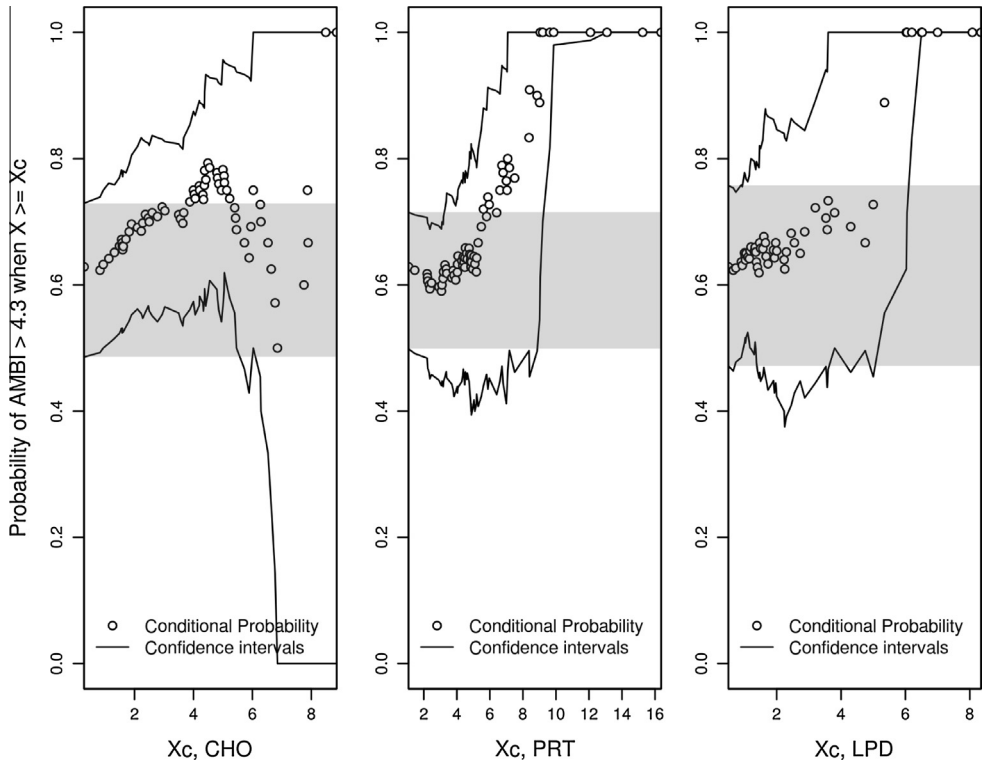

Fig. 7. Conditional probability plots of organic biopolymer concentrations in the sediment relative to AMBI. The statistically significant probability of obtaining a value higher than 4.3 of AMBI is shown when the confidence intervals (solid lines) do not overlap to the unconditional probability (gray box).

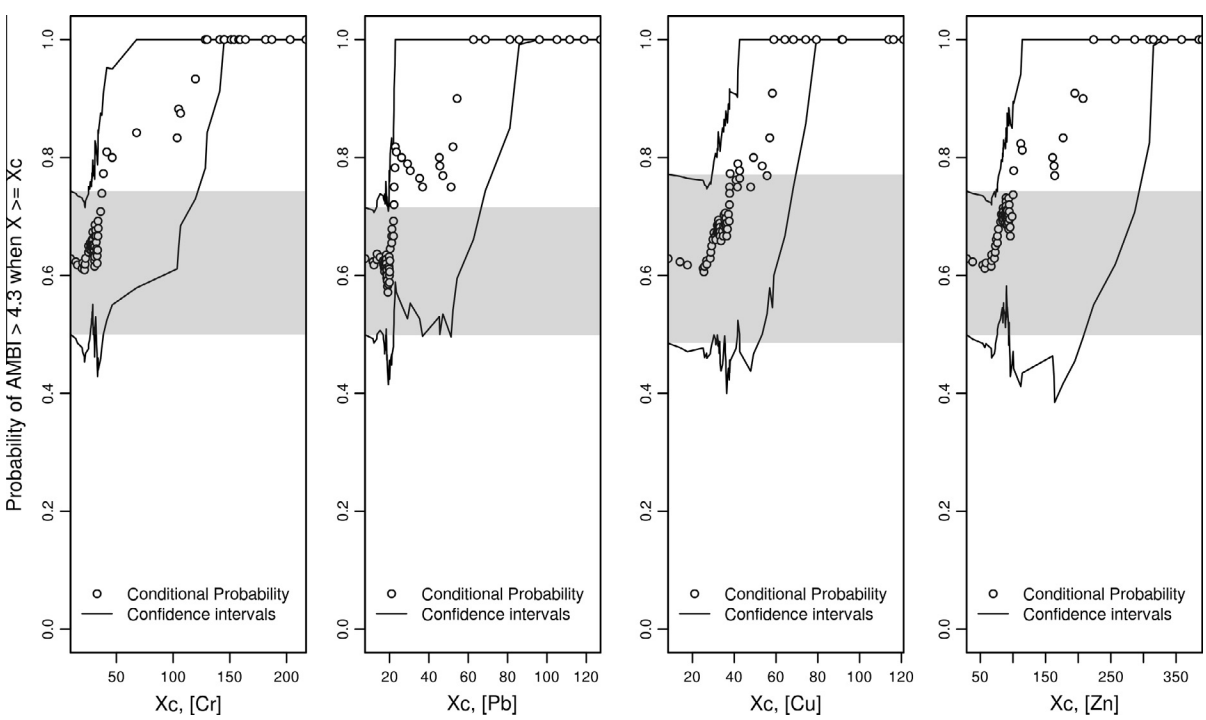

Fig. 8. Conditional probability plots of heavy metals sediment concentration relative to AMBI. The statistically significant probability of obtaining a value higher than 4.3 of AMBI is shown when the confidence intervals (solid lines) do not overlap to the unconditional probability (gray box).

threshold level is enough to cause and adverse effect on the biota (Abessa et al., 2008). Furthermore, it has been proven the enhanced toxicity of a single metal in presence of others (Clements, 2004; Norwood et al., 2007).

4.2. Ecological indices classification

Benthic indices (AMBI, BENTIX, H′ and M-AMBI) showed the same spatial trend in the ecological quality of the study area as

evidenced by the linear correlation assessment. This kind of analysis allows evaluation of the values given by the indices, independently of the ecological quality categories of each index (Blanchet et al., 2007). The tendency of the ecological quality given by ITI was incongruent with the results of the other indices as observed elsewhere (Salas et al., 2006; Ruellet and Dauvin, 2007; Afli et al., 2008; Muniz et al., 2012). Moreover, almost all the stations were classified as moderate by ITI, thus not properly reflecting the presumed ecological quality gradient of the study area. One possible explanation could be that the trophic structure of a benthic community depends mainly on the available resources (i.e., food or space) in unpolluted areas (Glémarec, 1993). However, in polluted areas, there are other intrinsic characteristics of the species (such as resistance, tolerance and opportunism) affecting the trophic community organization (Afli et al., 2008). Hence, the trophic community structure in polluted areas may not always correctly reflect the trophic status of the system. In addition, the diversity of the trophic community, which is not considered in the ITI formula, is appropriate to reflect and explain the perturbed conditions of the environment (Gaston et al., 1998; Brown et al., 2000). These observations, together with the difficulty of classifying the species into trophic groups, because of either lack of information or the nutritional plasticity, could lead to a low performance of the ITI for detecting disturbed or polluted zones in the study area.

Shannon diversity (H′) showed a similar pattern to those observed for other indices (excluded ITI) and was very useful to detect high impacted zones, such as the inner bay. However, in the adjacent coastal zone it showed a high variability, which would make the use of this index not fully appropriate. Similar to other univariate indices, diversity depends on natural factors (e.g. seasonal variability, habitat type, massive recruitment events or patchy distribution of species) or methodological aspects (sampling methodologies, sample size, etc.) (Martínez-Crego et al., 2010). Since in the present study no sampling methodology problems were experienced, index variation could be attributed to natural factors.

Although AMBI, BENTIX and M-AMBI showed the same trend in the classification, the low percentage in the similarity matrix between ecological quality statuses, suggests an inconsistency in the status thresholds of each index (Blanchet et al., 2007). Inconsistencies in the ecological status classification among different indices have already been documented in other locations (Ruellet and Dauvin, 2007; Pranovi et al., 2007; Tataranni and Lardicci, 2010); even some authors suggested the need for adjusting the thresholds for different types of environments (Blanchet et al., 2007; Zettler et al., 2007; Simboura and Reizopoulou, 2008). Particularly it is recommended to adjust the thresholds for transitional water zones (i.e. estuaries), according to the abiotic conditions and the abundance of tolerant species (Prato et al., 2009; Munari and Mistri, 2010). In this sense, it is noteworthy that the study is in a highly salinity transition area, but all samples stations are similarly affected.

Moreover, inconsistencies between the AMBI and BENTIX classification could be attributed at the different weight that each index displays for each ecologic group. In this sense, BENTIX exhibits the same weight for tolerant and opportunistic species (Simboura and Reizopoulou, 2008; Simboura and Argyrou, 2010), but the abundance for the tolerant species increase in natural stressed regions (i.e. estuaries) (Rosenberg et al., 2004). Hence, this index could understate the ecological quality of the estuarine systems, as observed in this study and by Blanchet et al. (2007). Furthermore, BENTIX was developed for an oligotrophic, eurihaline and microtidal region, with a uniform distribution and diverse benthic community (Simboura and Reizopoulou, 2008) that is why this index would not be fully appropriate for estuarine systems (Munari and Mistri, 2010).

However, AMBI showed an ecological quality gradient, being poor and/or bad in the inner bay, and moderate or poor in Pta. Carretas and Pta. Yeguas zones and the adjacent coastal zone. This index seems to have a better performance to evaluate the ecological status of the study area, in agreement with those observed by Prato et al. (2009) for brackish water ecosystems. Conversely, other authors state a lack of sensitivity of this index to discriminate among polluted areas where naturally stressed community exists, due to the dominance of tolerant species commonly observed for this type of environment (Chainho et al., 2007; Munari and Mistri, 2008; Zettler et al., 2007; Puente et al., 2008). Although in the present study there was a clear dominance of *Heleobia* cf. *australis*, an opportunistic species, its dominance could be rather related to the response to a chronic perturbation instead of a natural stress. Nevertheless, the dominance of one species resulted in a narrow range of variation in the gradient of AMBI.

M-AMBI correlated positively with AMBI, BENTIX and H′, but with low percentage of similarity among categories. This fact could be attributed to incongruences in the categories of ecological quality thresholds as well as to the reference values used. In this regard, it should be emphasized that, due to the lack of any pristine station or a historic condition to establish the reference values of high ecological status, the references used may be not appropriate. The reference condition of high ecological quality used to perform the M-AMBI, was based on a simulated situation, thus assuming that this would be the best scenario for the study area. However, since the entire study area is more or less impacted, the classification yielded by M-AMBI could overestimate the ecological quality, because it was considered as a reference an ecological status without the opportunistic species, as the best possible situation. But estuaries and transitional waters hosts many tolerant and opportunistic species adapted to the natural stress of the environment and potentially they could tolerate better the anthropogenic stress, underestimating the status given by biotic indices (phenomenon called Estuarine Quality Paradox by Dauvin (2007) and Elliot and Quintino (2007)). Therefore, the main problem of this index would arise in the determination of correct reference values, which are necessary to calculate it (Borja et al., 2012).

Despite these clear incongruences, the differences between the classification of these indices (AMBI, M-AMBI and BENTIX) were always observed between adjacent categories (mostly moderate to poor), and therefore the performance of the indices is less critical than for non-adjacent categories, as properly established by Borja et al. (2007).

4.3. Evaluation of the ecological indices vs. the environmental variables

The diversity and abundance of macrobenthos appeared to properly represent the gradient of disturbance of the study area, as they were negatively correlated with almost all the environmental structuring variables. Despite diversity can be used as an indicator of the environmental changes (Salas et al., 2006), the interpretation should be taken with caution, since as stated by Warwick and Clarke (1993) a disturbed environment can exhibit the same diversity than an undisturbed one, although with different species composition. Therefor, it is recommended the use of indices or methodologies that consider the identity of the species.

The multivariate index, M-AMBI did not show a significant correlation with any of the analyzed variables, thus suggesting a low performance in the study area. The other ecological indices (AMBI, BENTIX and ITI) in spite of being based in models of response to the organic enrichment, they did not display significant correlations with the sediment organic matter. However, CHO showed signifi-

cant correlations with AMBI and BENTIX, although in the radar plot only the AMBI showed an association with CHO, LPD and PRT. This fact suggests that the classification derived from AMBI would agree with the benthic trophic status, with higher concentrations in those stations with higher AMBI values. However, high CHO concentrations did not seem to have a direct significant correlation, because the probability of achieving a poor or bad ecological status by AMBI was equal to the unconditional probability of impact. On the other hand, PRT, LPD and heavy metals (Cr, Pb, Cu and Zn) could explain the biota impairment (interpreted by high values of AMBI). The effectiveness of AMBI to detect perturbation originated by other factors, such as heavy metals, anoxic conditions, dredging, was evidenced by other authors (Muxika et al., 2005).

The Conditional Probability Analysis appeared to be useful for establishing thresholds of effect on biota of the study area. For biopolymers of organic matter it was possible to establish trophic levels above which a negative effect on the biota would be expected. In this sense, Dell'Anno et al. (2002) stated threshold levels for CHO and PRT to classify the trophic status of the sediments, however, no threshold values for LPD was defined. So, taking the statistically significant values of the analysis, the threshold effect level could be set to 5.35 mg g^{-1} for lipids in sediment of the study area. In addition, the protein threshold level determined with this method (6.73 mg g^{-1}) was higher than the level for the hypertrophic status of Dell'Anno et al. (2002). Concerning to heavy metals, lower values than TEL determined by MacDonald et al. (1996) were found. In this sense, the TEL and PEL values were determined under certain conditions and evaluated independently of other variables. But the toxicity of a pollutant could be enhanced by the presence of others and also depends on environmental factors, such as pH or salinity (MacDonald et al., 2000; Clements, 2004). Thus the values herein obtained could be useful as the reference thresholds, at least for the Montevideo's coastal zone. Comparing these thresholds with the heavy metal concentrations (Cu, Cr, Pb and Zn) obtained by Muniz et al. (2004a,b, 2005) in Montevideo coastal zone in 1998, it is possible to conclude that in the inner zone of the bay and inside the harbor of Montevideo, these values were well above the threshold effect on biota (obtained herein). This observation is in concordance with the conclusions reached in these works. However, further studies would be necessary to corroborate the threshold levels.

4.4. Temporal assessment

The perturbation pattern on the Montevideo's coast, detected with the environmental data, was similar during the analyzed seasonal scale, despite of the natural abiotic fluctuation. In turn, an advantage in the use of ecological indices is that they should be independence of the natural variations, although this aspect has only been little studied (Salas et al., 2004; Reiss and Kröncke, 2005; Muniz et al., 2012).

In this study AMBI, BENTIX and ITI did not show considerable temporal variations, and only AMBI displayed a good spatial performance. The temporal variations in AMBI were always observed between adjacent categories, as also reported by Tataranni and Lardicci (2010). Indices based on ecological groups appeared to better assess the ecological quality, by avoiding the seasonal variability of the benthic communities (Salas et al., 2004; Reiss and Kröncke, 2005).

Temporal differences were higher with M-AMBI, probably due to the influence of the diversity in this index. In temperate regions, with marked temporal/seasonal fluctuations, it is frequently observed that radical changes in abundance exert deep effects on diversity, that in turn could be misinterpreted in terms of better/worse ecological quality status (Reiss and Kröncke, 2005). In any case, it should be considered the problem of determining the reference conditions for this index.

5. Conclusions

The temperate urban estuary herein analyzed presented an environmental quality status varying between poor and moderate, being poor in the inner portion of Montevideo Bay, and moderate/poor in the external portion of the bay and Pta. Carretas. Pta. Yeguas zone showed a decreased environmental quality when compared with previous studies. This last trend coincide with those previously detected with other variables and benthic indicators (Muniz et al., 2011).

The protein, lipid and heavy metal (Cu, Cr, Pb and Zn) sediment concentrations, together with AMBI appeared to be the most suitable set of variables to evaluate the environmental quality of the Montevideo coastal zone. AMBI provided a more integrated view of the ecological status of the study area, because it reduces the subjectivity and facilitates interpretation. Indeed, this index was able to demonstrate spatial status gradients and shows a better correlation (than other indices) with contaminant levels in spite of the naturally stressed conditions of the estuary. Therefore, the use of benthic indices represents a good complement to assess the ecological status, but their effectiveness should be evaluated before use. The high natural variability in the physicochemical variables of the study area did not affect the AMBI performance in the considered temporal scale.

Finally, a new threshold effect levels for heavy metals and organic matter biopolymers was established, which is at least site-specific and would be useful to determine tolerable levels of these stressors in the assessment or monitoring the study area. This in turn, could be useful in order to provide scientific tools for management plans and to establish more precise regulation actions to improve and/or maintain the environmental quality.

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