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Urbanization effects on different biological organization levels of an estuarine polychaete tolerant to pollution

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ABSTRACT

Estuarine species exposed to diffuse contaminants might trigger either positive or negative feedbacks in many biological scales. Their life history traits performing at different biological organization levels could propose an organism as a useful indicator of environmental pollution, mainly addressed as sensitive or tolerant species. To track the effects of contaminants from the molecular to the population level of the polychaete *Laeonereis acuta* we utilize a framework of biomarkers. For this purpose we assessed the *L. acuta* frequency of micronuclei at the molecular level, the body size and biomass at individual level, and the production-to-biomass ratio at population level in five urbanized and five non-urbanized estuaries in southern Brazil. *L. acuta* had significantly varying positive and negative feedbacks between urbanized and non-urbanized estuaries at multiple biological scales. These generalized effects in all biological organization scales indicate a pollution impact on the polychaete. The main responses accounted for individuals becoming lengthy and weighty, but with molecular damage. The *L. acuta* allocation of energy to body enlargement in polluted environments, and a consequent reduced population turnover, contradicts the expected from an opportunist species. The damages in DNA and the internal strategies of individuals, as antioxidant defense mechanisms, could favor resistance of the population and tolerance to pollutants. All of these characteristics induce bioaccumulation and could cause bottom-up pollution transfer compromising the estuarine food web. These results, ascertain that *L. acuta* could be considered as a tolerant species, instead of an opportunistic, and as a useful indicator of environmental pollution in estuaries.

1. Introduction

Coastal urbanization has increased the discharge of diffuse toxic substances into water bodies responsible for acute and chronic degradation of the estuarine biota. In advanced situations, this process causes elimination of sensitive species and dominance of the most tolerant or opportunistic individuals (Klerks and Weis, 1986; Bickham et al., 2000; D'Alessandro et al., 2015). Along their evolutionary history, tolerant species might have selected life traits with adaptive values (fitness) that are nowadays coupled with or benefited from contaminants (Kawecki and Ebert, 2004). Notwithstanding, species exposed to contaminants usually present negative responses in genetic, physiological, morphological and behavioral levels (Mersch et al., 1996; Fleeger et al., 2003; Schiedek et al., 2006; Catalano et al., 2012). The description of biological mechanisms under such evolutionary and ecological scales has been a current challenge to elucidate how species function as tolerants to environmental pollution.

Evaluation of the effects of contaminants usually involves an outlined bulk of biomarkers (Monserrat et al., 2007). To integrally comprehend those effects, the biomarkers need to be processed in many biological scales, as species may experience stress at different levels of biological organization throughout the medley from molecular to the community level. Among the implications to the environmental management of investigating many biological orga-

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nization levels, the assessment of changes in lower biological levels can be used to avoid further effects on higher levels, when they have not yet happened. However, the uses and responses of biomarkers in molecular or subcellular levels in invertebrates are still poorly known and fragmented (Dixon et al., 2002). Conversely, the expression of both qualitative and quantitative damage to DNA is very similar between invertebrates and higher complexity organisms (Dixon et al., 2002; Hagger et al., 2002; Jha, 2004). These similarities could be used to drive endeavors in researches with marine invertebrates.

Polychaetes numerically dominate the estuarine communities living in the water-sediment interface, have a short lifespan and are less motile than other benthic macroinvertebrates, and thus responsive to changes in their surrounding environment. All of these characteristics confer the generic status of good indicators of environmental health (Durou et al., 2007; Botter-Carvalho et al., 2011). The Nereididae polychaete *Laeonereis acuta* is widely spread in South American Atlantic estuaries and sheltered areas, from 2°S (Brazil) to 42°S (Argentina) (Orensanz and Gianuca, 1974; Pamplin et al., 2007). Early studies have indicated *L. acuta* to have an opportunistic behavior because of their relatively short life cycle and rapid sediment recolonization strategies (Netto and Lana, 1994; Omena and Amaral, 2000). Further studies highlighted the species as pertaining to a tolerant assemblage in urbanized estuaries (Pagliosa and Barbosa, 2006). This was suggested after their density and biomass were found to be related to the general state of the environment, both in water and sediments, than simply to modifications in the sediment. More recently, the potentiality of *L. acuta* as a tolerant bioindicator of pollution has stimulated the mapping of their histological and morphological alterations, as well as biochemical responses, accumulation, and biotransformation after exposure to contaminants (Geracitano et al., 2002; Ferreira-Cravo et al., 2008; Ventura-Lima et al., 2011). However, an integrated biomarkers assessment at different levels of biological organization of *L. acuta* under urbanization effects is still lacking.

The aim of this study was to assess the effects of diffuse pollution caused by urbanization in estuaries at different biological organization levels of the polychaete *L. acuta*. For this purpose we used the biomarkers frequency of micronuclei at subcellular level, body size and biomass at the individual level, and production-to-biomass ratio at the population level. This framework was applied in urbanized and non-urbanized estuaries. Thus, we expected *L. acuta* biomarkers to indicate the environmental health differences between estuaries types which would be detected in all levels of biological organization. It is hypothesized that *L. acuta* from urbanized estuaries will exhibit higher frequencies of micronuclei in cells, lower individual body sizes and biomass, as well as higher production-to-biomass ratio in populations when compared to those from non-urbanized estuaries.

2. Material and methods

The study was conducted from August to September in 2014 at ten estuaries located between the coordinates 25°5′S–48°3′W and 27°5′S–48°4′W (Fig. 1). The coastal region is typically composed of bights founded by quaternary sediments turning over marine, tidal, and river-lake plains crossed by estuaries. The climate is humid subtropical with rainfall well distributed throughout the year. The annual mean temperature is 20 °C with seasonal differences ranging from 17 to 22 °C. The tidal regime is microtidal with average amplitude of 0.83 m for spring tides and 0.15 m for neap tides (Cruz, 1998). The estuarine basins in south Brazil are generally well preserved areas (highest concentrations recorded: 1 μM of P, 52 μM of N, 27 mg/kg of Cu, 28 mg/kg of Pb, 105 mg/kg of Zn) interspersed with urbanized areas (highest concentrations recorded: 12 μM of P, 217 μM of N, 46 mg/kg of Cu, 56 mg/kg of Pb, 144 mg/kg of Zn) (Pagliosa, 2005; Pagliosa et al., 2006a,b, 2016; Rovai et al., 2013). Then, we performed a visual interpretation of aerial images complemented by field surveys, and used existent data of water and sediment quality to select. In this sense, we have determined two types of estuaries for our study in relation to the degree of anthropogenic environmental disturbance: (i) urbanized estuaries, whenever available, indicated by the water and sediment data, and when a great extent of the estuarine basins was composed of residential, commercial or industrial areas with clear signs of disturbed landscape; and (ii) non-urbanized estuaries, whenever available, indicated by the water and sediment data, and when the estuarine basin presents just a small or absence of signs of anthropic interventions and visually the physiognomy of the landscape is preserved. The study estuaries were spaced from 05 km to 200 km and belonged to a different watershed, avoiding confound effect of urbanization.

In order to assess the responses of *L. acuta* to environmental conditions, three sites were randomly established in shallow waters at the mouth of five urbanized and five non-urbanized estuaries. The mouths of the estuaries were chosen for sampling because they sink the continental material and might therefore represent the general health of these small systems. At each site, individuals of *L. acuta*, sediment and water were sampled. The effects of urbanization on different levels of biological organization of the polychaete were assessed using: (i) the frequency of micronuclei, at the molecular

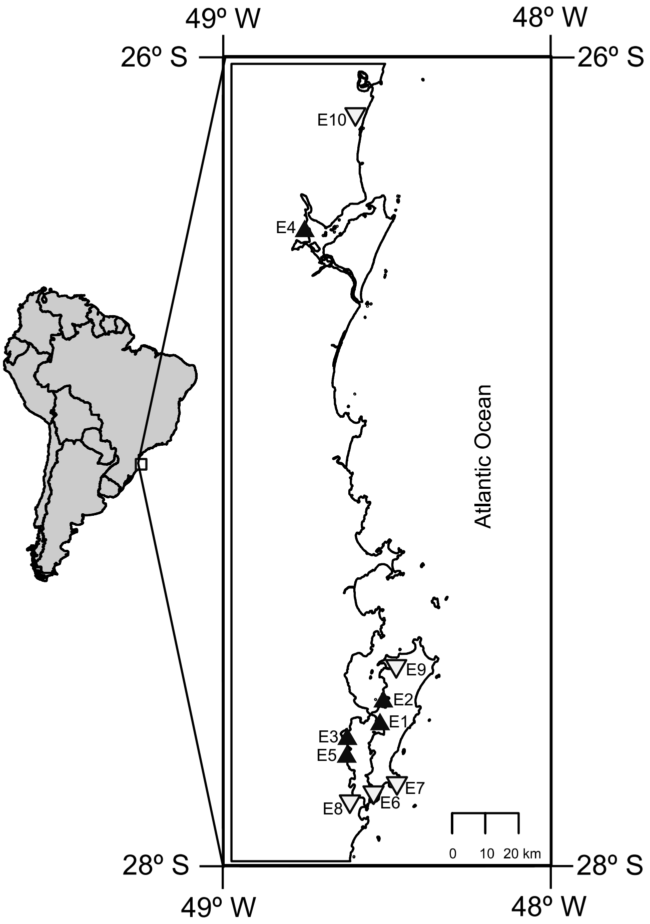

Fig. 1. Map indicating the 10 estuaries in Southern Brazil. E1-Costeira do Pirajubaé, E2–Itacorubi River, E3-Maruim River, E4-Saguaçu Bay, E5-Aririu River, E6-Ribeirão da Ilha, E7-Lagoinha do Leste, E8-Massiambu River, E9-Ratones River, E10-Sai Mirim River.

level; (ii) the body size and biomass, at the individual level; and (iii) the production-to-biomass ratio (P/B), at the population level.

2.1. Molecular level

At each site, multiple individuals of *L. acuta* were carefully and manually collected using a spatula and tweezers, and held in a container with sediment and water from the local source. In the laboratory, all containers were maintained with aeration. Then, we randomly chose 10 alive and unharmed organisms (no breakage during handling) from each sampling site to evaluate the frequency of micronuclei in cells. The posterior segments of alive individuals were removed using a scalpel and were kept for four days in aquarium (natural variability of light and temperature) receiving dehydrated brine shrimp (*Branchipus stagnalis*) as food. At the end of this period, the regenerated tissues were removed, disaggregated into acetic acid and methanol at a ratio of 3:1 and centrifuged at 900 rpm for four minutes. The bulk of the material resulting from the disaggregation of the 10 specimens was fixed in methanol and stained with May-Grünwald Giemsa 5% and diluted in phosphate buffer for 10 min. Micronuclei frequencies were evaluated under a light microscope (Olympus CX21SF1, 100× magnification) by counting one thousand cells per site (bulk of 10 individuals). Micronuclei were considered as the corpuscles of smaller diameters, entirely separated and which were similar in color and morphology to the main nuclei. The micronuclei test was adapted from Fenech (2000).

2.2. Individual level

From the container, we randomly select 10 other alive and unharmed organisms from each sampling site to measure for individual size and biomass. The body length was taken from the anterior prostomium to posterior pygidium of each individual under a Zeiss optical stereoscope (Discovery V12; 10 μm precision) coupled to a digital camera (AxioCam MRc 5). Additionally, the wet weight of each individual was measured (BEL Analytical balance; 0.0001 g precision and internal calibration M124AI 24 V 550 mA).

2.3. Population level

The biomass values of sampled individuals from each site were used to calculate empirically the production-to-biomass ratio of the population. We used the following formula adapted from the model of Brey (2012):

$$\log 10\, P/B = 7.947(-2.294 \log 10\, M - 2409.856 \times (1/(T + 273)) + 0.548 + 582851 \times (\log 10\, M \times (1/(T + 273)))$$

Where, P = production, B = mean population biomass, M = mean individual biomass (we used a global data bank on body composition of aquatic organisms to get the energy content (Joule) per mg of wet mass; Brey et al., 2010), and T = mean seasonal temperature (we used the historical, from 1960 to 2015, mean temperature value of 17.6 °C from the sampled months for all sites; INMET (2016). The value 0.548 is a product of the adapted model, since all organisms belong to Annelida (taxon = 1), are infaunal (mobility = 1), and occur in intertidal habitats (depth = 0 m).

The population P/B ratio is the renovation or replacement of biological material, thus being the quantity of matter and energy potentially available for higher trophic level. The empiric model of Brey (2012) used to calculate population productivity was built using sample-based data and was originally treated as a scale of area (mg/m^2). In order to validate the performance of the empirical model using individual-based data (i.e., sampling a number of individuals in a population instead of sampling individuals of the population living in a specified area), we used previously published information on *L. acuta* population (Omena and Amaral, 2000). The data set is made of two year-long monthly samples and with *L. acuta* populations structured in four different cohorts. We estimated the P/B ratios based on mean individual biomass (mg) and based on mean individual biomass per square meter (mg/m^2). The comparison between the P/B ratios calculated with sample-based data and individual-based data were found to be not significant (ANOVA, $F_{(1,40)} = 0.080$; $P = 0.778$). We therefore assumed that a production-to-biomass ratio using individual-based data could be used to represent population level test in our study.

2.4. Environmental data

At each estuary and site, samples of near-bottom water were collected in 500 mL polyethylene bottle, previously cleaned by immersion in 50% HCl, washed with deionized water and kept refrigerated at 4 °C. Determination of total nitrogen, total phosphorus and total phenols was performed by spectrophotometry according to Standard Methods (APHA, 1998), using a UV visible (FEMTO spectrophotometer 700 Plus). For the particle size analyses, we used homogenized sediment sub-samples of a standardized weight (50 g). The sand fraction was assessed by dry sieving using meshes among −1.5 and 4.0 Phy, and fine fractions were accessed via pipetting at 20 °C (Suguio, 1973). The aluminum, cadmium, chromium, copper and lead contents in sediments were obtained by contrAA 600 high resolution continuum source atomic absorption spectrometer (HR-CS GF-AAS, Analytik Jena AG, Jena, Germany) with a transversely heated graphite tube atomizer. The samples digestions were preceded with addition of 2.5 mL HNO3 67% and 7.5 mL HCl 37% for approximately 3.0 g, using a TOPwave IV laboratory microwave digestion system (Analytik Jena AG, Jena, Germany). The standard solution of Al, Cd, Cr, Cu and Pb were prepared from stock concentrate solution containing 1000 mg L^{-1} (Merck, Darmstadt, Germany) with nitric acid were used to prepare the external calibration curve. Additionally, to provide a basis for asserting the absence of sediment toxicity (as expected for non-urbanized estuaries) we contrasted the metal concentrations found with the Consensus-Based Threshold Effect Concentration (TEC; MacDonald et al., 2000). Similarly, to asserting sediment toxicity (as expected for urbanized estuaries) we contrasted the metal concentrations found with the Consensus-Based Probable Effect Concentration (PEC; MacDonald et al., 2000).

2.5. Data analysis

In order to assess the relationship between samples of the biomarkers at multiple biological scales of the polychaete and samples of environmental conditions of estuaries, a distance-based redundancy analysis (dbRDA; Legendre and Anderson, 1999; McArdle and Anderson, 2001) was used. In this analysis, the axes of ordinations are linear combinations of the environmental variables that best explain the biological variation. The Bray-Curtis coefficient and Euclidean distance were used on squared-root transformed biological and environmental data, respectively. To model the changes in each of the biological variables representing different biological organization levels (micronuclei frequency, body size, biomass, and P/B ratio) related to the environmental conditions, we used a canonical analysis of principal coordinates (CAP; Anderson and Wills, 2003). The CAP analyses how well the multivariate environmental data can predict the position of samples along a continuous values of each biomarker. CAP avoids over-parameterization (i.e., to avoid including too many axes and finding spurious relationships) by choosing the number of principal coor-

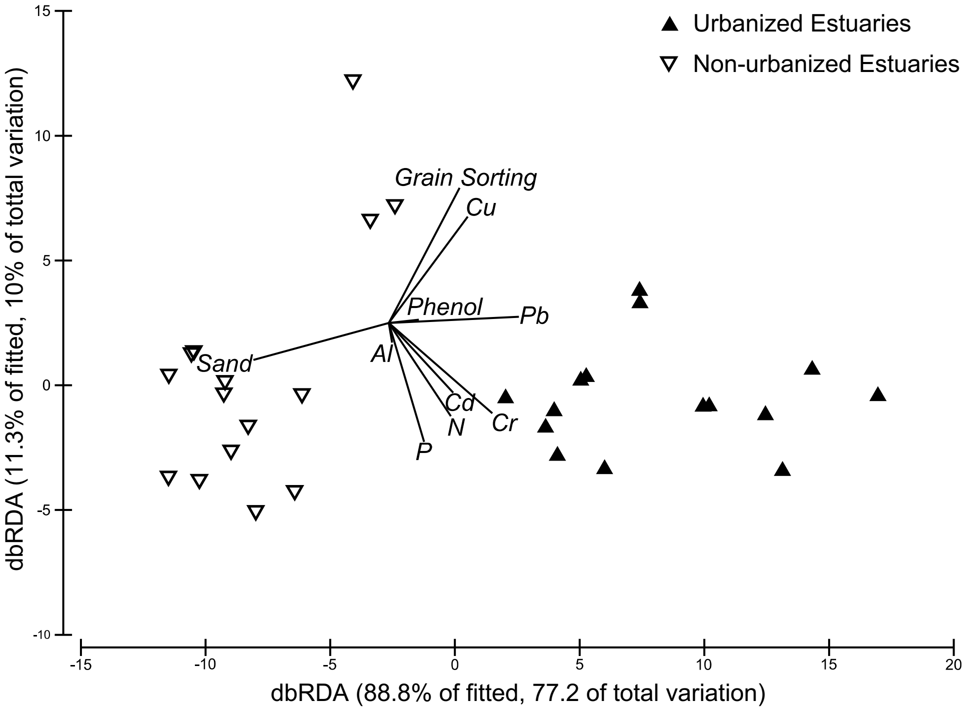

Fig. 2. Distance-based RDA ordination relating sediment and water parameters to *L. acuta* responses (micronuclei frequency, body size, biomass, and P/B ratio) in urbanized and non-urbanized estuaries.

dinate axes that minimize a leave-one-out residual sum of squares (Anderson and Robinson, 2003).

Additionally, the biological and environmental variables were individually evaluated using hierarchical nested analyses of variance. The factor "condition" was fixed (two levels: urbanized vs non-urbanized) and the factor "estuary" was random and nested within the condition factor (five levels: five estuaries within each estuary type). The three sites within each estuary provided the replicates. The nested design provides an estimate of the contribution of each estuary to the total variation among replicates within the urbanized and non-urbanized comparison. Partitioning of the variances associated with each factor permits unconfounded comparisons at any of the chosen factors (Underwood, 1997). Additionally, components of variation were calculated to estimate the percentage of variability attributed to each factor and for the residual by restricted maximum likelihood estimation (Pinheiro and Bates, 1996). Data were previously tested for heterogeneity of variance with the Cochran test and squared-root transformed whenever necessary. For the ANOVAs, we used the GAD package (Sandrini-Neto and Camargo, 2012) in the R environment (R Development Core Team, 2013), and for the dbRDA and CAP we used the PERMANOVA+ add-on PRIMER software (Anderson and Gorley, 2007).

3. Results

The spatial distribution of *L. acuta* samples was distinct between the urbanized and non-urbanized estuaries, while three replicates from a non-urbanized estuary appeared in an intermediate position (dbRDA with 89.6% of the explained variation; Fig. 2). Samples from urbanized estuaries were characterized by higher content of metals (lead, cadmium, and chrome) in sediment and higher concentrations of dissolved nutrients (phosphorus and nitrogen) and phenol in water. Otherwise, biological samples of non-urbanized estuaries were more related with sandy sediments and well sorted grains (low variance). Nevertheless, the intermediate group of non-urbanized samples appeared to group with content of copper and very poorly sorted grains (large variance).

All recorded metal concentrations in non-urbanized estuaries were lower than the Consensus-Based Probable Effect Concentration (PEC) and just one estuary (estuary 6) reported higher concentrations than the Consensus-Based Threshold Effect Concentration (TEC), indicating an absence of sediment toxicity (Fig. 3). Conversely, the chromium, copper, and lead concentrations in all sites of urbanized estuaries (except estuary 5 for copper) were higher than the TEC and some estuaries (mainly for chromium) were higher than the PEC, indicating the presence of sediment toxicity.

The analysis of variances of environmental variables showed significantly higher values for aluminum, cadmium, lead and sorting in sediment as well as nitrogen in water from urbanized estuaries than from non-urbanized ones (Tables 1 and 2; Figs. 3 and 4). The magnitude of the effects for all of those variables presented the greatest percentage of explanation related to the factor condition (all components of variation >50%). Neither environmental variable showed higher values in non-urbanized than urbanized estuaries and the percentage of sand, content of copper and chrome in sediment, and concentration of phosphorus and phenols in water were not different between the two estuary conditions. The lowest values of components of variation (between 27 and 42%) highlighted the low importance of factor conditions for these variables. Contrarily, all variables from sediment and water were significantly different between estuaries nested within condition. This means that the variation within a group of estuaries is different in general to the variation within the other group of estuaries. In general, the urbanized estuaries were more heterogeneous among themselves than non-urbanized estuaries (mean and error in Figs. 3 and 4). The magnitude of the effects of the factor estuary nested in factor condition was low when the factor condition for the same variable was significant (between 23 and 33%) and increased only a little when the factor condition was not significant (between 34 and 51%), indicating the low importance of the variation within

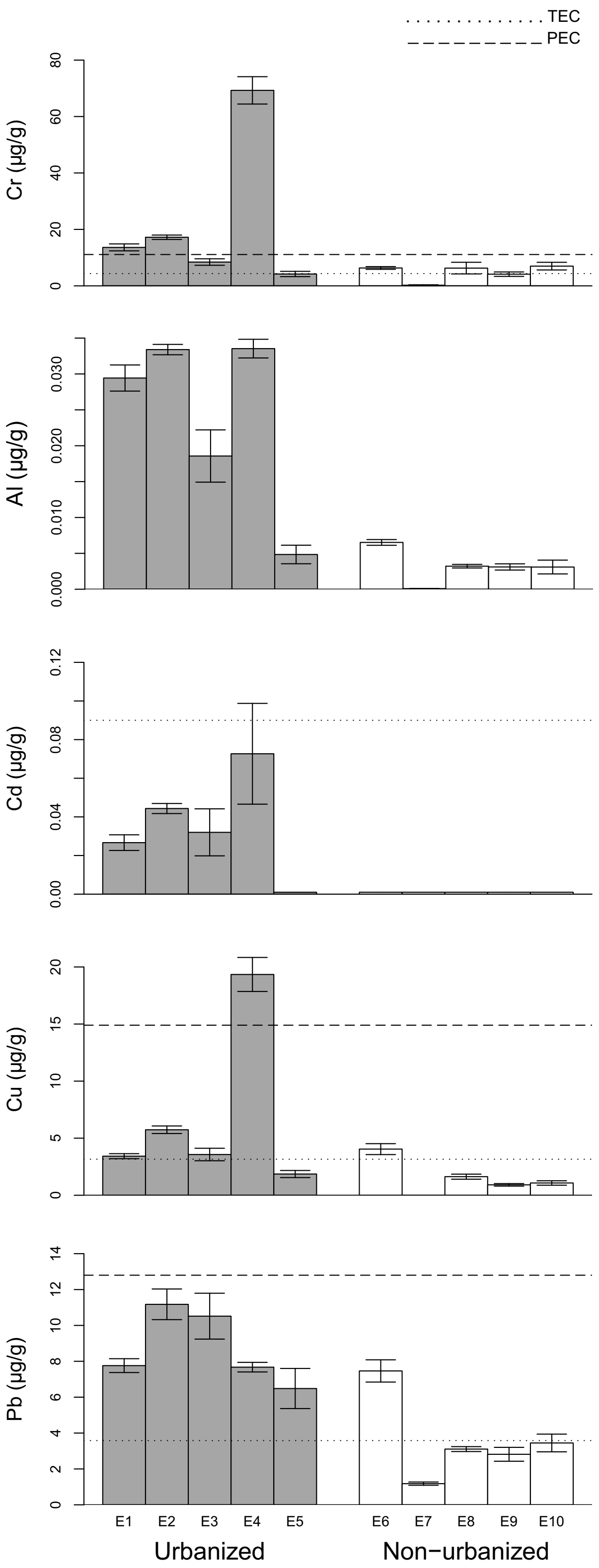

Fig. 3. Metal sediment toxicity (mean ± 1SD) in urbanized and non-urbanized estuaries. Underlines represent the Consensus-Based Threshold Effect Concentration (TEC) and the Consensus-Based Probable Effect Concentration (PEC), according to MacDonald et al. (2000). See Fig. 1 for original names of estuaries.

groups of estuaries to explain all variability found in the analysis of variance.

Table 1
Result of hierarchical nested analysis of variance and component of variation (CV) of metal from sediments in urbanized and non-urbanized (Condition) estuaries.

Source	Anova				CV (%)
	Df	MS	F-ratio	P-value	
Al					
Condition	1	0.071	15.418	**0.004**	57.038
Estuary(Condition)	8	0.005	34.818	**<0.001**	33.103
Residuals	20	<0.001			9.859
Cd					
Condition	1	0.138	12.686	**0.007**	50.059
Estuary(Condition)	8	0.011	8.806	**<0.001**	30.829
Residuals	20	0.001			19.112
Cr					
Condition	1	36.744	3.624	0.093	37.578
Estuary(Condition)	8	10.140	67.556	**<0.001**	51.490
Residuals	20	0.150			10.932
Cu					
Condition	1	12.989	4.536	0.065	41.701
Estuary(Condition)	8	2.864	91.362	**<0.001**	49.314
Residuals	20	0.031			8.985
Pb					
Condition	1	9.280	13.329	**0.006**	52.626
Estuary(Condition)	8	0.696	14.977	**<0.001**	32.375
Residuals	20	0.047			14.999

In bold $P < 0.05$, Df = degrees of freedom, MS = mean square.

Table 2
Result of hierarchical nested analysis of variance and component of variation (CV) of sediment and water parameters in urbanized and non-urbanized (Condition) estuaries.

Source	Anova				CV (%)
	Df	MS	F-ratio	P-value	
SEDIMENT					
Sand					
Condition	1	62.368	4.337	0.070	38.151
Estuary(Condition)	8	14.380	25.297	**<0.001**	45.767
Residuals	20	0.568			16.082
Grain Sorting					
Condition	1	3.765	12.778	**0.007**	49.717
Estuary(Condition)	8	0.295	7.851	**<0.001**	30.260
Residuals	20	0.037			20.023
WATER					
N					
Condition	1	0.020	16.980	**0.003**	49.880
Estuary(Condition)	8	0.001	3.211	**0.016**	23.153
Residuals	20	<0.001			26.967
P					
Condition	1	0.088	3.356	0.104	27.796
Estuary(Condition)	8	0.026	3.358	**0.013**	33.929
Residuals	20	0.008			38.275
Phenol					
Condition	1	0.010	2.473	0.155	26.587
Estuary(Condition)	8	0.004	9.900	**<0.001**	46.446
Residuals	20	<0.001			26.966

In bold $P < 0.05$, Df = degrees of freedom, MS = mean square.

All parameters presenting the different levels of biological organization of *L. acuta* were significantly different between the conditions urbanized and non-urbanized, and among the factor estuaries nested in conditions (Table 3; Fig. 5). The lower levels of biological organization (frequency of micronuclei at molecular level; body size and biomass at individual level) showed higher values in urbanized than in non-urbanized estuaries. Contrarily, the higher level of biological organization assessed, P/B ratio of population, was significantly higher in non-urbanized estuaries than in urbanized ones. The magnitudes of the effects of the factor condition were always high (between 54 and 66%) and of the factor estuary were always low (between 15 and 33%). These values of component of variation indicate that contribution of the variation within groups of estuaries is less important for the total spatial variability found than the variation between the urbanized and non-urbanized condition.

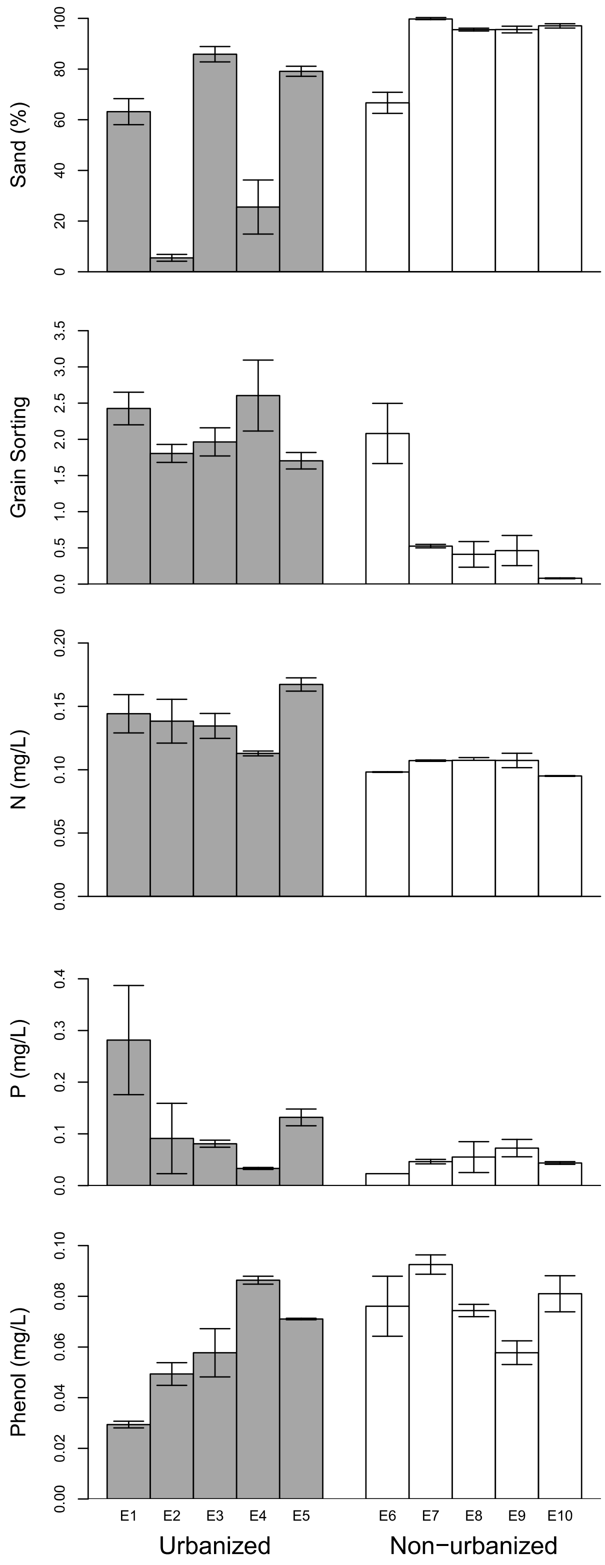

Fig. 4. Sediment and water variables (mean ± 1SD) in urbanized and non-urbanized estuaries. See Fig. 1 for original names of estuaries.

Table 3
Result of hierarchical nested analysis of variance and component of variation (CV) of *L. acuta* different levels of biological organization in urbanized and non-urbanized (Condition) estuaries.

Source	Anova				CV (%)
	Df	MS	F-ratio	P-value	
Micronuclei frequency (1000 cells^{-1})					
Condition	1	11.981	13.837	**0.005**	54.195
Estuary(Condition)	8	0.866	20.948	**<0.001**	33.006
Residuals	20	0.041			12.800
Body size (cm)					
Condition	1	1.778	35.095	**<0.001**	59.456
Estuary(Condition)	8	0.051	3.387	**0.013**	19.114
Residuals	20	0.015			21.430
Biomass (mg)					
Condition	1	7.783	66.136	**<0.001**	66.537
Estuary(Condition)	8	0.118	3.013	**0.021**	15.069
Residuals	20	0.039			18.394
P/B ratio (y^{-1})					
Condition	1	17.115	26.764	**<0.001**	60.415
Estuary(Condition)	8	0.639	10.422	**0.013**	25.306
Residuals	20	0.061			14.279

In bold $P < 0.05$, Df = degrees of freedom, MS = mean square.

The CAP analyses found a strong linear relationship among each *L. acuta* biological parameters and the sediment and water properties representative of the urbanized and non-urbanized estuaries (Fig. 6). The squared canonical correlation was high and positive for micronuclei frequency ($\delta^2 = 0.86$), body size ($\delta^2 = 0.79$) and biomass ($\delta^2 = 0.93$), and high and negative for the P/B ratio ($\delta^2 = 0.82$). Additionally, the CAP analysis for micronuclei frequency showed three replicated sites of the non-urbanized estuaries were intermediately grouped among sites of the urbanized estuaries.

4. Discussion

L. acuta had significantly varying positive and negative feedbacks between urbanized and non-urbanized estuaries at multiple biological scales. These results indicate that the biomarkers showed the differences in environmental health of the studied estuaries in southern Brazil quite well. In fact, the environmental conditions of urbanized estuaries were exceeding the consensus-based threshold effect concentration (TEC) and in some sites even surpassed the consensus-based probable effect concentration (PEC). Conversely, non-urbanized estuaries never reached the PEC levels and rarely surpassed the TEC levels. Then, we confirm our hypothesis that biomarkers can detect environmental health differences between estuaries types in all levels of biological organization studied. The detected generalized effects in all biological organization scales indicate a pollution impact on *L. acuta*. The population of the pollution tolerant polychaete is growing more, the individuals are increasing in body mass and body size, but the molecules are standing damaged. Furthermost, there is a comprehensive field investigation stressing changes in estuarine soft-bottom communities connected with changes in *L. acuta* population, expressed as increased abundance, size and biomass (Souza et al., 2016; Pagliosa and Barbosa, 2006; Souza et al., 2013; Brauko et al., 2015; Gusmão et al., 2016; Magalhães and Barros, 2011). On the other hand, there is a collection of laboratory experiments evidencing changes in *L. acuta* behavior, histology (coelom obliteration, separation of the cuticle from epidermis), tissue (loss of the digestive epithelium) and biochemical parameters (catalase, superoxide dismutase, lipid peroxidation, and glutathione *S*-transferase) caused by different pollutants (Geracitano et al., 2002; Ferreira-Cravo et al., 2008; Leão et al., 2008; Ventura-Lima et al., 2011). All of these evidences evoke two questions about *L. acuta* responses to pollution: (i) at the higher scales of biological organization (from individual to community), the polychaete has been efficiently occupying niche space which is

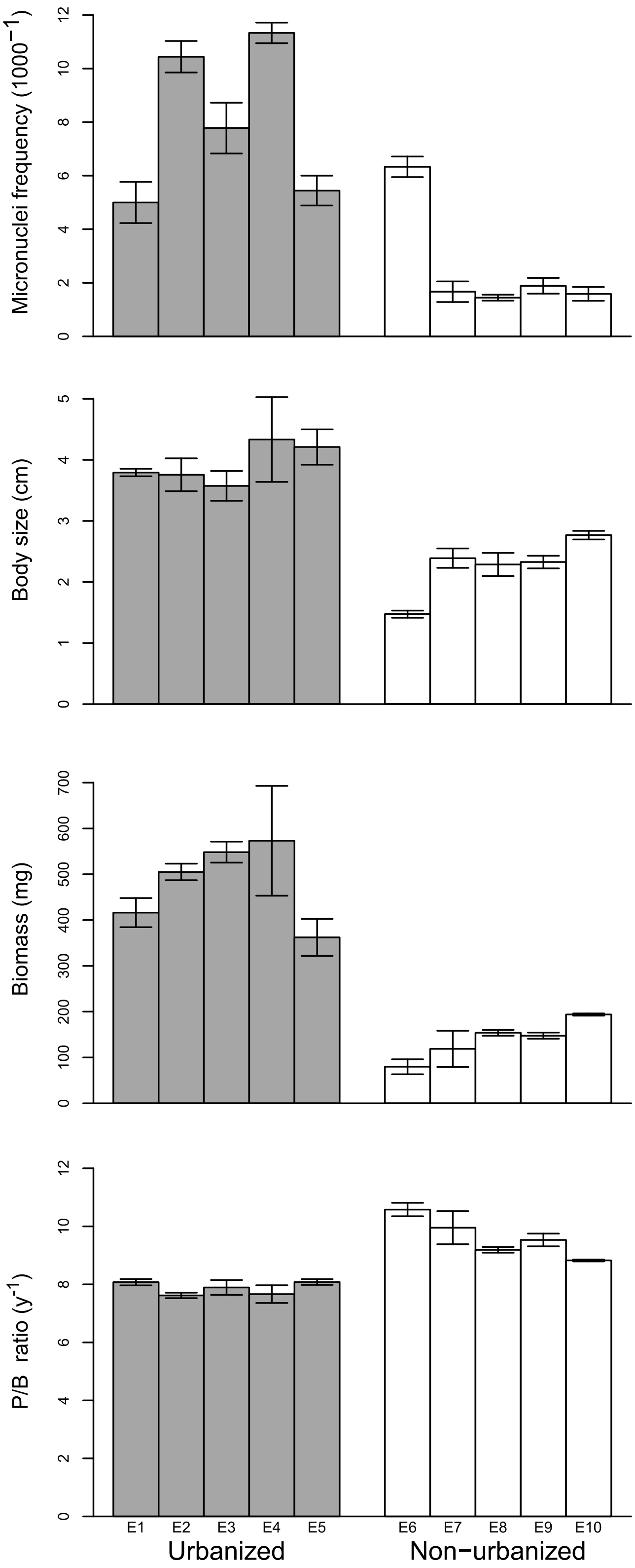

Fig. 5. Biomarkers of *L. acuta* different levels of biological organization (mean ±1SD) in urbanized and non-urbanized estuaries. See Fig. 1 for original names of estuaries.

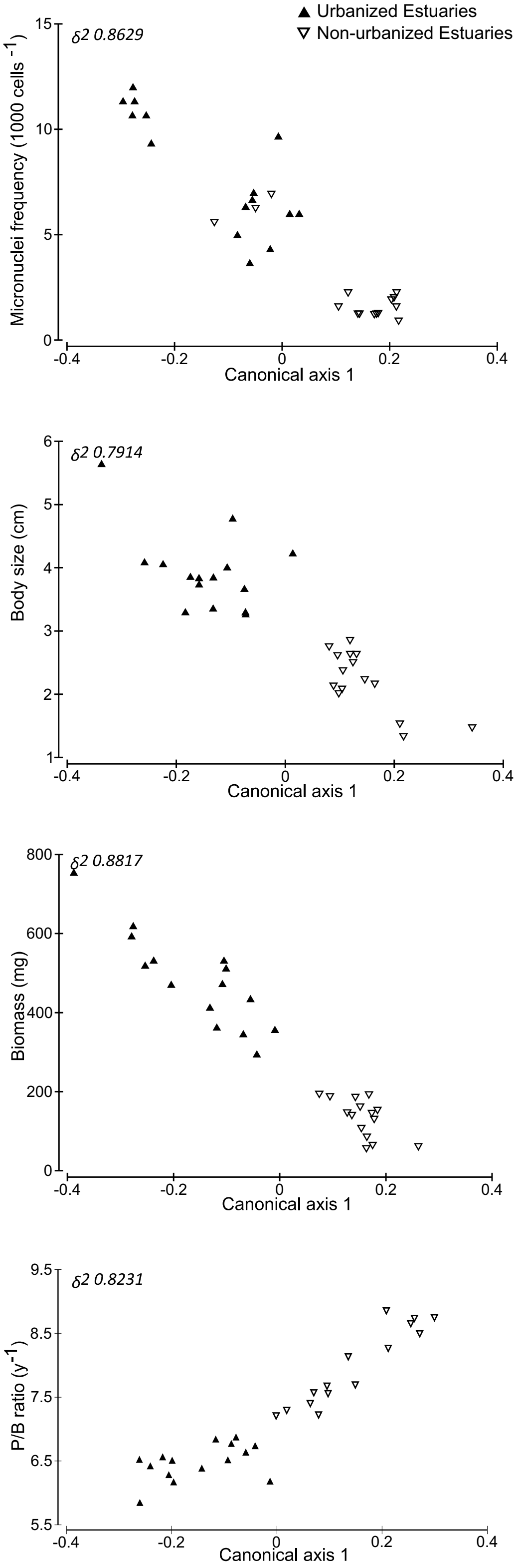

Fig. 6. Canonical analysis of principal coordinates (CAP) to model the relationships of sediment and water variables to *L. acuta* responses (micronuclei frequency, body size, biomass, and P/B ratio) in urbanized and non-urbanized estuaries.

vague for other species that are more sensitive to pollution. The advantage comes mostly from changing phenotypic expressions related with body size and biomass. As found in other invertebrates (Hoffmann et al., 2004), these changes in size and biomass could be rooted in the increased *L. acuta* chromosome numbers (2n = 38; ~36% more than others nereidids) and the consequent increased potential for chromosomal mutations related to inversions (4-fold more nucleoli per interphase cell as in other nereidids) (Ipucha et al., 2007). In fact, *L. acuta* is so different to other family species in many aspects that it stands in a separate and unresolved clade in phylogenetic analysis of the nereidids (Santos et al., 2006); (ii) at more basal levels of biological organization (from molecule to organ) the species developed strategies to deal with excessive pollutants and toxins. The substances are accumulated in regions of the body, tissues and cells, and mucus appears to be a key substance for primary protection, favoring the biotransformation of contaminants (Leão et al., 2008). The resultant permanence and resistance of the enlarged organism with accumulated contaminants in their body are eye-catching prey to consumers (Ieno et al., 2000), which might cause a bottom-up pollution transfer (biomagnification) along the estuarine food web.

The response mechanisms of organisms to pollution are usually rapidly and evidently observed at molecular and cellular scales but become rather difficult to define at higher levels due to evolutionary distance and environmental complexity (Smolders et al., 2004; Sulmon et al., 2015). Here, we detected organisms with micronucleus frequency, a type of DNA damage, three times higher in urbanized than in non-urbanized estuaries, indicating general genotoxicity of metals in the former (Sanchez-Galan et al., 1999; Ayllón et al., 2000; Andrade et al., 2004). Further, we recorded *L. acuta* DNA damage but no individual or population changes in the sites of one estuary typified as non-urbanized and presenting non-expected great concentrations of lead, copper, chromium and aluminum in sediments, indicating an early stage of genotoxicity. Metals exert genotoxic action alone or through synergistic action. In sub-lethal concentrations and chronic form, they could cause marked changes in antioxidant enzymes, suggesting the induction of oxidative stress with implications for cell repair systems (Livingstone, 2003; Nusetti et al., 2005) which affects the DNA molecule (Meneghini, 1997). Among all types of DNA damages, strand break and the subsequent micronucleus formation is the most challenging to the cells once the continuous formation of such breaks may contribute to genomic instability (Halazonetis et al., 2008).

Estuarine species living in urbanized areas are usually characterized by small organisms with low individual biomass and r-strategists (Elliott and Quintino, 2007). *L. acuta* is a typical r-strategist species with no parental care, and the presence of females with large mature oocytes and recruits throughout the year (Omena and Amaral, 2000; Santos et al., 2006; Martin and Bastida, 2006). Conversely, in the present study, the size and biomass of *L. acuta* individuals at urbanized estuaries were two- to three-fold higher than those of the non-urbanized. Possibly, the exposure of organisms to sublethal amounts of xenobiotics triggers adaptive responses providing greater ability to tolerance (Klerks and Weis, 1987; Adams, 2005). Polychaetes advantages of an increased size could come from the concentration of metals in the tissues tending to a decrease in the increased sized and age of organisms (Mendez and Páez-Osuna, 1998), meaning that larger organisms are more likely to successfully thrive under contamination. When subjected to low to moderate concentrations of xenobiotics, *L. acuta* responds by positively activating detoxifying enzymes in sufficient quantities to prevent damage caused by oxidative stress (Geracitano et al., 2004a,b). Additionally, the production of mucus substantially increases the antioxidant defense system (Moraes et al., 2006). Thus, living in polluted environment requires many metabolic responses that demands high-energy expenditure to organisms (Bayne et al., 1979; Smolders et al., 2004). In addition, the polychaete may take advantage strategy for surviving in areas with an increased organic material in sediments. Hence, for *L. acuta* population this excessive energy might be required to increase body size and biomass coupled with activating responses related, especially, to the antioxidant defense mechanisms. As a consequence of energy allocation to tolerate the environmental pollution in urbanized estuaries, the production to biomass ratio of *L. acuta* tended to decrease. That is the contrary to the expected to an opportunist species, when usually populations high density and biomass in polluted sites are supported by individuals with smaller size ranges and higher production values with a turnover ratio higher than in non-polluted sites or when pollution was ended (Alla et al., 2006; Méndez et al., 1997). All presented evidences in molecular, individual and populations ascertain *L. acuta* as a tolerant species, instead of an opportunist, and is a useful indicator of environmental pollution in estuaries.

Our results showed an environmental pollution impact in all levels of biological organization of *L. acuta* in urbanized estuaries, which indicate that even in non-heavy polluted estuaries, the synergisms of diffuse contaminants could cause a generalized biological effect. The main response in higher biological organization levels of the polychaete (above individual level) appears to be changes in their phenotype expression related with increased body size and biomass, and the consequent decreased P/B ratio. However, what appears to be a competitive advantage at higher biological levels is high cost at lower levels (bellow individual level), with DNA damage. We detected the effects of contaminants on molecular levels occur earlier, which might favor decision making by managers and stakeholder as preventive practices before the emergence of damages to populations or communities levels. All these life history characteristics of *L. acuta* point to a tolerance to pollution performance, which is a desirable feature for a useful bioindicator.

The approach used here to track the effects of contaminants at multiple biological scales using a framework of biomarkers showed accurate when contrasted with the environmental data. All used biomarkers are friendly and easy to use. The micronuclei frequency is a lower cost and more time efficient to use than other traditional molecular approaches, as proteomic ou metabolomic. The biomarkers at individual level are widespread used and do not need any additional material than the already used in benthic ecology. The empirical population P/B ratio is a powerful tool already implemented, but unfortunately scarcely used. All used biomarkers seem to be particularly effective to highlight differences in environmental health and could be an alternative method to determine estuarine pollution condition.

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