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Determination of organic contamination levels by the ABC Method (Abundance/Biomass Curves) in intertidal estuarine flats using hierarchical design

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ABSTRACT

This study evaluates the applicability and reliability of the ABC method as an indicator of the level of organic pollution in non-vegetated tidal flats in a subtropical estuary in southern Brazil. Following a hierarchical sampling design, the method was applied in two contaminated and two non-contaminated tidal flats near Paranaguá. It was conducted in three consecutive fortnights of a summer and a winter, aiming to analyze correlations between faunal responses and chemical indicators of contamination and spatio-temporal variability. Tidal flats closer to the city were classified as grossly contaminated. However, the responses on the non-contaminated area were highly heterogeneous, indicating that natural inputs of organic matter were confounded with pollution effects. In the AIC analysis applied to the *W*-statistics of the ABC curves, the best model showed correlations with some of the fecal steroids and total organic carbon. Contrary to expected, the ABC curves significantly varied at the smaller spatio-temporal scales due to the high local hydrodynamics and natural organic inputs. This suggests that the ABC method is indeed sensible to the contamination levels and can be used as an index of biotic quality. However, the method must be cautiously applied in estuaries subjected to natural organic enrichment.

Keywords: Macrobenthic fauna, Tidal flats, K and r-strategies

INTRODUCTION

The use of indexes is an alternative for impact studies since their synthetic, direct, and practical language is easily understood. Indexes and indicators of environmental quality can and should be used to guide research and policies of environmental programs (Pinto et al., 2009, Carter et al., 2017). In this context, benthic macrofauna has been widely integrated with environmental quality indexes since it is composed of relatively sedentary animals and long-life cycles with different levels of stress tolerance (Wildsmith et al., 2011; Dauvin et al. 2012; Carter et al., 2017; Huang et al., 2021; Huang et al., 2022). Many indexes based on the conditions and functions of the benthic communities have been proposed and applied in specific regions, particularly in temperate areas of the northern hemisphere. However, for such indicators to be in fact generalizable and applicable in real situations, studies are still needed in other areas, including tropical and subtropical ones.

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An example of a yet non-generalizable indicator is the Abundance Biomass Curves (ABC) method, proposed by Warwick (1986), based on the relationship between abundance and biomass of benthic macrofauna in response to contamination gradients. The ABC method has been applied even to planktonic, ichthyofaunal, mega- and meiobenthic communities (Warwick et al., 1990; Rogers et al., 2008, Viana et al., 2012, Jimenez et al., 2015; Tweedley et al., 2017). According to the method, the conditions of a community can be evaluated from the relationships between two *k*-dominance curves (Lambshead et al., 1983), one of abundance and one of biomass. In these curves, species are classified in the *x*-axis according to the order of importance (in logarithmic scale) and the percentage of dominance on the *y*-axis (cumulative scale). In this way, *k*-dominance curves are cumulative rankings of abundances and biomass plotted against a species ranking (more precisely, its logarithm). The advantage of dominance curves is that the distribution of abundance and biomass among species can be compared under similar conditions.

The curves typically respond to disturbances caused by organic enrichment associated with domestic sewage disposal. In these cases, the enriched environment is quickly dominated by r-strategist species in high abundance and low diversity, which have small size and high reproduction rates. When the environment is in equilibrium, on the other hand, *k*-strategist species of lower reproductive rate, greater size, and longevity dominate the environment in biomass and occur in greater diversity. In this situation, it is expected that the total biomass is larger, but distributed among a larger number of individuals. Thus, general trends of variation of ABC curves allow to categorize environments into three levels of pollution: unpolluted, moderately polluted, and heavily polluted (Warwick, 1986; Clarke, 1990). In a polluted environment it is expected that the total biomass is distributed only among a few species, making the abundance curve above the biomass curve. In unpolluted communities, the biomass curve is above the abundance curve. In moderately polluted communities, the curves are similar.

However, the choice of sample design may affect the interpretation of patterns of variation of the benthos structure (Schielzeth and Nakagawa, 2013; Underwood and Chapman, 2013; Brauko et al., 2015). For instance, the macrobenthic fauna that occupy habitats associated with top layers of the sediments may be more sensitive to variations in habitat heterogeneity associated with the sediment-water interface, as well as to differences in these habitat components at the patch and larger spatial scales (Zajac et al., 2013). The temporal variation makes the interpretation of spatial patterns even more complex and must be considered (Varfolomeeva and Naumov, 2012). Despite the evident influence of spatio-temporal variability on environmental quality assessments, few studies have incorporated more robust designs into ABC studies (eg. Drake and Arias, 1997; Geist et al., 2012; Tweedley et al., 2017), such as hierarchical sampling designs. These designs are considered an appropriate method to estimate the contribution of a series of progressively increasing spatial scales to the total variation among samples (Underwood and Chapman, 2013; Souza et al., 2013), yet to be tested in ABC quality assessments.

This paper evaluated the ABC method as an indicator of the level of pollution by organic enrichment in a subtropical estuary by using a hierarchical space-time sample design. For this purpose, macrofauna sampling and chemical indicators of organic contamination were conducted on intertidal flats submitted to the discharge of urban effluents and away from the source of discharge.

METHODS

Study area

The Paranaguá Estuarine Complex (PEC) is one of the largest and most preserved coastal areas along the South American coast, covering 612 km^2 (Figure 1). The surveys were conducted in the polyhaline Cotinga sub-estuary, which measures about 20 km long and is located near the estuary mouth. Nearly 34% of the surface area of the sub-estuary is covered by intertidal mangroves and marshes or remain unvegetated (Noernberg et al., 2006). The inner sector of the sub-estuary receives most of the anthropogenic input of sedimentary organic matter or sewage-derived material from Paranaguá city (Souza et al., 2013). The waste of nearly 50% of the city's population undergoes treatment, while the rest is released in natura to the environment (Companhia de Águas do Brasil: CAB, 2010). A compressed gradient of sewage contamination from the inner sector to the outer part of the sub-estuary was evidenced by *Escherichia coli* sediment concentrations (Kolm et al., 2002) and levels of fecal steroids, which are highly stable sewage organic markers (Martins et al., 2010). The sewage-derived impacts indicated by coprostanol levels and the AMBI benthic quality index may vary from high to moderate and are confined to Paranaguá city vicinity (Abreu-Mota et al., 2014; Brauko et al., 2016).

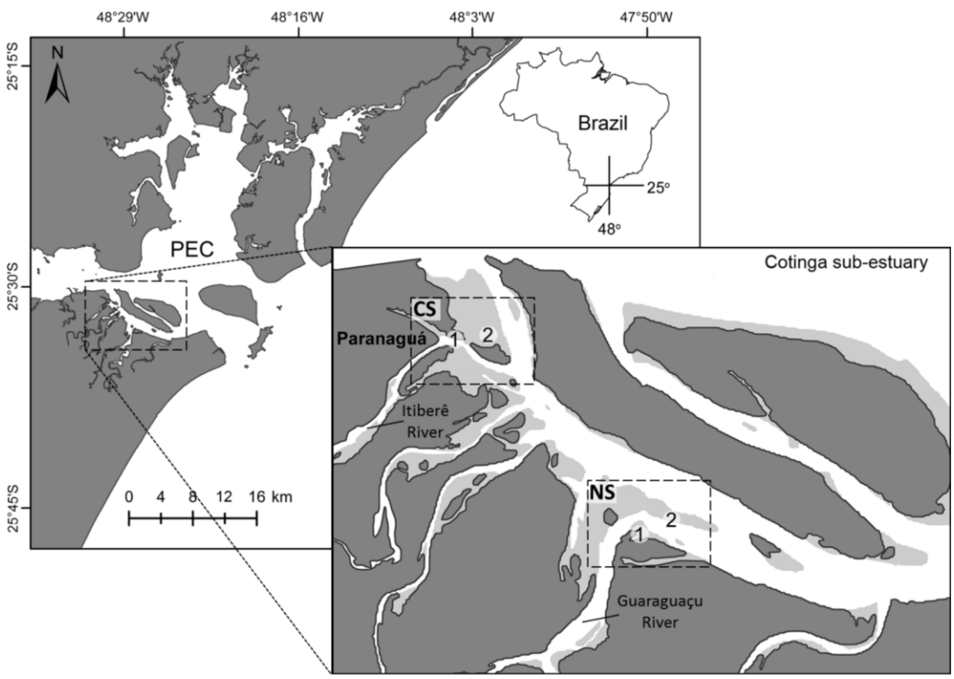

Figure 1. Study sites in Paranaguá Estuarine Complex (PEC), Brazil. Indices were applied to tidal flats 1 and 2, sampled at the Contaminated (CS) and Non-Contaminated sites (NS) of Cotinga sub-estuary in 2011.

Sample collection and processing

Sampling was performed in 2011 during austral summer (January and February) and winter (June and July), at low spring tides. The sampling design was based on a five-factor model (two temporal and three spatial factors). The temporal factors were: Season (two levels, Summer and Winter) and Fortnight (three levels, F1 to F3) within each Season. Spatial factors included: four Plots (four levels, P1 to P4 arranged parallel to the waterline, 10 m apart) with three replicates each, nested within two Tidal Flats (two levels, T1 and T2, 102 m apart), which were, in turn, nested within two Conditions (two levels, Contaminated and Non-Contaminated - 103 m apart). The temporal and spatial factors were arranged orthogonally (Figure 2). Season and Condition were fixed, and all other factors were random.

Faunal samples were collected using a PVC corer (10 cm diameter, 15 cm deep, 78.5 m^2). All samples were sieved through a 0.5 mm mesh, fixed in 6% formaldehyde, and preserved in 70% ethanol. In the laboratory, the organisms were counted and identified to the lowest possible taxonomic level, usually species. The dry biomass of these organisms was determined from the difference between the initial and final weights after oven drying at 60°C for 48 hours. Taxa considered rare, with a sample *n* less than 11 in all samples were excluded from the analysis due to not affecting major community trends.

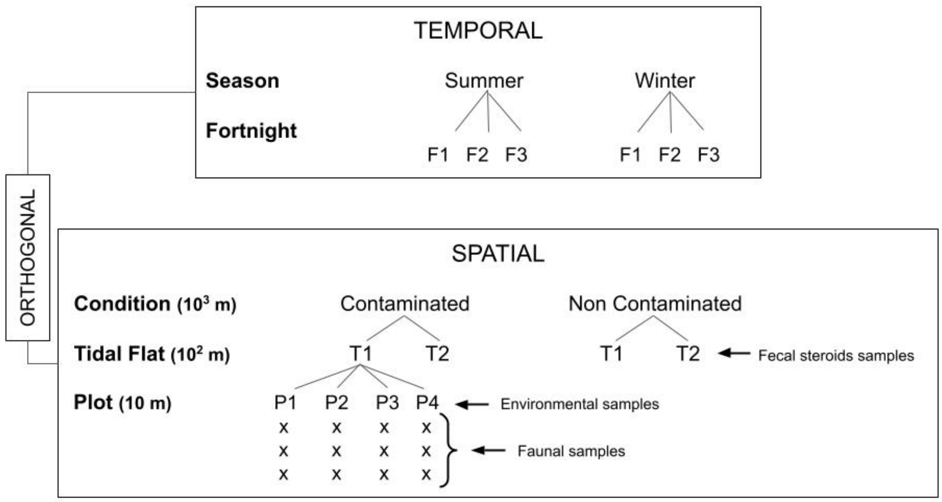

Figure 2. Sampling design diagram. Temporal and spatial scales correspond to the factors of the linear model. Two Seasons were included (Summer and Winter), with three consecutive Fortnights per Season (F1, F2, and F3). In each Fortnight, two Conditions were sampled (Contaminated and Non-Contaminated) 103 m apart, with two Tidal flats per Condition (T1 and T2) 102 m apart, four Plots per Tidal flat (P1, P2, P3 e P4) (10 m apart), and three replicates each (12 m plot).

For the redox discontinuity layer (RDL), 3 measurements were taken at each sampling point with the help of a ruler, which consisted of the threshold or transition depth of the lighter sediment (oxygen rich) for the darker sediment (poor in oxygen). Sediment from each plot was also taken to determine the following physico-chemical parameters: total nitrogen (TN), total organic carbon (TOC), calcium carbonate (CaCO3), sedimentary organic matter (OM) and mud contents, as well as mean grain size and sorting. The concentrations of TN were obtained according to Grasshoff et al. (1983), and TOC was determined with the oxidation method described by Strickland and Parsons (1972). Sediment samples were processed according to Suguio (1973), and granulometric parameters were determined on the R software (R Core Team, 2009) using the package rysgran (Gilbert et al., 2012). Calcium carbonate (CaCO3) and total organic matter contents were determined using acid digestion and furnace combustion at 550ºC for 1 h, respectively.

We additionally sampled the sediment from each tidal flat for fecal steroids analysis, using the method described by Kawakami and Montone (2002). Briefly, sediments were extracted in a Soxhlet apparatus, and the steroid 5a-cholestane was added as a surrogate. The extract was concentrated using rotoevaporation and cleaned up using column chromatography with deactivated alumina and ethanol. The extracts were then evaporated to dryness and derivatized with BSTFA (bis (trimethylsilyl) trifluoroacetamide) with 1% TMCS (trimethylchlorosilane). The organic extracts were analyzed using gas chromatography with an Agilent HP 7890A coupled with a flame ionization detector (FID) and a fused silica capillary column coated with 5% diphenyl/dimethylsiloxane (30 m, 0.32 mm ID and 0.25 m film thickness) for fecal steroids. Instrument specifications and calibration procedures are described by (Montone et al., 2010). The detection limits (DLs) were <0.01 mg g-1 for all analyzed compounds. Measured concentrations of target steroids in the IAEA-417 reference material were within 90 and 110% of the certified values provided by the International Atomic Energy Agency (IAEA).

The ABC curves were generated for the averages of each fortnight of summer and winter, under contaminated and non-contaminated conditions. W coefficients were also generated for each replicate sampled, indicating the ratio or distance between biomass and abundance. This coefficient was proposed by Clarke (1990) as a statistical treatment for the ABC method, with the following formula:

$$W = \sum_{i=1}^{s} (Bi - Ai)/[50(S -1)$$

Where Bi is the biomass of species i, Ai is the abundance of species i, and S is the number of species.

The values of W vary from -1 to +1, and positive values occur when the biomass curve is above the abundance curve, that is, in pristine conditions. The negative values of *W* occur when the abundance curve is above the biomass curve, indicating a polluted environment (Clarke and Gorley, 2006). Moderately polluted environments present a *W* very close to zero since the abundance and biomass curves overlap practically all their extensions.

To explore the correlations between ABC responses and abiotic variables, multiple regressions were performed using the *W* coefficient as the response variable. The predictive variables were: coprostanol (COP) and cholesterol (COL) content in the sediment, coprostanol/cholesterol (COP/COL) and coprostanol/(coprostanol+cholestanol) (COP/COP+COLA), calcium carbonate ($CaCO_3$), total nitrogen (NT) and total organic carbon (TOC) of the sediment, mud content, mean grain size, sorting and total organic matter (OM). The Akaike Information Criterion (AIC) (Akaike, 1974) was then used to select the best model of correlations. AIC is a methodology for selecting models, where more than one model fits the parameters and shows which models are significant. Before the analysis, the abiotic variables were either logit transformed for proportional data (Warton and Hui, 2011) or square-root transformed for continuous data to fulfill linear model assumptions.

The spatio-temporal variability of the W coefficient was estimated at multiple scales using a nested ANOVA following the linear mixed model:

$$X= \mu + Si + F(S)_{j(i)} + Ck + T(C)_{l(k)} + P(T(C))_{m(l(k))} + SCik + ST(C)_{il(k)} + SP(T(C))_{im(l(k))} + F(S)Cj(i)k + F(S)T(C)j(i)l(k) + F(S)P(T(C))_{j(i)m(l(k))} + en_{(ijklm)}$$

Where S = Seasons; F(S) = Fortnights, nested in Seasons; C = Conditions; T(C) = Tidal flats, nested in Conditions; P(T(C)) = Plots, nested in Tidal flats.

The PRIMER 6 software (Clarke and Gorley, 2006) was used to obtain ABC curves and W coefficients. Statistical analyses were performed with the PERMANOVA+ add-on (Anderson et al., 2008) and the vegan package (Oksanen et al., 2008) in the R software (R Core Team, 2009).

RESULTS

The fauna varied considerably between seasons and conditions of contamination (Table 1). *Tubificinae* sp. 1, also known as oligochaete worms, was very abundant during the winter in both conditions and almost absent in the summer. The abundance of *Capitella* sp. was reduced in the uncontaminated condition, as well as *Tubificinae* sp. 1, *Laeonereis culveri*, *Heteromastus* sp., and *Streblospio benedicti*. The opposite occurred with *Heleobia australis*, generically named snail mud, and *Sigambra* sp., which were more abundant under non-contaminated condition. As for total biomass, the species that contributed most were the larger bivalves, represented by *Anomalocardia flexuosa*—commonly known as maçunim, vôngole, or berbigão—, *Macoma constricta* (tarioba branca), and *Tellina versicolor*. Low biomass values were found for the numerically dominant species *Tubificinae* sp. 1, *Laeonereis culveri*, and *Heleobia australis*. Curve responses varied between contaminated and non-contaminated areas. All the curves classified the contaminated shoals as very polluted, with the line of abundance almost always above the biomass line (Figure 3). In Winter Fortnight 2, the abundance and biomass curves overlapped, but a high degree of contamination was evidenced by the *W* coefficient (Figures 3 and 4). In the summer, there was a gradual improvement of the environmental quality, evidenced by the decrease in the distance between the curves, which was not repeated in the winter fortnights.

However, the responses of the curves in the non-contaminated area were highly heterogeneous, varying from unpolluted to heavily polluted throughout the study periods. The classification "unpolluted" was only attributed by the curves in the first fortnight of winter. The shoals were classified as very polluted in two and three fortnights of summer and winter, respectively. The other shallows of the non-contaminated area were classified as moderately polluted.

Table 1. Abundance and biomass (10-3 g) of the ten most abundant species during winter and summer in Contaminated and Non-contaminated conditions.

Contaminated				
	Winter		Summer	
Species	Abundance	Biomass (10^{-3} g)	Abundance	Biomass (10^{-3} g)
Tubificinae sp. 1	7408	173	6555	140
Laeonereis culveri	3872	332	1222	452
Heleobia australis	898	15	155	42
Tubificinae sp. 2	3603	121	1	2
Sigambra sp.	512	133.7	189	99
Capitella sp.	372	91	701	85
Heteromastus sp.	387	156	129	61
Streblospio benedicti	467	113	107	29
Anomalocardia flexuosa	262	1097	50	1023
Glycinde multidens	190	403	80	63
Non-contaminated				
	Winter		Summer	
Species	Abundance	Biomass (10^{-3} g)	Abundance	Biomass (10^{-3} g)
Heleobia australis	1406	142.7	1677	158
Sigambra sp.	775	89.2	359	72
Heleobia australis	619	49.8	469	46
Tubificinae sp. 1	146	82.7	106	98
Heteromastus sp.	130	57	34	22
Anomalocardia flexuosa	120	1352	92	2573
Tubificinae sp. 2	10	24	0	0
Laeonereis culveri	97	59	89	55
Streblospio benedicti	52	23.1	11	11
Capitella sp.	9	13	5	6

Multiple regressions generated several correlation models between the *W* coefficient and the abiotic variables. The AIC analysis indicated that the best model explained approximately 67% of *W* (global R = 0.6037, adjusted R2 = 0.6037, df = 19, P<0.0001), which includes coprostanol/cholestanol ratio (indicating the presence of organic enrichment by sewage), the presence of cholesterol and coprostanol, grain size, and total nitrogen concentrations. The other models had a set of variables less correlated to *W*, and their respective AIC values can be observed in Table 2.

PERANOVA showed significant differences in the spatiotemporal variability of the ABC curves (represented by the *W* coefficients) in the Lowlands scale (102 m) and the interactions between the Forties and Points (101 m) (Table 3). The variation components (CV%) confirmed the high importance of the scale of tidal flats and the interaction between Points and Fortresses for the variability of the W coefficients.

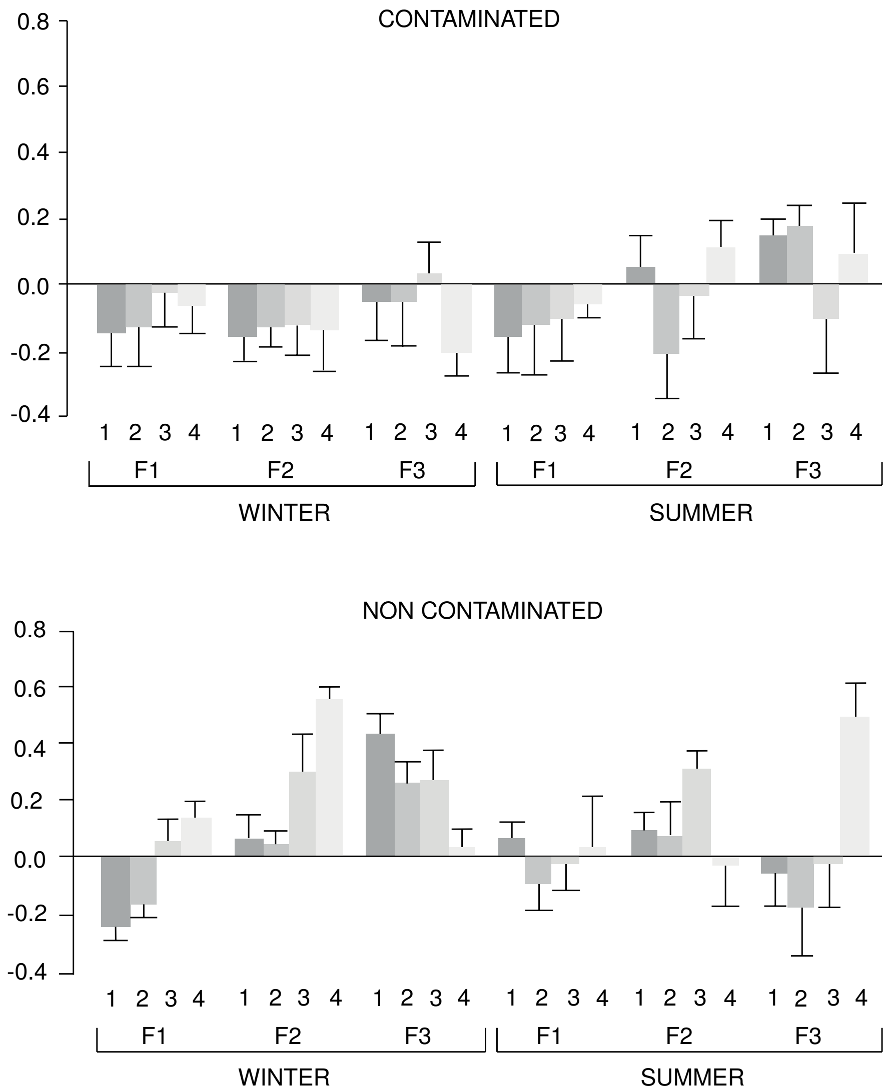

Figure 3. Mean of the *W* coefficients for each point in summer and winter for Contaminated and Non-contaminated conditions. B1 and B2 correspond to the shoals and Q1, Q2, and Q3 in the sampled fortnights.

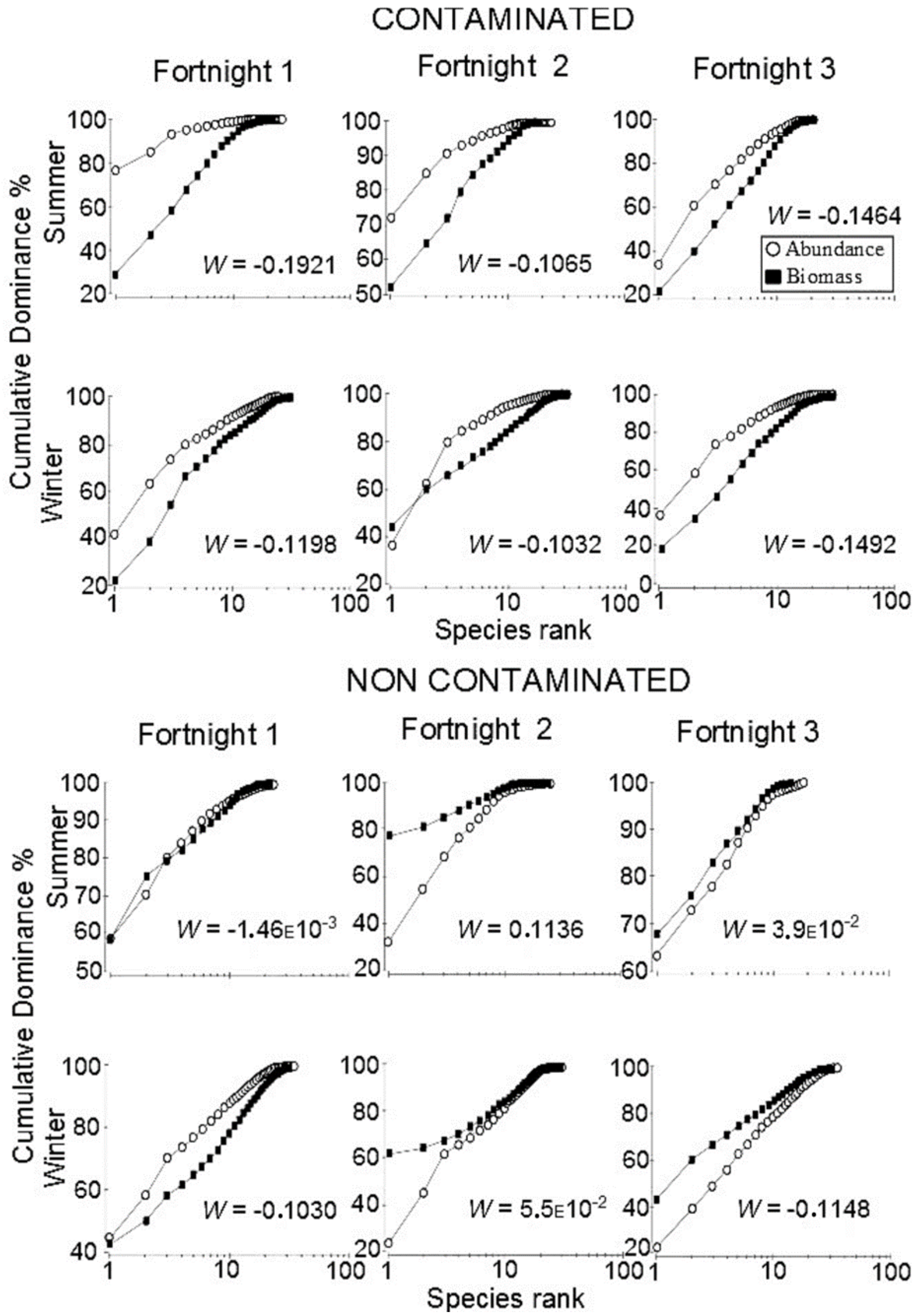

Figure 4. ABC curves in every winter and summer fortnight in Contaminated and Non-Contaminated shallows. Species classified in terms of biomass and abundance in the *x*-axis (logarithmic scale), and percentage of dominance (cumulative scale) in the *y*-axis.

Table 2. Model selection table ranked by Akaike information criteria (AIC) for effects of various abiotic variables on *W*-statistic showing the final predictive model in bold (with estimates, standard error, F-, and *p*-value), variables dropped against the full model and selected variables. ΔAIC= difference in AIC value between given model or variable and the best overall model.

Model	Intercept	TOC	CaCO3	COL	COP	COP/ (COP+COLA)	COP/COL	Grain
285	0.15290			0.02911	-0.03144	-0.2423		
349	**3.50400**			**0.02690**	**-0.03065**	**-0.2752**		**-1.878**
29	0.05665			0.02479	-0.02926	-0.2013		
317	0.28920			0.03071	-0.03148	-0.4309	0.02143	
1309	0.04633			0.02757	-0.02961	-0.2591		
1373	3.67300			0.02482	-0.02835	-0.2986		-2.045
287	0.06248		0.05218	0.02902	-0.02959	-0.2357		
286	0.20700	-0.04715		0.02965	-0.03237	-0.2343		
381	3.63200			0.02850	-0.03069	-0.4628	0.02133	-1.874
797	-0.09710			0.02852	-0.02991	-0.2413		
413	0.17230			0.02947	-0.03129	-0.2320		

Model	Intercept	Mud	TN	OM	Sort	d.f	AICc	ΔAIC
285	0.15290		0.05821			6	-31.4	0.00
349	**3.50400**		**0.07123**			**7**	**-30.7**	**0.75**
29	0.05665					5	-28.9	2.52
317	0.28920		0.06366			7	-28.9	2.56
1309	0.04633		0.05871		0.1392	7	-28.5	2.91
1373	3.67300		0.07300		0.1697	8	-28.0	3.38
287	0.06248		0.05338			7	-28.0	3.46
286	0.20700		0.06735			7	-27.8	3.61
381	3.63200		0.07663			8	-27.8	3.63
797	-0.09710		0.05612	-0.0597		7	-27.7	3.73
413	0.17230	-0.004021	0.05598			7	-27.7	3.75

Final model statistics

# 349	d.f.	Res. S.E.	R^2	Adj. R^2	Fvalue	Pvalue
	19	0.09929	0.6727	0.6037	9.761	0.0001825

Table 3. PERANOVA results for the macrofauna associations in the different scales investigated. p-values calculated by means of the Monte Carlo permutation test. The scales include: period (S), fortnight (F), condition (C), tidal flats (T), and point (P).

	df	MS	F	*p*	CV(%)
S	1	0.00	0.29	0.91	0.00
C	1	1.77	1.65	0.28	4.69
F(S)	4	0.27	2.88	0.10	3.42
T(C)	2	0.89	5.08	**<0.01**	10.42
SxC	1	0.50	2.03	0.21	3.82
P(T(C))	12	0.11	0.83	0.62	0.00
SxT(C)	2	0.06	1.00	0.44	0.01
F(S)xC	4	0.23	2.46	0.14	5.31
SxP(T(C))	12	0.09	0.69	0.75	0.00
F(S)x(T(C))	8	0.10	0.75	0.64	0.00
F(S) x P(T(C))	48	0.13	2.29	**<0.01**	21.71
RES	192	0.06			50.62
Total	287				

DISCUSSION

The ABC method responses were clearly congruent in the contaminated area but ambiguous in the non-contaminated area. Most points in the uncontaminated area were classified as moderately polluted, although the chemical indicators of organic contamination indicated concentrations below the contamination limit described in the literature (Grimalt et al., 1990; Jeng and Han, 1994; Mudge and Seguel, 1999). In both contaminated and non-contaminated areas, small species were dominant, which may have confounded the results of the curves. The susceptibility of the method to the presence of dominant species possibly caused the uncontaminated areas to be classified as moderately polluted. Therefore, the presence of individuals of small size and high abundance species such as *Heleobia australis* and *Sigambra* sp. in the tidal flats of the uncontaminated areas may have led to the classification of the environment as moderately polluted.

The absence of dominant species in biomass can result in changes in the positioning of the curves, causing the biomass curve to be below the abundance curve in regions considered as non-polluted (Dauer et al., 1993). In addition, the low abundance of large bivalves such as *Anomalocardia flexuosa* and *Macoma constricta* may have influenced the classification of non-contaminated areas, as their absence displaces the biomass curve below the abundance curve, a characteristic situation of polluted environments. The presence or absence of organisms that are more sensitive to impacts, such as many species of echinoderms and mollusks, may also tend to produce the overall result of the curves as well as the size of the sampler used (Dauer et al., 1993; Salas et al., 2004).

The benthic macrofauna is largely influenced by changes in salinity, temperature, and dissolved oxygen (Lv et al., 2016a; Zhang et al., 2023). The increase of precipitation occurred in the summer may have directly influenced these factors and resulted in the gradual improvement of the environmental quality evidenced by the decrease in the distance between the curves in this period.

The use of the ABC curves in the sublittoral has contributed consistently and congruently to the classification of environmental quality (Frontalini et al., 2011; Semprucci et al., 2013; Lv et al., 2016b; Zhang et al., 2016; Liu et al. 2018). Cai et al. (2013) have shown that the *W* coefficient responds satisfactorily to the organic contamination gradients in sub-littoral sheltered environments from unconsolidated funds. On the other hand, ABC curves may be less efficient in tidal flats, where biomass and diversity tend to be low, even if the fauna occurs at high densities (Beukema, 1988).

The values of *W* were correlated to cholesterol and coprostanol levels in the environment, as well as the ratio coprostanol/cholesterol (COP/COL) (an indicator of fecal steroids) and total nitrogen (TN), suggesting that the patterns observed in the curves are associated with organic enrichment from sewage disposal. However, there is a high natural background input represented by organic matter from adjacent mangroves in the region (Abreu-Mota et al., 2014). Organic enrichment in the Cotinga sub-estuary acts as an effective force in the structuring of macrobenthos; however, organic matter of natural origin can show the same effect of discharging sewage into the uncontaminated area on the structure of benthic fauna (Souza et al., 2013). Comparative studies between the ABC method and other macrobenthic fauna-based indexes (Shannon-Weaver, AMBI, M-AMBI) showed that *W* coefficient associated with ABC curves was less sensitive to levels of organic contamination. However, the ABC method has been identified as one of the most sensitive indices in the detection of chemical contamination by several vectors, such as Pb, Cd, Cu, Ni, Hg, Zn, DDD, and TBT (Wetzel et al., 2012; Taupp and Wetzel, 2013).

Adequate sampling replication is a prerequisite for the use of the ABC method since dominant species in biomass are represented by few individuals (Warwick, 1986; Warwick and Pearson, 1987). The application of a hierarchical sampling design with due replication should minimize the effects generated by the sampling of dominant species in biomass. Nevertheless, the performance of the curves was not totally satisfactory in the non-contaminated area, highly influenced by organic matter of natural origin and high abundance of small species.

Contrary to what was expected, the main range of variation of the ABC curves along space and time was not the Contamination (103 m), but that of Tidal Flats (102 m) and that of the interaction between Points (101 m) and Fortnights. The high variability of *W* between the points over the fortnights indicates a highly dynamic environment. The influence of estuarine gradients involving hydrological and sedimentary factors clearly conditions the responses of macrobenthic communities (Mannino and Montagna, 1997). Such natural variability overlapped the effects of contamination on the structure of macrobenthic communities (Gaston et al., 1998), which was reflected in the curves. The ABC method ignored the taxonomic position of species, so polluted or disturbed conditions indicated by this method should be viewed with caution if the species considered responsible were not polychaetes (Warwick and Clarke, 1994).

The *W* values confirm that tidal flats in the contaminated area are more polluted than tidal flats in non-contaminated areas. On the other hand, there was a high variability in the scale of tidal flats. This is because the shoals in the contaminated area are in different stages of contamination (Souza et al., 2013), which was evidenced by the significant value of *p* in the scales of shoals. The fact that the scale of classification of pollution in the ABC method only presents three levels, (unpolluted, moderately polluted, and very polluted) hinders the decision making.

Although there is a numerical value (*W*), the environmental classification limits of the method are not clearly defined. When a large number of ABC curves are plotted, problems can occur, and some authors have proposed an index that measures the area between the two curves to solve them (Beukema 1988; Clarke 1990; McManus and Pauly 1990; Meire and Dereu, 1990).

Our results indicate that the application of the ABC method for the detection of impacts by organic pollution was not totally satisfactory in the case of a subtropical estuary with sources of natural organic matter associated with coastal vegetation. Despite the consistent classification of contaminated areas, some spots in the uncontaminated area were inconsistently classified as moderately or heavily polluted. The high background organic matter input of natural origin promoted the incorrect diagnosis of disturbance in the contamination-free shoals, which resulted in the significant variability of the smaller spatial scales (from 101 m to 102 m) rather than the larger scale (103 m), or the scale contamination. The dominance of small species also influenced these ambiguous responses. The curves are excessively dependent on the first species classified, which can increase the dominance curves over the biomass curves.

The ABC method is not necessarily more sensitive than diversity indices in detecting perturbations and is less sensitive than multivariate methods (such as multi-dimensional scaling analysis, MDS) in differentiating the structure of macrobenthic communities (Warwick and Clarke, 1991). However, it holds the advantage of providing an absolute response rather than a comparative measure of contamination induced perturbations, that is, it provides an index that gives us a measure of how the environment is without having to compare indices of biomass and abundance separately (Warwick, 1993).

CONCLUSION

The ABC method responded to organic contamination in the study region. However, the curves show a rough scale of environmental classification, besides responding to inputs of natural organic matter not only of human origin. Thus, ABC curves should be applied with caution in estuarine environments subject to organic matter discharges of natural origin, which represent natural background variability of the "noise" type and may not be the most suitable index for decision makers in these environments.

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AUTHOR CONTRIBUTIONS

S.K.B: Conceptualization; Methodology; Software; Investigation; Formal Analysis; Writing – Original draft; Writing – Review & editing.

K.M.B: Supervision; Conceptualization; Methodology; Formal Analysis; Investigation; Writing – Review & editing.

P.C.L: Supervision; Resources; Project Administration; Funding Acquisition; Writing – Review & editing.

REFERENCES

Akaike, H. 1974. A new look at the statistical model identification. *IEEE Transactions on Automatic Control*, 19(6), 716–723.

Abreu-Mota, M. A., Moura Barboza, C. A., Bícego, M. C. & Martins, C. C. 2014. Sedimentary biomarkers along a contamination gradient in a human-impacted sub-estuary in Southern Brazil: A multi-parameter approach based on spatial and seasonal variability. *Chemosphere,* 103, 156–163.

Anderson, M., Gorley, R. N. & Clarke, R. K. 2008. *Permanova+ for Primer: Guide to Software and Statistical Methods*. Plymouth, PRIMER-E.

Beukema, J. J. 1988. An evaluation of the ABC-method (abundance/biomass comparison) as applied to macrozoobenthic communities living on tidal flats in the Dutch Wadden Sea. *Marine Biology*, 99(3), 425–433.

Brauko, K. M., Muniz, P., Martins, C. de C., & da Cunha Lana, P. 2016. Assessing the suitability of five benthic indices for environmental health assessment in a large subtropical South American estuary. Ecological *Indicators*, 64, 258–265.

CAB – Águas de Paranaguá. *Esgoto*. Available from: https://igua.com.br/paranagua. Access date: June 5, 2014.

Cai, W., Meng, W., Liu, L. & Lin, K. 2013. Evaluation of the ecological status with benthic indices in the coastal system: the case of Bohai Bay (China). *Frontiers of Environmental Science & Engineering*, 8, 737–746.

Carter, J. L., Resh, V. H. & Hannaford, M. J. 2017. Macroinvertebrates as Biotic Indicators of Environmental Quality. In: Lamberti, G. A. & Hauer, F. R. (Ed.). *Methods in Stream Ecology* (3º ed, vol 2: Ecosystem Function, pp. 293–318).

Clarke, K. R. 1990. Comparisons of dominance curves. *Journal of Experimental Marine Biology and Ecology*, 138(1), 143–157.

Clarke, K. R. & Gorley, R. N. 2006. *PRIMER v6: user manual/tutorial (Plymouth routines in multivariate ecological research)*. Plymouth, Primer-E Ltd.

Dauer, D. M., Luckenbach, M. W. & Rodi Jr, A. J. 1993. Abundance biomass comparison (ABC method): effects of an estuarine gradient, anoxic/hypoxic events and contaminated sediments. *Marine Biology*, 116(3), 507–518.

Dauvin, J. C., Alizier, S., Rolet, C., Bakalem, A., Bellan, G., Gesteira, J. L., Grimes, S., De-La-Ossa-Carretero, J.A. & Del-Pilar-Ruso, Y. 2012. Response of different benthic indices to diverse human pressures. *Ecological Indicators*, 12(1), 143–153.

Drake, P. & Arias, A. M. 1997. The effect of aquaculture practices on the benthic macroinvertebrate community of a lagoon system in the Bay of Cadiz (southwestern Spain). *Estuaries*, 20(4), 677–688.

Frontalini, F., Semprucci, F., Coccioni, R., Balsamo, M., Bittoni, P. & Covazzi-Harriague, A. 2011. On the quantitative distribution and community structure of the meio and macrofaunal communities in the coastal area of the Central Adriatic Sea (Italy). *Environmental Monitoring and Assessment*,180(1-4), 325–344.

Gaston, G. R., Rakocinski, C. F., Brown, S. S. & Cleveland, C. M. 1998. Trophic function in estuaries: response of macrobenthos to natural and contaminant gradients. *Marine and Freshwater Research*, 49(8), 833–846.

Geist, S. J., Nordhaus, I. & Hinrichs, S. 2012. Occurrence of species-rich crab fauna in a human-impacted mangrove forest questions the application of community analysis as an environmental assessment tool. *Estuarine, Coastal and Shelf Science*, 96, 69–80.

Gilbert, E. R., Camargo, M. G. & Sandrini-Neto, L. 2012. *rysgran: Grain size analysis, textural classifications and distribution of unconsolidated sediments*. R package version 2.

Grasshoff, K., Ehrhardt, M. & Kremling, K. 1983. *Methods of seawater analysis* (2a ed). Weinheim, Verlag Chemie.

Grimalt, J. O., Fernandez, P., Bayona, J. M. & Albaiges, J. 1990. Assessment of fecal sterols and ketones as indicators of urban sewage inputs to coastal waters. *Environmental Science & Technology*, 24(3), 357–363.

Huang, Y., Li, Y., Chen, Q., Huang, Y., Tian, J., Cai, M., Huang, Y. Yang, J., Yang, Y. Du, X., Liu, Z. & Zhao, Y. 2021. Effects of reclamation methods and habitats on macrobenthic communities and ecological health in estuarine coastal wetlands. *Marine Pollution Bulletin*, 168, 112420.

Huang, Y., Huang, Y., Du, X., Li, Y., Tian, J., Chen, Q., Huang, Y., Lv, W. Yang, Y., Liu, Z. & Zhao, Y. 2022. Assessment of macrobenthic community function and ecological quality after reclamation in the Changjiang (Yangtze) River Estuary wetland. *Acta Oceanologica Sinica*, 41(11), 96–107.

Jeng, W. L. & Han, B. C. 1994. Sedimentary coprostanol in Kaohsiung harbour and the Tan-Shui estuary, Taiwan. *Marine Pollution Bulletin*, 28(8), 494–499.

Jimenez, H., Dumas, P., Bigot, L. & Ferraris, J. 2015. Harvesting effects on tropical invertebrate assemblages in New Caledonia. *Fisheries Research*, 167, 75–81.

Kawakami, S. K. & Montone, R. C. 2002. An efficient ethanol-based analytical protocol to quantify fecal steroids in marine sediments. *Journal of the Brazilian Chemical Society*, 13, 226–232.

Kolm, H. E., Schoenenberger, M. F., Piemonte, M. D. R., Souza, P. S. D. A., Mucciatto, M. B. & Mazzuco, R. 2002. Spatial variation of bacteria in surface waters of Paranaguá and Antonina Bays, Paraná, Brazil. *Brazilian Archives of Biology and Technology*, 45(1), 27–34.

Lambshead, P. J. D., Platt, H. M. & Shaw, K. M. 1983. The detection of differences among assemblages of marine benthic species based on an assessment of dominance and diversity. *Journal of Natural History*, 17(6), 859–874.

Liu, Z., Chen, M., Li, Y., Huang, Y., Fan, B., Lv, W., Yu, P., Wu, F. & Zhao, Y. 2018. Different effects of reclamation methods on macrobenthos community structure in the Yangtze Estuary, China. *Marine Pollution Bulletin*, 127, 429–436.

Lv, W., Huang, Y., Liu, Z., Yang, Y., Fan, B. & Zhao, Y. 2016a. Application of macrobenthic diversity to estimate ecological health of artificial oyster reef in Yangtze Estuary, China. *Marine Pollution Bulletin*, 103(1-2), 137–143.

Lv, W., Liu, Z., Yang, Y., Huang, Y., Fan, B., Jiang, Q. & Zhao, Y. 2016b. Loss and self-restoration of macrobenthic diversity in reclamation habitats of estuarine islands in Yangtze Estuary, China. *Marine Pollution Bulletin*, 103(1-2), 128–136.

Mannino, A. & Montagna, P. A. 1997. Small-scale spatial variation of macrobenthic community structure. *Estuaries*, 20(1), 159–173.

Martins, C. C., Braun, J. A., Seyffert, B. H., Machado, E. C. & Fillmann, G. 2010. Anthropogenic organic matter inputs indicated by sedimentary fecal steroids in a large South American tropical estuary (Paranaguá estuarine system, Brazil). *Marine Pollution Bulletin*, 60(11), 2137–2143.

McManus, J. W. & Pauly, D. 1990. Measuring ecological stress: variations on a theme by RM Warwick. *Marine Biology*, 106(2), 305–308.

Meire, P. M. & Dereu, J. 1990. Use of the abundance/biomass comparison method for detecting environmental stress: some considerations based on intertidal macrozoobenthos and bird communities. *Journal of Applied Ecology*, 27(1), 210–223.

Montone, R. C., Martins, C. C., Bícego, M. C., Taniguchi, S., Da Silva, D. A. M., Campos, L. S. & Weber, R. R. 2010. Distribution of sewage input in marine sediments around a maritime Antarctic research station indicated by molecular geochemical indicators. *Science of the Total Environment*, 408(20), 4665–4671.

Mudge, S. M. & Seguel, C. G. 1999. Organic contamination of San Vicente Bay, Chile. *Marine Pollution Bulletin*, 38(11), 1011–1021.

Naumov, A. D. 2006. *Clams of the White Sea: ecological and faunistic analysis*. St. Petersburg, Russian Academy of Sciences.

Noernberg, M. A., Lautert, L. F. C., Araújo, A. D., Marone, E., Angelotti, R., Netto Jr, J. P. B. & Krug, L. A. 2006. Remote sensing and GIS integration for modelling the Paranaguá estuarine complex-Brazil. *Journal of Coastal Research*, 39, 1627–1631.

Oksanen, J., Kindt, R., Legendre, P., O'hara, B., Simpsons, G. L., Solymos, P., Stevens, M. H. H. & Wagner, H. 2008. *The vegan package*, Available from: http://vegan.r-forge.r-project.org/. Access date: 2024 Mar 15.

Pinto, R., Patrício, J., Baeta, A., Fath, B. D., Neto, J. M. & Marques, J. C. 2009. Review and evaluation of estuarine biotic indices to assess benthic condition. *Ecological Indicators*, 9(1), 1-25.

R Core Team. 2009. *R: A language and environment for statistical computing*. Vienna, The R Foundation.

Available from: http://www.R-project.org. Access date: 2013 Feb 15.

Rogers, S. I., Somerfield, P. J., Schratzberger, M., Warwick, R., Maxwell, T. A. & Ellis, J. R. 2008. Sampling strategies to evaluate the status of offshore soft sediment assemblages. *Marine Pollution Bulletin*, 56(5), 880–894.

Salas, F., Neto, J., Borja, A. & Marques, J. 2004. Evaluation of the applicability of a marine biotic index to characterize the status of estuarine ecosystems: the case of Mondego estuary (Portugal). *Ecological Indicators*, 4(3), 215–225. DOI: https://doi.org/10.1016/j.ecolind.2004.04.003

Schielzeth, H. & Nakagawa, S. 2013. Nested by design: model fitting and interpretation in a mixed model era. Methods in Ecology and Evolution, 4(1), 14–24.

Semprucci, F., Frontalini, F., Covazzi-Harriague, A., Coccioni, R. & Balsamo, M. 2013. Meio-and macrofauna in the marine area of the Monte St. Bartolo Natural Park (Central Adriatic Sea, Italy). *Scientia Marina*, 77(1), 189–199.

Souza, F. M., Brauko, K. M., Lana, P. C., Muniz, P. & Camargo, M. G. 2013. The effect of urban sewage on benthic macrofauna: A multiple spatial scale approach. Marine Pollution Bulletin, 67(1), 234–240.

Strickland, J. D. H. & Parsons, T. R. 1972. *A practical handbook of seawater analysis* (2a ed, vol. 167). Ottawa, Fisheries Research Board of Canada.

Suguio, K. 1973. *Introdução à Sedimentologia*. São Paulo, Blücher, Edusp.

Taupp, T. & Wetzel, M. A. 2013. Relocation of dredged material in estuaries under the aspect of the Water Framework Directive—A comparison of benthic quality indicators at dumping areas in the Elbe estuary. *Ecological Indicators*, 34, 323–331.

Tweedley, J. R., Warwick, R. M., Hallett, C. S. & Potter, I. C. 2017. Fish-based indicators of estuarine condition that do not require reference data. *Estuarine, Coastal and Shelf Science*, 191, 209–220.

Underwood, A. J., & Chapman, M. G. (2013). Design and Analysis in Benthic Surveys in Environmental Sampling. *Methods for the Study of Marine Benthos*, 1–45.

Varfolomeeva, M., & Naumov, A. 2012. Long-term temporal and spatial variation of macrobenthos in the intertidal soft-bottom flats of two small bights (Chupa Inlet, Kandalaksha Bay, White Sea). *Hydrobiologia*, 706(1), 175–189.

Viana, A. P., Frédou, F. L. & Frédou, T. 2012. Measuring the ecological integrity of an industrial district in the Amazon estuary, Brazil. *Marine Pollution Bulletin*, 64(3), 489–499.

Warton, D. I. & Hui, F. K. 2011. The arcsine is asinine: the analysis of proportions in ecology. *Ecology*, 92(1), 3–10.

Warwick, R. M. 1986. A new method for detecting pollution effects on marine macrobenthic communities. *Marine Biology*, 92(4), 557–562.

Warwick, R. M. 1993. Environmental impact studies on marine communities: pragmatical considerations. *Australian Journal of Ecology*, 18(1), 63–80.

Warwick, R. M. & Clarke, K. R. 1991. A comparison of some methods for analysing changes in benthic community structure. *Journal of the Marine Biological Association of the United Kingdom*, 71(1), 225–244.

Warwick, R. M. & Clarke, K. R. 1994. Relearning the ABC: taxonomic changes and abundance/biomass relationships in disturbed benthic communities. *Marine Biology*, 118(4), 739–744.

Warwick, R. M., Platt, H. M., Clarke, K. R., Agard, J. & Gobin, J. 1990. Analysis of macrobenthic and meiobenthic community structure in relation to pollution and disturbance in Hamilton Harbour, Bermuda. *Journal of Experimental Marine Biology and Ecology*, 138(1), 119–142.

Warwick, R. M. & Pearson, T. H. 1987. Detection of pollution effects on marine macrobenthos: further evaluation of the species abundance/biomass method. *Marine Biology*, 95(2), 193–200. DOI: https://doi.org/10.1007/BF00409005

Warwick, R. M., & Clarke, K. R. 1996. Relationships between body-size, species abundance and diversity in marine benthic assemblages: facts or artefacts? *Journal of Experimental Marine Biology and Ecology*, 202(1), 63–71.

Wentworth, C. K. 1922. A scale of grade and class terms for clastic sediments. *The Journal of Geology*, 30(5), 377–392.

Wetzel, M. A., Von Der Ohe, P. C., Manz, W., Koop, J. H. & Wahrendorf, D. S. 2012. The ecological quality status of the Elbe estuary. A comparative approach on different benthic biotic indices applied to a highly modified estuary. *Ecological Indicators*, 19, 118–129.

Wildsmith, M. D., Rose, T. H., Potter, I. C., Warwick, R. M. & Clarke, K. R. 2011. Benthic macroinvertebrates as indicators of environmental deterioration in a large microtidal estuary. *Marine Pollution Bulletin*, 62(3), 525–538.

Zajac, R. N., Vozarik, J. M. & Gibbons, B. R. 2013. Spatial and temporal patterns in macrofaunal diversity components relative to sea floor landscape structure. *PloS one*, 8(6).

Zhang, J., Zhang, S., Zhang, S., Du, Y. & Xu, F. 2016. What has happened to the benthic mollusks of the Yellow Sea in the near half century? Comparison on molluscan biodiversity between 1959 and 2007. *Continental Shelf Research*, 113, 21–29.

Zhang, Y., Wan, H., Zhao, Y., Ding, J., Zhu, Z., Zhang, H. & Liu, Z. 2023. Macrobenthic functional group analysis of ecological health of the intertidal artificial oyster reefs in the Yangtze Estuary, China. *Frontiers in Marine Science*, 9, 1059353.