Universidade Federal do Paraná

Danilo Cândido Vieira

THE IMPORTANCE OF VERTICAL AND HORIZONTAL DIMENSIONS OF THE SEDIMENT MATRIX IN STRUCTURING NEMATODES ACROSS SPATIAL SCALES

Dissertação apresentada como requisito parcial à obtenção do grau de Mestre em Sistemas Costeiros e Oceânicos. Curso de Pós-Graduação em Sistemas Costeiros e Oceânicos, Centro de Estudos do Mar, Setor de Ciências da Terra, Universidade Federal do Paraná.

Orientador:. Dr. Gustavo Fonseca

Pontal do Paraná

2013

"The importance of vertical and horizontal dimensions of the sediment matrix in structuring nematodes across spatial scales"

POR

Danilo Candido Vieira

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TERMO DE APROVAÇÃO

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Resumo: Pesquisas intensivas têm sido realizadas para desvendar padrões espaciais de comunidades infaunais bentônicas. Embora é reconhecido que organismos bentônicos são espacialmente estruturados ao longo das dimensões horizontal e vertical do sedimento, pouco se sabe como essas duas dimensões interagem entre si. Este estudo investigou a interdependência entre as dimensões horizontal e vertical na estruturação de assembleias de nematóides marinhos. Para isto, testamos se a similaridade na composição de espécies de nematóides ao longo da dimensão horizontal é dependente da camada vertical do sedimento. Para testar esta hipótese, secções verticais de 3 cm de sedimento (15 cm de profundidade) foram coletadas de forma independente em dois bancos não vegetados em três estuários. Os dados indicaram que as assembleias que vivem nas camadas superiores são mais abundantes, ricas em espécies e menos variável, em termos de presença de espécies/ausência e abundância relativa, do que as assembleias que vivem nas camadas mais profundas. Os resultados também mostraram que mais importante que a profundidade do sedimento, o potencial redox foi a variável mais importante explicando 12% da variabilidade da fauna na dimensão horizontal. A fauna de camadas oxigenadas foi mais homogênea do que a das camadas mais reduzidas. Em contraste com estudos anteriores que sugeriam uma fauna específica de camadas anóxicas, observou-se que as espécies identificadas nas camadas mais profundas eram mais casuais, i.e. caracterizadas principalmente por espécies errantes. O mecanismo proposto é que nas camadas superficiais oxigenadas, as espécies têm grandes chances de serem deslocadas e colonizarem novos locais por transporte passivo, enquanto nas camadas mais profundas e anóxicas, elas são restritas à dispersão ativa a partir de sedimentos vizinhos. Tal restrição no potencial de dispersão juntamente com as condições ambientais adversas levam a uma maior aleatoriedade na presença de espécies, resultando em uma alta variabilidade entre assembleias ao longo da dimensão horizontal.

Abstract: Intensive surveys have been conducted to unravel spatial patterns of benthic infauna communities. Although it has been recognized that benthic organisms are spatially structured along the horizontal and vertical dimensions of the sediment, little is known on how these two dimensions interact with each other. In this study it has been investigated the interdependence between the vertical and horizontal dimensions in structuring marine nematodes assemblages. For this, we tested whether the similarity in nematode species composition along the horizontal dimension was dependent on the vertical layer of the sediment. To test this hypothesis, three centimeters interval sediment samples (15 cm depth) were taken independently from two bedforms in three estuaries. Results indicated that assemblages living in the top layers are more abundant, species rich and less variable, in terms of species presence/absence and relative abundances, than assemblages living in the deeper layers. The results further showed that more important than sediment depth, redox potential was the most important variable explaining 12% of the variability of the species composition. The fauna inhabiting the more oxygenated layers were more homogeneous across the horizontal scales than those from the reduced layers. In contrast to previous studies, which suggested that reduced layers are characterized by a specific set of tolerant species, the present study showed that species assemblages in the deeper layers more casual, i.e. characterized mainly by vagrant species. The proposed mechanism is that at the superficial oxygenated layers, species have higher chances of being resuspended and displaced over longer distances by passive transports, while at the deeper anoxic layers they are restricted to active dispersion from the above and nearby sediments. Such restriction in the dispersion potential together with the unfavorable environmental conditions leads to randomness in the presence of species resulting in the high variability between assemblages along the horizontal dimension.

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The importance of vertical and horizontal dimensions of the sediment matrix in structuring nematodes across spatial scales

A importância das dimensões vertical e horizontal da matriz de sedimento na estruturação de nematoides nas diferentes escalas espaciais

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1 - Introduction

The sediment is a three-dimensional habitat for a vast number of infauna species. In the sediment, these benthic organisms are spatially structured through of a variety of environmental factors, such as granulometry, salinity and oxygen and food availability, along both the horizontal and vertical dimensions [1–3]. Specially for nematodes, the most abundant and species rich taxa of marine sediments [4,5], at the horizontal dimension the variability of the fauna at the scale of centimeters is as large as at the scale of meters to hundreds of kilometers [6–8]. At the vertical dimension, changes in nematode community structure occurs at the scale of few centimeter, due to a more pronounced change in environmental factors, such as food resources and oxygen availability [3,9,10]. Such abrupt change along the vertical dimension, causes significant decrease in nematodes densities, number of species and changes in species composition [3,11,12]. In fact, only few meiofaunal taxa can persist to extreme reduced conditions at the deeper layers, and although nematodes are considered a group very tolerant to such conditions, this tolerance is considered species-specific. [2,13]. Reduced layers impose therefore a strong habitat selection for the fauna. Although the vertical pattern is already well established in the meiobenthic literature [3,9,14,15], nothing has been done to understand how the vertical and horizontal patterns are interacting with each other. For instance, we still do not know whether superficial and deep dwelling species show similar spatial patterns at the horizontal scale. This lack of knowledge is, at least in part, consequence of the dependent sampling design traditionally used in infauna studies [3,16–18]. In this design, vertical

subsamples are taken from the same corer restricting thus the comparisons of vertical layers from multiple sites along horizontal scales.

Oxygen is recognized as a major structuring factor of metazoan communities in marine sediments, along both horizontal and vertical dimensions [19,20]. The availability of oxygen to benthic system depends on the oxygen demand for organic matter degradation and on the supply through several transport mechanisms [19]. Surface sediments are generally more oxygenated than deeper sediments where oxi-reduction reactions predominate. The depth of the oxygenated layer is variable depending on the balance between hydrodynamic regime, bioturbation and organic degradation [21,22]. For example, in high-energy environments, such as sandy beaches, the oxygenated layer can reach depths greater than 20 cm, because of the high drainage maintained by the strong hydrodynamics regime. Meanwhile, in low-energy environments, such as muddy estuarine sediments, the oxygenated layer is restricted to few millimeters, because a consequence of high organic loads and weak hydrodynamics regime [1,23].

Based on the current evidences of the vertical distribution of the fauna and of the redox profile, it can be hypothesized that the species poor assemblages inhabiting the more reduced layers of the sediment will be characterized by few tolerant species. If this pattern proves to be consistent at multiple sites (horizontal scale), we can expect that the deep samples will have the same set of tolerant species and high similarity in the multivariate analysis. The species rich superficial assemblages in comparison would be expected to be more heterogeneous with the composition of

species highly dependent on local disturbances and colonization rates that occur at random [24,25].

In order to test the interdependence between the vertical and horizontal dimensions in structuring marine nematodes assemblages, this study analyzed the nematode vertical profile using an independent sampling design over multiple spatial scales. Estuarine bedforms were selected to test our hypothesis. Estuarine bedforms are sandy environments formed from patterns of sediment transport governed by hydrodynamic forces such as tidal currents, river discharge and wind driven currents. Given the high organic conditions of estuaries, reduced conditions is usually present at the deeper layers of the sediment [26].

2 - Materials and methods

2.1 - Study area and sampling design

Three estuarine systems (Una do Prelado, Cananéia, Guaratuba) were sampled along the southeast coast of Brazil in February 2011 (Fig. 1). The region is influenced by a mean tidal range of 0.76 m. At all estuaries, bedforms were exposed and submerged at low and high tide, respectively.

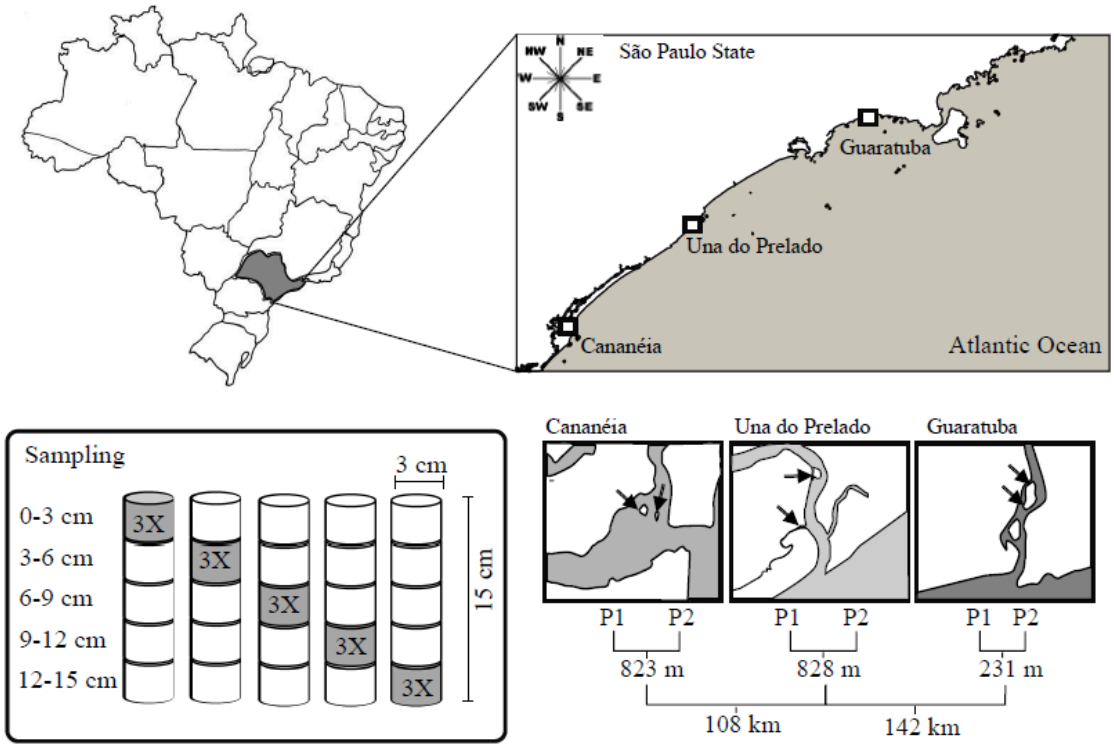

Figure 1**. Map of the sampling sites and schematic view of the sampling design**. Arrows represent the unvegetated bedforms. P1 and P2 represent the respective plots sampled in each estuary.

A hierarchical sampling design was used to determine variation in community structure at 3 horizontal scales ranging from meters to hundreds of kilometers, and 5 vertical sediment layers (Fig. 1). In each of the three estuaries two unvegetated bedforms (plots) were sampled (scale of hundreds of meters) during low tide and similar meteorological conditions. During sampling, no visual differences in terms of

sediment's surface (e.g. ripples formation, depressions, etc.) were observed between bedforms of the different estuaries. At each plot, random samples several meters apart from each other were collected with a corer (3 cm diameter, 15 cm length) to obtain meiofauna and redox potential measures. In order to analyze the vertical distribution of the meiofauna, five layers of sediment (0-3, 3-6, 6-9, 9-12, 12-15 cm), each from a different corer, were sampled three times. In total, 90 samples were analyzed. The fauna samples were immediately fixed in 4% formaldehyde solution. Redox potential of the sediment was measured with electrodes inserted into the middle of each sediment layer. Two additional samples for analysis of organic matter and granulometry were obtained with a corer of 5 cm in diameter. In order to increase the volume of sediment collected for the granulometry analysis, each layer was collected three times. For logistical constraints, only one sample for organic matter and granulometry was taken.

2.2 - Sample processing

Meiofauna samples were washed through a 45 µm sieve, extracted by flotation with a solution of colloidal silica (LUDOX TM-50) with density of 1.18 $g.cm^{-3}$ [14]. The samples were stained with Rose Bengal and major taxonomic groups were counted under a stereomicroscope. 10 % of the nematodes were randomly picked for identification, unless densities were smaller than 120, then all individuals were identified. Nematodes were first transferred to anhydrous glycerol (5%) and then mounted on permanent slides. Nematodes were identified to genus level [5] and separated into morphospecies [27].

Organic matter samples were dried at 80 °C until reaching a constant weight. They were then re-weighed and organic material was combusted in a muffle furnace at 550 °C for 4 h [28]. Wet, dry and ash-free dry weight values were used to calculate water content and organic content of the sediment through the difference between wet and dry weight and between dry and ash-free dry weight, respectively. Granulometric analysis was carried out using an automatic sieve shaker with different mesh sizes (1.000, 0.500, 0.125, 0.063 and 0.063mm) for 20 min. The dry weight of each fraction was determined and the proportion that each fraction contributed to total mass was calculated [29]. Sediment statistical parameters were calculated using the SysGran v3 software [30].

2.3 - Data analysis

Abiotic data was analyzed by means of principal component ordination (PCA). Redox potential was analyzed by means of analysis of variance using mixed models design (mixed-ANOVA; Table 1). In order to differentiate the oxidation degree of the different layers of the sediment, redox potential values were separated in four classes: "strongly oxidized" (>100mV), "oxidized" (0mV<x<100mV), "reduced" (0mv<x<-100mV) and "strongly reduced" sediments (<-100mV) [31,32].

As univariate descriptors of the fauna we used abundance and species richness of nematodes. These data were also treated statistically by mixed-ANOVA, preceded by Cochran's test for homogeneity of variances. ANOVA was performed in the R environment with the aid package GAD [31]. *Posteriori* Student-Newman-Keuls (SNK) multiple comparisons tests were used to investigate differences among means.

Table 1. Summary of the ANOVA mixed models design used to analyze the data sets. Abrev: abbreviation; Type of factor: Random (R) or Fixed (F); Respective numerator, denominator and degrees of freedom (df) used to calculate the F-ratios.

Source of variation	Abrev.	Type	Numerator	Denominator	df
Estuary	E	R	1*E	1*P(E)	2
Layer	L	F	1*L	1*E x L	4
Plot(Estuary)	P(E)	R	1*P(E)	1*Res	3
Estuary* Layer	E*L		1*E x L	1*P(E) x L	8
Plot(Estuary)* Layer	P(E)*L		1*P(E) x La	1*Res	12

The analytical design used for the multivariate analyses of the fauna was the same used for the univariate measures (Table 1). All tests were applied on a Bray–Curtis similarity matrix underlying the classification of samples (factors). Prior to the analysis, data were standardized and transformed when necessary. Since our data set was characterized by many samples having few individuals or even no individuals, increasing significantly the variability of the data, we added a dummy variable (weight 1) to the matrix [34]. Permutational multivariate analysis of variance - PERMANOVA [35] was used to test for differences in community structure. Differences in multivariate aspects of community structure between layers, and between four classes of redox potential were assessed by multidimensional scaling ordination (MDS). Similarity percentage analysis procedure (SIMPER) was used to identify the species making the greatest contribution to differences between clusters observed in the MDS plot.A distance-based multivariate linear model (DistLM) using forward selection was performed to determine the proportion of total variation on species composition data explained of by each abiotic variable (software PRIMER 6 & PERMANOVA). Correlation values between abiotic variables higher than 0.9 (considered redundant) were omitted for the DistLM procedures. Eight environmental variables were included in the

regression analysis: Redox potential, pore water, organic matter, grain size asymmetry, %sand, %silt, medium sand and very fine sand. P–values were obtained using 999 permutations of the raw data.

The degree of variability in the composition and relative abundance of species in the community was assessed through permutational multivariate dispersion (PERMDISP,[36]) for each sediment layer at the three spatial scales: all estuaries together, within estuaries and within plots. To test whether the dispersion of the data varied according to the redox potential of the sediment, regression analyses were conducted for each spatial scale.

3 - Results

3.1- Environmental characterization

No differences in redox potentials were found between estuaries ($p>0.05$; Table 2), whereas plots within estuaries and sediment layers differed significantly ($p<0.05$). At all estuaries redox potential decreased with increasing sediment depth (Fig. 2). Post-hoc SNK-tests showed significant differences between L1 and L5, and no difference between the intermediate layers (L2, L3 and L4; Table 2).

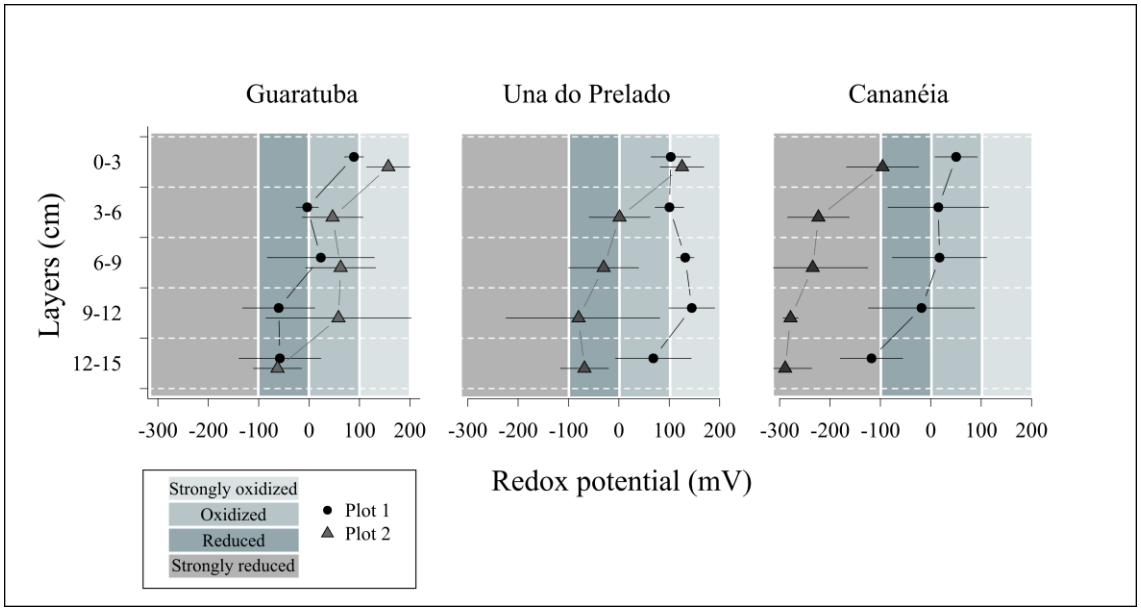

Figure 2. **Vertical profile of redox potential values of the two bedforms sampled at each estuary.** Bars represent standard deviation from the mean (n=3 for each point).

PCA analysis on the abiotic parameters showed that PC1 and PC2 accounted for 36.4% and 20.4% of total variation present, respectively, and it showed no clustering of estuaries or layers. Data was instead clustered into plots within each estuary (Fig. 3). At Guaratuba and Una do Prelado, differences between plots were mainly driven by differences in organic matter and redox potential. At Cananéia plots differed by the presence of medium sand in Plot 1 and very fine sand in Plot 2.

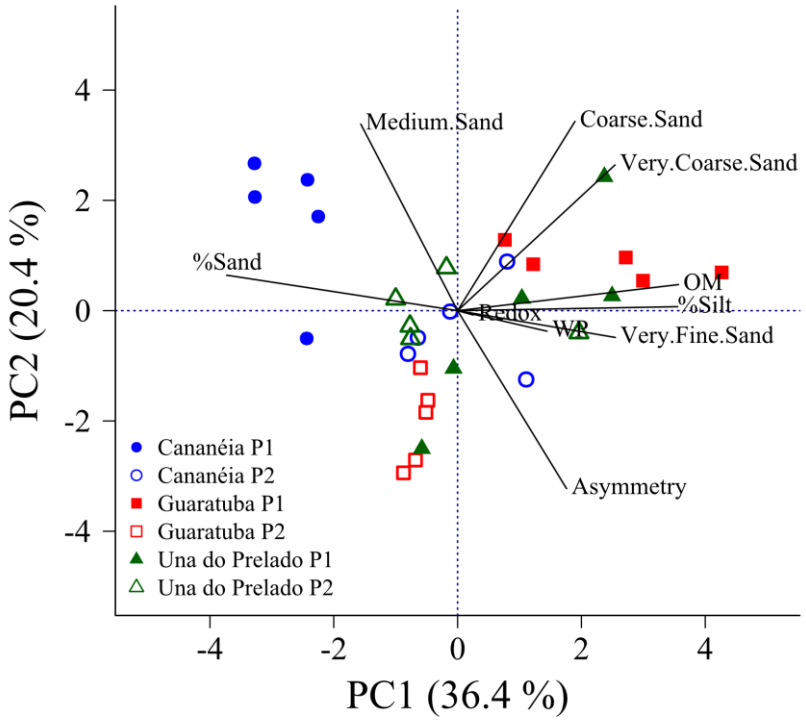

Figure 3. **Principal component analysis.** PCA of the main abiotic parameters evaluated at Guaratuba, Una do Prelado e Cananéia. Filled and empty symbols represent Plots 1 and 2, respectively.

3.2 - Abundance and species richness

Nematode abundance and species richness did not differ between estuaries (Table 2). Significant differences for both univariate parameters were observed for the interaction effect layers between plots (P(E)*L; Table 2). At Guaratuba differences in species richness between plots were restricted to the deepest layer, while at Cananéia significant differences were found at all depths.

In general, nematode abundances and species richness were highest at the sediment surface and decreased gradually with depth (Fig. 4), with the exception of Plot 1 at Guaratuba estuary where the abundance showed an alternating pattern between layers (Fig. 4A). At the surface, nematode abundance varied between 52 and 900 ind. 10 cm², while at the deepest layer varied from 0 to 571 ind. 10 cm^2. Species richness varied from 7 to 17 and between 0 and 14 in the top and bottom layer, respectively.

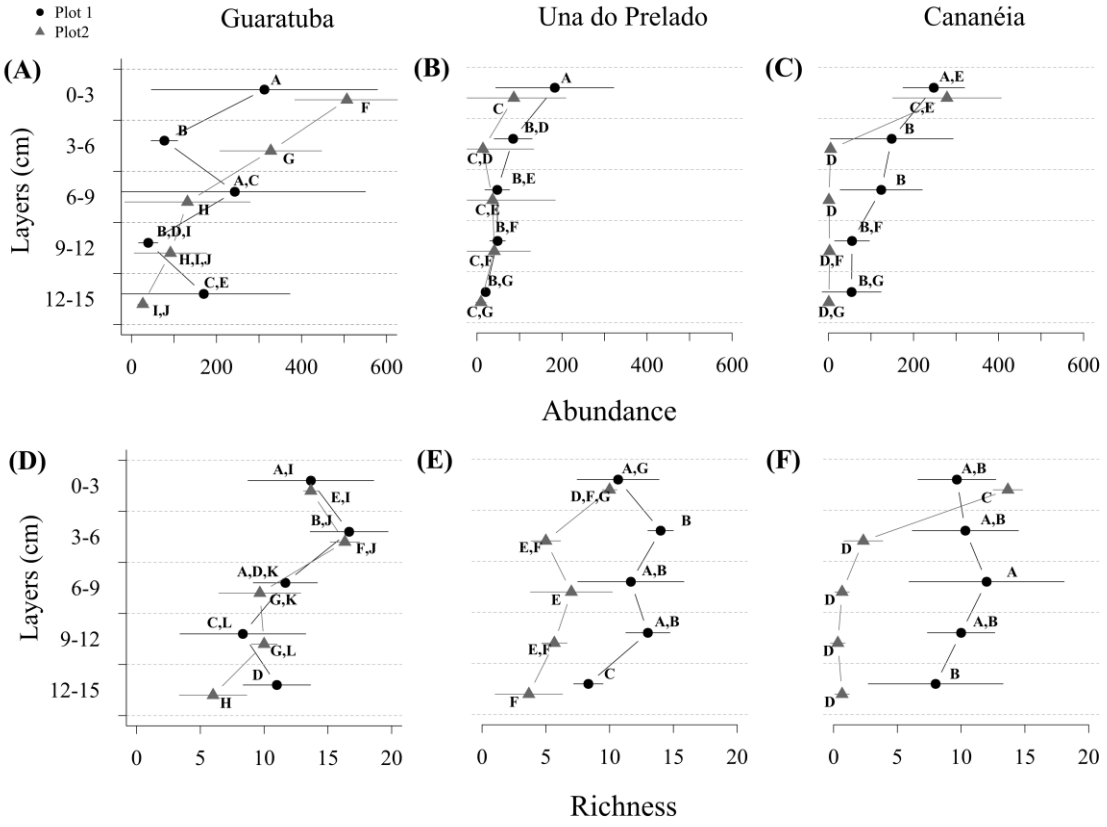

Figure 4. **Vertical profile of univariate descriptors of the fauna.** (A, B, C) Nematode abundances and (D, E, F) species richness in all sampled sites. Bars represent standard deviation from the mean (n=3 for each point). Different letters represent significant differences found after post-hoc tests

3.2 - Community structure

Like for the univariate measures, species composition based on presence/absence data did not differed between estuaries. Differences between plots within estuaries were dependent on the vertical layer analyzed, on the same way differences between layers were dependent on the plot (interaction effect P(E)*L; Table 2). While at Cananéia plots were significantly different from each other at all sediment layers, at Una do Prelado significant differences between plots were restricted to the deeper layers (3-15 cm) (Appendix 1).

The MDS plot on presence/absence of nematode species showed no clustering for layers (Fig. 5a). However, when this analysis was repeated using the four classes of redox potential, a cluster among samples classified as "strongly oxidized" was observed (Fig. 5b). Results from SIMPER analysis based on presence/absence data revealed that the differences between "strongly oxidized" samples and the other categories were mainly due to the higher frequency of *Viscosia* sp.1, *Pomponema* sp.1, *Microlaimus* sp.4, *Cobbia* sp.1 and *Microlaimus* sp.5. (Table 3). The average similarity of the samples decreased with increasing depth. Strongly oxidized samples were about ten times more similar to each other than samples marked as "strongly reduced" (37.45% vs 3.47%).

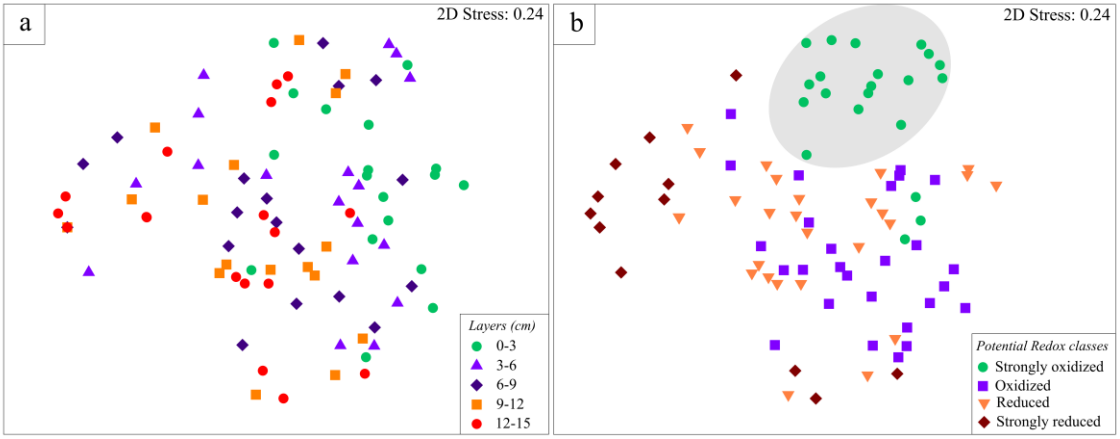

Figure 5. **non-metric MDS ordination plot**. Based on Bray-Curtis dissimilarities of presence/absence of species. (a) five sediment layers; (b) four classes of oxidation of sediment,

Table 2. Analysis of variance (ANOVA) and Permutational multivariate analysis of variance (PERMANOVA). Bold lettering identifies those P values that are significant (<0.05). Df: degress of freedom; MS: mean squares

Variable	Source of variation	DF	MS	F	P
Redox potential	Estuary	2	244071.2	1.55	0.345
	Layer	4	60741.2	41.73	**0.000**
	Plot(Estuary)	3	157247.3	36.30	**0.000**
	Estuary*Layer	8	1455.5	0.22	0.980
	Plot(Estuary)*Layer	12	6538.4	1.51	0.146
	Residual	60	4332.0		
Richness species	Estuary	2	183.6	1.04	0.455
	Layer	4	90.6	4.26	**0.039**
	Plot(Estuary)	3	177.1	20.97	**0.000**
	Estuary*Layer	8	21.2	0.80	0.614
	Plot(Estuary)*Layer	12	26.5	3.14	**0.002**
	Residual	60	8.4		
Abundance	Estuary	2	260.5	2.43	0.236
	Layer	4	297.8	17.04	**0.001**
	Plot(Estuary)	3	107.4	6.56	**0.001**
	Estuary*Layer	8	17.5	0.55	0.797
	Plot(Estuary)*Layer	12	31.7	1.94	**0.048**
	Residual	60			
PERMANOVA	Estuary	2	26246	1.63	0.06
Community	Layer	4	6050.4	3.32	**0.003**
	Plot(Estuary)	3	16079	17.08	**0.001**
	Estuary*Layer	8	1819.6	1.04	0.402
	Plot(Estuary)*Layer	12	1738.9	1.86	**0.001**
	Residual	60	942.2		

Table 3. SIMPER analysis showing species ranked according to average Bray-Curtis dissimilarity between classes of potential redox. The list of species was limited to a cumulative percentage dissimilarity of 50%, i.e. when 50% of the dissimilarity was reached, remaining species were skipped. Abreviations: Contrib% - Percentage of contribution to similarity; Cum% - Cumulative percentage of contribution to similarity

Species	Frequency	Contrib%	Cum.%
Strongly oxidized	Average similarity: 37.45%		
Viscosia sp.1	0.76	13.53	13.53
Pomponema sp.1	0.71	11.67	25.2
Microlaimus sp.4	0.71	10.91	36.11
Cobbia sp.1	0.67	10.41	46.53
Microlaimus sp.5	0.67	9.51	56.03
Oxidized	Average similarity: 34.04%		
Sabatieria sp.3	0.89	20.9	20.9
Pomponema sp.1	0.63	11.35	32.25
Viscosia sp.1	0.56	8.4	40.65
Trochamus sp.1	0.56	8.08	48.74
Odontophora urotrix	0.56	7.74	56.48
Reduced	Average similarity: 31.38%		
Pomponema sp.1	0.81	27.4	27.4
Odontophora urotrix	0.67	16.59	43.99
Sabatieria sp.3	0.63	13.02	57.01
Strongly reduced	Average similarity: 3.47%		
Pomponema sp.1	0.13	19.42	19.42
Spirinia sp.1	0.2	18.77	38.19
Sabatieria sp.3	0.2	11.97	50.16

3.3 - Correlations between the environmental variables and the fauna

Forward DistLM showed that redox potential was the most important variable explaining, 12% and 33% of the total variation in abundance and species richness, respectively ($p<0.001$). After including other environmental factors these models explained respectively 24% and 42%. (Appendix 2). Redox potential also explained 12% of the total variation observed in species composition ($p<0.001$); followed by organic matter, medium and very fine sand, and the percentages of silt and sand, which all together explained 35% of the fauna variability (Appendix 2).

3.5 – Relationship between community variability, sediment layer and redox potential

Dispersion of the multivariate data set was significantly lower at the first sediment layer and did not differ between the deeper layers when considering all estuaries together (Fig. 6A). Although not always significant, analysis for each estuary separately also showed lower dispersion at the top most sediment layer (Fig. 6B). At the smallest spatial scale (within plots), no significant differences were observed (Fig. 6C). This analysis was consistent whether the data was analyzed by means of presence/absence or relative abundances. Pairwise analyzes between sediment layers within each spatial scale are listed in Appendix 3.

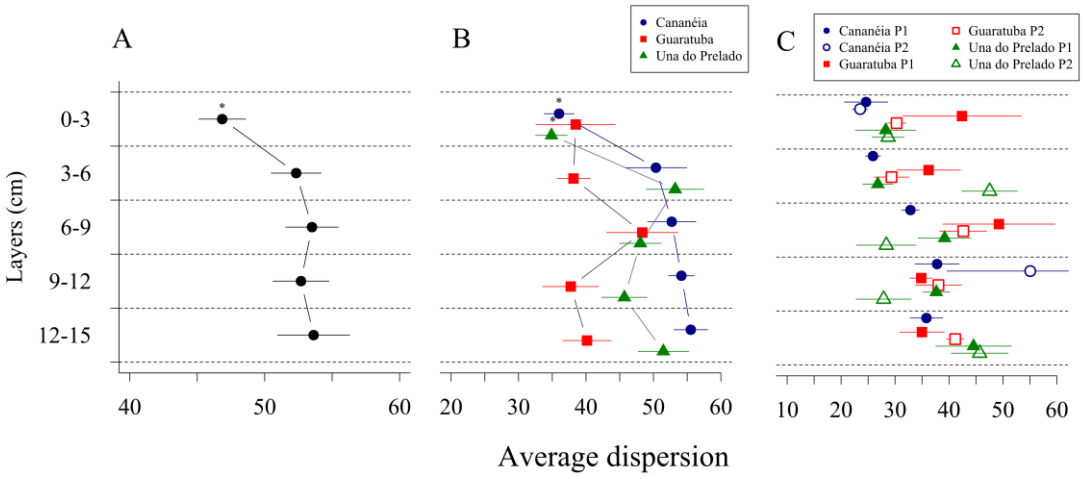

Figure 6. **Average dispersion of the community structure.** Results of PERMDISP analysis on presence-absence data along the depth gradient calculated for all estuaries together (A) and separated (B) and for each plot within estuary (C).

Since the redox potential was the main factor selected by DistLM to explain the variability in species composition, we tested whether the dispersion of the data was correlated to redox values at the different spatial scales. For the presence/absence data set, dispersion was negatively correlated with redox only at the larger scales (Fig. 7A, B, C). When analyzing the data set based on the relative abundance, the negative relationship was significant at all scales (Fig. 7D, E, F).

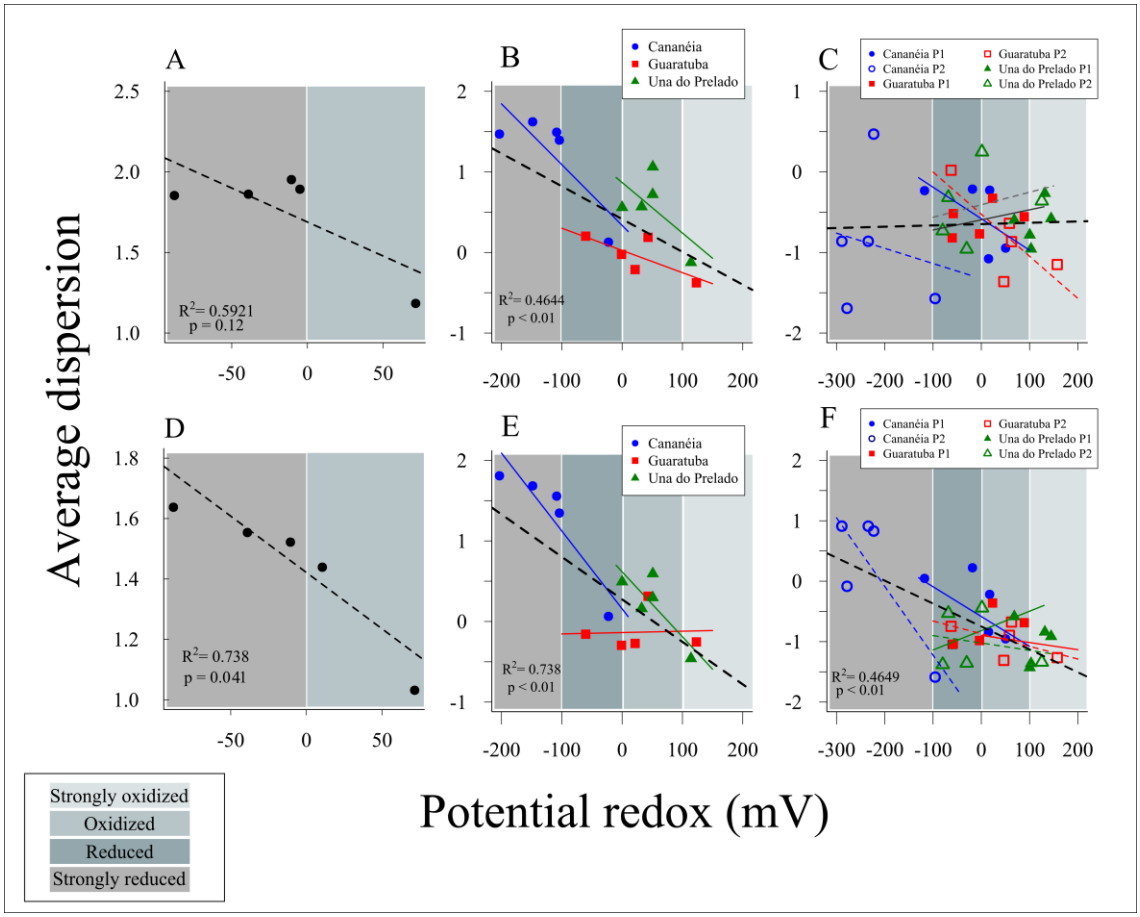

Figure 7. **Linear regression of average multivariate dispersion against sediment redox potential.** A, B and C represent results from presence/absence data; D, E, and F represent results from relative abundance data. This analysis was performed to the different sources of variation: all estuaries together (A, D) and separated (B, E), and for each plot within estuary (C, F).

4 - Discussion

The present study rejected our hypothesis that assemblages inhabiting more reduced layers of the sediment are more similar at horizontal dimension, due to habitat selection driving the spatial distribution. We observed that assemblages living in the top more oxygenated layers are in fact more abundant, species rich and at the same time are actually less variable, in terms of species presence/absence and relative abundances, than assemblages living in the deeper reduced layers. Although we observed species typically related to reduced sediments like, *Sabatieria* sp.3 and *Spirinia* sp.1 in samples classified as "strongly reduced", this sediment class showed very low similarity between the assemblages suggesting that there is not a specific set of species living under these conditions. These findings contradicts previous assumptions that the range of tolerance to extreme reduced conditions is species-specific [2,3,11,13,37]. It is important to note however that all previous studies were either experimental manipulations of a reduced set of species, or did not strictly compare the multivariate dispersion of each sediment layer.

The present data also indicates that community patterns in the sediment are better explained by changes in redox potential than sediment depth *per se*. All three parameters of the fauna (abundance, species richness and community similarity) were better explained by differences in redox potentials. The importance of redox in structuring benthic communities along the vertical dimension is well known [12], however this study shows that redox can influence the fauna at both dimension, vertical and horizontal. Basically, we can hypothesize that at the more oxygenated superficial sediment, organisms have the opportunity to colonize and/or migrate on a

wide range of depths within the sediment (Fig. 8) and random displacement of organisms can result in an unpredictable distribution pattern [38]. The most probable mechanism causing this pattern is that at surface layers, water current promotes passive redistribution, and the benign conditions in the sediment permits that many species establish and coexist (Fig. 8). There are already evidences that dispersal of nematodes occurs mainly through passive processes, via hydrodynamic forces [38–41]. At the deeper layers, in contrast, species are not exposed to hydrodynamism and species are chiefly arriving by active migration and is therefore limited from the above set of species. The colonization of this layer will be mainly dependent of migration rates, localized environmental conditions and species interactions. Empirical evidences supporting that the colonization processes may operate differently in superficial and deep layers comes from a series of previous experiments on meiofauna[40].

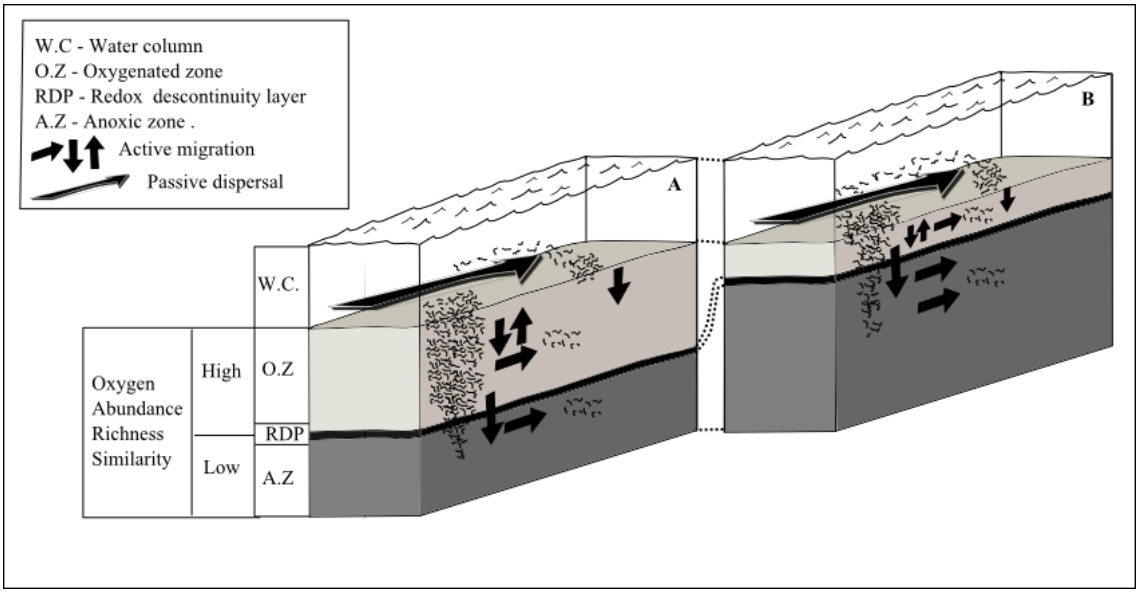

Figure 8. **Schematic illustration.** Representation of the three dimensions of the sediment matrix emphasizing potential interacting processes structuring the infauna under two hypothetical redox gradients: (A) Large oxygenated layer; (B) small oxygenated layer

The patterns above were detected however when comparing communities at large scales, i.e. between estuaries and plots within estuaries. At the smaller scale, within plots, the increasing variability in species composition with increasing redox potential or sediment depth was much less evident. The negative relationship was only detected on the relative abundance data set, suggesting that community changes at the small scale are more subtle and not perceived with presence/absence transformation. At the small horizontal scale there was little turnover and most of the species occurred with different relative abundances. Probably at this scale biotic interactions [42,43] and stochasticity are more important in structuring the fauna than redox potential alone. Small scale variability in nematode composition is in fact less predictable than at larger scales [6,8,44].

Although the current community patterns at both horizontal and vertical dimensions were mainly driven by redox and just weakly explained by organic matter content, one cannot exclude the possibility of the role of food quality [45,46]. Especially in food limited environment, like the deep sea, food quality is known to drive vertical and horizontal patterns of the meiofauna [47]. However, evidence for highly productive areas like estuaries is still inconclusive.[1].

Another important fact to be discussed is the continuous decrease in nematode abundances and species richness with increasing depth in the sediment. Significant differences were restricted between the uppermost (0-3 cm) and deepest layer (12-15 cm), the intermediate layers (3-6, 6-9 and 9-12 cm) were highly variable and did not differ from between each other. This high variability could be due to the inherent characteristics of estuarine bedforms, since they are highly dynamic environments,

and/or a consequence of the independent sampling design adopted. It is well accepted that communities inhabiting a particular depth of the substrate are in some way influenced by communities above and below it, once they share the same sediment column [48]. As such, studies using a dependent sampling design [e.g. 2,13,14,19], would artificially reinforce differences between layers because of the reduced variability sampled. The independent sampling design used in the present study better characterizes the spatial patterns of the fauna and inevitably increases the variability between replicates and thus the vertical changes in communities would not be as evident as previously expected. We strongly recommend that an independent sampling design should be adopted if horizontal and vertical patterns are intended to be investigated simuntaneously.

5 - Acknowledgments

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7 - Appendices:

Appendix 1 *- Pairwise comparisons of PERMANOVA*

Pairwise tests P-value based on Monte Carlo (MC) of community structure for different sources of variation. Bold lettering identifies those P-values that are significant (<0.05). L1, L2, L3, L4 and L5.

Source of Variation	Comparison level	Group	t	P(MC)
Layer	All estuaries together	L1, L2	1.6197	0.063
		L1, L3	1.7627	0.066
		L1, L4	2.0495	**0.035**
		L1, L5	2.0654	**0.022**
		L2, L3	1.4249	0.123
		L2, L4	1.899	**0.024**
		L2, L5	1.8444	**0.026**
		L3, L4	1.1676	0.313
		L3, L5	1.7517	**0.024**
		L4, L5	1.4419	0.126
Plot(Estuary)	Cananéia	P1, P2	5.1617	**0.001**
	Guaratuba	P1, P2	2.9881	**0.001**
	Una do Prelado	P1, P2	3.9271	**0.001**
Plot(Estuary)*Layer	Cananéia P1	L1, L2	1.1862	0.299
		L1, L3	1.4741	0.112
		L1, L4	1.912	0.098
		L1, L5	1.6095	**0.095**
		L2, L3	1.1727	0.372
		L2, L4	1.611	0.1
		L2, L5	1.3463	0.394
		L3, L4	0.8014	0.778
		L3, L5	0.93413	0.52
		L4, L5	0.83971	0.596
	Cananéia P2	L1, L2	2.1351	0.101
		L1, L3	3.7498	0.118
		L1, L4	5.0858	0.081
		L1, L5	3.9643	0.115
		L2, L3	1.2024	0.293
		L2, L4	1.2769	0.315
		L2, L5	1.2024	0.292
		L3, L4	1.0198	0.908
		L3, L5	1.029	0.602
		L4, L5	1.0198	0.901
	Guaratuba P1	L1, L2	1.3675	0.192
		L1, L3	1.2179	0.2
		L1, L4	1.8769	0.095
		L1, L5	1.3553	0.197
		L2, L3	1.0718	0.433
		L2, L4	2.0206	0.094
		L2, L5	1.1967	0.426
		L3, L4	1.2295	0.192
		L3, L5	0.68901	0.787
		L4, L5	0.8508	0.597
	Guaratuba P2	L1, L2	1.1778	0.205
		L1, L3	1.8719	0.104
		L1, L4	1.9792	0.101
		L1, L5	2.0077	0.106
		L2, L3	1.3323	0.103
		L2, L4	1.5463	0.103
		L2, L5	1.7178	0.095
		L3, L4	1.3009	0.18
		L3, L5	1.1846	0.293
		L4, L5	1.2833	0.103
	Una do Prelado P1	L1, L2	1.9251	0.115

Appendix 1 continued

Source of Variation	Comparison level	Group	t	P(MC)
Plot(Estuary)*Layer		L1, L3	1.816	0.097
		L1, L4	2.2869	0.092
		L1, L5	2.1055	0.091
		L2, L3	1.004	0.594
		L2, L4	1.6133	0.11
		L2, L5	2.0603	0.088
		L3, L4	0.99405	0.718
		L3, L5	1.3865	0.19
		L4, L5	1.2572	0.183
	Una do Prelado P2	L1, L2	1.1764	0.205
		L1, L3	2.1521	0.095
		L1, L4	2.5785	0.096
		L1, L5	2.0427	0.11
		L2, L3	1.3699	0.119
		L2, L4	1.43	0.094
		L2, L5	1.0915	0.311
		L3, L4	1.2023	0.295
		L3, L5	1.3892	0.311
		L4, L5	1.3667	0.403
Plot(Estuary)*Layer	Cananéia L1	P1,P2	2.5414	**0.015**
	Cananéia L2	P1,P2	2.2437	**0.022**
	Cananéia L3	P1,P2	2.7413	**0.02**
	Cananéia L4	P1,P2	3.3081	**0.006**
	Cananéia L5	P1,P2	2.5861	**0.019**
	Guaratuba L1	P1,P2	1.4098	0.156
	Guaratuba L2	P1, P2	2.0296	**0.031**
	Guaratuba L3	P1, P2	1.8405	0.052
	Guaratuba L4	P1, P2	1.7225	0.09
	Guaratuba L5	P1, P2	1.3976	0.153
	Una do Prelado L1	P1, P2	1.4812	0.129
	Una do Prelado L2	P1, P2	2.3121	**0.019**
	Una do Prelado L3	P1, P2	2.4354	**0.02**
	Una do Prelado L4	P1, P2	2.399	**0.022**
	Una do Prelado L5	P1, P2	2.0304	**0.026**

Appendix 2 *- Results of Distance-based multivariate analysis for a linear model (DistLM).*

Results of forward distance-based multivariate analysis for a linear model (DistLM). SS = sum of squares; F= pseudo-F; P= p- value; Prop= proportion of explanation; Cumul= Cumulative proportion of explanation; res.df= residual degree of freedom.

	Variable	Adj R^2	SS	F	P	Prop.	Cumul.	res.df
Abundance	Redox	0.115	250100	12.58	**0.00**	0.13	0.13	88
	% Silt	0.141	70164	3.64	**0.05**	0.04	0.16	87
	Very fine sand	0.188	110220	6.04	**0.02**	0.06	0.22	86
	Assymetry	0.197	36214	2.01	0.17	0.02	0.23	85
	Medium sand	0.202	26919	1.5	0.22	0.01	0.25	84
Richness	Redox	0.326	752.5	44.05	**0.00**	0.33	0.33	88
	% Silt	0.346	60.361	3.64	0.08	0.03	0.36	87
	% Sand	0.378	87.431	5.55	**0.02**	0.04	0.4	86
Presence/absence	Redox	0.111	26139	12.06	**0.00**	0.12	0.12	88
	Organic Matter	0.188	18539	9.37	**0.00**	0.09	0.21	87
	Medium sand	0.243	13524	7.33	**0.00**	0.06	0.27	86
	% Silt	0.261	5540.6	3.08	**0.00**	0.03	0.29	85
	% Sand	0.297	9294.5	5.43	**0.00**	0.04	0.34	84
	Very fine sand	0.304	3082.8	1.82	0.07	0.01	0.35	83
	Assymetry	0.309	2586.2	1.54	0.13	0.01	0.36	82
	Water pore	0.311	2098.8	1.25	0.24	0.01	0.37	81
Presence/absence for layer								
Layer 1	Medium sand	0.187	9501.8	4.92	**0.00**	0.24	0.24	16
	% silt	0.289	5548.8	3.28	**0.01**	0.14	0.37	15
	Organic Matter	0.431	6423.4	4.74	**0.00**	0.16	0.53	14
	Assymetry	0.551	5086.2	4.77	**0.00**	0.13	0.66	13
	Very fine sand	0.649	3856.7	4.62	**0.00**	0.1	0.75	12
	Redox	0.654	956.48	1.16	0.34	0.02	0.78	11
	Water pore	0.654	0	0	1	0	0.78	11
Layer 2	Medium sand	0.127	8957.7	3.46	**0.00**	0.18	0.18	16
	Water pore	0.25	8060.6	3.63	**0.00**	0.16	0.34	15
	Organic Matter	0.348	6283.4	3.25	**0.00**	0.12	0.46	14
	Assymetry	0.418	4624.1	2.68	**0.01**	0.09	0.55	13
	% sand	0.517	5247	3.67	**0.00**	0.1	0.66	12
	Redox	0.518	1478.8	1.04	0.41	0.03	0.69	11
	Very fine sand	0.517	5247	3.67	**0.00**	0.1	0.66	12
Layer 3	Medium sand	0.1	8041.1	2.88	**0.00**	0.15	0.15	16
	Very fine sand	0.194	7200	2.88	**0.00**	0.14	0.29	15
	Assymetry	0.293	6773.5	3.09	**0.00**	0.13	0.42	14
	% silt	0.371	5355.7	2.75	**0.01**	0.1	0.52	13
	Water pore	0.445	4699.8	2.73	**0.00**	0.09	0.61	12
	Redox	0.492	3311.6	2.1	**0.02**	0.06	0.67	11
	Organic Matter	0.492	0	0	1	0	0.67	11
Layer 4	Very fine sand	0.101	7914.4	2.92	**0.00**	0.15	0.15	16
	Redox	0.205	7388.5	3.08	**0.00**	0.14	0.3	15
	Water pore	0.319	7239.1	3.53	**0.00**	0.14	0.44	14
	Assymetry	0.493	8861.9	5.79	**0.00**	0.17	0.61	13
	Medium sand	0.52	2494.8	1.72	0.12	0.05	0.66	12
	Organic Matter	0.549	2428.4	1.79	0.1	0.05	0.71	11
Layer 5	Very fine sand	0.114	8955	3.18	**0.00**	0.17	0.17	16
	Assymetry	0.194	6625.7	2.59	**0.01**	0.12	0.29	15
	Redox	0.276	6196.1	2.69	**0.01**	0.11	0.4	14
	% silt	0.346	5206.7	2.51	**0.01**	0.1	0.5	13
	% sand	0.371	3023.6	1.51	0.09	0.06	0.56	12
	Organic Matter	0.427	3937.8	2.16	**0.00**	0.07	0.63	11
	Medium sand	0.427	6727.7	3.7	**0.00**	0.12	0.63	11

Appendix 3 *- Pairwise comparisons of PERMDISP*

Pairwise tests of Permutational analysis of multivariate dispersions (PERMDISP) under presence/absence species of nematodes at different sources of variation. Bold lettering identifies those P-values that are significant (<0.05). L1, L2, L3, L4 and L5 correspond respectively to vertical strata 0-3, 3-6, 6-9, 9-12, 12-15 cm.

Source of Variation	Comparasion level	Group	T	P
Layer	All estuaries together	L1,L2	2.88	**0.01**
		L1,L3	2.58	**0.01**
		L1,L4	2.54	**0.01**
		L1,L5	2.43	**0.04**
		L2,L3	0.23	0.86
		L2,L4	0.36	0.74
		L2,L5	0.37	0.72
		L3,L4	0.12	0.92
		L3,L5	0.14	0.89
		L4,L5	0.03	0.98
Estuary*layer	Cananéia	L1,L2	3.15	**0.01**
		L1,L3	4.10	**0.00**
		L1,L4	6.19	**0.00**
		L1,L5	4.13	**0.00**
		L2,L3	0.20	0.83
		L2,L4	0.52	0.55
		L2,L5	0.16	0.89
		L3,L4	0.35	0.69
		L3,L5	0.05	0.96
		L4,L5	0.41	0.61
	Guaratuba	L1,L2	0.46	0.69
		L1,L3	1.25	0.25
		L1,L4	1.03	0.41
		L1,L5	1.43	0.24
		L2,L3	1.01	0.38
		L2,L4	0.70	0.50
		L2,L5	1.21	0.27
		L3,L4	0.55	0.64
		L3,L5	0.03	0.99
		L4,L5	0.70	0.55
	Una do Prelado	L1,L2	3.14	**0.02**
		L1,L3	2.47	**0.03**
		L1,L4	2.00	0.07
		L1,L5	1.75	0.11
		L2,L3	0.90	0.38
		L2,L4	1.28	0.22
		L2,L5	1.19	0.26
		L3,L4	0.43	0.63
		L3,L5	0.41	0.66
		L4,L5	0.02	0.98
Plot(Estuary)*Layer	Cananéia L1	P1,P2	1.43	0.30
	Cananéia L2	P1,P2	2.37	0.10
	Cananéia L3	P1,P2	1.07	0.72
	Cananéia L4	P1,P2	2.36	0.40
	Cananéia L5	P1,P2	0.66	0.80
	Guaratuba L1	P1,P2	0.93	0.90
	Guaratuba L2	P1, P2	1.84	0.21
	Guaratuba L3	P1, P2	0.93	0.59
	Guaratuba L4	P1, P2	0.37	0.81
	Guaratuba L5	P1, P2	1.57	0.30
	Una do Prelado L1	P1, P2	1.15	0.53
	Una do Prelado L2	P1, P2	2.47	0.11
	Una do Prelado L3	P1, P2	1.53	0.20

Appendix 3 continued.

Source of Variation	Comparasion level	Group	T	P
Plot(Estuary)*Layer	Una do Prelado L4	P1, P2	0.53	0.71
	Una do Prelado L5	P1, P2	0.40	0.90
	Cananéia P1	L1, L2	0.36	0.62
		L1, L3	1.42	0.59
		L1, L4	1.32	0.50
		L1, L5	0.79	0.80
		L2, L3	1.80	0.12
		L2, L4	1.65	0.32
		L2, L5	0.95	0.50
		L3, L4	0.02	1.00
		L3, L5	0.01	1.00
		L4, L5	0.02	1.00
	Cananéia P2	L1, L2	2.95	0.11
		L1, L3	1.34	0.40
		L1, L4	0.23	0.61
		L1, L5	1.34	0.38
		L2, L3	1.79	0.22
		L2, L4	2.93	0.10
		L2, L5	1.79	0.19
		L3, L4	1.41	0.40
		L3, L5	0.00	0.76
		L4, L5	1.41	0.39
	Guaratuba P1	L1, L2	1.22	0.59
		L1, L3	0.43	0.81
		L1, L4	0.10	0.89
		L1, L5	0.92	0.49
		L2, L3	0.85	0.62
		L2, L4	0.18	0.81
		L2, L5	0.61	0.48
		L3, L4	1.03	0.89
		L3, L5	0.33	0.70
		L4, L5	0.84	0.68
	Guaratuba P2	L1, L2	0.84	0.52
		L1, L3	0.75	0.62
		L1, L4	1.05	0.40
		L1, L5	7.59	0.10
		L2, L3	1.21	0.51
		L2, L4	1.41	0.29
		L2, L5	6.15	0.10
		L3, L4	0.39	1.00
		L3, L5	2.46	0.09
		L4, L5	1.39	0.50
	Una do Prelado P1	L1, L2	0.41	0.71
		L1, L3	1.46	0.42
		L1, L4	1.14	0.49
		L1, L5	0.87	0.50
		L2, L3	1.05	0.49
		L2, L4	0.56	0.70
		L2, L5	0.42	0.79
		L3, L4	0.74	0.59
		L3, L5	0.68	0.72
		L4, L5	0.06	1.00
	Una do Prelado P2	L1, L2	1.18	0.29
		L1, L3	1.20	0.40
		L1, L4	0.76	0.63
		L1, L5	0.06	1.00
		L2, L3	3.28	0.10
		L2, L4	2.76	0.09
		L2, L5	0.81	0.73
		L3, L4	0.69	0.49
		L3, L5	0.94	0.74
		L4, L5	0.61	0.69