Benthic macrofaunal structure and secondary production in tropical estuaries on the Eastern Marine Ecoregion of Brazil

Lorena B Bissoli [1] , **Angelo F Bernardino** [Corresp. 1]

[1] Department of Oceanography, Universidade Federal do Espírito Santo, Vitoria, ES, Brazil

Corresponding Author: Angelo F Bernardino
Email address: angelo.bernardino@ufes.br

Estuaries are highly productive and support diverse benthic assemblages, but few estimates of benthic production are available for most ecosystems. In tropical estuaries mangroves and tidal flats are typical habitats with high spatial heterogeneity of benthic macrofaunal assemblages. However, accessing differences and similarities of benthic assemblages within estuarine habitats and between regional ecosystems may provide scientific support to management of those ecosystems. Here we studied three tropical estuaries in the Eastern Marine Ecoregion of Brazil to assess the spatial variability of benthic assemblages from vegetated (mangroves) and unvegetated (tidal flats) habitats. A nested sampling design was used to determine spatial scales of variability in benthic macrofaunal density, biomass and secondary production. Habitat differences in benthic assemblage composition, biomass, density and secondary production were significant, but also varied between estuaries. Macrofaunal secondary production differed between estuaries and between tidal flat and mangrove habitats, and those differences were related to the composition of benthic assemblages. High benthic production were associated with tidal flats in estuaries with presumable less human impacts, although benthic assemblages from mangrove sediments had similar production irrespective of human disturbances. Given variable levels of human impacts and predicted climate change effects on tropical estuarine assemblages in Eastern Brazil, our data support the use of benthic secondary production to address long-term changes and improved management of estuaries in Eastern Brazil.

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Lorena Bonno Bissoli[1], Angelo Fraga Bernardino[1*]

1. Grupo de Ecologia Bêntica, Departamento de Oceanografia, Universidade Federal do Espírito Santo, CCHN, Av. Fernando Ferrari, 514, Goiabeiras, Vitória, ES, Brazil, 29075910

*Corresponding author
Angelo Bernardino
angelo.bernardino@ufes.br

ABSTRACT

Estuaries are highly productive and support diverse benthic assemblages, but few estimates of benthic production are available for most ecosystems. In tropical estuaries mangroves and tidal flats are typical habitats with high spatial heterogeneity of benthic macrofaunal assemblages. However, accessing differences and similarities of benthic assemblages within estuarine habitats and between regional ecosystems may provide scientific support to management of those ecosystems. Here we studied three tropical estuaries in the Eastern Marine Ecoregion of Brazil to assess the spatial variability of benthic assemblages from vegetated (mangroves) and unvegetated (tidal flats) habitats. A nested sampling design was used to determine spatial scales of variability in benthic macrofaunal density, biomass and secondary production. Habitat differences in benthic assemblage composition, biomass, density and secondary production were significant, but also varied between estuaries. Macrofaunal secondary production differed between estuaries and between tidal flat and mangrove habitats, and those differences were related to the composition of benthic assemblages. High benthic production were associated with tidal flats in estuaries with presumable less human impacts, although benthic assemblages from mangrove sediments had similar production irrespective of human disturbances. Given variable levels of human impacts and predicted climate change effects on tropical estuarine assemblages in Eastern Brazil, our data support the use of benthic secondary production to address long-term changes and improved management of estuaries in Eastern Brazil.

KEYWORDS: Macrofauna, Secondary production, Estuaries, Mangroves, Tidal flats.

INTRODUCTION

Estuaries are productive ecosystems that commonly support large densities and biomass of benthic organisms (Kennish 2002). The benthic macrofauna has an important role on estuarine productivity through sediment bioturbation, trophic linkages and facilitating biogeochemical processes (Ysebaert et al. 1998, Herman et al. 1999, Nilsen et al. 2006; Kristensen & Kostka 2005, Kristensen 2008, Kristensen et al. 2014). Given the strong linkage between benthic dynamics and estuarine ecosystem functioning, spatial and temporal changes in sediment composition and organic matter between estuarine habitats are of interest to understand ecosystem productivity (Edgar & Barrett 2002, Kristensen et al. 2014, Morais et al. 2016).

Spatial patterns of macrofaunal assemblages reflect a number of stressors that act at a number of spatial scales (Edgar & Barrett 2002, Barros et al. 2008, Blanchet et al. 2014, Giménez et al. 2014). Sediment grain size, organic matter quality and quantity, plant cover, and disturbance (e.g. hidrology) are frequently associated with macrofaunal spatial heterogeneity in estuaries. Spatial changes in macrofaunal assemblages that occur between vegetated and unvegetated estuarine habitats have also been previously quantified in some areas (Lana & Guiss 1991, Netto & Lana 1997, Checon et al. 2017; Bernardino et al., 2018). Although an increased abundance, biomass and production of macrofauna has been reported for estuarine vegetated habitats, (Lana & Guiss 1991, Heck et al. 1995, Sheridan 1997, Dolbeth et al. 2007, Kon et al. 2010), patterns of benthic diversity and assemblage composition have been less clearly associated with differences in habitat. Another important and often confusing factor leading to faunal spatial heterogeneity in estuaries is salinity, which has a central role on the structure of benthic assemblages (Ysebaert & Herman 2002, Barros et al. 2012, Mariano & Barros 2014). In tropical estuaries several species exhibit a restricted distribution along the salinity gradient whereas others present an opposite trend (Krull et al. 2014, Mariano & Barros 2014, Morais et al. 2016).

Benthic secondary production is an important ecological parameter to understand ecosystem dynamics as it allows energy flow estimates within ecosystems and represents the formation of community biomass by growth through time (Dolbeth et al. 2005, 2012, Benke 2010). Benthic secondary production is an indicator of both population dynamics (biomass, life span and body-size) and also biotic interactions and environmental variability within ecosystems (Waters & Crawford 1973, Dolbeth et al. 2012). These indicators vary with estuarine environmental changes and therefore influence secondary production. For example, temperature can influence growth rates and reproduction, leading to an increase in production in warmer waters (Tumbiolo & Downing 1994). So, changes in water temperature, nutrient and oxygen availability, and also habitat heterogeneity including variations in sediment grain size and vegetation are likely to have an effect on secondary production (Edgar et al. 1994, Heck et al. 1995, Edgar & Barrett 2002, Dolbeth et al. 2003, Rodrigues et al. 2006).

Benthic secondary production can be used to represent the functional responses of fauna subjected to long-term environmental and local anthropogenic impacts (Benke 2010, Dolbeth et al. 2012). For example, eutrophication affects production in different ways where nutrient enrichment can promote algal blooms that temporarily enhance macrofauna production and abundance. On the other hand, post-bloom periods can cause collapse of the system and decline of community production (Kennish 2002, Dolbeth et al. 2003, Dolbeth et al. 2012). In addition, eutrophication can also lead to hypoxia events or increase its extent and severity, producing an adverse effect in benthic biomass and production that can have negative consequences for the whole system (Sturdivant et al. 2014). Climate change is also a concern, as it can increase the frequency and intensity of extreme climate events, including rises in temperature and events of floods or droughts (Dolbeth et al. 2011).

The spatial patterns of secondary production in mangroves and unvegetated estuarine tidal flats are largely unknown, especially for tropical estuaries (Alongi 2002, Lee 2008). In South America, although the Brazilian coast has hundreds of estuarine systems, benthic production has only been evaluated on epibenthic assemblages (i.e. crabs and gastropods) on the Amazon Ecoregion, or focused on specific populations in some localities (Pagliosa & Lana 2000, Koch & Wolff 2002, Costa & Soares-Gomes 2015, Bernardino et al. 2016). However, secondary production in temperate estuaries followed long-term changes in temperature and productivity (Dolbeth et al. 2011), suggesting that benthic assemblages may also be used to monitor tropical estuaries.

Given the increasing human and climatic impacts on estuarine ecosystems, understanding spatial patterns of estuarine benthic secondary production may be invaluable to monitoring and conservation of these ecosystems (Alongi 2002, Kennish 2002). This study investigated benthic secondary production, biomass and density at variable spatial scales in vegetated and unvegetated habitats from three tropical estuaries on the Eastern Brazil Marine Ecoregion. We tested the hypothesis that spatial variations in benthic communities occurs between vegetated and unvegetated habitats (scales of habitat) and among estuaries (scales of estuary). We expected to find higher production of benthic fauna within mangrove forests in response to higher organic availability and higher faunal biomass when compared to unvegetated tidal flat habitats.

MATERIAL & METHODS

Study area

The study was carried out in three tropical estuaries with a microtidal, semidiurnal tides within the Eastern Brazil Marine Ecoregion (Spalding et al. 2007; Fig. 1). The northernmost estuary, Piraquê-Açu-Mirim estuary (PAE; 19°57’S 40°09’W) is within a municipal conservation unit and covered by extensive and well-developed mangrove forests with an area of over 19 km^2 (Servino et al. in review). The Vitória Bay estuarine system (VIB; 20º16’S 40º20’W) is the largest estuary on the region with approximately 18 km^2 of mangrove forests and surrounded by a densely populated metropolitan area with high levels of sewage input and industrial activities (Jesus et al. 2004). The southernmost estuary, Benevente estuary (BEN, 20º48'S 40º39'W), has well preserved mangrove forests that cover an area of approximately 4.6 km^2 with minor urban settlement (Pereira et al. 2009, Petri et al. 2011). Mangrove forests of the three estuaries are composed by *Rhizophora mangle*, *Laguncularia racemosa* and *Avicennia schaueriana* species.

Sampling and sample processing

Benthic macrofaunal assemblages were sampled on a nested spatial design on vegetated (V - mangrove forests) and unvegetated (NV - tidal flats) habitats on the mesohaline sectors (Area 1 - A1; salinity range between 18 and 5; Venice System 1958) of the three estuaries (VIB, PAE and BEN, ICMBIO permit N 24700-1). We also sampled on the polyhaline sector (Area 2 - A2; salinity range between 30 and 18; Venice System 1958) in two of the estuaries (PAE and BEN), in order to test for larger scale variability. Salinity sectors in the sampled estuaries were measured with either a multiparameter or with semi-continuous (5-20 days) deployment of data-loggers (Bernardino et al. unpubl. data). Sampling occurred in one sampling event in each estuary between August and September 2014, during low tides and on the dry season. PAE and BEN estuaries were sampled in two areas (separated by at least 1 km) in different salinity sectors. Each area was divided in two sites distanced in the scale of hundreds of meters containing adjacent vegetated and unvegetated habitats (Appendix Fig. A1). Three sampling plots were randomly established in each habitat and site, parallel to the waterline and separated by tens of meters. Three replicate faunal samples were sampled within each plot, distanced by approximately 1 meter from each other using a PVC corer with 15 cm

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diameter (0.0177 m^2 area) and to a sediment depth of 10 cm. VIB was sampled only in the mesohaline sector following the same sampling design of site, habitat, plot and replicate. Additionally, one composite sediment sample was collected at each plot for sediment analysis (grain size, total organic matter and chlorophyll-a), by mixing three samples of 7 cm diameter and 5 cm depth. Superficial water temperature and salinity were measured in each sampling area.

Faunal samples were preserved in 4% formalin and posteriorly washed through a 1 mm sieve and the retained material was stored in 70% ethanol. In the laboratory, samples were sieved through a stacked series of sieves (1, 1.4, 2, 2.8 and 4 mm), using the methods described by Edgar (1990a). Macrofauna was sorted in each sieve size and identified at family level, considering that this level of identification is satisfactory to identify differences in macrofaunal assemblages (Warwick 1988, Chapman 1998, Olsgard et al. 1998). During sorting of samples, the plant material was separated for plant biomass (plant detritus and living roots) determination (dry weight) after drying at 60°C during 48 hours.

Sediment subsamples were treated with hydrogen peroxide (H_2O_2), to eliminate organic matter, and mud content was determined by wet sieving samples through a 0.063 mm mesh size. After drying, the sediment >0.063 mm was sieved through a series of sieves and grain size was classified following the Wentworth scale (Suguio 1973). Sediment total organic matter (TOM) content was estimated by weight loss after combustion at 500ºC during 4 hours. Chlorophyll-*a* (Chl-*a*) and phaeopigments were extracted from the sediment with acetone and analyzed using a spectrophotometer before and after acidification with HCl (Lorenzen 1967, Quintana et al. 2015).

Faunal biomass and secondary production

After identification, macrofauna was wet weighed within each taxonomic group, generally family, by each sieve size (1, 1.4, 2, 2.8 and 4 mm) after identification. Macrofaunal biomass (mg wet weight m^{-2}) was converted into ash-free dry weight (mg AFDW m^{-2}) using the conversion factors compiled in Brey (2001) and Brey et al. (2010). Shells of mollusks were excluded from biomass estimation. Conversion factors from Brey (instead of estimate by methodology used by Edgar (1990a)) were chosen to avoid overestimation of AFDW and consequently of production, mainly in the larger sieve size, since some individuals with elongated bodies and low weights were retained in the sieves.

The secondary production of benthic macrofauna was estimated using the general equation $P = 0.0049*B^{0.80}*T^{0.89}$ of Edgar (1990a), which relates daily macrobenthic production P (µg day^{-1}) to ash-free dry weight B (µg) and water temperature T (°C). Temperature was standardized at 23.5°C, which was the mean water temperature measured in the estuaries during faunal sampling. Production was calculated for each taxon (Polychaeta, Oligochaeta, Kalliapseudidae, Other Crustacea, Mollusca and Others) within each sieve size and total production per sample was calculated as the sum of these values. The annual production to biomass ratio (P/B) for each habitat in each estuary was calculated from mean production divided by the mean macrofaunal biomass. This parameter can be considered a measurement of biomass turnover rate (Dolbeth et al. 2012).

Data analysis

The spatial variability in benthic macrofaunal density, biomass and secondary production were evaluated at multiple spatial scales in different salinities and habitats using a nested and orthogonal analysis of variance (ANOVA). Habitat was defined as a fixed factor and orthogonal to spatial factors (estuary, area, site, plot). Spatial factors were treated as random and included three estuaries, areas (N=2) nested in estuary, sites (N=2) nested in area, plots (N=3) nested in site and samples (N=3) collected at plots. Spatial differences on sediment properties and plant biomass were assessed by ANOVA across scales of estuary, area (nested in estuary) and site (nested in area), due to the lack of sample replication at plots. This ANOVA also included habitat factors orthogonal to spatial factors since both vegetated and unvegetated habitats were sampled. Two different ANOVAs were performed for macrofaunal density, biomass and secondary production and for each sediment property. One ANOVA compared only the mesohaline sector (A1) of the three estuaries, while the other ANOVA compared the two areas (A1 and A2) of the estuaries of BEN and PAE. A Cochran's test was performed previously to each ANOVA to assess homogeneity of variances and when necessary data was transformed. A posteriori Student-Newman-Keuls (SNK) tests were applied on the factors or interactions significantly different in ANOVA to determine the differences.

Differences on macrofaunal assemblages were assessed by three Permutational Multivariate Analysis of Variance (9999 permutations, Anderson et al. 2008). The first PERMANOVA compared only the mesohaline sector (A1) of the three estuaries. The others PERMANOVAs compared the mesohaline and the polyhaline sectors (A1 and A2) of the BEN and PAE estuaries, each estuary separately. A non-metric multidimensional scaling (nMDS) performed using Bray-Curtis dissimilarity matrix and square-root transformed data was used to visualize variation in macrofauna assemblages. A Similarity Percentage analysis (SIMPER) was used to identify the taxa that most contributed to dissimilarities among habitats. The relationships between sediment properties (TOM, Chl-*a*, Mud, plant biomass) and density of macrofauna were investigated using a Canonical Correspondence Analysis (CCA). In this analysis, the density of the top 5 dominant taxa (comprising over 90% of total density) was used, instead of complete data of density. And also in this analysis, the sum of density data of macrofauna replicates samples was used so that the number of samples from density and sediment properties was the same. All statistical analyses were performed in the software R (R Core Team 2015). PERMANOVA was carried out using the software PRIMER 6 with the PERMANOVA+ add-on package (Clarke & Gorley 2006, Anderson et al. 2008).

RESULTS

Sediment properties and plant material

The sediment was predominantly composed of mud in all estuaries and habitats (Fig. 2). When comparing only the area 1 of the three estuaries, the sediment mud content, mean grain size and total organic matter differed significantly among sites and in the interaction between habitat and site (Table 1a). These results represent spatial variation at local scales. Chl-*a* and phaeopigments differed significantly between habitats and estuary, respectively, with higher sediment Chl-*a* at unvegetated habitats (SNK, $p < 0.05$) and lower phaeopigments in the BEN estuary (SNK, $p < 0.001$; Table 1a, Fig. 2). Plant biomass differed significantly among estuaries and in the interaction between habitat and site (Table 1a). VIB presented over 2 times higher total plant

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biomass (4217 ± 3097.6 $g.m^{-2}$) when compared to similar sectors in the BEN and PAE estuaries (592 ± 516.5 $g.m^{-2}$ and 1663.2 ± 1206.2 $g.m^{-2}$, respectively).

Comparing the two areas of BEN and PAE estuaries, significant differences in the sediment mean grain size, mud content, total organic matter and plant biomass were found among sites and in the interaction between habitat and site (Table 1b). Total plant biomass also presented significant differences between estuaries, and plant biomass in PAE (1555 ± 1323.7 $g.m^{-2}$) was higher than BEN (579.7 ± 521.5 $g.m^{-2}$; SNK, $p < 0.05$). Chl-*a* differed significantly in the interaction between habitat and area, with the area 2 (mesohaline sector) with less Chl-a than area 1 in PAE and BEN (SNK, $p < 0.05$). Phaeopigments differed at the estuary scale, with higher values in PAE when compared to BEN (SNK, $p < 0.01$).

Macrofaunal density, biomass and secondary production

A total of 23,833 individuals belonging to 46 taxa were sampled at the three estuaries, of which 12,266 individuals were Kalliapseudidae (Tanaidacea). BEN estuary had a total of 16,389 individuals, distributed in 31 taxa. In PAE estuary 2,617 individuals were collected and distributed in 34 taxa. VIB had a total of 4,827 individuals, distributed in 23 taxa. Within the mesohaline sector (A1) of the three estuaries, total macrofaunal density was significantly different at the plot and estuary spatial scales, and in their interactions with habitat (Table 2a). The BEN estuary presented higher macrofaunal density in unvegetated habitats (SNK, $p < 0.01$), but this pattern was opposite to the VIB and PAE estuaries that had higher densities in vegetated habitats, although, the differences were not statistically significant (Fig. 3a). Macrofaunal densities varied over 40-fold between unvegetated habitats in area 1 at BEN and PAE estuaries (33,022 ± 14,709 ind.m^{-2} and 784 ± 641 ind.m^{-2}, respectively; Fig. 3a). Kalliapseudidae (Tanaidacea) was dominant in unvegetated tidal flats in the mesohaline sector at BEN estuary, whereas Polychaeta and Oligochaeta were more abundant in similar habitats at PAE and VIB estuaries. Vegetated habitats in the three estuaries had higher densities of Oligochaeta and Polychaeta. However, BEN presented high densities of Kalliapseudidae in the polyhaline sector (A2) in the vegetated habitat (Fig. 4a).

Significant differences in macrofaunal biomass and estimated secondary production were observed only in the interaction between habitat and estuary when comparing the mesohaline sector of the three estuaries (Table 2a). Biomass and production followed patterns of macrofaunal density and were higher at unvegetated habitats in the mesohaline sector at BEN, contrasting with higher values in vegetated habitats at the other two estuaries (SNK, $p < 0.05$; Fig. 3b, c). The lowest macrofaunal biomass (100.7 ± 206.4 mg AFDW m^{-2}) and production (3.4 ± 5.2 mg m^{-2} day^{-1}) were observed at unvegetated tidal flats in the PAE estuary (Fig. 3b, c).

Macrofaunal density was also significant different in the interactions between habitat and site and between habitat and plot when comparing the mesohaline and polyhaline sectors (A1 and A2) at BEN and PAE estuaries (Table 2b). Macrofaunal biomass and secondary production had significant differences in the scales of site and in the interaction between habitat and site, and secondary production also differed significantly among estuaries and in its interaction with habitat (Table 2b), with higher total macrofaunal secondary production in BEN (77.4 ± 69.3 mg m^{-2} day^{-1}) than in PAE (25.1 ± 62 mg m^{-2} day^{-1}; SNK $p < 0.05$).

The contribution from each macrofaunal group to total assemblage biomass and secondary production varied greatly between estuaries and habitats (Fig. 4). Large individuals including Bivalve molluscs and Brachyuran crabs contributed greatly to benthic biomass and production in vegetated habitats at the three estuaries despite their low density (Figs. 4 & 5). At vegetated habitats in VIB estuary, Mollusca (mainly Mytilidae and Solecurtidae) contributed to most of the biomass (1832.5 ± 2780.5 mg AFDW m^{-2}) and production (28.3 ± 37.8 mg m^{-2} day^{-1}; 65% and 48%, respectively), with Oligochaeta and Polychaeta representing second and third groups respectively. At vegetated habitats in the mesohaline sector of the PAE estuary, Mollusca (mainly Mytilidae; 2864.6 ± 8115.6 mg AFDW m^{-2}, 35.1 ± 82.9 mg m^{-2} day^{-1}) and Crustacea (mainly Panopeidae; 1199.4 ± 4331.9 mg AFDW m^{-2}, 15.6 ± 49.3 mg m^{-2} day^{-1}) were the most representative groups in biomass and production. Crustaceans (mainly Ocypodidae; 1897.8 ± 3682.9 mg AFDW m^{-2}, 28.3 ± 46.5 mg m^{-2} day^{-1}) contributed to over 70% of the macrofaunal biomass and production in vegetated habitats of the mesohaline sector at the BEN estuary with Polychaeta as the second group.

At the polyhaline sector in vegetated habitats at BEN, Crustacea (mainly Panopeidae; 1620.4 ± 3717.0 mg AFDW m^{-2}, 23.1 ± 47.3 mg m^{-2} day^{-1}) contributed to most of the biomass and secondary production (63% and 49%, respectively), with Kalliapseudidae (434.2 ± 646.2 mg AFDW m^{-2}, 11.7 ± 15.4 mg m^{-2} day^{-1}) and Mollusca representing second and third groups respectively. At vegetated habitats in the polyhaline sector of the PAE estuary, Crustacea (mainly Ocypodidae; 1793.3 ± 4431.3 mg AFDW m^{-2}, 24.0 ± 53.1 mg m^{-2} day^{-1}) was the most representative group in biomass (93%) and production (80%), with Polychaeta and Oligochaeta as second and third groups respectively.

In general, macrofaunal biomass and production of estuarine habitats were mainly derived from large size classes (Fig. 5b, c). Vegetated habitats had over 70% of its production from large size classes (> 4 mm), whereas unvegetated habitats had more variable contribution (40-85%) of the other size classes from 1 to < 4 mm (Fig. 5b, c). At unvegetated habitats in the mesohaline sector of VIB (329.4 ± 759.3 mg AFDW m^{-2}, 6.6 ± 12.6 mg m^{-2} day^{-1} of Mollusca) and PAE (51.3 ± 193.2 mg AFDW m^{-2}, 1.3 ± 4.2 mg m^{-2} day^{-1} of Mollusca) estuaries, Mollusca (mainly Solecurtidae) and Polychaeta (mainly Capitellidae) contributed significantly to total macrofaunal biomass and production (Fig. 4b, c). In the polyhaline sector of the PAE, Polychaeta (mainly Magelonidae and Goniadidae; 38.5 ± 38.2 mg AFDW m^{-2}, 1.6 ± 1.5 mg m^{-2} day^{-1}) contributed most to total biomass and production in unvegetated habitats (over 75%, Fig. 4b, c). Kalliapseudidae was the dominant taxa at unvegetated habitats in BEN estuary (7315.7 ± 5343.6 mg AFDW m^{-2}, 126.8 ± 86.8 mg m^{-2} day^{-1} in the mesohaline sector; 4191.2 ± 3303.8 mg

AFDW m^{-2}, 79.7 ± 54.8 mg m^{-2} day^{-1} in the polihaline sector) and contributed greatly to biomass and production (over 90%; Fig. 4b, c).

The mean estimated community annual production to biomass ratio (P/B) varied among estuaries and habitats. The highest P/B ratio was observed at unvegetated habitats at PAE estuary (12.6 and 15 y^{-1}, for A1 and A2 respectively), whereas vegetated habitats in this estuary had the lowest P/B ratio (5.3 and 5.7 y^{-1}, for A1 and A2 respectively). P/B ratios did not vary significantly between habitats or areas at BEN (6.4 and 6.6 y^{-1} in L1 for V and NV; 6.7 and 7.1 y^{-1} in L2, for V and NV habitats respectively) and VIB estuaries (7.5 y^{-1} and 9.3 y^{-1} for V and NV habitats respectively).

Assemblage composition

Macrofaunal assemblages differed markedly between vegetated and unvegetated habitats and between estuaries (Table 3). The numerically dominant taxa in vegetated habitats in the three estuaries were Oligochaeta and Capitellidae (>60%), except in the polyhaline sector at BEN where Kalliapseudidae was also dominant. In the unvegetated habitats the numerically dominant taxa were more variable among the estuaries. At BEN estuary Kalliapseudidae and Oligochaeta (>98%) were dominant in the mesohaline sector and Kalliapseudidae and Capitellidae (98%) were dominant in the polyhaline sector. In unvegetated habitats at VIB Spionidae and Capitellidae (>80%) were more abundant, whereas at PAE estuary Capitellidae and Oligochaeta (almost 70%) dominated in unvegetated habitats in the mesohaline sector and Capitellidae and Magelonidae in the polyhaline sector. Although differences among the dominant taxa between unvegetated habitats at BEN, VIB and PAE, all three estuaries had most taxa shared between them.

The macrofaunal assemblage composition was significantly different in several spatial scales within the mesohaline sector of the three estuaries (PERMANOVA; Table 4). These significant differences occurred in the interaction among habitat and all the spatial scales analyzed (estuary, site and plot) and the spatial scales within estuaries (site and plot). Comparing areas 1 and 2 in BEN, significant differences in the macrofaunal assemblage composition occurred in the scale of site and plot and in their interactions with habitat (PERMANOVA; Table 5). At the PAE estuary, the significant differences occurred only in the interaction among habitat and plot (PERMANOVA; Table 5). Faunal distribution patterns in nMDS ordination evidenced differences between unvegetated and vegetated habitats in all the estuaries and also evidenced the differences in the samples within each habitat, mainly in unvegetated habitats (Fig. 6). Macrofaunal assemblages at unvegetated habitats had lower similarity among estuaries, if compared to vegetated habitats, which were more similar among estuaries. However, samples from Area 2 in the vegetated habitat in BEN estuary, where Kalliapseudidae was dominant, created a group of samples closer to the samples from unvegetated habitat. This was the main difference noticed between areas in the macrofaunal assemblages in the MDS, where the similarity between areas was evidenced as no clear spatial differences between samples from different areas were found. This vision of the samples reinforces the PERMANOVA results, where no significant differences occurred between areas.

Dissimilarities were high (>60%) between habitats inside each estuary and among estuaries in the unvegetated habitat (SIMPER). Kalliapseudidae, Oligochaeta, Capitellidae and Ampharetidae were the taxa that most contributed to the observed differences among habitats in the mesohaline sector of BEN (SIMPER; Appendix Table A1). At VIB and PAE, Oligochaeta, Spionidae, Capitellidae, Nereididae and Pilargidae were the taxa that most contributed to the observed differences among habitats in the mesohaline sector (SIMPER; Appendix Table A1). The dissimilarity between unvegetated habitats among estuaries within the mesohaline sectors occurred mainly due to differences in abundance of Kalliapseudidae (BEN), Spionidae (VIB) and Oligochaeta (PAE; (SIMPER; Appendix Table A2).

Relationships between sediment properties and macrofaunal assemblages

Macrofaunal assemblages were related to sediment mud content, TOM, plant biomass and Chl-*a*, with the first and second canonical axes explaining 25% and 13.2% of the variation in the data, respectively (CCA; Fig. 7). These relationships also explained the differences in assemblage composition between vegetated and unvegetated habitats. The CCA evidenced differences between habitats and estuaries and three groups of samples were formed in the CCA. The first group was corresponding to unvegetated habitat in VIB, the second group to unvegetated habitats in BEN with some samples from vegetated habitats in the polyhaline sector in this estuary, and the third group was formed by samples from both habitats in PAE, vegetated habitat in VIB and the other samples from vegetated habitat in BEN. Vegetated habitats of the three estuaries were related to higher TOM content, higher plant biomass and to higher densities of Oligochaeta and Capitellidae. Nereididae was also a family with high densities at vegetated habitats in PAE. Unvegetated habitats were more heterogeneous between estuaries, with VIB exhibiting higher Chl-*a* and dominated by Spionidae, whereas at PAE Capitellidae was dominant. At BEN, Kalliapseudidae was abundant at unvegetated sediments and in some samples from vegetated habitats in the polyhaline sector, with high mud content and relative low plant biomass and TOM content.

DISCUSSION

Macrofaunal assemblage composition, abundance and secondary production exhibited different patterns of spatial variability within the three estuaries on the Eastern Brazil Marine Ecoregion. We observed marked differences in macrofaunal densities between the estuaries, but with inconsistent patterns between vegetated and unvegetated habitats. At the BEN estuary, spatial differences included a high dominance of Kalliapseudidae in unvegetated habitats in a similar pattern with subtropical estuaries (Lana & Guiss 1991, Leite et al. 2003, Pagliosa & Barbosa 2006, Pennafirme & Soares-Gomes 2009). However, tanaidaceans were not sampled at the PAE and were very rare at VIB estuaries, suggesting that these tanaidaceans may be occasional opportunists on tidal flats (Nucci et al. 2001, Leite et al. 2003). In contrast to our hypothesis vegetated and unvegetated habitats at PAE and VIB estuaries had statistically similar macrofaunal densities, but supports that abundance is not strictly related to the presence or absence of vegetation (Schrijvers et al. 1995, Sheridan 1997, Yu et al. 1997; Alfaro 2006). As observed elsewhere, macrofaunal densities can be highly variable between estuaries and among estuarine habitats and the macrofaunal abundances from Eastern Brazil estuaries are in the range of values of other tropical and temperate ecosystems (Appendix Table A3).

Macrofaunal assemblage composition had higher similarity within mangrove forests if compared to tidal flat assemblages. Mangrove sediments were composed mainly by Oligochaeta and Capitellidae despite the differences in urbanization among estuaries. These taxa are typically dominant in sediments with high organic content and detritus, and are widely present at other tropical and subtropical mangroves (Schrijvers et al. 1995, Sheridan 1997, Netto & Lana 1999, Dittmann 2001, Netto & Galluci 2003, Demopoulos & Smith 2010). Mangrove derived detritus and sedimentation patterns in nearby sediments can also have indirect effects in the composition and abundance of macrofauna (Netto & Lana 1999, Netto & Galluci 2003; Sweetman et al., 2010; Bernardino et al., 2018).

Macrofaunal estuarine assemblages may change in response to variable levels of disturbance (Lindegarth & Hoskin 2001). The three sampled estuaries have wide differences in ecosystem quality, suggesting that habitat dissimilarity between estuaries were mostly related to local impacts including pollution. For example, Kalliapseudidae was a dominant group in tidal flats of BEN estuary suggesting higher estuarine ecosystem quality (Pagliosa & Barbosa 2006). However, Spionidae and Capitellidae were dominant both in VIB and PAE estuaries. VIB is a heavily polluted region whereas the PAE estuary is located in a conservation area, but still with detectable organic pollutants (Grilo et al. 2013). As a result, the macrofaunal assemblage composition of the three estuaries include a broad range of tolerant (pollution), rare and opportunist taxa in response to multiple ecosystem changes, both natural and human. Given variable levels of local impacts, we could not identify consistent patterns of benthic macrofaunal assemblages from intertidal vegetated and unvegetated habitats as recently observed for subtidal habitats in Eastern Brazil (Barros et al. 2012; Mariano & Barros 2014).

The density and composition of macrofauna varied at small spatial scales within estuaries (among plots and also in the interaction between habitat and plot), evidencing a patchy distribution (Underwood & Chapman 1996, Underwood et al. 2000, Chapman & Tolhurst 2004, Morais et al. 2016). Mean grain size, mud content, TOM and plant biomass also varied at the same spatial scales, and likely influenced macrofaunal assemblages. We did not evidence significant differences in macrofaunal density, biomass and secondary production between salinity sectors in two of the estuaries analyzed. Although salinity is considered a major factor influencing the distribution of organisms and community structure in estuaries (Attrill 2002, Elliott & Whitfield 2011), we observed that local-scale environmental drivers were more significant for our intertidal assemblages. In the present study we did not find a strong evidence for salinity changes over macrofaunal structure and production (Ysebaert et al. 2003), but we only sampled at one period in the year. Higher abundances in oligohaline and mesohaline regions have been reported for both tropical and subtropical estuaries in Brazil and those seasonal differences need to be investigated further (Mariano & Barros 2014, Morais et al. 2016).

In general, estuarine macrofaunal biomass in the Eastern Brazil Marine Ecoregion was comparable to other temperate estuaries (Appendix Table A3). Macrofaunal biomass and secondary production were higher in vegetated habitats in the mesohaline sector at PAE and VIB, suggesting that mangrove forests are an important source of organic material to the local benthic assemblages (Edgar 1990b, Sprung 1994, Heck et al. 1995, Dolbeth et al. 2003; Bernardino et al., 2018). However, habitat structure may also increase benthic biomass and secondary production by creating microhabitats and offering protection from predators (Edgar 1990b, Kon et al. 2010). These differences may be important at regional scales, creating significant changes in benthic secondary production among estuaries. In our study, higher biomass and production at unvegetated habitats occurred due to the high densities of

Kalliapseudidae, which have continuous reproduction and fast growth (Fonseca & D'Incao 2003, Leite et al. 2003, Pennafirme & Soares-Gomes 2009). So, it seems that regional changes in the composition of benthic assemblages may also temporally lead to significant changes in benthic production, and long-term studies could help identify seasonal and inter-annual patterns (Dolbeth et al. 2003).

Macrofaunal biomass and production were driven by variable taxonomic groups and size classes. Unvegetated habitats at BEN estuary had higher biomass and production in both areas given high Kalliapseudidae densities. These tanaidaceans are deposit and suspension feeders and offer direct trophic links to fishes, birds and other crustaceans (Lana & Guiss 1991, Pagliosa & Barbosa 2006, Pennafirme & Soares-Gomes 2009), evidencing its importance to estuarine food webs at BEN. Other mollusks and crustaceans markedly contributed to total biomass and production despite relative low densities in vegetated and unvegetated habitats. Mytilidae contributed to mangrove benthic biomass at VIB and PAE estuaries, and are important human food resources (Nishida & Leonel 1995; Nalesso et al. 2005). Brachyurans were also important to biomass and production of mangrove sediments evidencing their importance as a food source and to overall ecosystem health (Koch & Wolff 2002, Cannicci et al. 2008).

The benthic biomass turnover rate (P/B ratio) was variable between habitats and estuaries. At PAE and VIB estuaries, the P/B ratio was higher or slightly higher in unvegetated habitats suggesting higher turnover rates of benthic production at tidal flats (Edgar et al. 1994, Sprung 1994). The lower P/B ratio in vegetated habitats occurred due to the dominance in biomass and production of bivalves and crustaceans (crabs) that were larger individuals with slow growth rates and longer life spans (Sprung 1994, Edgar & Barrett 2002). At BEN estuary, P/B ratio was relatively similar between tidal flats and mangroves. As higher P/B ratios suggest higher population resilience to environmental perturbations (Tumbiolo & Downing 1994), highly productive estuarine habitats including tidal flats at BEN estuary may indicate target areas for estuarine conservation in Eastern Brazil. Mangroves also provide essential processes and services for estuarine systems and as evidenced in this study, they supporting a similar benthic production within the three estuaries and show great resilience to pollution and other impacts.

The Eastern Brazil Marine Ecoregion is experiencing loss of mangrove forests and climate change impacts to estuaries (Bernardino et al. 2015; Bernardino et al., 2018; Servino et al., in review). As in other estuarine ecosystems, macrofaunal assemblages are highly variable with respect to taxa composition and abundance. However, secondary production, which is a measure of ecosystem function, may provide an important information of ecosystem change that could be used to track ecosystem health (Dolbeth et al. 2011), as our data suggest. The implementation of long-term monitoring series that includes macrofaunal secondary production may increase our understanding of ecosystem functioning in Eastern Brazil and provide management actions towards areas with higher ecosystem quality (Dolbeth et al. 2011, Dolbeth et al. 2012).

CONCLUSIONS

In summary, we found that macrofaunal assemblages varied at multiple spatial scales, between vegetated and unvegetated habitats and among estuaries. Macrofaunal density varied at the scale of individual samples, whereas biomass and secondary production differed between the interaction of habitats and estuary suggesting that estuarine benthic ecosystem functioning varies markedly at regional scales. Mangrove and tidal flat habitats had distinct patterns of production to biomass ratio, evidencing larger individuals with longer time spans at vegetated habitats which may promote higher resilience to environmental perturbations in urban estuaries in Eastern Brazil. Benthic secondary production may offer an alternative metric to evaluate estuarine ecosystem health among estuaries in Eastern Brazil, and should be incorporated in long-term assessments to support management of local impacts and future climate change effects.

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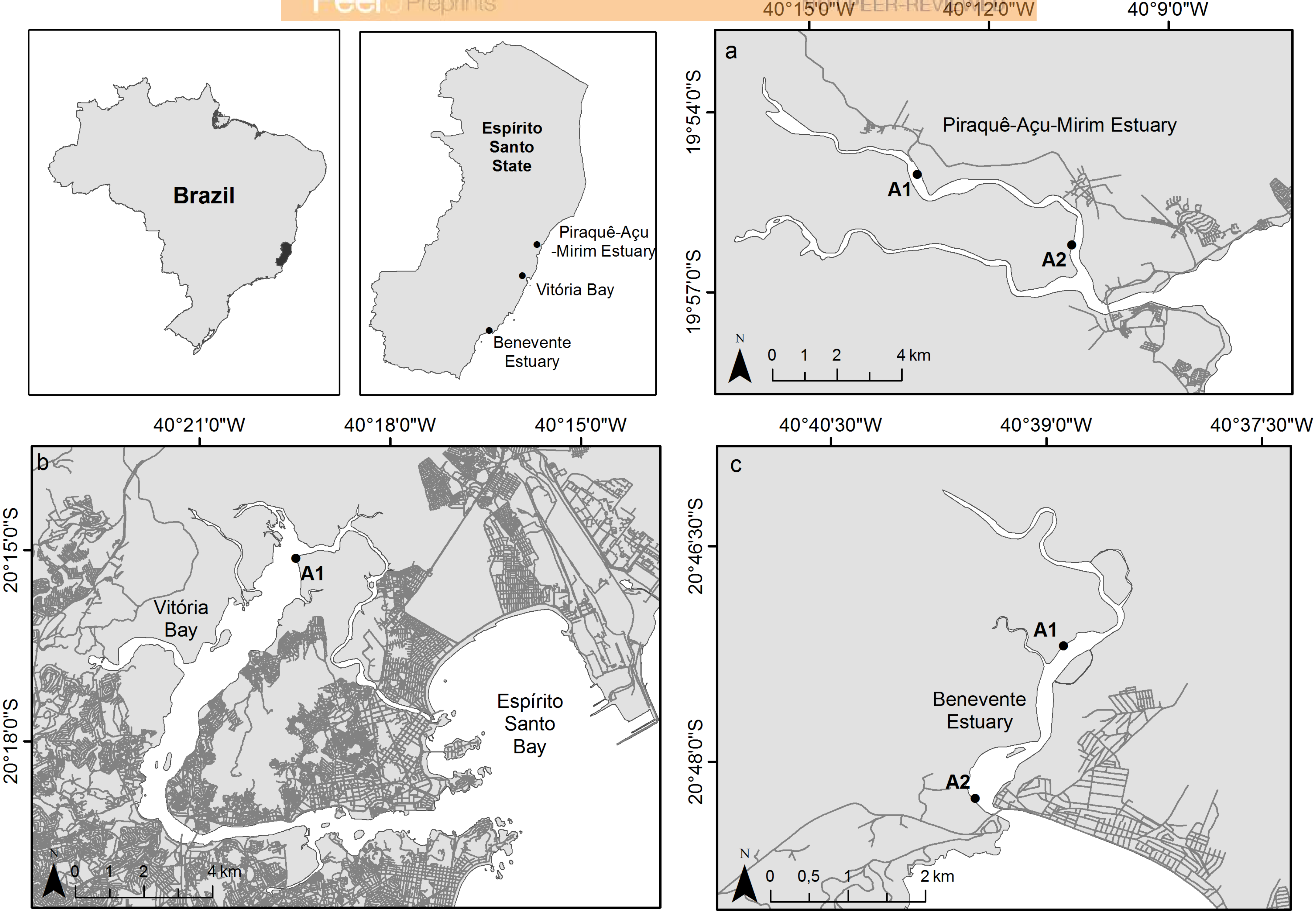
Figure 1(on next page)

Map of study sites

Fig 1 - Study area indicating the three sampled estuaries and study areas. (a) Piraquê-Açu-Mirim estuary, (b) Vitória Bay, (c) Benevente estuary. A1= area 1 (mesohaline sector); A2 = area 2 (polyhaline sector).



Brazil
Espírito
Santo
State
Piraquê-Açu
-Mirim Estuary
Vitória Bay
Benevente
Estuary
40°15'0"W
40°12'0"W
40°9'0"W
a
19°54'0"S
19°57'0"S
Piraquê-Açu-Mirim Estuary
A1
A2
N
0 1 2 4 km
40°21'0"W
40°18'0"W
40°15'0"W
b
20°15'0"S
20°18'0"S
A1
Vitória
Bay
Espírito
Santo
Bay
N
0 1 2 4 km
40°40'30"W
40°39'0"W
40°37'30"W
c
20°46'30"S
20°48'0"S
A1
Benevente
Estuary
A2
N
0 0,5 1 2 km

Figure 2(on next page)

Sediment grain size, TOM, pigments and detritus biomass

Fig 2 - Sediment properties and plant material at sampled estuaries. Means (±SD) of (a) mud content (%), (b) mean grain size (Φ), (c) TOM (%), (d) chlorophyll-a (µg.g-1), (e) phaeopigments (µg.g-1) and (f) plant biomass in DW (dry weight) (g.m-2). V = vegetated habitat, NV = unvegetated habitat, A1 = area 1, A2 = area 2.

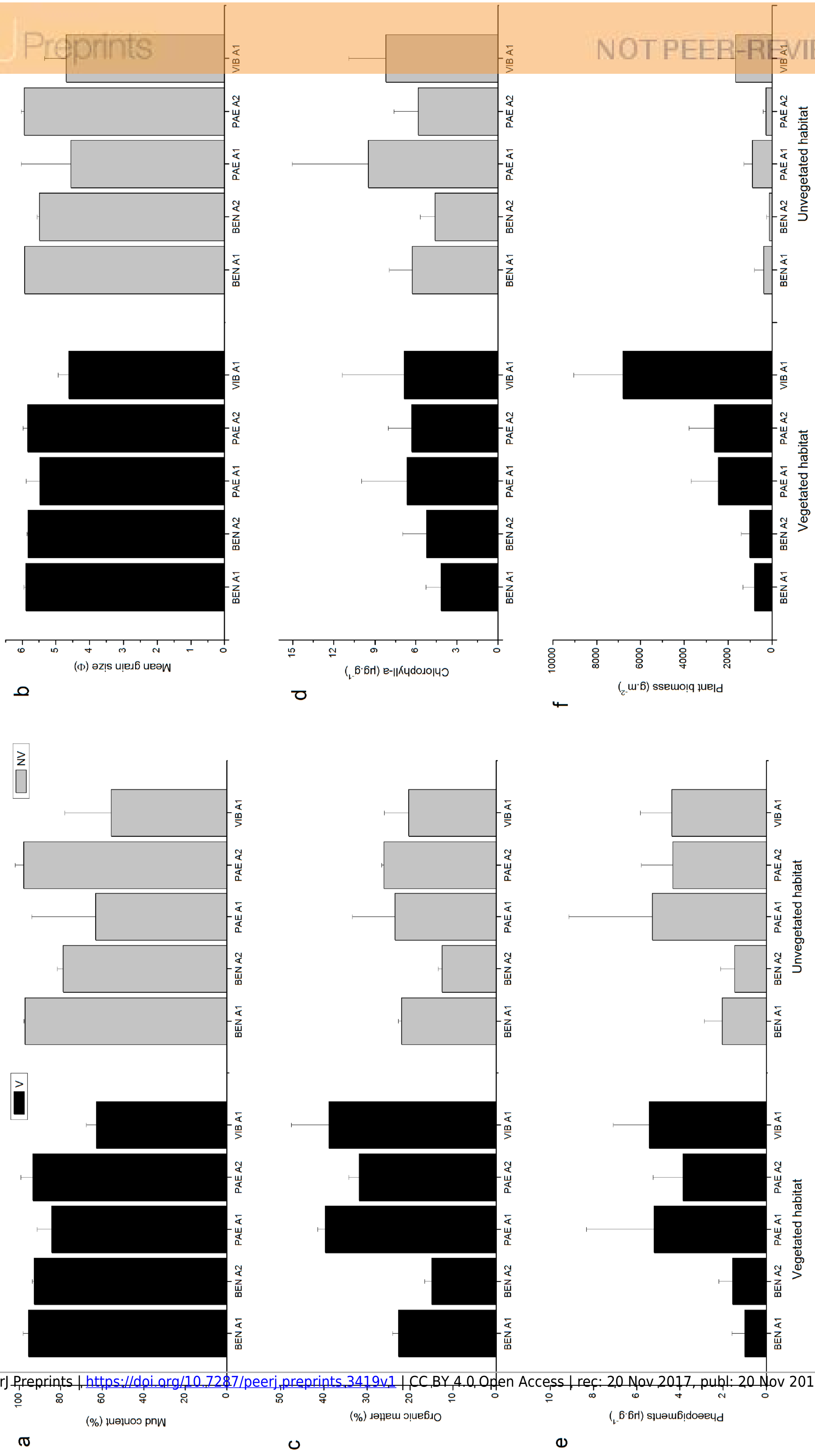

a
b
c
d
e
f
Mud content (%)
Mean grain size (Φ)
Organic matter (%)
Chlorophyll-a (µg.g-1)
Phaeopigments (µg.g-1)
Plant biomass (g.m-2)
V
NV
BEN A1
BEN A2
PAE A1
PAE A2
VIB A1
Vegetated habitat
Unvegetated habitat

Figure 3 (on next page)

Macrofaunal density, biomass and production

Fig 3 - Macrofauna at sampled estuaries. Means (±SD) of macrofaunal (a) density (ind.m-2), (b) biomass (mg AFDW m-2) and (c) production (mg m-2 day-1). V = vegetated habitat, NV = unvegetated habitat, A1 = area 1, A2 = area 2.

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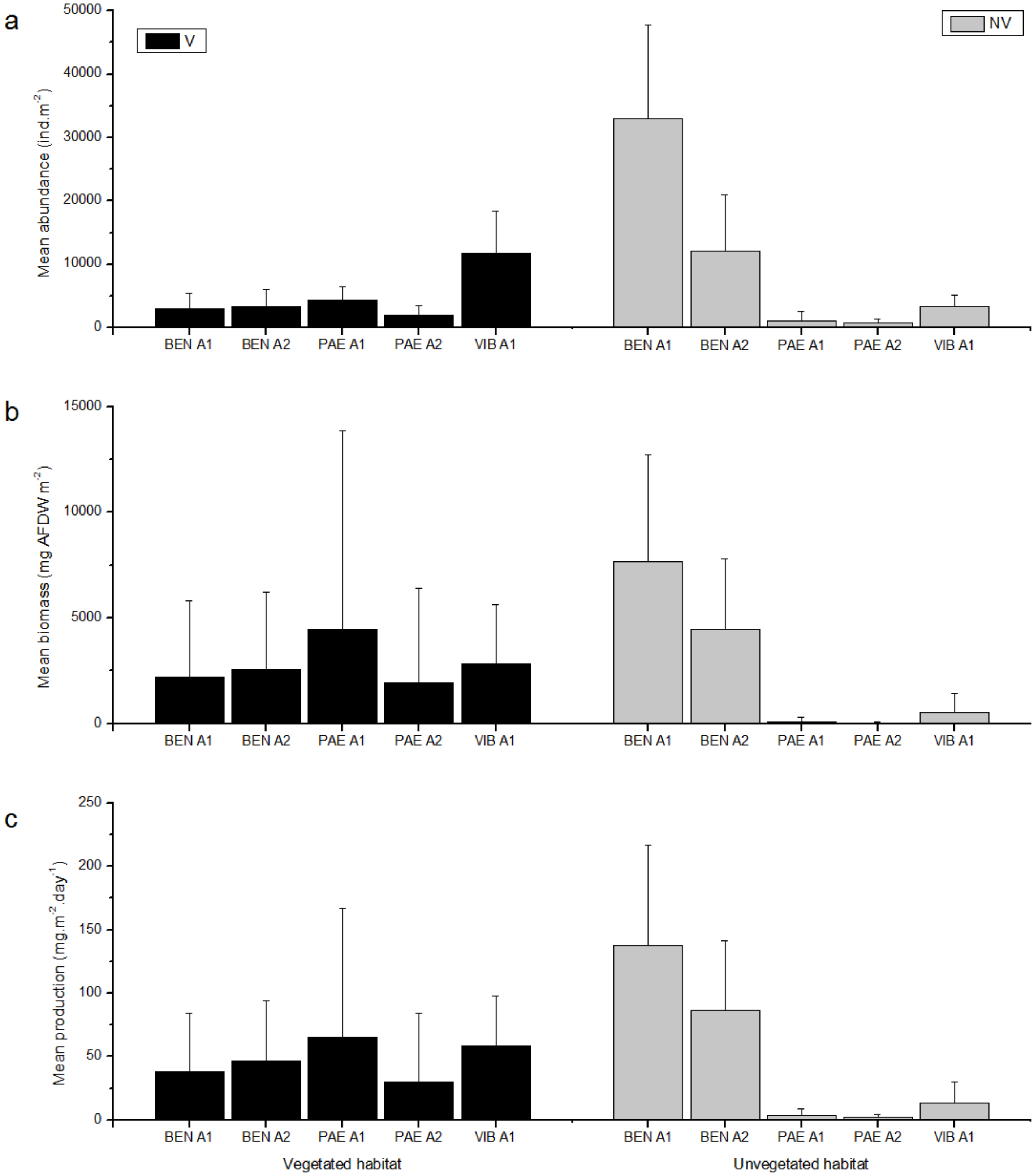
a
b
c
Mean abundance (ind.m⁻²)
Mean biomass (mg AFDW m⁻²)
Mean production (mg.m⁻².day⁻¹)
V
NV
BEN A1
BEN A2
PAE A1
PAE A2
VIB A1
Vegetated habitat
Unvegetated habitat

Figure 4(on next page)

Relative macrofaunal abundance, biomass and production per taxa

Fig 4 - Relative (a) abundance, (b) biomass and (c) production of macrofaunal taxa at sampled estuaries. V = vegetated habitat, NV = unvegetated habitat, A1 = area 1, A2 = area 2.

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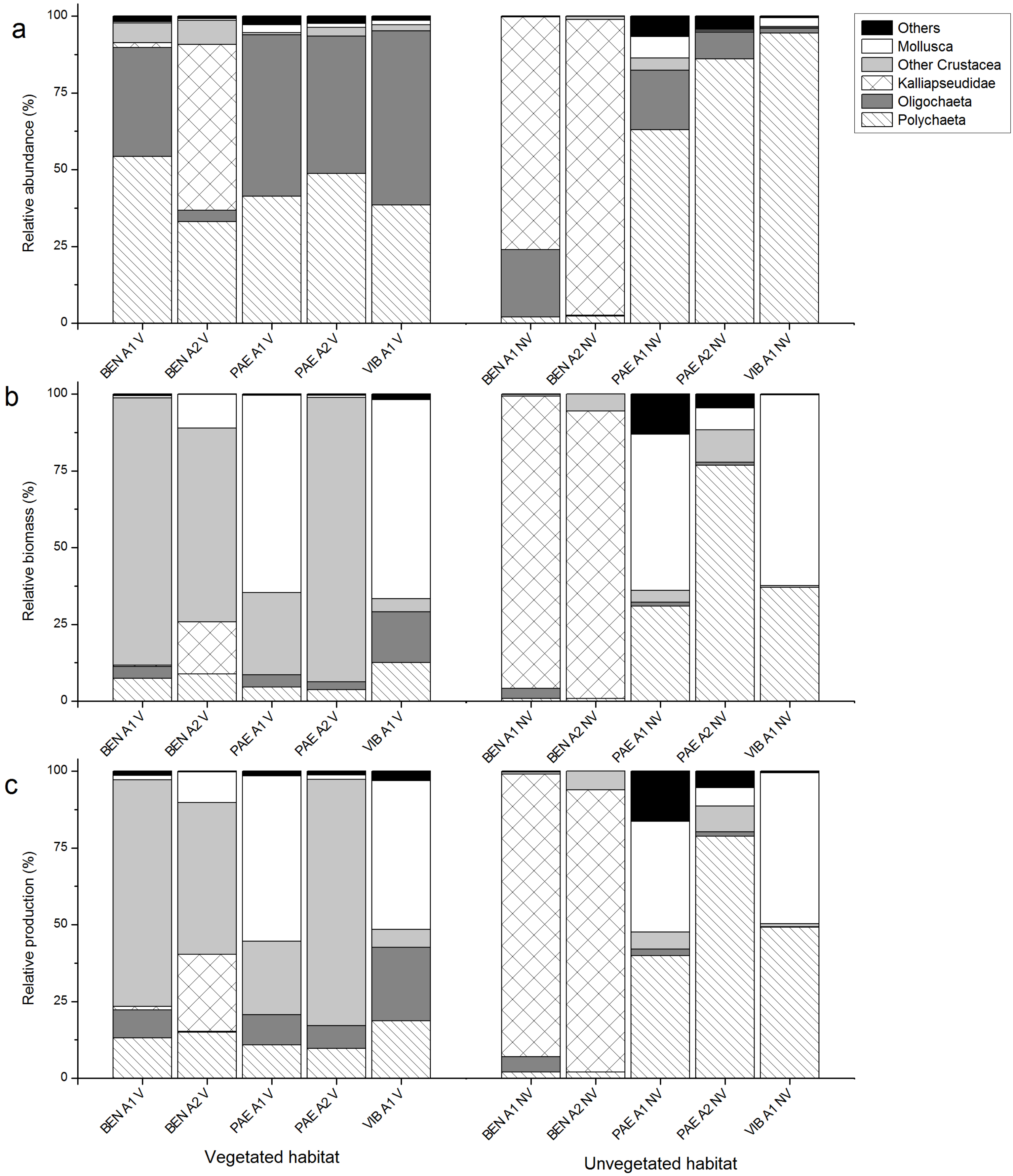
a
b
c
Relative abundance (%)
Relative biomass (%)
Relative production (%)
0
25
50
75
100
Others
Mollusca
Other Crustacea
Kalliapseudidae
Oligochaeta
Polychaeta
BEN A1 V
BEN A2 V
PAE A1 V
PAE A2 V
VIB A1 V
BEN A1 NV
BEN A2 NV
PAE A1 NV
PAE A2 NV
VIB A1 NV
Vegetated habitat
Unvegetated habitat

Figure 5(on next page)

Relative macrofaunal abundance, biomass and production per size classes

Fig 5 - Relative (a) abundance, (b) biomass and (c) production of macrofaunal per size classes at sampled estuaries. V = vegetated habitat, NV = unvegetated habitat, A1 = area 1, A2 = area 2.

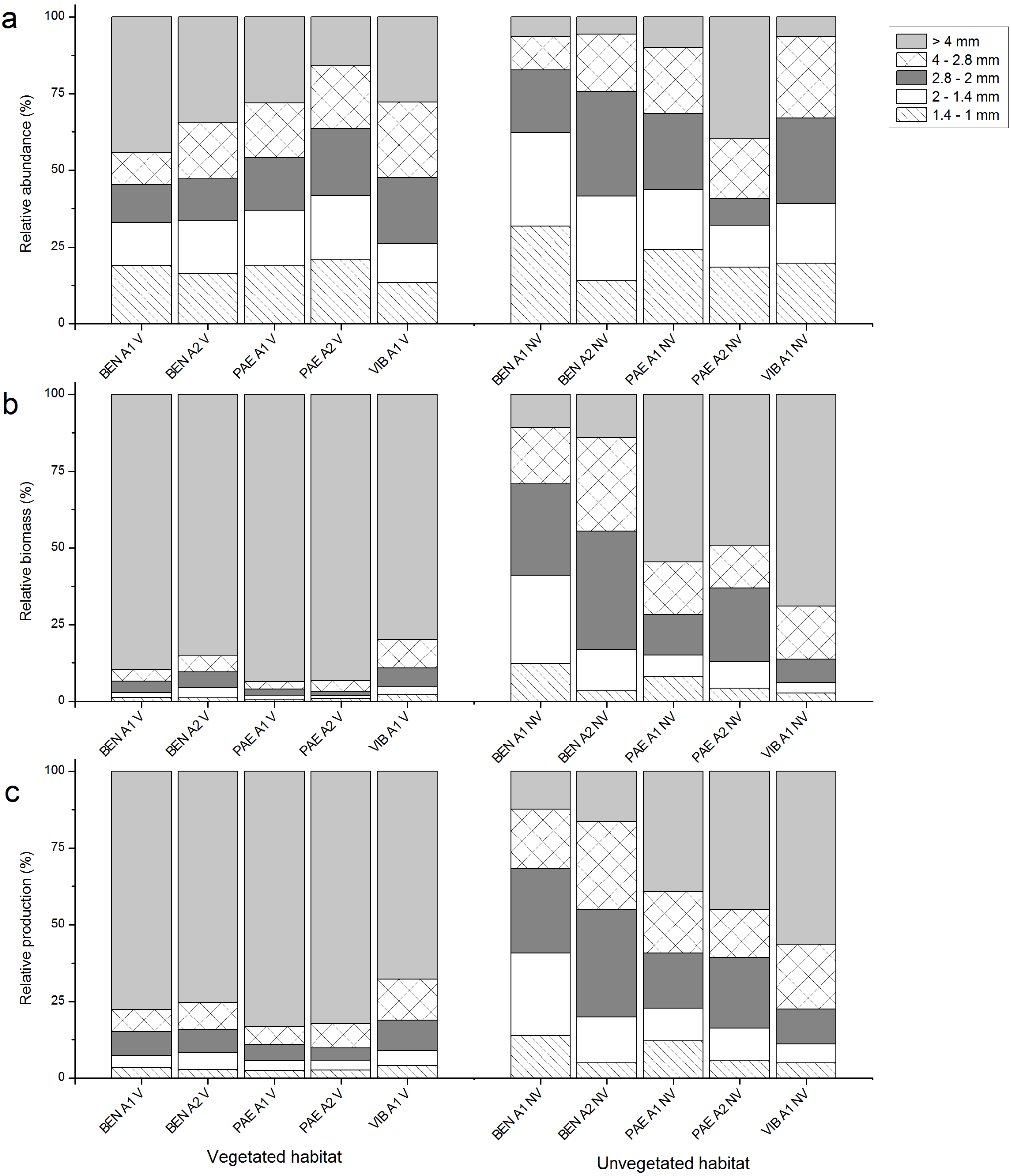
a
b
c
Relative abundance (%)
Relative biomass (%)
Relative production (%)
0
25
50
75
100
> 4 mm
4 - 2.8 mm
2.8 - 2 mm
2 - 1.4 mm
1.4 - 1 mm
BEN A1 V
BEN A2 V
PAE A1 V
PAE A2 V
VIB A1 V
BEN A1 NV
BEN A2 NV
PAE A1 NV
PAE A2 NV
VIB A1 NV
Vegetated habitat
Unvegetated habitat

Figure 6 (on next page)

nMDS of macrofaunal assemblages across habitats and estuaries

Fig 6 - Non-metric multidimensional scaling (nMDS) ordination plot of macrofaunal assemblages from vegetated (V) and unvegetated (NV) habitats at mesohaline and polyhaline sectors (A1 and A2) in the studied estuaries.

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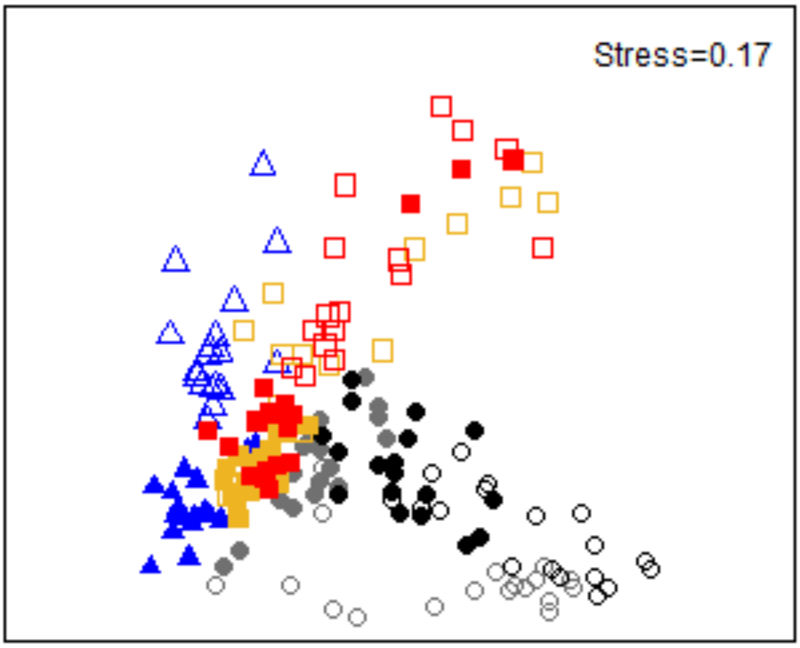

Stress=0.17

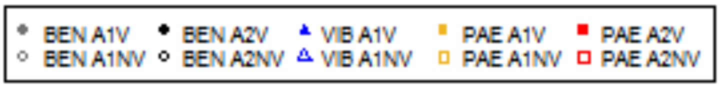
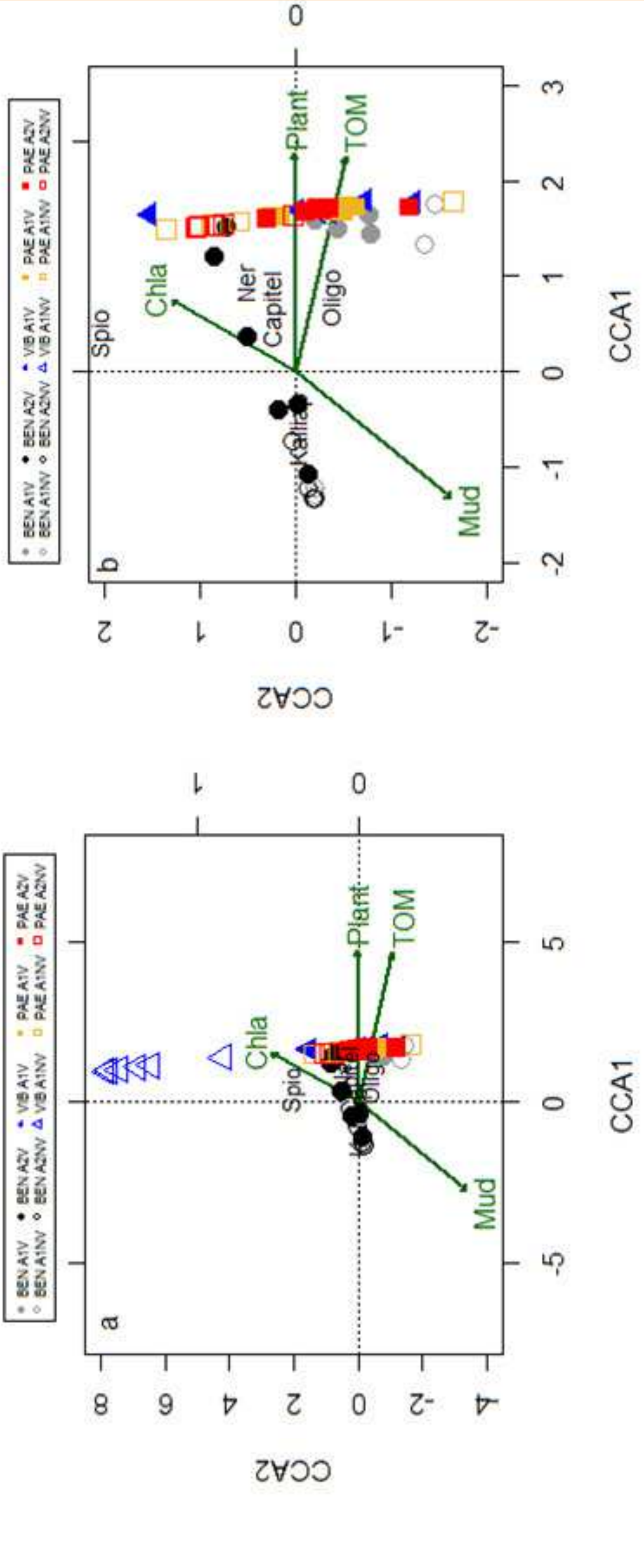

BEN A1V
BEN A1NV
BEN A2V
BEN A2NV
VIB A1V
VIB A1NV
PAE A1V
PAE A1NV
PAE A2V
PAE A2NV

Figure 7(on next page)

Canonical correspondence analysis of macrofaunal assemblages and environmental variables

Fig 7 - Canonical correspondence analysis (CCA) for macrofaunal taxa and environmental properties. a) Complete CCA with samples from mesohaline and polyhaline sectors (A1 and A2) of the estuaries, b) Detailed CCA with selected taxa. Taxa: Kalliap = Kalliapseudidae, Capitel = Capitellidae, Ner = Nereididae, Spio = Spionidae, Oligo = Oligochaeta. Environmental variables: TOM = total organic matter, Mud = mud content, Plant = plant biomass, Chla = Chlorophyll-*a*.



a
b
CCA1
CCA2
Chla
Plant
TOM
Mud
Spio
Oligo
Ner
Capitel
BEN A1V
BEN A2V
VIB A1V
PAE A1V
PAE A2V
BEN A1NV
BEN A2NV
VIB A1NV
PAE A1NV
PAE A2NV

Table 1(on next page)

ANOVA results of sediment properties

Table 1 - ANOVA results for sediment properties and plant material a) comparing A1 of BEN, PAE and VIB estuaries and b) comparing both A1 and A2 of BEN and PAE estuaries. H = habitat, E = estuary, A = area, S = site, df = degrees of freedom, MS = mean square. * Significant values.

a) ANOVA comparing area 1 of BEN, PAE and VIB estuaries

		Mean grain size			Mud content			TOM		
Source	**df**	**MS**	**F**	***p***	**MS**	**F**	***p***	**MS**	**F**	***p***
H	1	0.64	0.680	0.50	710.88	1.813	0.31	1244.64	4.448	0.17
E	2	5.11	3.474	0.17	4190.30	4.729	0.12	283.85	1.596	0.34
HxE	2	0.95	0.635	0.59	392.01	0.465	0.67	279.85	5.528	0.10
S(E)	3	1.47	6.115	0.003*	886.16	8.709	0.0004*	177.85	11.505	<0.0001*
HxS(E)	3	1.49	6.204	0.003*	843.80	8.293	0.0006*	50.62	3.275	0.04*
Residual	24	0.24			101.75			15.46		

		Chlorophyll-*a*			Phaeopigments			Plant biomass		
Source	**df**	**MS**	**F**	***p***	**MS**	**F**	***p***	**MS**	**F**	***p***
H	1	39.04	24.060	0.04*	0.06	0.160	0.73	90.50	6.448	0.13
E	2	27.90	1.944	0.29	4.02	479.926	0.0002*	79.93	60.220	0.004*
HxE	2	1.62	1.447	0.36	0.35	2.915	0.20	14.03	1.727	0.32
S(E)	3	14.35	1.487	0.24	0.01	0.030	0.99	1.33	1.525	0.23
HxS(E)	3	1.12	0.116	0.95	0.12	0.437	0.73	8.13	9.337	0.0003*
Residual	24	9.65			0.28			0.87		

b) ANOVA comparing both areas 1 and 2 of BEN and PAE estuaries

		Mean grain size			Mud content			TOM		
Source	**df**	**MS**	**F**	***p***	**MS**	**F**	***P***	**MS**	**F**	***p***
H	1	0.96	5.178	0.26	626.71	44.170	0.10	468.17	1.767	0.41
E	1	1.34	0.525	0.54	484.13	0.273	0.65	1799.32	7.352	0.11
HxE	1	0.19	0.209	0.69	14.19	0.021	0.90	264.91	3.218	0.21
A(E)	2	2.55	2.560	0.19	1773.19	4.162	0.11	244.73	6.024	0.06
HxA(E)	2	0.89	1.160	0.40	667.78	1.930	0.26	82.31	2.127	0.23
S(A(E))	4	0.99	6.204	0.0008*	426.06	6.148	0.0009*	40.63	5.238	0.002*
HxS(A(E))	4	0.77	4.787	0.004*	346.02	4.993	0.003*	38.69	4.988	0.003*
Residual	32	0.16			69.30			7.76		

		Chlorophyll-*a*			Phaeopigments			Plant biomass		
Source	**df**	**MS**	**F**	***p***	**MS**	**F**	***p***	**MS**	**F**	***p***
H	1	10.83	22.550	0.13	0.05	3.292	0.32	11.57	62.541	0.08

E	1	48.96	3.996	0.18	1.45	146.699	0.007*	5.36	22.107	0.04*
HxE	1	0.48	0.035	0.87	0.01	0.359	0.61	0.19	0.300	0.64
A(E)	2	12.25	1.363	0.35	0.01	1.461	0.33	0.24	1.844	0.27
HxA(E)	2	13.78	25.616	0.005*	0.04	3.031	0.16	0.62	0.996	0.45
S(A(E))	4	8.99	1.445	0.24	0.01	0.244	0.91	0.13	2.795	0.04*
HxS(A(E))	4	0.54	0.086	0.99	0.01	0.455	0.77	0.62	13.159	<0.0001*
Residual	32	6.22			0.03			0.05		

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Table 2(on next page)

ANOVA results of macrofaunal density, biomass and secondary production

Table 2 - ANOVA results for macrofaunal density, biomass and secondary production a) comparing the mesohaline sector (A1) of BEN, PAE and VIB estuaries and b) comparing both sectors A1 and A2 (mesohaline and polyhaline, respectivelly) of BEN and PAE estuaries. H = habitat, E = estuary, A = area, S = site, P = plot, df = degrees of freedom, MS = mean square. * Significant values.

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a) ANOVA comparing the mesohaline sector (Areas 1) of BEN, PAE and VIB estuaries										
		Density			**Biomass**			**Secondary production**		
Source	**df**	**MS**	**F**	***p***	**MS**	**F**	***p***	**MS**	**F**	***p***
H	1	311266.70	0.253	0.66	0.20	0.369	0.61	6.43	0.421	0.58
E	2	689817.25	19.504	0.02*	0.32	4.388	0.13	8.95	5.596	0.10
HxE	2	1230614.84	23.040	0.02*	0.54	20.178	0.02*	15.29	26.162	0.01*
S(E)	3	35368.39	1.372	0.30	0.07	3.427	0.052	1.60	2.982	0.07
P(S(E))	12	25774.72	4.120	<0.0001*	0.02	1.400	0.19	0.54	1.616	0.11
HxS(E)	3	53413.02	1.431	0.28	0.03	1.061	0.40	0.58	0.990	0.43
HxP(S(E))	12	37327.63	5.967	<0.0001*	0.03	1.682	0.09	0.59	1.779	0.07
Residual	72	6255.48			0.01			0.33		

b) ANOVA comparing both sectors (mesohaline and polyhaline) of BEN and PAE estuaries										
		Density			**Biomass**			**Secondary production**		
Source	**df**	**MS**	**F**	***p***	**MS**	**F**	***p***	**MS**	**F**	***p***
H	1	3,38	0,061	0,85	0,001	0,007	0,95	0,0004	<0.0001	1,00
E	1	60,37	16,311	0,06	0,08	7,353	0,11	728,57	32,587	0,03*
HxE	1	54,96	12,077	0,07	0,13	9,830	0,09	602,80	18,656	0,0496*
A(E)	2	3,70	2,483	0,20	0,01	0,433	0,68	22,36	0,466	0,66
HxA(E)	2	4,55	1,867	0,27	0,01	0,761	0,52	32,31	0,915	0,47
S(A(E))	4	1,49	2,837	0,06	0,02	4,358	0,01*	47,99	5,189	0,007*
HxS(A(E))	4	2,44	3,318	0,04*	0,02	3,166	0,04*	35,33	4,013	0,02*
P(S(A(E)))	16	0,53	1,745	0,05	0,005	0,912	0,56	9,25	1,082	0,38
HxP(S(A(E)))	16	0,73	2,441	0,004*	0,006	0,931	0,54	8,80	1,030	0,43
Residual	96	0,30			0,006			8,55		

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Table 3(on next page)

Macrofaunal density and relative abundance of top 5 ranked taxa in all estuaries

Table 3 - Mean density (ind.m^{-2}) and relative abundance (%) of the most representative taxa in vegetated (V) and unvegetated (NV) habitats in areas 1 and 2 (A1 and A2) in the sampled estuaries.

Taxa	Density ind.m^{-2} (±SD)	Rel. ab. (%)	Taxa	Density ind.m^{-2} (±SD)	Rel. ab. (%)
BEN A1 V			**BEN A1 NV**		
Oligochaeta	1070 (862)	36	Kalliapseudidae	25028 (18207)	76
Capitellidae	728 (501)	24	Oligochaeta	7235 (13440)	22
Polychaeta sp1	355 (1002)	12	Capitellidae	276 (291)	0.8
Ampharetidae	348 (423)	12	Nereididae	182 (181)	0.6
Polychaeta sp2	151 (488)	5	Polychaeta sp1	163 (319)	0.5
BEN A2 V			**BEN A2 NV**		
Kalliapseudidae	1802 (2814)	54	Kalliapseudidae	11623 (9097)	96
Capitellidae	716 (368)	21	Capitellidae	191 (277)	2
Ampharetidae	298 (224)	9	Aoridae	82 (126)	0.7
Crustacea sp1	163 (203)	5	Nereididae	72 (51)	0.6
Oligochaeta	126 (221)	4	Oligochaeta	41 (79)	0.3
VIB A1 V			**VIB A1 NV**		
Oligochaeta	6701 (5356)	57	Spionidae	2323 (1628)	69
Capitellidae	2288 (1967)	19	Capitellidae	505 (390)	15
Nereididae	1073 (1126)	9	Nereididae	248 (250)	7
Spionidae	549 (1013)	5	Pilargidae	82 (89)	2
Polychaeta sp2	257 (649)	2	Bivalvia não ID	72 (75)	2
PAE A1 V			**PAE A1 NV**		
Oligochaeta	2307 (1510)	53	Capitellidae	512 (949)	50
Capitellidae	1252 (655)	29	Oligochaeta	201 (320)	19
Nereididae	242 (142)	6	Nemertea	50 (75)	5
Pilargidae	113 (140)	3	Pilargidae	41 (121)	4
Ampharetidae	113 (111)	3	Aoridae	35 (55)	3
PAE A2 V			**PAE A2 NV**		
Oligochaeta	898 (589)	45	Capitellidae	355 (420)	45
Capitellidae	640 (935)	32	Magelonidae	198 (197)	25
Nereididae	119 (130)	6	Oligochaeta	69 (102)	9
Pilargidae	97 (108)	5	Nemertea	35 (65)	4
Spionidae	41 (61)	2	Goniadidae	25 (48)	3

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Table 4(on next page)

PERMANOVA results of macrofaunal assemblages at mesohaline sites across estuaries

Table 4 - PERMANOVA results calculated from the Bray–Curtis dissimilarity matrix for the macrofauna assemblages from mesohaline sites (A1) from all three estuaries BEN, PAE and VIB. H = habitat, E = estuary, S = site, P = plot. * Significant values.

Source	df	MS	Pseudo-F	P(perm)
H	1	34861	1.895	0.23
E	2	24587	5.153	0.06
S(E)	3	4771.7	3.025	0.0001*
HxE	2	18394	4.366	0.02*
P(S(E))	12	1577.5	2.414	0.0001*
HxS(E)	3	4213.5	2.593	0.0016*
HxP(S(E))	12	1625	2.486	0.0001*
Residual	72	653.61		

Table 5(on next page)

PERMANOVA results of macrofaunal assemblages across mesohaline and polyhaline sectors from BEN and PAE estuaries

Table 5 - PERMANOVA results calculated from the Bray–Curtis dissimilarity matrix for the macrofauna assemblages at the different scales investigated in the mesohaline (A1) and polyhaline (A2) in BEN and PAE estuaries. H = habitat, A = area, S = site, P = plot. * Significant values.

		BEN			PAE		
Source	**df**	**MS**	**Pseudo-F**	**P(perm)**	**MS**	**Pseudo-F**	**P(perm)**
H	1	44209	12,293	0,26	29630	5,101	0,25
A	1	16637	2,628	0,34	6937,7	3,497	0,34
S(A)	2	6329,7	4,370	0,002*	1984	1,247	0,29
HxA	1	3596,2	0,546	0,66	5808,8	1,718	0,25
P(S(A))	8	1448,5	3,54	0,0001*	1590,6	1,184	0,24
HxS(A)	2	6581,5	4,395	0,006*	3380,9	1,691	0,10
HxP(S(A))	8	1497,7	3,660	0,0001*	1999,8	1,489	0,0492*
Residual	48	409,17			1343,6		