# Effects of PAHs on meiofauna from three estuaries with different levels of urbanization in the South Atlantic (#75419)

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# Effects of PAHs on meiofauna from three estuaries with different levels of urbanization in the South Atlantic

**Renan B da Silva** Equal first author, [1] , **Giovanni A P Dos Santos** Corresp., Equal first author, [1] , **Ana Luiza L de Farias** [1] , **Débora A A França** [1] , **Raianne A Cavalcante** [1] , **Eliete Zanardi-Lamardo** [2] , **José R B de Souza** [1] , **André M Esteves** [1]

[1] Campus Recife, Center for Biosciences, Department of Zoology, Av. Professor Moraes Rego, s/n. 50670-901, Federal University of Pernambuco, Recife, Pernambuco, Brazil

[2] Campus Recife, Technology and Geoscience Center, Department of Oceanography, Av. Arquitetura s/n. 50740-550, Federal University of Pernambuco, Recife, Pernambuco, Brazil

Corresponding Author: Giovanni A P Dos Santos
Email address: giopaiva@hotmail.com

Suggested to be the final receivers of human pollution, estuarine environments are impacted by the surrounding urbanization and by the compounds carried by the rivers' waters that flow from the continent. Among the contaminants that can reach estuaries and directly affect marine conservation, are polycyclic aromatic hydrocarbons (PAHs), which are considered highly deleterious to organisms residing in these environments. Thus, this research investigated the meiofauna of three estuaries exposed to different levels of urbanization/conservation and consequently different levels of pollution by PAHs, in order to assess how these compounds and environmental factors affect the distribution, structure and diversity of these interstitial invertebrates. A total of 16 major meiofauna groups were identified, with Nematoda being the dominant taxon (75%), followed by Copepoda and Polychaeta (9% each). It was possible to observe significant differences in all diversity indices studied in the estuaries. With the exception of average density, the diversity indices were higher in the reference estuary, Goiana estuarine system (GES), with a richness of 8.75 ± 0.5, Shannon index of 2.06 ± 0.06, and evenness of 0.49 ± 0.05. On the other hand, the Timbó estuarine system (TES) had the lowest Shannon index value (1.36 ± 0.17) and richness (5.33 ± 0.55), while the Capibaribe estuarine system (CES) had the lowest value of evenness (0.34 ± 0.04). These last two estuaries (TES and CES) present intermediate and high levels of urbanization, respectively. The ecological quality assessment (EcoQ) in the estuaries was classified from Poor to Moderate, and the estuary with the lowest demographic density in its surroundings was better ranked (GES). A significant correlation was observed between the environmental variables and the density of the meiofaunistic community, with PAHs and pH contributing the most to the variation of organisms in the studied areas. The granulometry of the estuaries was very characteristic, where the most polluted environments were dominated by very fine sand

and silt, while the reference environment was dominated by medium grains. The highest concentrations of PAHs were found in the most urbanized estuaries, these compounds directly affected the structure of the interstitial benthic community. The metrics used in the present study proved to be adequate for assessing the environmental quality and conservation of the investigated estuaries.

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[a] Department of Zoology, Federal University of Pernambuco, Recife, Pernambuco, Brasil

[b] Department of Oceanography, Federal University of Pernambuco, Recife, Pernambuco, Brasil

*Corresponding author:

Giovanni dos Santos

Email address: Giopaiva@hotmail.com

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[a] Department of Zoology, Federal University of Pernambuco, Recife, Pernambuco, Brasil

[b] Department of Oceanography, Federal University of Pernambuco, Recife, Pernambuco, Brasil

*Corresponding author:

Giovanni dos Santos

Email address: Giopaiva@hotmail.com

## Abstract

Suggested to be the final receivers of human pollution, estuarine environments are impacted by the surrounding urbanization and by the compounds carried by the rivers' waters that flow from the continent. Among the contaminants that can reach estuaries and directly affect marine conservation, are polycyclic aromatic hydrocarbons (PAHs), which are considered highly deleterious to organisms ~~residing~~ in these environments. ~~Thus,~~ this research investigated the meiofauna of three estuaries exposed to different levels of urbanization/conservation and consequently different levels of pollution by PAHs, in order to assess how these compounds and environmental factors affect the distribution, structure and diversity of these interstitial invertebrates. A total of 16 major meiofauna groups were identified, with Nematoda being the dominant taxon (75%), followed by Copepoda and Polychaeta (9% each). It was possible to observe significant differences in all diversity indices studied in the estuaries. With the exception of average density, the diversity indices were higher in the reference estuary, Goiana estuarine system (GES), with a richness of 8.75 ± 0.5, Shannon index of 2.06 ± 0.06, and evenness of 0.49 ± 0.05. On the other hand, the Timbó estuarine system (TES) had the lowest Shannon index value (1.36 ± 0.17) and richness (5.33 ± 0.55), while the Capibaribe estuarine system (CES) had the lowest value of evenness (0.34 ± 0.04). These last two estuaries (TES and CES) present intermediate and high levels of urbanization, respectively. The ecological quality assessment (EcoQ) in the estuaries was classified from Poor to Moderate, and the estuary with the lowest demographic density in its surroundings was better ranked (GES). A significant correlation was observed between the environmental variables and the density of the meiofaunistic community, with PAHs and pH contributing the most to the variation of organisms in the studied areas. The granulometry of the estuaries was very characteristic,

where the most polluted environments were dominated by very fine sand and silt, while the reference environment was dominated by medium grains. The highest concentrations of PAHs were found in the most urbanized estuaries, these compounds directly affected the structure of the interstitial benthic community. The metrics used in the present study proved to be adequate for assessing the environmental quality and conservation of the investigated estuaries.

Keywords: Meiofauna; PAHs; Tropical Estuary; Urbanization; EcoQ; Pollution

## Highlights

Meiofauna proved to be an excellent ecological tool for the assessment of anthropic impacts in areas with different levels of urbanization and polluted by PAHs.

The evaluation of ecological quality (EcoQ) must be integrated with the diversity indices, mainly equitability, as it is associated with the constancy of the fauna in the areas.

A progressive gradient of PAH concentrations was found, ranging from less to more urbanized areas.

There was a clear correlation between PAH concentration with organic matter and fine sediment fractions.

## Introduction

Estuaries are coastal water bodies that present wide environmental variation in variables such as salinity, pH and sediment granulometry. Although they are semi-enclosed environments, estuaries are directly connected to the ocean and are influenced by continental drainage and evaporation (Elliott and Whitfield, 2011), as well as being influenced by alternating tides (Jones et al., 2020). These environments, recognized as natural nurseries (Courrat et al., 2009), are also affected by urban expansion and regional processes, such as heat islands caused by the reduction of riparian vegetation, climatic factors, recent sedimentation and local hydrodynamics (Cui et al., 2021; Hoque et al., 2020; Scanes et al., 2020).

Even if estuarine environments are ecologically important and provide services for biological (Adams et al., 2006) and economic maintenance (Cai and Li, 2011; Glaser, 2003), their close proximity to urban areas leaves them vulnerable to the entry and chronic deposition of potentially toxic compounds (Gabriel et al., 2020; Han et al., 2020; Wang et al., 2021). Among the pollutants, are polycyclic aromatic hydrocarbons (PAHs), which are organic compounds resulting from the mishandling of petroleum-based compounds (Stogiannidis and Laane, 2015). These are found in two forms: petrogenics, originating directly from petroleum derivatives (crude oil, fuels, lubricants) that flow into affluents and water bodies; and pyrolytics, which arise from the partial burning of petroleum derivatives. Both types have their own characteristics in terms of chemical composition and toxicity level (Abdel-shafy and Mansour, 2016; Zakaria et al., 2002).

Highly industrialized and urbanized areas, where there are several enterprises, are more propitious to the release of these pollutants, since PAHs reach coastal and estuarine environments mainly through the release of effluents and untreated domestic sewage, as well as urban runoff (Domínguez et al., 2010; Elmquist et al., 2007; Zakaria et al., 2002). These multiple sources carry a mix of polycyclic aromatic hydrocarbons, which accumulate in the sediments and cause problems for the surrounding fauna, since ~~HPAs~~ are toxic, mutagenic and carcinogenic (Engraff et al., 2011; USEPA, 2017).

All the fauna that inhabits an estuary comes into contact with PAHs and the possible disturbances that they can cause. Among the affected organisms are benthic invertebrates, mainly meiofauna. Although the topic of conservation is poorly explored in terms of intra-sedimentary benthic organisms, it is important to highlight that the conservation of marine environments is achieved efficiently, based on these tiny animals, in the face of anthropic impacts. Monitoring early changes across meiofauna by establishing indicators or sentinels at the base of food webs and ecosystem functions, rather than among their end members, allows for more efficient monitoring and timely conservation response (Ingels et al., 2021). As they share a close relationship with the sediment, do not present larval dispersion and are sensitive to environmental changes, these organisms are widely used as indicators of ecological impacts and for biomonitoring, (Hyland et al., 2005; Pusceddu et al., 2007). ~~Among the tools used,~~ in addition to diversity indices, meiofauna enables the application of ecological quality status (EcoQ), an index widely used both in studies of open habitats (Chen, 2018) and semi-enclosed environments (Semprucci et al., 2016), ~~and here will be used in estuaries~~.

The aim of the present study was to characterize the ~~meiofaunistic groups present in~~ three estuaries with different levels of urbanization, ~~on the East coast of the South Atlantic~~, ~~relating~~ meiofauna to concentrations of polycyclic aromatic hydrocarbons (PAHs) and environmental variables. ~~Considering that meiofauna has been shown to be a good indicator of different global impacts (Schratzberger and Ingels, 2018), showing especially detailed responses to different pollutants (Semprucci et al., 2016),~~ the hypothesis is that (i) environmental factors and (ii) polycyclic aromatic hydrocarbon concentrations significantly correlate with the spatial variation of: density; richness and diversity of meiofauna.

## Materials and methods

### Study area

The studied areas are urban estuaries of the Goiana, Timbó and Capibaribe rivers, located on the East coast of South America, Northeastern Brazil (Figure 1). The Goiana estuarine system (GES) is located at 7° 32' 43.2” S, 34° 51' 50.2” W. A study by the state environmental agency considered this estuary to be poorly urbanized (CPRH, 2005). The Timbó estuarine system (TES), is located at 7° 53' 45.8” S, 34° 51' 35.9” W. This estuary has an intermediate level of urbanization. The use of the biotic index (AMBI) identified the estuary of the Timbó river as slightly disturbed (Valença and Santos, 2012). The Capibaribe estuarine system (CES) is located at 8◦ 04'03” S and 34◦ 52'16” W, in the innermost part of Recife’s Port. It is formed by the confluence of the Tejipió, Jiquiá, Jordão and Pina rivers and the Southern arm of the Capibaribe river. Based on the AZTI biotic index, this estuary was classified as highly disturbed (Valença and Santos, 2012).

## Sampling

The samples were obtained in January and February 2016, during the summer (dry) period. The collection was carried out during this season in order to avoid the period of high rainfall that leads to the enrichment of pollutants, including PAHs, in estuarine systems (Boonyatumanond et al., 2006; Zakaria et al., 2002; Zhang et al., 2017). The estuaries were sampled at three points, in each of them four releases (replicas) were obtained using a Van Veen bottom sampler.

The sediment for the study of the meiofauna was obtained (in each release) with the aid of a 5cm tall cylinder with an internal diameter of 3.6 cm (area of 10 cm$^2$). Samples were preserved with 4% buffered formaldehyde (Giere, 2009).

For the analysis of chemical and physical parameters and PAHs, sediment fractions were separated within each replica. In order to avoid contamination of the fraction destined to identify PAHs, a stainless-steel spatula was used to scrape the surface of the sediment (~2cm) and a sterile aluminum container was used to store it until PAH processing and reading. Avoiding punctual variation and chemical agglomeration, the samples were homogenized before the spectrophotometric analysis and PAH characterization. Salinity, temperature, dissolved oxygen and pH were measured using a JFE Advantech type CTD probe, Rinko Profiler model.

## Treatment of biological samples in the laboratory

To separate the fauna from the sediment, running water and sieves (300 and 45µm coupled meshes, respectively) were used. The sediment remaining in the 45µm mesh underwent a process of ten manual elutriations and the supernatant from each elutriation was removed and fixed with 4% buffered formalin (Giere, 2009). Meiofauna was identified with the aid of a stereomicroscope at the level of large groups (Higgins and Thiel, 1988).

## Analyzes related to sediment variables

Organic matter was calculated from weight loss following ignition at 450°C for 5 hours. Granulometry was determined following Suguio (1973), using the wet method to sieve and separate the silt/clay fraction. The remaining sediment was sieved in a shaker after being dried and weighed and fractionated through sieves with openings of 2 mm to 0.062mm.

The methods for analyzing PAH concentrations are described in Souza et al. (2021). Briefly, the samples were analyzed by gas chromatography (GC - Agilent Technologies, model 7820A) coupled with mass spectrometry (MS - Agilent Technologies, model 5975C), in the selected ion monitoring mode (SIM). The values presented here refer to the sum of the PAHs (∑PAH).

## Data analysis

Meiofauna density data were transformed into fourth root and the similarity matrix was calculated using the Bray-Curtis index. To visualize the similarity patterns, they were ordered by the non-metric multidimensional scaling technique (nMDS). To test the significance of the visualized patterns, a permutational ANOVA (PERMANOVA) was applied, using the estuary areas (GES, TES and CES) as a factor. Before being used in correlation analyses, all abiotic data were transformed (Log(V+1)) and normalized.

To identify community structure, the following indices were calculated: Shannon Wiener (H'), Pielou (J) and richness values (S). To integrate these ecological indexes of meiofauna with environmental variables and PAH concentrations, the distance method based on a linear model (DISTLM) was applied.

The environmental quality status (EcoQ) was obtained through meiofauna group richness in the estuaries. For this, we used the limits proposed by Danovaro et al. (2004) and adapted by Semprucci et al. (2016) that define environmental quality as follows: group richness ≤ 4, bad; between 5 and 7 groups, poor; between 8 and 11 groups, moderate; between 12 and 15 groups, good; ≥ 16 groups, high.

Multivariate analyzes were performed using the software: PRIMER v6 with the addition of the PERMANOVA+ package (Gorley and Clarke, 2008).

## Results

### Environmental variables

Estuary salinity ranged from 23 - 36, and the average temperature was 29.2 ± 0.1 ºC (average ± SE). Organic matter varied greatly between estuaries, ranging from 1.37 - 16.28% (pseudo-F = 21.61; $p = 0.014$). The Goiana estuarine system (GES) presented lower concentrations and differed from the other estuaries ($p < 0.045$). The registered pH ranged from 5.79 - 8.5, where significantly more acidic measurements were recorded for the Capibaribe estuarine system (CES), differing from the others ($p < 0.0003$). Interestingly, dissolved oxygen was at least three times higher in the CES, differing in the pairwise comparison that was registered in the GES and in the Timbó estuarine system (TES) ($p < 0.02$) (ESM 1).

Regarding PAHs, a total of 17 different types were identified, of which 16 are listed as harmful to health by the United States Environmental Protection Agency (US EPA) (Keith, 2015). Concentrations ranged from 0.55 ± 0.67 (average ± SE) in GES, to 674.81 ± 331.31 in CES (Table 1). In the GES, only three PAHs were registered, 2-Methyl Naphthalene, Fluorene and Phenanthrene. The vast majority of PAHs were common to CES and TES, with the exception of Acenaphthene which only appeared in ~~the~~ CES. The highest individual concentrations of PAHs were ~~of~~ the following compounds: Fluoranthene, Indeno [1,2,3-cd] pyrene, Pyrene, Benzo[a]pyrene and Benzo[b]fluoranthene, together accounting for 56.2% of the total concentration of PAHs recorded.

The granulometry ~~registered~~ in the estuaries ranged from gravel to silt, with the average sand fraction being more abundant in the estuary less impacted by PAH, while in the most polluted areas the fractions of fine sediments and silt were dominant. When comparing the sediment fractions between the areas, the amount of very fine sand was significantly lower in ~~the~~ GES, whereas the silt fraction in ~~the~~ CES was significantly higher compared to GES (ESM 2).

The sedimentary matrix was classified as poorly selected in the most polluted estuaries (CES and TES), however, in the reference estuary, only one station was classified as poorly selected, the grains in the other two reference area stations were classified as moderately selected and were dominated by medium grains.

Meiofauna

A total of 22,152 individuals were identified, ~~distributed in~~ 16 meiofauna groups: Nematoda, Copepoda, Rotifera, Turbellaria, Tardigrada, Gastrotricha, Ostracoda, Halacaroidea, Nauplius, Oligochaeta, Cnidaria, Polychaeta, Amphipoda, Sipuncula, Kinorhyncha, Priapulida. Three of them ~~being~~ exclusive to GES (Sipuncula, Kinorhyncha, Priapulida), and another one exclusive to TES (Amphipoda). Richness varied significantly across estuaries (pseudo-F = 13,537; $p$ = 0.0001), ranging from 3 in the TES to 11 in the GES. On average, the richness in the GES was ~~8.75~~ ± ~~0.5~~, followed by ~~7.83~~ ± 0.46 in the CES and ~~5.33~~ ± ~~0.55~~ in the TES, where the latter presented the lowest average and differed from the others in the pairwise comparison (p < 0.0014).

Meiofauna density (ind./10cm²) varied significantly from one estuary to another (pseudo-F = 9.7861; $p$ = 0.0002), ~~where the density~~ in the CES ~~was~~ significantly higher than that in the other estuaries (p < 0.0065). Average densities (±SE) were 1069.17 ± 194.37 in CES; 452.17 ± 74.47 in GES and 324.67 ± 91.79 in TES.

Among the taxa, Nematoda were the most ~~dominant, and~~ when combined with Copepoda and Polychaeta they accounted for more than 90% of the organisms ~~considered in this study~~ (figure 2). The high-density value within the CES was mainly due to the presence of Nematoda and Polychaeta, whereas Nematoda and Copepoda were the most abundant groups in the GES and TES (Figure 2).

Although appearing in smaller ~~amounts~~ (less than 2%), ~~it is possible to highlight the highest averages~~ of Tardigrada and Gastrotricha in the GES, Ostracoda in the CES and Rotifera in both the TES and CES (Figure 3).

### Multivariate analyses.

The PERMANOVA analysis and the pairwise comparison revealed that community structure differed significantly between estuaries (~~ESM~~ 3).

The ~~non-metric multi-dimensional analysis (~~nMDS~~)~~ showed a greater association between the CES and TES ~~points in relation~~ to the GES ~~points~~ (Figure 4). The Spearman's correlation applied to the nMDS (vectors) indicated that most organisms that were positively correlated with GES were also negatively correlated with TES (eg. Turbellaria, Tardigrada and Gastrotricha). The Rotifera and Polychaeta taxa correlated positively with both CES and TES.

### ~~Indices and Environmental Quality Status (EcoQ)~~

Shannon's diversity (H) ranged from 2.31 in the GES to 1.02 in the TES, this index differed between estuaries (ESM 4), ~~where it was higher in the GES therefore, differing from the other two locations~~. ~~As with Shannon's diversity,~~ evenness varied significantly from one estuary to another, with the value of this index being significantly lower in the CES compared to ~~pairs~~ (ESM 4). Based on meiofauna richness, the ecological quality status (EcoQ) of the studied stations was classified from Poor (S = 1-4) to Moderate (S = 8-11). While the quality of the three GES stations was characterized as Moderate, the three TES stations were characterized as Poor. Two of the CES estuary stations had Moderate EcoQ and one was classified as Poor EcoQ (ESM 5).

### Correlation between meiofauna and environmental variables

Environmental data explained 60.93% of the meiofauna community variation (DistLM - Best) (Table 6). Of the environmental data tested, salinity ($p = 0.5$) and some sediment fractions did not significantly influence community variation (coarse sand: $p = 0.46$; fine sand: $p = 0.28$). It ~~is~~ possible to highlight that the sum of PAHs and pH contributed to more than 39% of the total variation of meiofauna (ESM 6). The dbRDA, ~~which demonstrates the relationship between environmental parameters and the structure of the fauna community,~~ demonstrated a clear separation between the least polluted area (GES) and the other areas (CES and TES), as well as the relevance of PAH and pH for the distinction between different groups (Figure 5).

The total concentration of PAH significantly affected the abundance of some taxa, reducing the Tardigrada ($p < 0.001$) and Gastrotricha ($p < 0.001$), but favoring the increase of Nematoda ($p = 0.001$) and Polychaeta ($p < 0.0001$). In contrast, higher pH values correlated positively and significantly with Tardigrada ($p = 0.002$) and Gastrotricha ($p = 0.04$) abundance. However, this relationship was inverted for Nematoda and Polychaeta, since they showed a direct and positive relationship with the high acidity registered in the more urbanized estuary (pH: 5.81 ± 0.02).

When only testing individual PAH concentrations, the correlation analysis explained 59.40% of the total faunal variation. All PAHs significantly influenced organism variation in the estuaries, with Benzo[b]fluoranthene and Anthracene best explaining the variation, accounting for 46.44% of the total variation (~~ESM~~ 7). The first two axes of the dbRDA ~~generated in this analysis~~ explained about 86.3% of the PAH distribution within the estuaries. From the vectors in the dbRDA, it is possible to observe a positive correlation between PAHs and the more urbanized estuaries (CES and TES), whereas the correlations with the less urbanized estuary (GES) were all negative (Figure 6).

Some of the environmental variables correlated significantly with the ~~estuary~~ diversity indices. The density of organisms correlated significantly and positively with total ~~PAH~~, dissolved Oxygen, temperature and silt, but ~~correlated~~ negatively with the sandy and coarse fraction of the sediment. Shannon diversity correlated negatively and significantly with organic matter and very fine and very coarse sand. These sediment fractions were negatively and significantly correlated with richness ~~as well~~. Evenness was significantly negatively affected by ∑ PAH, dissolved oxygen, organic matter, as well as very fine sand and silt (~~see ESM~~ 8).

## Discussion

### Origin of PAH pollution in estuaries with different urbanizations levels

The variation and distribution of organisms in marine environments is linked to the environmental factors of each location. ~~In~~ estuarine environments, ~~there is a~~ continuous ~~oscillation~~ ~~of~~ environmental parameters (i.e., salinity, organic matter, granulometry, etc.), making them ~~heterogeneous~~ in time and space. These oscillations are associated with the characteristics of the areas, through which the converging rivers pass during their course forming this ecosystem. Common impacts on tributary courses are: waste derived from urbanization, agriculture and industrialization in surrounding areas, as well as the misuse of their banks resulting in leaching and silting processes.

~~The Polycyclic Aromatic Hydrocarbons~~ (PAHs~~)~~ that reach rivers' waters, ~~derived from these processes mentioned above~~, are transported and deposited ~~onto~~ estuarine ~~sediment~~ mainly through association with salts, sediment grains and organic matter ~~found in this environment~~, which can directly affect the resident biocenosis (Maciel et al., 2015). These events raise concerns, especially in more populated areas due to the higher incidence and recurrence of impacts and human pollution ~~that reach~~ estuaries. ~~Our~~ study area ~~with the lowest rate of autochthonous anthropic influence~~, used as a reference point due to the lower level of urbanization, has a population density of 150.72 inhab/km $^2$ and considerably lower concentrations of PAHs compared to the other sites. Population densities in estuaries with medium and high levels of urbanization showed values that were 22 and 46 times higher, respectively (IBGE, 2020).

~~Nonetheless,~~ although the impacts related to urbanization in ~~our less urbanized~~ area were lower, activities such as aquaculture and the burning of sugar cane are carried out throughout this estuary basin. Such activities have already been identified as ~~sources~~ ~~for the entry of PAHs~~ into the Goiana estuarine system ~~(GES), although~~ they are developed in areas that are more adjacent to the continent, they reach the estuarine region, even if in low intensities (Arruda-santos et al., 2018). Estuaries such as Ohiwa, New Zealand, suffer human impacts similar to those observed in the more conserved area of this study (i.e., rural pastures and less inhabited areas), ~~as well as having~~ similar total PAH concentrations (Goiana: 1.7 ng/g $^{-1}$; Ohiwa : 3.0 ng/g $^{-1}$) (Hack et al., 2007). ~~As observed in the reference area of the present study, the Ohiwa estuary was considered unpolluted by PAHs and demonstrated no correlation between these pollutants and the composition of the meiofauna community (Hack et al., 2007).~~ Based on the Canadian classification of the Sediment Quality Guidelines (SQC), high sediment quality was recorded in the GES, since the PAH concentrations observed in this estuary were lower than the threshold for inclusion in the rare category (REL) (ECM, 2007; He et al., 2014). The pollution in this estuary was not observed to influence meiofauna community structure (supplementary material table), however these concentrations indicate the entry of PAHs into this area, ~~thus reaffirming~~ the importance ~~of monitoring estuarine environments~~ ~~with little impact~~.

The Timbó estuarine system (TES), which has an intermediate level of urbanization, suffers from a lack of sanitation and the release of untreated effluents, generating an increase in organic matter concentration in this environment (Santana et al., 2017). The increase in organic matter particles becomes a negative factor for estuary health, since ~~they are~~ associated with and facilitate the deposition and accumulation of PAHs in the sediment (Medeiros and Caruso Bícego, 2004). As a consequence, there is a loss of biodiversity, disruption of the food web, as well as a decrease in ecosystem services provided by ~~intrasedimentary~~ organisms (Gambi et al., 2020; Louati et al., 2013). The PAH concentrations obtained in ~~the estuary with intermediate urbanization~~ were similar to those found in other urban estuaries such as the Guan River, China (He et al., 2014), and in the West and South-central region of Cuba (Martins et al., 2018; Tolosa et al., 2009). As in the Guan River, most PAHs in the TES had individual concentrations that, according to the SQC, would have a low probability of causing negative effects on benthic fauna (ECM, 2007). However, the presence of Benzo[a]anthracene, Dibenz[a,h]anthracene and Fluoranthene compounds can become alarming if they continue to be released into this area, ~~since, based on their~~ individual concentrations, ~~sediment quality approaches~~ the threshold effect level (TEL) in benthic organisms (ECM, 2007; He et al., 2014).

Among the studied sites, the Capibaribe estuarine system (CES) had the highest concentrations of PAHs. As seen in previous studies, this area presents characteristics that facilitate the retention of organic matter and ~~the~~ PAHs ~~themselves~~ (Araújo et al., 2011; Maciel et al., 2015). The waters of this estuary are connected to Recife's Port, which receives numerous vessels annually, becoming a vector area for the release of PAHs (Porto do Recife, 2021). The PAH concentrations observed in ~~the most polluted estuary of this study~~ are similar to those analyzed in urban and highly industrialized estuaries, such as the Yangtzen River in eastern China (Li et al., 2012), as well as in port areas in the Adriatic Sea (Baldrighi et al., 2019). The meiofauna abundances reported in the aforementioned port area were similar to our results~~, where~~ the abundance of sensitive taxa (Tardigradas and Gastrotricha) decreased due to the high values of PAHs, while the abundance of Nematoda and Polychaeta, ~~considered tolerant taxa~~ increased (Dal Zotto et al., 2016; Moreno et al., 2011; Pusceddu et al., 2007). It was possible to observe that the concentrations of six of the 17 PAHs were high enough to exceed the threshold effect level (TEL) in at least one station of ~~the most polluted estuary~~ (PAHs: Acenaphthylene, Phenanthrene, Fluoranthene, Benzo[a]anthracene, Benzo [a]pyrene and Dibenz[a,h]anthracene). The levels of PAHs recorded in ~~the~~ CES ~~are~~ even more alarming than the findings for the TES, and are thus consequently more harmful ~~to~~ the resident fauna (ECM, 2007; He et al., 2014). Within the CES samples, a positive correlation was observed between Turbellaria and all PAHs, whereas Rotifera and Ostracoda were only affected by Acenaphthene, Anthracene and Naphthalene

### Environmental factors shaping the distribution of meiofauna and diversity in estuaries with different levels of urbanization

Meiofauna have been used for decades as bioindicators to assess human impacts on coastal ecosystems (Sandulli, 1986; Vincx and Heip, 1987), and even today they are still used as a biomonitoring tool (Moreno et al., 2009; Semprucci et al., 2015). ~~These organisms are intra-sedimentary and closely related to the benthos~~, having no free-swimming or dispersal abilities and remaining in their environments despite the presence of stressors (Kennedy and Jacoby, 1999). Meiofauna respond quickly to physical and chemical environmental fluctuations and evolve community structures in direct association with environmental conditions (Schratzberger et al., 2004). As such, the fauna present in each area ~~becomes a reflection of~~ the surrounding environmental conditions, ~~in addition to demonstrating possible deficiencies in the ecosystem functions of specific locations~~ (Balsamo et al., 2012). Due to meiofauna community responses to a disturbance event, ~~especially during times of increasing frequency and intensity of environmental change caused by humans~~, ~~these communities are~~ currently considered to be ~~vital tools that can be used~~ to measure disturbance effects on habitats ~~as they interact critically with environmental characteristics~~ (Schratzberger and Somerfield, 2020).

Conservation strategies aim to protect habitats, in addition to understanding the loss of fauna and the ecosystem services they provide to the environment (Ingels et al., 2021). ~~The changes that marine environments experience, as a result of~~ anthropogenic actions~~,~~ affect meiofauna and cause decreases in diversity and richness, resulting, mainly, in the loss of more sensitive and rare taxa. When this occurs, individuals with broader niches can proliferate, as they are more resistant or even opportunistic (Pusceddu et al., 2007; Supp and Ernest, 2014). Nematoda (76.56%) stood out in the s~~tudy~~ estuary with the highest level of pollution, followed by Polychaeta (13.53%). On the other hand, the density of Copepoda, which respond more sensitively to areas impacted by anthropogenic action (Soetaert et al., 1995), was 15 times lower than that of Nematoda.

Nematoda dominance in the most polluted estuary did not deviate from patterns observed in coastal marine environments in southern Italy and northern Iran (Bertocci et al., 2019; Zarghami et al., 2019), in the deep sea of the Gulf of Mexico (Baguley et al., 2006) and in estuaries present in southeastern India and northern Taiwan (Cai and Li, 2011; Chinnadurai and Fernando, 2007). As in the most polluted estuary in this study, in the aforementioned studies, Nematoda were denser in areas with greater anthropogenic activity and, notably, with high concentrations of organic matter and PAHs. This is due to the opportunistic characteristics that some colonizing Nematoda genera present, together with their diet composed of bacteria that proliferate from the decomposition of organic content (Bongers and Ferris, 1999; Schratzberger and Ingels, 2018).

The most urbanized estuary of this study (CES) presented fine granulometry, favoring the presence of Polychaeta, due to the greater retention of organic matter and consequently nutrient availability for this taxon (Gowda et al., 2009; Alves et al., 2013). The high abundance of this group has already been reported in another anthropized estuary, located in Mondego (Portugal), where Polychaeta were the second most abundant group, following Nematoda (Alves et al., 2013). While the results of this paper suggest positive and significant correlations of Polychaeta with ∑PAH, the same was not observed for the coast of Galicia, where no correlations were found between Polychaeta density, environmental parameters and pollution by PAHs (Veiga et al., 2009). When exposed to acute impacts, such as the Deepwater Horizon accident, there was a decrease in Meiofauna Polychaeta at points close to the accident, however macrofauna Polychaeta families (e.g.: Spionidae, Capitellidae, Maldanidae) were dominant at the spill site (Baguley et al., 2015; Jewett et al., 1999; Washburn et al., 2016). The estuary most polluted by PAHs housed more than 90% of the total Polychaeta recorded in the present study, among which there was a slight predominance of the Spionidae family (data not shown). This family is commonly associated with environmental disturbances in marine environments, as its abundance increases when exposed to environmental stress (Dean, 2008), in addition to presenting opportunistic species, with rapid colonization capacities and tolerance both to oil pollution and its derivatives (i.e., PAHs).

The fauna pattern observed in estuaries with less impact (GES) and intermediate impact (TES) of PAHs presented similar meiofauna proportions in natural estuarine environments classified by Coull, 1999, where Nematoda dominated with 60-90% and Copepoda presented 10- 40% of the total abundance. In the GES and TES, Nematoda dominated with 66.09 and 80.17%, respectively, followed by Copepoda with 17.66 and 13.27%. The relative abundance of Copepoda in the estuary less impacted by PAHs was higher compared to the others. Furthermore, the presence of taxa such as Tardigrada and Gastrotricha in polluted estuaries was much lower and even null compared to that observed in the GES. The decrease in sensitive taxa in more urbanized estuaries ~~reinforces~~ the negative effect of pollution from constant anthropogenic activities, such as: effluent and sewage discharge, port and industrial activities, which are all factors that favor the entry of PAHs into estuaries (Damme et al., 1984; Maciel et al., 2015; Zeppilli et al., 2015).

### Ecological quality assessment using EcoQ and variation of meiofauna diversity

The quality classification in the estuaries ranged from poor to moderate, with a prevalence of moderate in the estuary with fewer PAHs, while the poorest classification was made in the estuary with an intermediate concentration of PAHs. Richness similar to that found in the TES were observed in the Venice lagoon in Italy, differing only in that in the TES all the stations were classified as Poor EcoQ, while in Venice the EcoQ fluctuated between Bad and Poor (Pusceddu et al., 2007).

As in the TES, the main factors highlighted for the bad EcoQ evaluation in Venice were: sewage entry, as well as residues from the industrial district present in the surrounding area, which has released trace elements into the estuary for decades (Hg, Zn, Pb, Cu, in addition to the PAHs) (Semprucci et al., 2016; Zonta et al., 2007). Such impacts correspond to what is reported in the most polluted estuaries of this study, which justifies both the low abundance (or absence) of sensitive organisms, as well as the low value in environmental indices (i.e., richness, density and Shannon index) and poor EcoQ at its stations.

The lower level of urbanization and anthropogenic effluent release, compared to other estuaries, are not the only factors indicative of superior ecological quality in the reference estuary. The sedimentary structure composed mainly of medium grains and concentrations of organic matter 3 to 6 times lower than, implies that there will be a lower accumulation of PAHs in the reference area (Evans et al., 1990; He et al., 2014). In fact, the concentration in the GES was at least 250 and 1225 times lower compared to the TES and CES, respectively. In addition to this fact, many of the taxa found in polluted estuaries were extremely rare and with low equitability. These organisms did not account for even 0.5% of the total abundance, both in the estuary with an intermediate PAH impact (Oligochaeta, Turbellaria, Nauplius, Halacaroidea, Amphipoda, Tardigrada, Cnidaria), and in the estuary with the greatest impact (Oligochaeta, Cnidaria, Nauplius, Gastrotricha, Halacaroidea) (Zeppilli et al., 2015).

Although the EcoQ of the most polluted estuary (CES) is comparable to that of the least polluted area (GES), the distribution of taxa in the CES was significantly less equitable compared to the other estuaries. As EcoQ is closely associated with richness, it is worth noting that the equitability that sustains this richness can be used as a parameter to establish the reliability and strength of this status. As such, even if the EcoQ level advances due to the emergence of an organism by chance, the inconsistency of this individual in the other samples should be seen as weakening the status.

The uniformity of the fauna distribution was negatively and significantly affected by the high concentrations of organic matter, very fine sand and silt, in addition to the high concentrations of PAHs, present in estuaries ~~inserted into~~ urban areas ~~which were consequently, more polluted~~. The decrease in the values of environmental indices, such as~~:~~ Shannon diversity, richness and evenness~~,~~ are ~~solid~~ indications of the presence of environmental stress in studies ~~with benthic fauna~~ (Damasio et al., 2020; Janakiraman et al., 2017; Warwick et al., 1990). Thus, these results further reinforce the ecological damage in our areas impacted by higher concentrations of PAHs, since they present lower values of the aforementioned environmental quality indices, when compared to the reference area. ~~Moreover, the urbanization gradient, which we highlight in the present research, brings up a very important concept when working with the meiofauna community, these organisms once again prove to be an adequate tool to detect early changes in impacted areas (Ingels et al., 2021)~~

Abiotic data model of tropical estuarine fauna exposed to different levels of urbanization

~~The fluctuation of environmental factors had a strong influence on the distribution of meiofauna organisms, as well as on the variation of the diversity indices present in each estuary.~~ ~~As an example of this,~~ ~~there are the~~ taxa Turbellaria, Tardigrada, Gastrotricha, Halacaroidea, Sipuncula and the Equitability index (J'), ~~which~~ correlated negatively with the fractions of very fine sediment and silt~~,~~ ~~which~~ ~~were mainly present in~~ the most polluted estuaries. On the other hand, in the less polluted estuary, medium and moderately selected grains predominated, resulting in larger interstitial spaces and favoring meiofauna diversity (Giere, 2009). Organisms such as Tardigrada and Gastrotrincha, which were abundant in the reference estuary, are adapted to liv~~ing~~ in interstitial spaces, whereas in areas where the sediment is muddy, such as in the CES and TES, these groups ~~seek~~ to adapt to epibiont life and become less diverse and abundant (Giere, 2009). Additionally, matrices that are rich in coarse grains accumulate less PAHs (Tolosa et al., 2009) and organic matter, where the latter directly influences the accumulation of PAHs in the sediment (Maciel et al., 2015; Wang et al., 2021).

In this study, larger proportions of fine grains and silt were registered in the estuaries with higher concentrations of PAHs and the relationship between these factors has been reported by other authors as well (Egres et al., 2019; Zanardi-Lamardo et al., 2019). Most fine grains exhibit a greater contribution of organic matter, which consequently contributes to the accumulation of PAHs and directly affects meiofauna, either through ingestion or direct contact with contaminants (Arruda-santos et al., 2018; Stogiannidis, E.; Laane, 2015; Tremblay et al., 2005). Even though PAHs best explained the distribution of the fauna, it is possible to affirm that grain size also plays an important role in the heterogeneity and diversity of the groups (He et al., 2014).

Sediment acidity also strongly influenced organism distribution. Extremely low pH values were found in the CES (average pH – 5.81 ± 0.02), which were approximately 1.45 times lower than those observed in the GES (average pH 8.4 ± 0.04) and TES (average pH – 8.39 ± 0.06) estuaries. The difference regarding estuary acidity can be directly linked to the individual processes and dynamics of each environment, such as sewage dumping and the decomposition of the organic matter present at each location. Wastewater from sewage or organic matter enrichment stimulates microbial activity and respiration at different proportions, causing a decrease in pH (Gallert and Winter, 2005).

Additionally, it has already been shown that most industrial effluents and sewage that reach estuarine waters have very low pHs (Wallace et al., 2014), which directly changes the pH of these environments. However, groups such as Nematoda and Polychaeta were apparently not ~~negatively~~ affected by the accentuated acidification in the CES and the high values of organic matter observed at this location. On the other hand, Tardigrada and Gastrotrincha were more abundant in the GES, which had a more alkaline pH and significantly lower organic matter values compared to the other estuaries.

PAH concentrations are often ignored in publications involving estuaries, even though they are ~~often~~ associated with environmental disturbances (Iburg et al., 2020; Louati et al., 2013; Martinec et al., 2014). However, these ubiquitous substances reach estuaries ~~through the various human activities mentioned throughout this study,~~ and require attention, monitoring and public policies to mitigate their damage to the environment. It is worth noting that estuarine environments are subject, not only to PAHs, but to several other contaminants. In previous studies, sediment analy~~z~~es detected both heavy metal (Noronha et al., 2011; Silva et al., 2011) and fertilizers (Noriega et al., 2019) in the urbanized estuaries (CES and TES) studied here.

The effect of pollutants, especially PAHs, on meiofauna is clear. Studies using meiobenthos are ~~extremely viable~~, both financially and scientifically. However, public policies rarely use the diversity of intra-sedimentary microscopic benthos as ecological tools. This study shows that not only ~~do~~ some taxa clearly disappear in the presence of PAHs, ~~but also that richness (EcoQ) proves to be a useful tool for quick and efficient access to ecological quality.~~

## Conclusion

The PAH pollution followed the urbanization gradient, with the concentrations of 5 compounds found to be above the Fauna Impact Threshold (TEL) in the most urbanized estuary. Among the meiofauna groups, Tardigrada and Gastrotricha showed greater sensitivity to PAH impact and, in contrast, Nematoda and Polychaeta were more resistant. Thus, these differences among the ~~phylum~~ show that meiofauna can be used as a good early satge biomonitoring tool for marine conservation, even as a way of predicting possible ecological losses in areas that, although in low quantity, suffer from an anthropic impact, especially regarding PAHs. Such compounds ~~proved to be aggravating~~ the loss of biodiversity within estuaries, ~~in addition to being more harmful to equitability.~~ On the other hand, PAH pollution favored ~~an increase in organism density, due to the facilitation of~~ the emergence of opportunistic groups of Nematoda and Polychaeta in the most polluted place. The use of EcoQ ~~made it possible to understand the environmental quality of the study areas, especially when examined together~~ with the diversity indices, particularly equitability, which can demonstrate the stability of the richness pattern in a specific area. Among the environmental data, the PAHs most influenced the distribution of organisms in the three estuaries suffering from different levels of urbanization/conservation. Furthermore, the autochthonous pollution in the most urbanized estuaries was reflected both in the high organic enrichment and in the accentuated acidification in one of estuaries. Granulometry also played an important role in the heterogeneity of the groups, especially the finer grains, by both reducing interstitial spaces and by more easily associating organic matter with PAH pollution.

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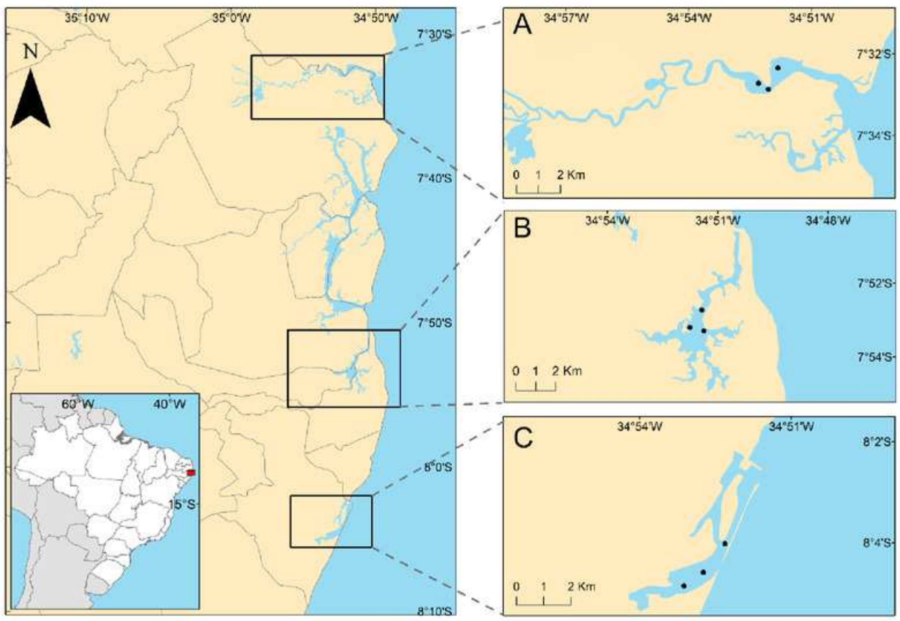
# Figure 1

Positions of sampling stations in the estuaries

Positions of sampling stations in the estuaries of the present study, located on the northeastern coast of Brazil. A: Less urbanized area – Goiana estuarine system (GES); B: Intermediate urbanization area - Timbó estuarine system (TES); C: More urbanized area - Capibaribe estuarine system (CES).

**Figure. 1.** Positions of sampling stations in the estuaries of the present study, located on the northeastern coast of Brazil. A: Less urbanized area – Goiana estuarine system (GES); B: Intermediate urbanization area - Timbó estuarine system (TES); C: More urbanized area - Capibaribe estuarine system (CES).



# Figure 2

Density of the most abundant groups registered in each estuary

Density of the most abundant groups registered in each estuary. Median (solid black line), Average (in red), quartiles and upper/lower limits. (A) Nematoda density, (B) Copepoda density, (C) Polychaeta density, (D) Density of the other groups recorded in the estuaries. (E) Pie chart indicating the relative group densities. GES, Goiana estuarine system; TES, Timbó estuarine system; CES, Capibaribe estuarine system.

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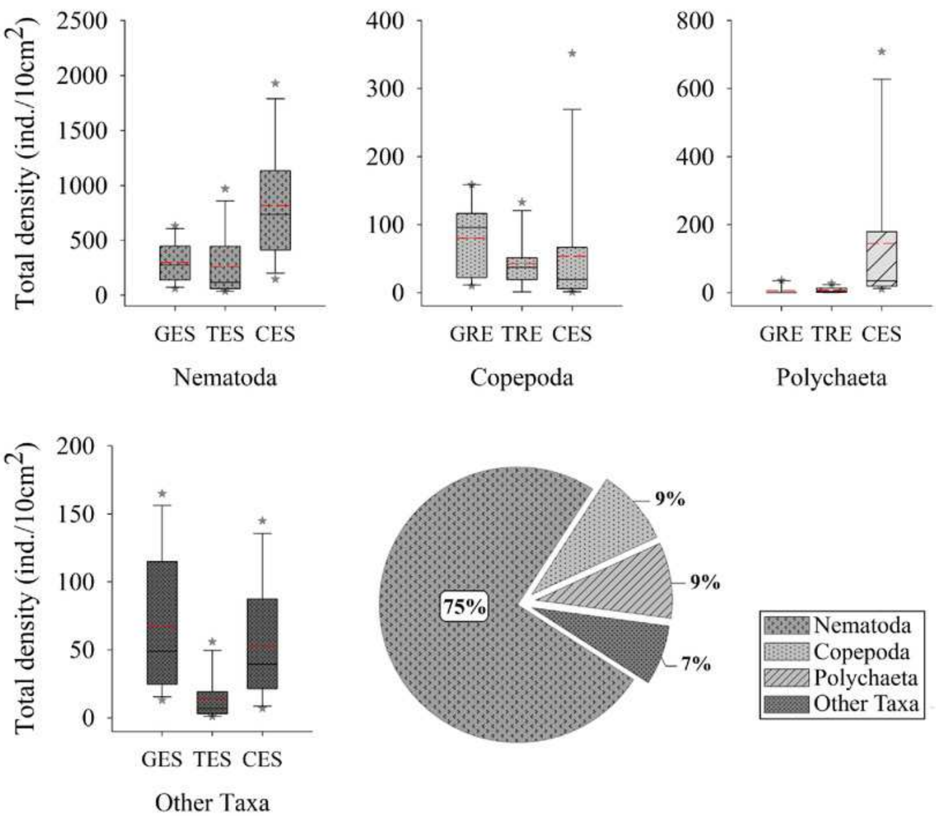

# Figure 3

Density of the least abundant groups recorded in each estuary (< 2% of the total density)

Density of the least abundant groups recorded in each estuary (< 2% of the total density). Median (solid black line), Average (in red), Quartiles and upper/lower limits. GES, Goiana estuarine system; TES, Timbó estuarine system; CES, Capibaribe estuarine system.

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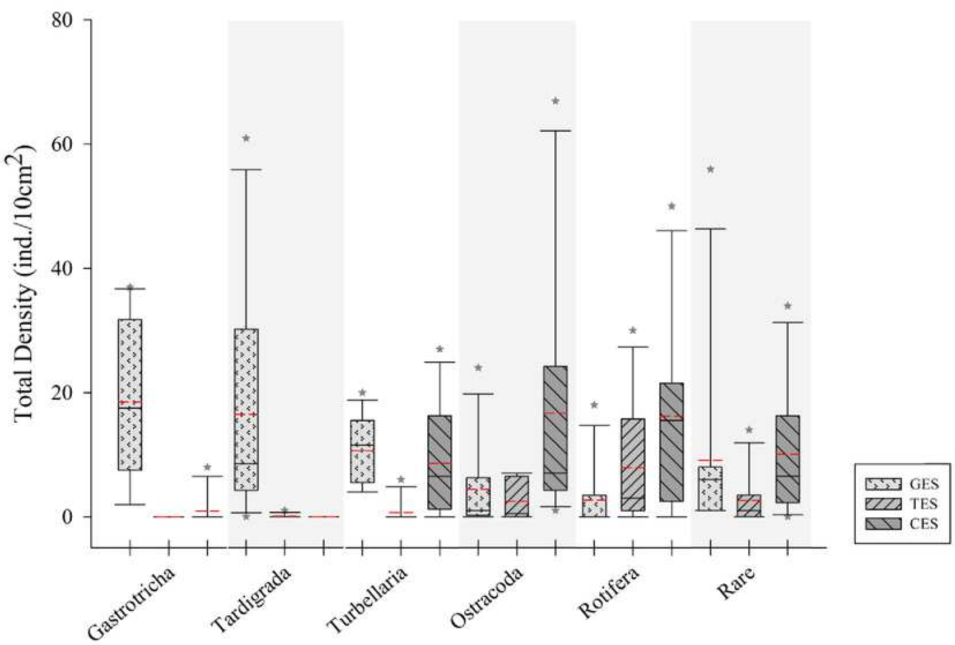
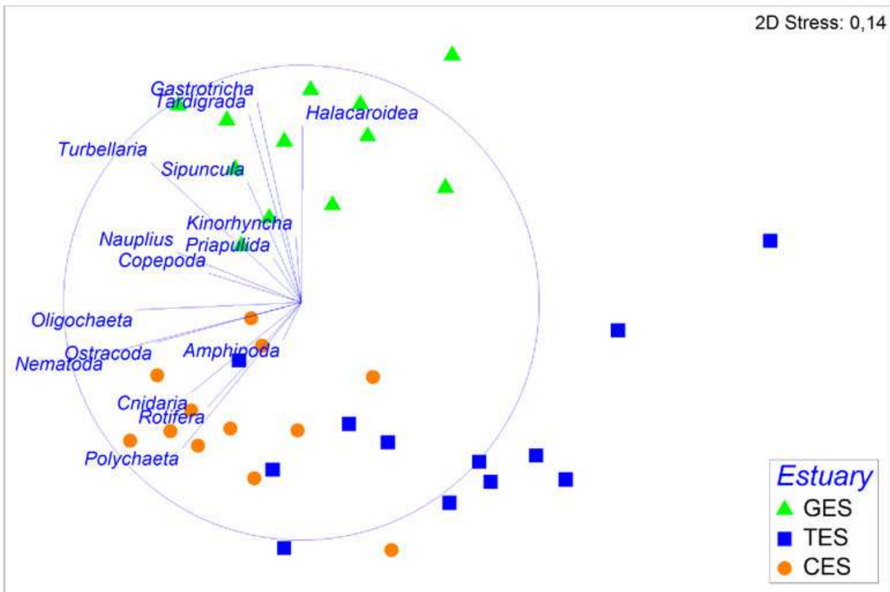


# Figure 4

Non-metric multidimensional scaling (nMDS) based on the density of meiofauna groups

Non-metric multidimensional scaling (nMDS) based on the density of meiofauna groups (standardized on the 4th root, using Bray - Curtis). GES, Goiana estuarine system; TES, Timbó estuarine system; CES, Capibaribe estuarine system.

**Figure. 4.** Non-metric multidimensional scaling (nMDS) based on the density of meiofauna groups (standardized on the 4th root, using Bray - Curtis). GES, Goiana estuarine system; TES, Timbó estuarine system; CES, Capibaribe estuarine system.

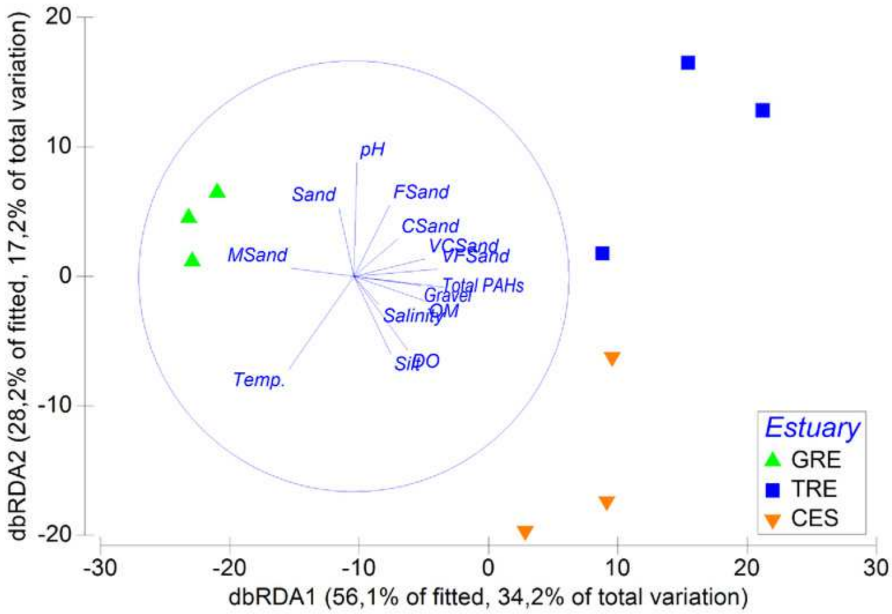


# Figure 5

Mean values (±SE) of richness within each estuary and in their respective stations, in addition to the ecological quality corresponding to the richness of each station

Analysis of dbRDA (DISTLM), correlation between environmental data (including ∑PAHs) in the three estuaries tested. GES, Goiana estuarine system; TES, Timbó estuarine system; CES, Capibaribe estuarine system.

**Figure. 5.** Analysis of dbRDA (DISTLM), correlation between environmental data (including ∑PAHs) in the three estuaries tested. GES, Goiana estuarine system; TES, Timbó estuarine system; CES, Capibaribe estuarine system.

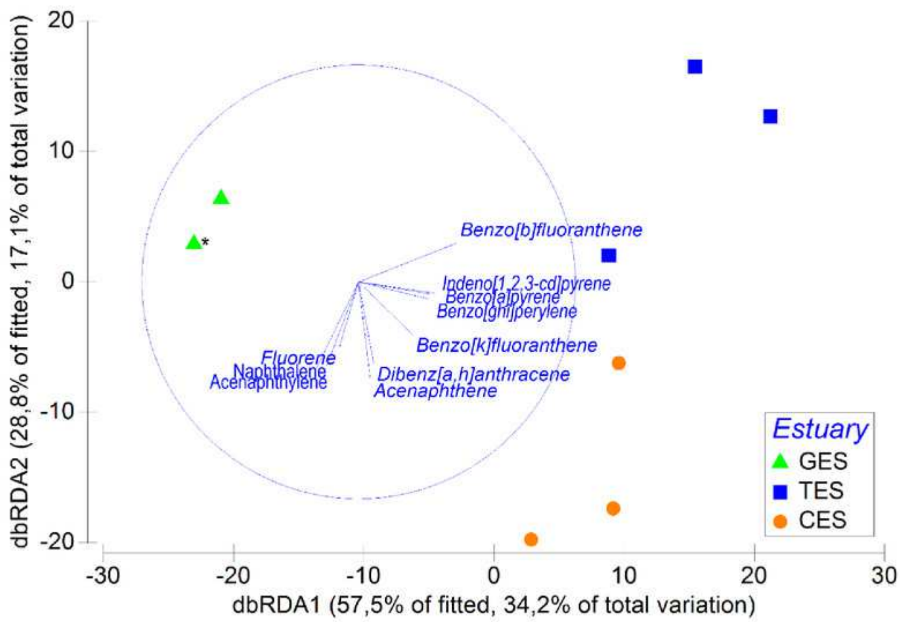


# Figure 6

Group of environmental variables, selected by the DISTLM analysis, that most correlate with the estuarine fauna

Analysis of dbRDA (DistLm), correlation between individual PAH concentrations in the three estuaries tested. The overlap of factors was restricted in order to include variables that have a correlation greater than 0.3. GES, Goiana estuarine system; TES, Timbó estuarine system; CES, Capibaribe estuarine system. * Two points are overlapping on GES as they do not present PAHs.

**Figure. 6.** Analysis of dbRDA (DistLm), correlation between individual PAH concentrations in the three estuaries tested. The overlap of factors was restricted in order to include variables that have a correlation greater than 0.3. GES, Goiana estuarine system; TES, Timbó estuarine system; CES, Capibaribe estuarine system. * Two points are overlapping on GES as they do not present PAHs.



## Table 1 (on next page)

Comparison of environmental data in estuaries, values expressed in average (± SE).

Average values (± SE) of the environmental variables of the three estuaries. Values of P (perm) < 0.05 are in bold. The letters represent groups of significant differences between the studied areas. GES, Goiana estuarine system; TES, Timbó estuarine system; CES, Capibaribe estuarine system. ∑PAH, sum of polycyclic aromatic hydrocarbons; DO, dissolved oxygen; OM, organic matter; Temp, Temperature.

| Area/Station | ∑PAH ng/$g^{-1}$ | DO mg/L | pH | MO mg/g | Temp. C$^{o}$ | Salinity |
|---|---|---|---|---|---|---|
| GES | 0.55 ± 0.67 [a] | 4.45 ± 0.44 [a] | 8.4 ± 0.04 [a] | 1.87 ± 0.35 [a] | 29.13 ± 0.08 | 27.33 ± 2.16 |
| TES | 139.17 ± 71.43 [b] | 5.43 ± 0.49 [a] | 8.39 ± 0.06 [a] | 6.88 ± 1.12 [b] | 29.03 ± 0.04 | 30.67 ± 3.56 |
| CES | 674.81 ± 331.31 [b] | 17.67 ± 2.04 [b] | 5.81 ± 0.02 [b] | 11.77 ± 3.04 [b] | 29.47 ± 0.27 | 27.57 ± 3.78 |
| *p* | **0.01** | **0.024** | **0.039** | **0.014** | 0.103 | 0.349 |
| *Pseudo-F* | 21.712 | 63.793 | 4146.9 | 21.609 | 3.381 | 1.364 |
| *PermDisp* | 0.9214 | 0.3889 | 0.071 | 0.7611 | 0.0245 | 0.7779 |

## Table 2(on next page)

Comparison of granulometric fractions in estuaries, values expressed in average (± SE).

Average values (± SE) of the granulometric fractions in the three estuaries. Result of PERMANOVA, PERMIDISP on the granulometric matrix of estuaries. Values of P(perm) < 0.05 are in bold. The letters represent groups of significant differences between the study areas and each granulometric size. GES, Goiana estuarine system; TES, Timbó estuarine system; CES, Capibaribe estuarine system; VCS, very coarse sand; CS, coarse sand; MS, medium sand; FS, fine sand; VFS, very fine sand.

| Estuary | Gravel | Sand | VCS | CS | MS | FS | VFS | Silt |
|---|---|---|---|---|---|---|---|---|
| | % | | | | | | | |
| GES | two | 96 | 2.13 | 16.03 | 43.23 | 33.96 | 0.67 | 1.97 |
| | ± 2.27 | ± 3.28 [a] | ± 1.45 | ± 9.62 | ± 12.5 | ± 24.61 | ± 0.11[a] | ± 1.0 [a] |
| TES | 5.13 | 83.9 | 5.8 | 13.77 | 19.7 | 27.83 | 16.87 | 10.97 |
| | ± 4.7 | ± 3.4 [b] | ± 3.01 | ± 5.98 | ±5.27 | ± 7.27 | ± 8.72 [b] | ± 4.52 [ab] |
| CES | 9.17 | 64 | 5.9 | 13.67 | 17.93 | 15.1 | 11.5 | 26.73 |
| | ± 6.63 | ± 14.99 [b] | ± 3.48 | ± 6.88 | ± 7.48 | ± 2.18 | ± 4.67 [b] | ± 10.7 [b] |
| *p* | 0.384 | **0.02** | 0.459 | 0.956 | 0.258 | 0.482 | **0.043** | **0.048** |
| *Pseudo-F* | 1.1259 | 5.2086 | 1.1466 | 5.74E-02 | 2.913 | 0.62938 | 7.8821 | 9.3009 |
| *PermDisp* | 0.8707 | 0.2695 | 0.8873 | 0.1701 | 0.7261 | 0.1298 | 0.0522 | 0.4192 |

## Table 3(on next page)

Comparison of estuarine meiofauna community structure, as well as pairwise comparison.

Results of PERMANOVA, PERMIDISP and PAIR-WISE tests on the structure of the meiofauna communities in the study estuaries. The analysis factor was the area (Estuary). Values of P (perm) < 0.05 are in bold. GES, Goiana estuarine system; TES, Timbó estuarine system; CES, Capibaribe estuarine system; df, degrees of freedom; MS, mean squares; Res, residual.

| PERMANOVA | df | MS | Pseudo-F | P(perm) |
|---|---|---|---|---|
| Estuary | 2 | 6748.9 | 16.251 | **0.0001** |
| Res | 33 | 460.53 | | |
| Total | 35 | | | |
| PERMIDISP | 2 | | 1,6914 | 0,2831 |
| | | | PAIR-WISE | |
| Groups | | | t | P(perm) |
| GES, TES | | | 4.1003 | **0.0001** |
| GES, CES | | | 4.9839 | **0.0001** |
| TES, CES | | | 3.1187 | **0.0003** |

## Table 4(on next page)

Comparison among ecological indices cataloged in estuaries: Richness (S), Density (N), Shannon index (H') and Evenness (J').

PERMANOVA and PAIR-WISE results for the ecological indices in the study estuaries. The analysis factor was the area (Estuary). Values of P(perm) < 0.05 are in bold. GES, Goiana estuarine system; TES, Timbó estuarine system; CES, Capibaribe estuarine system; df, degrees of freedom; MS, mean squares; Res, residual.

| | | PERMANOVA | | | | | PAIR-WISE | |
|---|---|---|---|---|---|---|---|---|
| Ecological index | Source | df | MS | pseudo-F | P (perm) | Groups | t | P (perm) |
| Richness - S | Estuary | 2 | 143.02 | 14.31 | **0.0002** | GES, TES | 4.4379 | **0.0007** |
| | Res. | 33 | 9.995 | | | GES, CES | 1.4402 | 0.1621 |
| | Total | 35 | | | | TES, CES | 3.7744 | **0.0018** |
| Density - N | Estuary | 2 | 850.14 | 9,7861 | **0,0002** | GES, TES | 1.6754 | 0.1060 |
| | Res. | 33 | 86.872 | | | GES, CES | 3.1014 | **0.0065** |
| | Total | 35 | | | | TES, CES | 4.2221 | **0.0002** |
| Shannon - H' | Estuary | 2 | 118.86 | 8,2434 | **0,0024** | GES, TES | 3.3466 | **0.0053** |
| | Res. | 33 | 14.418 | | | GES, CES | 3.4752 | **0.0040** |
| | Total | 35 | | | | TES, CES | 0.43717 | 0.6684 |
| Evenness - J' | Estuary | 2 | 81.323 | 6,264 | **0,0061** | GES, TES | 1.0207 | 0.3174 |
| | Res. | 33 | 12.983 | | | GES, CES | 3.0416 | **0.0082** |
| | Total | 35 | | | | TES, CES | 2.7726 | **0.0130** |

## Table 5(on next page)

Average values of richness in estuaries and their stations, used to generate the EcoQ ecological quality index.

Mean values (±SE) of richness within each estuary and in their respective stations, in addition to the ecological quality corresponding to the richness of each station. GES, Goiana estuarine system; TES, Timbó estuarine system; CES, Capibaribe estuarine system; Med, average; EcoQ, environmental quality status; St, Station.

| Estuary | Med. (±SE) Estuary | Station | Med. (±SE) Station | EcoQ |
|---|---|---|---|---|
| GES | 8.75 ± 0.5 | St1 | 9 ± 0.54 | Moderate |
| | | St2 | 9 ± 0.2 | Moderate |
| | | St3 | 8.25 ± 0.52 | Moderate |
| TES | 5.33 ± 0.55 | St1 | 6.25 ± 0.69 | Poor |
| | | St2 | 4.75 ± 0.24 | Poor |
| | | St3 | 5 ± 0.35 | Poor |
| CES | 7.83 ± 0.46 | St1 | 8.5 ± 0.14 | Moderate |
| | | St2 | 6.25 ± 0.38 | poor |
| | | St3 | 8.75 ± 0.24 | Moderate |

# Table 6(on next page)

DISTILM, indicating which of the environmental variables best explain organisms' distribution in estuaries.

Group of environmental variables, selected by the DISTLM analysis, that most correlate with the estuarine fauna. The BEST procedure was used on similarity matrices based on meiofauna density. RSS, Residual Sum of Squares; No. Vars, number of variables; ∑PAHs, sum of Polycyclic Aromatic Hydrocarbons; DO, dissolved oxygen; VCSand, very coarse sand; OM, organic matter; CSand, coarse sand; MSand, medium sand.

| $R^2$ | RSS | No. Vars | Variable Selection |
|---|---|---|---|
| 0.26226 | 21170 | 1 | ∑PAHs |
| 0.39501 | 17360 | 2 | ∑PAHs; pH |
| 0.47378 | 15100 | 3 | DO; pH; VCSand |
| 0.52768 | 13553 | 4 | DO; pH; VCSand; Silt |
| 0.56147 | 12584 | 5 | DO; pH; OM; VCSand; Silt |
| 0.59516 | 11617 | 6 | DO; pH; OM; Sand; VCSand; Silt |
| **0.60939** | 11209 | 7 | DO; pH; Sand; VCSand; Csand; MSand; Silt |

# Table 7(on next page)

DISTILM, indicating which PAH's (individual concentration) best explain organisms' distribution in estuaries.

Group of PAHs, selected by the DISTLM analysis, which most correlated with estuarine fauna. The BEST procedure was used on similarity matrices based on meiofauna density. RSS, Residual Sum of Squares; No. Vars, number of variables; BbF, Benzo[b]fluoranthene; A, Anthracene; DA, Dibenz[a,h]anthracene; ghi, Benzo[ghi]perylene; BaA, Benzo[a]anthracene; BkF, Benzo[k]fluoranthene; IP, Indeno[1,2,3-cd]pyrene; AY, Acenaphthylene; F, Fluorene.

| $R^2$ | RSS | No. Vars | Variable Selection |
|---|---|---|---|
| 0.24024 | 21801 | 1 | BbF |
| 0.46448 | 15367 | 2 | A; BbF |
| 0.50893 | 14091 | 3 | A; BbF; DA |
| 0.54333 | 13104 | 4 | A; BbF; DA; ghi |
| 0.57024 | 12332 | 5 | A; BaA; BkF; IP; DA |
| **0.59441** | 11639 | 6 | AY; F; A; BbF; DA; ghi |

## Table 8 (on next page)

Spearman correlation, between environmental data and diversity indices registered in estuaries.

Spearman correlation values between environmental data and diversity indices registered in estuaries: Density (ind./10cm$^2$), Shannon Index (H), meiofauna richness (S) and group equitability (J). Significant values are represented by: *p<0.05, **p<0.01, ***p<0.001.

| | Density (ind./10cm$^2$) | Shannon (H) | Richness (S) | Equitability (J) |
|---|---|---|---|---|
| ∑ PAH | **0.450**** | -0.161 | -0.111 | **-0.422*** |
| Salinity | 0.004 | -0.095 | -0.075 | -0.067 |
| DO | **0.354*** | -0.194 | -0.141 | **-0.407*** |
| pH | -0.196 | 0.036 | -0.011 | 0.271 |
| OM | 0.214 | **-0.349*** | -0.32 | **-0.491**** |
| Temp. | **0.457*** | 0.228 | 0.258 | -0.224 |
| Gravel | 0.108 | -0.188 | -0.185 | -0.063 |
| Sand | **-0.389** | 0.185 | 0.148 | 0.316 |
| VCSand | **-0.237** | **-0.450**** | **-0.468**** | -0.138 |
| CSand | -0.265 | -0.208 | -0.182 | -0.05 |
| MSand | -0.27 | 0.174 | 0.163 | 0.315 |
| FSand | -0.157 | -0.064 | -0.079 | -0.021 |
| VFSand | -0.148 | **-0.456**** | **-0.409*** | **-0.608***** |
| Silt | **0.437**** | -0.205 | -0.155 | **-0.489**** |