UNIVERSIDADE FEDERAL DO ESPÍRITO SANTO
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PROGRAMA DE PÓS-GRADUAÇÃO EM OCEANOGRAFIA AMBIENTAL

CÍNTIA DA SILVA VARZIM

DETERMINAÇÃO DE IMPACTOS POR ENRIQUECIMENTO ORGÂNICO EM UM ESTUÁRIO TROPICAL

VITÓRIA
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Dissertação de Mestrado apresentada ao Programa de Pós-Graduação em Oceanografia Ambiental da Universidade Federal do Espírito Santo, como requisito parcial para obtenção do título de Mestre em Oceanografia Ambiental. Orientador: Prof. Dr. Angelo Fraga Bernardino.

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"Uso de Isótopos Estáveis na Determinação de Impactos Por Enriquecimento Orgânico em um Estuário Tropical."

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RESUMO

Os estuários são importantes áreas de transição entre terra e mar, caracterizados pela alta produtividade biológica, vital às áreas costeiras adjacentes. Encontram-se sob intensa exploração humana, tanto no setor urbano quanto industrial, através do despejo de efluentes não tratados, com conseqüências para o ecossistema. Considerando a quantidade de esgoto lançado nesses ecossistemas e a necessidade de monitorar os impactos sofridos, é importante utilizar diferentes metodologias para determinar a presença de poluentes e os seus efeitos ao longo da cadeia alimentar estuarina. Neste projeto foi avaliado o impacto do enriquecimento orgânico na comunidade bentônica da Baía de Vitória, na cidade de Vitória, Espírito Santo, Brasil, utilizando assinaturas de isótopos estáveis ($\delta^{13}C$ e $\delta^{15}N$) aliados aos dados de esteróis marcadores geoquímicos coprostanol e epicoprostanol, bem como a razão entre ambos, a fim de validar os dados fornecidos pelas análises isotópicas. As assinaturas isotópicas para nitrogênio na fauna dos pontos contaminados foi enriquecida em relação aos pontos não contaminados. A distribuição de grupos tróficos em estações contaminadas e não contaminadas apresentou diferença em relação ao fator contaminação, mostrando que assinaturas isotópicas são capazes de indicar alterações ambientais em estuários tropicais.

Palavras-chave: bentos, estuário, Baía de Vitória, isótopos estáveis, poluição.

ABSTRACT

Estuaries are important transitional ecosystems between land and sea, with high biological productivity and are vital for the adjacent coastal areas. They are under intense human pressure, mainly urban and industrial, due to the dumping of untreated effluents with impacts on the ecosystem. Considering the wide quantity of sewage released in these ecosystems and the necessity of monitoring the effects of these impacts, it is important to use different methods to determine the presence of pollutants and their effects on the estuarine food chain. We evaluated the organic enrichment impact on the benthic food web in Vitória Bay estuarine complex, Vitória, Espírito Santo, Brazil. We used stable isotopes ($\delta^{13}C$ and $\delta^{15}N$) from benthic macrofauna, with geochemical markers as coprostanol and epicoprostanol and their ratio, to validate isotopic analysis results. The macrofaunal nitrogen isotopic signatures at contaminated sites were enriched when compared to the non-contaminated. Macrofaunal trophic niche amplitude at most contaminated sites was smaller if compared to the non-contaminated sites. Our results suggest that stable isotopic signatures from benthic macrofauna could be used to evidence environmental impacts from organic enrichment at tropical estuaries under heavily polluted conditions.

Key-words: benthic, estuary, Vitória Bay, stable isotopes, pollution.

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CAPÍTULO 1

1.1. Introdução geral

Estuários são ecossistemas de importância ecológica e econômica que, apesar da variedade de serviços ambientais que propiciam para a humanidade, encontram-se seriamente ameaçados em todo o mundo (Bouillon et al., 2008; Obade et al., 2009). Os impactos humanos têm distanciado os estuários e ecossistemas costeiros de sua base histórica como ecossistemas ricos, diversificados e produtivos (Lotze et al., 2006). O intenso impacto antrópico nas áreas costeiras altera ambientes estuarinos de variadas formas, como através da poluição de origem industrial e urbana e de modificações físicas que visam adaptar os estuários às necessidades humanas (Schettini et al., 2000; Cooper, 2002; Elliot & Quintino, 2007).

Ambientes estuarinos tropicais provêm importante habitat e recursos para comunidades diversas de organismos bentônicos (Alfaro, 2005). São compostos por espécies de hábitos interligados às condições ambientais, podendo servir como indicadores ambientais (López-Gappa et al., 1990). A distribuição espacial desses organismos está relacionada com fatores ambientais diversos incluindo estressores antrópicos (Barros et al., 2008) como o lançamento de esgotos domésticos, com ou sem tratamento prévio, que alteram o ambiente e, por conseguinte, as condições ideais para a sobrevivência dos organismos (Carreira et al., 2001).

As comunidades macrobentônicas são compostas por espécies que podem assimilar um conjunto de condições ambientais ao decorrer do seu tempo de vida (Nalesso et al., 2005). Tais organismos permanecem junto ao substrato pelo menos durante parte do seu ciclo de vida, associados aos tipos de fundo que integram os componentes da diversidade aquática capaz de caracterizar a qualidade ecológica do ambiente (Barbour et al., 1999). Dentre estes organismos, os poliquetas possuem o papel de indicadores de poluição orgânica (Del-Pilar-Ruso et al., 2009).

A mais global das perturbações ambientais é o enriquecimento das águas marinhas, condição que, por causas naturais ou artificiais, resulta em mudanças em fatores químicos, físicos e biológicos que, por sua vez, têm efeitos diretos e indiretos na fauna presente (Pearson & Rosenberg, 1978). As águas residuais municipais constituem fonte de descarga de grandes montantes de compostos orgânicos ao meio marinho, cujas partículas podem ser transportadas e resultarem incorporadas ao sedimento (Maldonado et al., 2000). Uma das principais causas de deterioração da qualidade da água e do aumento da carga de nutrientes em áreas costeiras e internas é a poluição causada por resíduos humanos e animais (Leeming et al., 1994).

Marcadores químicos constituem uma ferramenta amplamente utilizada para a determinação da contaminação ambiental (Venkatesan & Mirsadeghi, 1992) considerando, entre outros, esteróis como o coprostanol e o epicoprostanol por não serem naturais de sedimentos marinhos, mas provenientes de material fecal humano (Martins et al., 2005). A presença de coprostanol é um indicador de contaminação de causa antrópica, já que ele é o principal esterol de origem fecal humana (Leeming et al., 1994; Martins et al. 2005).

Isótopos estáveis de elementos como carbono, nitrogênio e enxofre têm sido utilizados em estudos diversos acerca de ecologia costeira, empregando-se comumente análises com os isótopos de carbono e de nitrogênio (Carvalho, 2008). Tais análises podem fornecer informações sobre a dinâmica do fluxo de carbono e da posição trófica dos consumidores nas cadeias alimentares (Mazumder et al., 2015). A distribuição natural destes elementos reflete a história dos processos metabólicos e físicos no ambiente, o que é uma ferramenta válida para estudos que considerem estas variantes (Pereira, 2007). Os isótopos $\delta^{13}C$ e $\delta^{15}N$ permitem caracterizar a descarga de esgoto e o efeito deste processo na cadeia alimentar (Rogers, 1999). Análises desses isótopos na composição orgânica sedimentar na biota marinha também fornecem uma visão detalhada da ciclagem dos elementos de ecossistemas marinhos, permitindo distinguir a fonte orgânica e a posição trófica da fauna na cadeia alimentar marinha (Alongi, 1998; Fry, 2006).

Devido à expansão da indústria de petróleo na região de Vitória e consequente tráfego marinho, alterações na entrada de água doce se tornaram um dos vários impactos antropogênicos que afetam a Baía de Vitória nos últimos cinqüenta anos (Zalmon et. al, 2011). A ausência de planejamento das atividades humanas causa prejuízos ambientais que levam a mudanças na comunidade biológica e na qualidade dos serviços ecossistêmicos do sistema estuarino de Vitória (Grilo et al. 2016). Como resultado da crescente urbanização na cidade de Vitória e considerando a capacidade de assimilação de matéria pelos organismos bentônicos e pelo próprio substrato, esperamos encontrar diferenças significativas na assinatura isotópica da fauna bêntica e do sedimento ao longo da área analisada. O presente trabalho teve por objetivo, portanto, identificar as assinaturas isotópicas de sedimento e de fauna em grupos majoritários de invertebrados bentônicos nas estações amostradas, comparando eventuais mudanças espaciais com indicadores geoquímicos de poluentes. Dessa forma, objetivamos validar a eficácia do uso de isótopos estáveis como ferramenta para detectar impactos do enriquecimento orgânico decorrente da ação antrópica no ambiente e os efeitos desse processo no sistema estuarino da Baía de Vitória.

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CAPÍTULO 2

Use of Stable Isotopes in Determining Impacts of Organic Enrichment in the Benthic Food Chain at a Tropical Estuary

Abstract

Estuaries are important transitional ecosystems between land and sea, characterized by high biological productivity and vital for the adjacent coastal ecosystems. They are under intense human exploitation, mainly urban and industrial, through the dumping of untreated effluents with impacts on the ecosystem. Considering the wide quantity of sewage released in these ecosystems and the necessity to monitor the effects of these impacts, it is important to test different methods to identify the presence of pollutants and their effects on the estuarine food chain. We evaluated the organic enrichment impacts on the benthic communities in the Vitória Bay estuary, by contrasting spatial patterns of stable isotopes signatures ($\delta^{13}C$ and $\delta^{15}N$), with geochemical markers for sewage contamination. Coprostanol concentrations ranged from 0.14 to 13.8 $\mu g.g^{-1}$ (2.95 ± 3.98) along the estuarine complex. Deposit-feeders and omnivores had wide $\delta^{15}N$ signatures, suggesting different trophic positions at the study area. Carbon isotopic signatures of carnivore, deposit-feeders and omnivores showed depletion at heavily impacted sites. Our results indicated that the benthic fauna in contaminated sites exhibited less niche amplitude when compared to non-contaminated areas, suggesting that organic contamination impact benthic food webs and may be used as an indicator of pollution in coastal ecosystems.

Key-words: benthic, estuary, Vitória Bay, stable isotopes, pollution.

2.1. Introduction

Estuaries are transitional areas between land and sea (Kathiresan and Bingham, 2001) ecologically and economically important but at the same time seriously endangered globally (Bouillon et al., 2008; Obade et al., 2009; Olds et al. 2016). Human impacts in estuaries and coastal ecosystems altered biodiversity and productivity (Lotze et al., 2006). The intense anthropogenic impacts on estuaries are originated from pollution, coming from industrial and urban activities or by physical modifications in order to adapt the estuaries to human necessities (Schettini et al., 2000; Cooper, 2002; Elliot & Quintino, 2007).

Tropical estuaries provide important structure and resources to several communities of benthic organisms (Alfaro, 2005) and they are composed by species with habits linked to environmental conditions which serve as environmental indicators (López-Gappa et al., 1990). Spatial distribution of these organisms is connected to several environmental factors including anthropogenic stressors (Barros et al., 2008) like the dump of domestic sewage, with or without previous treatment, that alters environmental and conditions to the organisms survivor (Carreira et al., 2001). Ecosystem impact assessments of estuarine ecosystems are commonly based on the structure, function, and processes of benthic assemblages and overall indicators of human activities (Muniz et al., 2012). In this context, the polychaeta group has the role of indicator of organic pollution (Del-Pilar-Ruso et al., 2009) and bivalves also constitute important biomarkers of anthropogenic impact (Montagna & Kalke, 1995).

Organisms that belong to benthic communities naturally respond to spatial and temporal changes quickly and are able to show signs of degradation (Warwick, 1993). In general, the ratio of sensitivity to tolerance of benthic species was used to develop the biotic indices in order to estimate the environmental responses to anthropogenic activities (Borja et al., 2000). The macrobenthic communities are composed by species which can integrate a set of environmental conditions through their lifetime (Nalesso et al., 2005) remaining close to the substrate at least for a period of their life cycle, associated to types of bottom that ensemble the compounds of aquatic diversity, able to characterize the environmental ecological quality (Barbour et al., 1999).

The most universal environmental perturbation is the enrichment of marine waters, by natural or artificial causes, that results in changing factors chemical, physical and biological, with direct and indirect effects on the fauna (Pearson & Rosenberg, 1978). One of main causes of deterioration of water quality and the increase of nutrients in coastal and estuarine waters is the pollution caused by human waste (Leeming et al., 1994). At South America, urban sewage is one of the most important sources of marine pollution due to a high quantity of cities without facilities to treat sewage (Martins et al., 2010).

Chemical markers constitute a tool to determine environmental contamination by addition of sewage (Venkatesan & Mirsadeghi, 1992). They are compounds from natural or anthropogenic origin (Abreu-Mota et al. 2014). Among chemical markers, sterols as coprostanol (5β-cholestan-3β-ol) and epicoprostanol (5β-cholestan-3α-ol) are not natural of marine sediments but are present in human fecal material (Martins et al., 2005). Measures of coprostanol concentrations are useful to track domestic inputs close to its source (Maldonado et. al. 2000). Stable isotopes $\delta^{13}C$ and $\delta^{15}N$ from sedimentary organic matter also allow us to characterize the sewage discharge and the effects of this process in the food chain (Rogers, 1999). Analysis of these isotopes in sedimentary organic composition of marine biota can also provide a detailed view of the cycling of elements of marine ecosystems, allowing distinguish the organic source and the trophic position of fauna in marine food chain (Alongi, 1998; Fry, 2006).

Stable isotopes of elements such as carbon and nitrogen have been used in several studies about coastal ecology (Carvalho, 2008). Such analysis may provide insights about the dynamics of carbon flow and trophic position of consumers at food chains (Mazumder et al., 2015). The natural distribution of these elements reflects the history of metabolical and physical processes in the environment, which is a valid tool to studies that consider these variants (Pereira, 2007). Ecosystems impacted by sewage show variations in stable isotope signatures of carbon and nitrogen which therefore may indicate effects on benthic food chain (West et al., 2006). Couch (1989) has developed a study with benthic meiofauna, analysing assimilation of *Spartina alterniflora* and benthic micro-algae by harpacticoid copepods and nematodes using carbon and nitrogen stable isotope ratios. Iken et al. (2001) analyzed food web structure of benthic meio- ,macro- and megafauna and evidenced high competition for food at a very limited food system. Benthic macrofauna were analyzed by Sampaio et al. (2010) to trace organic sources of carbon and nitrogen at a coastal area under organic enrichment. Using carbon and nitrogen isotopic data of benthic fauna and macroalgae, Mayir et al. 2011 characterized benthic food web at a relative preserved marine ecosystem.

The discharge of municipal residual waters constitutes an important source of organic compounds of anthropogenic origin to the estuarine and marine ecosystems (Maldonado et al., 2000). The contamination of these systems is a relevant subject for human and environmental health (Grilo et al. 2013). The dumping of sewage, mostly not treated, is the main cause of environmental degradation that Victoria bay has suffered in recent decades and characterizes this bay, along with factors as also intense port activity, as eutrophic environment (Jesus et al., 2004).

Due to expanding the petroleum industry at region of Vitória, the marine traffic has increased and the changes on the input of fresh water became one of the many anthropic impacts that affect the Vitoria Bay during the last half century (Zalmon et. al., 2011). The aim of this study was to test $\delta^{13}C$ and $\delta^{15}N$ isotopic signatures from estuarine sediments and macrobenthic assemblages offer as a good proxy to identify sewage impacts in Vitória Bay estuarine complex. Isotopic signatures were compared to geochemical sewage markers to verify if these two data would be congruent about indicating spatial contamination.

2.2. Material and methods

2.2.1. *Study area and sampling*

Vitória Bay is at the metropolitan area of Vitória city, Espírito Santo State, Brazil and comprehends approximately 23 km² of mangrove (Chagas et al. 2006), compounding a system with two coastal whater entrances, the Baía de Vitória itself (BV) and the Canal da Passagem (CP) (Sterza & Fernandes, 2006). This channel communicates the northwest Vitória Bay to a coastal embayment, through the Baía do Espírito Santo (BV) (Jesus et al., 2004). This system is formed by the runoff of many rivers, the Rio Santa Maria da Vitória has a midsize, while Format-Marine, Bubu, Aribiri, Córrego Piranema and the Canal da Costa, are narrow rivers (Veronez-Jr., 2009) (Fig. 1).

The average salinity within all study area ranged between 23.1 and 36.2 and the average temperature, ranged between 22.1 to 27°C. The regions corresponding to Baía de Vitória and Canal da Passagem presented polihaline condition and the other regions, Canal do Porto and Baía do Espírito Santo, euhaline.

Benthic macroinvertebrates (>0.5 mm) were sampled with two replicates with a Day Grab (about 15L of capacity) at 11 sites (Fig. 1). Replicate contents were summed. The samples were then sieved on board with a 0.05 mm sieve and preserved in 4% formaldehyde solution. Sediment samples for pollutant analysis were obtained concomitantly to the fauna from the undisturbed sediment surface (2 cm), preserved in cleaned and decontaminated aluminum foil with ice and kept frozen in the laboratory to the preparation and send for analysis (Hadlich et al., in prep).

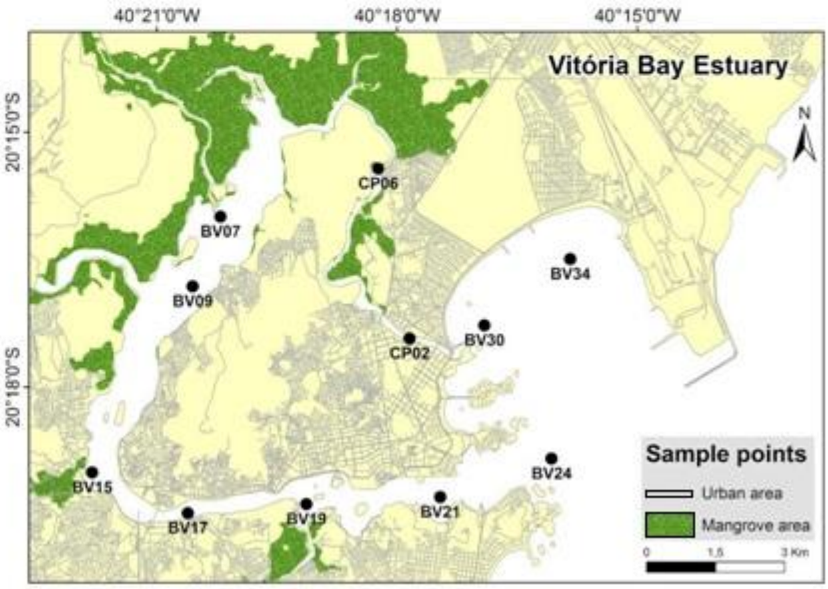

Figure 1. Distribution of sites at the Vitória Bay estuary and Baía do Espírito Santo.

2.2.2 *Laboratorial Analysis*

Macrofauna was sorted and identified at the laboratory following standard protocols. The macrofauna at the study area was dominated by Polychaeta (Annelida), Mollusca and Crustacea (Table 1), as observed in other studies (Nalesso et. al., 2005, Ramos et al., 2010). Thus, we selected the most dominant groups (Polychaeta and Mollusca) and identified all individuals to family taxa (Table 2).

Table 1. Number of benthic organisms sampled from dominant macrofaunal groups at points and respective region from study area.

	BV 07, BV 09, BV 15 Baía de Vitória	BV 17, BV 19, BV 21 Canal do Porto	BV 24, BV 30, BV 34 Baía do Espírito Santo	CP 02, CP 06 Canal da Passagem	Total
Annelida (Polychaeta)	1813	1277	223	376	**3689**
Mollusca	561	221	18	76	**876**
Arthropoda (Crustacea)	121	91	99	37	**348**
Others	62	76	2	80	**220**
Total	**2557**	**1665**	**342**	**569**	**5133**

The twelve most dominant families at study area were selected for isotopic analysis (Table 2). Feeding habits were obtained from bibliography. At the Polychaeta group we found Capitellidae and Spionidae, deposit-feeders; Goniadidae, carnivore; Onuphidae and Nereididae, omnivores (Jumars et al. 2015); Cirratulidae, deposit-feeder (Amaral & Pardo, 2004) and Orbiniidae, deposit-feeder (Dean & Blake, 2015). At Mollusca group we found Hydrobiidae, deposit-feeder (Kabat & Hershler, 1993); Mytillidae, suspension-feeder (Mayir et al. 2011); Solecurtidae, suspension-feeder; Tellinidae, deposit-feeder (Arruda et al., 2003) and Veneridae, suspension-feeder (Narchi, 1972; Arruda et al. 2003).

Table 2. Twelve dominant macrofaunal families sampled at study area and selected for isotopic analysis, in each site. Empty spaces indicate the absence of the correspondent family to the respective site.

Feeding Habit	Family	BV 07	BV 09	BV 15	BV 17	BV 19	BV 21	BV 24	BV 30	BV 34	CP 02	CP 06
Carnivore	Goniadidae	X	X	X	X	X		X				X
Depositivores	Capitellidae	X	X	X	X	X	X				X	X
	Cirratulidae		X	X	X	X	X					
	Hydrobiidae	X										X

	Orbiniidae			X	X		X	X	X		X	
	Spionidae	X	X	X	X	X	X	X	X	X	X	X
	Tellinidae	X			X	X	X		X		X	X
Omnivores	Onuphidae		X	X		X	X	X		X		X
	Nereididae	X		X		X	X				X	
Suspensivores	Mytilidae				X	X					X	
	Solecurtidae	X	X	X								X
	Veneridae	X		X		X					X	X

Stable isotopic signatures from sediment samples were obtained after drying at 60ºC and carbonate removal by the drop wise addition of 1M HCl; reducing until fine powder using a mortar and pestle. After acidification, macrofaunal organisms were dried in drying oven at 60ºC. Within a given sample, individuals from the same families were pooled to ensure that sample mass was enough to enable isotope analysis. All samples were analyzed using a stable isotope ratio mass spectrometer. The isotopic compositions ($\delta^{13}C$ and $\delta^{15}N$) are measured on animal samples to a final dry weight from 0.5 to 2 mg, following Levin & Carolyn (2012) and sediment samples following the same protocol.

In order to calculate Isotopic ratios for carbon and nitrogen, is used as reference for carbon the Vienna Pee Dee Belemnite (VPDB, $\delta^{13}C$ = 0 ‰) and for nitrogen the atmospheric nitrogen ($\delta^{15}N$ = 0 ‰) (Peterson & Fry, 1987). It is common to see at ecological studies isotopic compositions in terms of three values, which are parts per thousand differences from a standard:

$\delta X = [(R_{sample} / R_{standard}) - 1] \times 10^3$, where X is ^{13}C, ^{15}N. R is the corresponding ratio $^{13}C/^{12}C$, $^{15}N/^{14}N$. The δ values are measures of the amounts of heavy and light isotopes in a sample. Measurement precision typically is 0.2 ‰ (Peterson & Fry, 1987).

Considering the depletion registered for $\delta^{13}C$ at samples previously fixed with formaldehyde 4 % (Manetta et al., Syväranta et al., 2011), correction for these artifacts were performed by adding 1 ‰ to $\delta^{13}C$ macrofaunal signatures (Bernardino et al., 2010; Demopoulos et al. 2007). To visualize the spatial isotopic signatures at sediment, two maps were elaborated with ArcGIS (Geographic Information System)

(Fig 4 and 5), containing three range of values obtained by calculating three mathematically uniform intervals comprising all values between the lowest and the highest $\delta^{13}C$ and $\delta^{15}N$ at sediment.

2.2.3. *Study sites*

Samples for geochemical analyzes were frozen-dried and sent to the Organic Geochemistry Laboratory of Sea Studies Center at the Federal University of Paraná to follow analysis as proposed by Martins et al. (2008).

Fecal sterol, only, should not be considered an unambiguously attributed to fecal matter inputs, so it does not provide an accurate assessment of the contamination (Grimalt et al., 1990; Martins et al., 2010). Thus, is suggested using ratios involving coprostanol with different sterols, so we used the coprostanol concentration obtained as an indicator of pollution and the ratio II: epicoprostanol/coprostanol, proposed by Grimalti et al. (1990). This ratio provides the status of treated or not treated sewage, where smaller values than 0.2 $\mu g.g^{-1}$ indicates untreated sewage.

2.2.4. *Statistical analysis*

A two-dimensional non-metric scaling (MDS) was performed based on a Euclidean dissimilarity matrix (normalized variables, Primer 6.0) and used to visualize variation between stable isotope signatures of feeding guilds and under impact conditions. A One-Way Analysis of Variance (One-Way ANOVA) was used to test differences between feeding guilds to $\delta^{13}C$ and $\delta^{15}N$. In the aim to see how pollution condition influences the isotopic niche distribution, Stable Isotope Bayesian Ellipses (SIBER) analysis, were performed. SIBER analysis gives a comparison of isotopic niche width, what could be more assertive than analysis with descriptive metrics and it is possible to be applied to sets of data with different sample sizes (Jackson et al. 2011). This methodology allows quantify trophic diversity at food webs, including data that may indicate niche diversification (Layman et al. 2007) and provide, to this study, perspectives about how the macrofaunal structure is affected by organic enrichment.

We used the original metrics described by Layman et al. (2007), formulated using Bayesian inference, a methodology that allow to generate robust measures of isotopic niche width of both community members and entire communities, as a more honest descriptor of community structure than usual descriptive metrics and informing isotopic niche width in populations, functional groups as trophic guilds and communities (Jackson et al. 2011). This methodology is important at this study because, as described by the developer, could be applied to entire communities by taking the means of members and the uncertainty in the means with small sample size it is not a factor with introduces artifacts to the analyzes.

As exposed by Layman et al. (2007), their proposed analytical approach asses to calculate ‘‘community-wide’’ measures of trophic structure and are based on six metrics. 1. $\delta^{15}N$ Range (NR), distance between the two species with the most enriched and most depleted $\delta^{15}N$ values. It is one representation of vertical structure within a food web. 2. $\delta^{13}C$ range (CR), distance between the two species with the most enriched (maximum) and most depleted (minimum) $\delta^{13}C$ values. 3. Total area (TA), convex hull area encompassed by all species in $\delta^{13}C$ – $\delta^{15}N$ bi-plot space. It is a measure of the total amount of niche space occupied, and thus a proxy for the total extent of trophic diversity within a food web. 4. Mean distance to centroid (CD), average Euclidean distance of each species to the $\delta^{13}C$ – $\delta^{15}N$ centroids, which are the mean $\delta^{13}C$ and $\delta^{15}N$ value for all species in the food web. 5. Mean nearest neighbor distance (MNND): mean of the Euclidean distances to each species' nearest neighbor in bi-plot space, and thus a measure of the overall density of species packing. 6. Standard deviation of nearest neighbor distance (SDNND), a measure of the evenness of species packing in bi-plot space less influenced than MNND by sample size.

These metrics were applied to present study with all fauna samples and six sites of study area which have contrasting conditions of sewage pollution: the higher (4.0, 5.27 and 13.8 $\mu g.g^{-1}$) and lower (0.04, 0.2, and 0.14 $\mu g.g^{-1}$) concentration of coprostanol. Thus, it aims results for two conditions: contaminated and non-contaminated. Analysis was conducted in the R statistical computing package (R Development Core Team, 2007). At this study, these measurements show the difference between niche spreading at two opposite conditions.

SIBER (Stable Isotope Bayesian Ellipses in R) was performed considering all macrofauna $\delta^{13}C$ and $\delta^{15}N$ signatures at study area. As contaminated condition were used three heavily contaminated sites signatures (CP 02, BV 15 and BV 19) and as non-contaminated condition, the three lowest coprostanol concentration sites (BV 09, BV 30 and BV 34) corresponding to not polluted results.

2.3. Results and Discussion

2.3.1 *Study sites*

Definition of pollution conditions (i.e. sewage contamination) in the study area was carried out from the absolute concentrations of the sterol coprostanol, which indicated seven out of eleven are contaminated (BV 15, BV 17, BV 19, BV 21, BV 24, CP 02 and CP 06. Fig. 2)

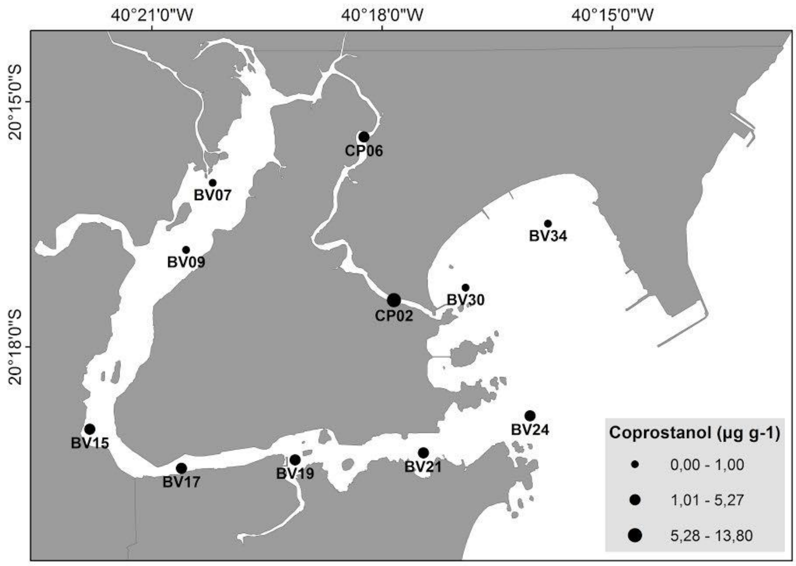

Figure 2. Coprostanol concentration (µg.g^{-1}) at study area sampled points. Values >1.0 µg .g^{1} indicate sewage contamination (Grimalti, 1990).

Coprostanol concentrations varied from 0.14 to 13.8 µg.g^{-1} (2.95 ± 3.98) at study area. The two sites less impacted by sewage contamination, BV 30 (0.14 µg.g^{-1}) and BV 34 (0.08 µg.g^{-1}), are on the marine area near the estuary, at the Tubarão Port, where is situated an industrial complex. BV 07 (0.38 µg.g^{-1}) and BV 09 (0.20 µg.g^{-1}), not heavily impacted sites, are located at the inner areas of Baía de Vitória, where receive contribution of freshwaters from St. Maria da Vitoria and Bubu Rivers, respectively. The higher impacted site, CP 02 (13.8 µg.g^{-1}), is at the communication channel between Baía de Vitória and Baía do Espírito Santo, the Canal da Passagem, where domestic sewage, most portion *in situ*, is discharged from all the neighborhood around, besides other discharge effluents from the own local sanitation company (Jesus et al. 2004).

Sites BV 17, BV 19 and BV 21 presented 2.17, 5.27 and 1.40 $\mu g.g^{-1}$ coprostanol concentrations, respectively, at the same area, Canal do Porto. These may due to the influence of different inputs from rivers, BV 17 is close to Marinho river, BV 19, to Aribiri River and BV 21, to the coastal area. These values may be considered low indices of contamination when compared to other Brazilian ecosystems, where it is possible to find an extreme of 40 $\mu g.g^{-1}$, as demonstrate for Carreira et al. (2014) studying the Guanabara Bay, at Rio de Janeiro, Brazil (Table 3).

Results for Ratio II (epicoprostanol/coprostanol) indicate only one site where we cannot claim that sewage is absolutely not treated, BV 09 (0.25), so all the study area except this site indicated presence of not treated sewage (Fig. 3). The lowest value for ratio II (0.01) is at CP 02 site, where we found the higher concentration of coprostanol. This suggests that this high sewage contamination is caused by not treated sewage, discharged from urban area around the channel, as mentioned above. The Canal do Porto sites analyzed follow the same pattern of high concentration of coprostanol and lower value of ratio II, indicating directly relation between discharges of not treated sewage and impacts reflected at sediment.

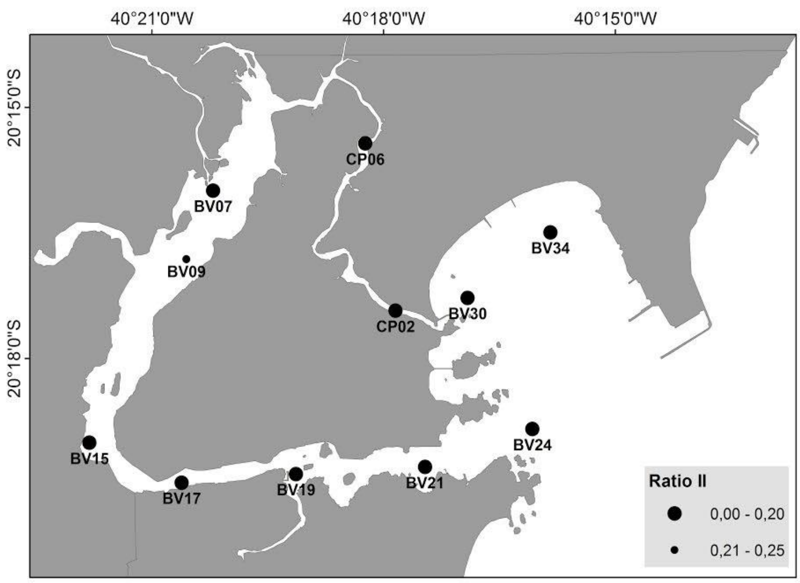

Figure 3. Ratio II (epicoprostanol/coprostanol) at study area sampled points. Values <0.20 indicate not treated sewage (Grimalti, 1990).

Table 3. Coprostanol concentration (µg.g-[1]) at study area and other Brazilian estuarine and coastal areas. DL: detection limit.

Local	Concentration (µg.g-1)	Reference
Vitória bay estuarine complex, ES	0.2 to 13.8	Present study
Cotingachannel, PR	<DL to 1.69	(Abreu et al., 2014)
Guanabara bay, RJ	0,01 to 40,0	(Carreira et al., 2004)
Sepetiba bay, RJ	0,01 to 0,42	(Carreira et al., 2009)
Guarajá bay, PA	0,06 to 7,93	(Gomes et al., 2015)
Vitória bay, ES	0,2 to 5,2	(Lehrback et al. 2016)

2.3.2. *Isotopic analysis*

2.3.2.1 Sediment

The spatial sedimentary $\delta^{15}N$ values ranged from 5 to 6.8 ‰, with an average of 5.8 ± 0.5 ‰ (Table 4), which is comparable to found at other estuarine sediments (4.6 ‰ ± 2.0, Owens, 1987; from 4.1 to 7.5 ‰, Barcellos, 2016).

In estuaries, there has been observed an increase in average $\delta^{13}C$ values from the inner estuary to the lower estuary (Barcellos et al. 2016). Average $\delta^{13}C$ in Vitória Bay estuarine complex were 25.5 ‰ (Canal do Porto), < -25.5 ‰ (Baía do Espírito Santo) and < -26.2 ‰ (Canal da Passagem), thus this trend was not observed to $\delta^{13}C$ averages at the present study. The Canal do Porto and Baía do Espírito Santo has similar sedimentary $\delta^{13}C$, indicating similar sources of carbon at this two areas. Average sedimentary $\delta^{15}N$ in the compartments of the study area increased from the polyhaline (inner estuary) to the euhalyne (oceanic portion) zones: 5.3 ‰ (Baía de Vitória), <5.8 ‰ (Canal do Porto), <6.0 ‰ (Baía do Espírito Santo), <6.2 ‰ (Canal da Passagem).

The sediment $\delta^{13}C$ signatures varied from -27 to -24.2 ‰ (-25.7 ± 0.77) (Table 5), similar to what was described by Barcellos et al. (2016), ranging from -28 to -24 ‰ at an urban tropical Brazilian estuary under influence of domestic and industrial wastes. Yu et al. (2010) observation found a bigger amplitude of signatures (-28.59 to -22.60 ‰) in comparison to our results, at a tropical estuary under significant sewage contamination. Gao et al. (2012) described at Bohay Bay, China, sediment $\delta^{13}C$ from -18.23 to -25.69 ‰, and thus, suggested that anthropogenic activities have a significant influence on the geochemistry of organic matter sediments besides natural processes. The values at the present study are lower than the $\delta^{13}C$ typical of marine-derived organic matter (-18 to -22 ‰) (Ramaswamy et al., 2008) and higher than sedimentary $\delta^{13}C$ signatures at fresh water ambient, as described by Amorim et al. (2009) in their study realized far 850 km from estuarine area (bank of Amazon River, Brazil) where the signatures ranged between -31 and -23 ‰. Thus, the distribution features of $\delta^{13}C$ identified at present study suggest that organic matter in surface sediments contents both mixed origins, derived from continental and marine, under anthropogenic impacts influence.

At CP 02, the most contaminated site, with high coprostanol concentrations (13.8 $\mu g.g^{-1}$) and the lowest indices of ratio II (0.01), sedimentary $\delta^{15}N$ (5.6 ‰) was similar to average of all sampled sites (5.8 ± 0.5 ‰). Sedimentary $\delta^{13}C$ was also similar between CP 02 (-25.6 ‰) and average of all sampled sites (-25.7 ± 0.77 ‰). CP 06, at same area than CP 02, had higher $\delta^{15}N$ (6.8 ‰) when compared to all other sites (values range from 5 to 6.8 ‰), what shows enrichment of 1 ‰ in comparison to overall mean (5.8 ± 0.5 ‰). Sediments at the Canal da Passagem had depleted $\delta^{13}C$ average (-26.2 ± 0.78 ‰) when compared to other areas: Baía de Vitória (-25.7 ‰ ± 0.77), Canal do Porto (-25.5 ‰ ± 0.49) and Baía do Espírito Santo (-25.5 ‰ ± 1.42), and enriched $\delta^{15}N$ (6.2 ±0.85 ‰) when compared to other areas at study site: Baía de Vitória (5.3 ‰ ± 0.2), Canal do Porto (5.8 ‰ ± 0.8), Baía do Espírito Santo (6.0 ‰ ± 0.21). Heavily contaminated sites, CP 02, BV 15 and BV 19, showed similar $\delta^{15}N$ signatures (5.6, 5.5 and 5 ‰, respectively), depleted in relation to other nitrogen isotopic results at study less contaminated sites as BV 21 (5.8 ‰), BV 24 (6.1 ‰), BV 17 (6.2 ‰), CP 06 (6.8 ‰) and one non-contaminated site (BV 34, 6.6 ‰). Heavily contaminated sites CP 02, BV 15 and BV 19 presented $\delta^{13}C$ -25.6 ‰, -26.1 ‰, -25.3 ‰, varying less than 0.05 ‰ to average carbon isotopic sediments average (-25.7 ‰ ± 0.77). Site BV 34, with the smaller coprostanol concentration (0.08 $\mu g.g^{-1}$), a non-contaminated site, have high $\delta^{15}N$ signature (6.2 ‰), same as Canal da Passagem media and, the most depleted $\delta^{13}C$ value (-27 ‰), of all sites. BV 30, which presented low coprostanol concentration (0.14 $\mu g.g^{-1}$) had similar $\delta^{15}N$ (5.8 ‰) to the average of all sites and the more enriched $\delta^{13}C$ (-24.2 ‰). BV 09, a non-contaminated site, as well as the two last mentioned, presented low $\delta^{15}N$ (5.1 ‰) and $\delta^{13}C$ -25.5 ‰ value, close to media (-25.7 ‰ ± 0.77) for all sites.

Our values in all study area (-25.7 ‰ ± 0.77 and 5.8 ‰ ± 0.5) were lower than typical discharge primary treatment facilities, which presents an average $\delta^{13}C$ of -23.2 ‰ ± 0.1 and $\delta^{15}N$, 2.5 ‰ ± 0.2 (Waldron et al., 2001). Components at $\delta^{15}N$ sewage may ranges from 3 to 7.2 ‰ (Tucker, 1999), similar to our results (5.8 ± 0.5 ‰), found at sediment under untreated sewage influence. However, sewage is compound from terrestrial sources, range from $\delta^{13}C$ -30 to -23±3 ‰ and $\delta^{15}N$ 5 to 18 ‰ (Hu et al., 2006), whit a typical $\delta^{13}C$ of domestic sewage mix of -26.7 ‰ (Barcellos, 2016). Our $\delta^{13}C$ results (-27 to -24.2 ‰) are slightly depleted in relation to the typical sign of domestic sewage. It is expected that sedimentary $\delta^{15}N$ values increase with urbanization degree (McClelland et al., 1997; McClelland & Valiela, 1998), and indicates that environmental changes, as well as the $\delta^{15}N$ isotopic signatures are more affected than $\delta^{13}C$ by biochemical processes (Ogrinc et. al, 2005). However, we could not detect marked changes sedimentary $\delta^{15}N$ signatures between the extreme contaminated and non-contaminated sites.

Table 4. Nitrogen isotopic values for the sediment and families in the study area (‰).

$\delta^{15}N$	Sediment	Goniadidae	Capitellidae	Cirratulidae	Hidrobiidae	Orbiniidae	Spionidae	Tellinidae	Onuphidae	Nereididae	Mytilidae	Solecurtidae	Veneridae
Site													
BV 07	5.3	13.2	10.2	7.0	9.0	8.1	10.6	9.7		12.4		6.6	5.8
BV 09	5.1	9.9		5.6			6.8		8.4	11.0	4.5	4.4	5.0
BV 15	5.5	9.4	6.0			7.0	4.4	3.8	8.0	9.5		3.1	5.0
BV 17	6.6	10.5	7.2	5.8		7.3	5.4						
BV 19	5	8.6	5.9	4.9			4.4	3.4	6.6	5.8			4.8
BV 21	5.8		7.4	6.9		7.3	7.3	5.2	7.2	10.3			
BV 24	6.1		10.5				6.6		10.1				
BV 30	5.8	12.9		9.7			6.8	8.8	11.1				
BV 34	6.2	8.8					10.2		10.7				
CP 02	5.6		6.4	5.8		8.1	5.6	3.7		4.6		3.2	3.9
CP 06	6.8	9.3	6.5			6		3.7	7.5				

Table 5. Carbon isotopic values for the sediment and families in the study area (‰).

$\delta^{13}C$	Sediment	Goniadidae	Capitellidae	Cirratulidae	Hidrobiidae	Orbiniidae	Spionidae	Tellinidae	Onuphidae	Nereididae	Mytilidae	Solecurtidae	Veneridae

Site													
BV 07	-25.8		-21.6	-21.4	-16.8		-18.5	-18.2				-21.4	-22.4
BV 09	-25.5	-20.0		-25.4			-20.2	-19.9	-20.4	-19.4	-21.4	-21.0	-21.8
BV 15	-26.1		-21.0			-27.1	-19.5		-20.5				-20.4
BV 17	-26.1	-17.9	-19.2			-19.8	-19.5						
BV 19	-25.3	-17.2	-23.3	-19.3			-18.9	-19.1	-18.7	-18.7			-27.0
BV 21	-25.2		-18.4	-19.4		-19.3	-18.9	-18.4	-18.8	-17.5			
BV 24	-25.2		-16.7				-18.5		-18.7				
BV 30	-24.2	-21.6		-15.4			-17.9	-16.7	-20.2				
BV 34	-27	-18.6					-16.3		-14.7				
CP 02	-25.6		-20.2	-21.4		-20.0	-19.9	-20.8		-18.5		-20.8	-21.3
CP 06	-26.7	-20.3	-22.0			-22.7		-23.0	-21.0				

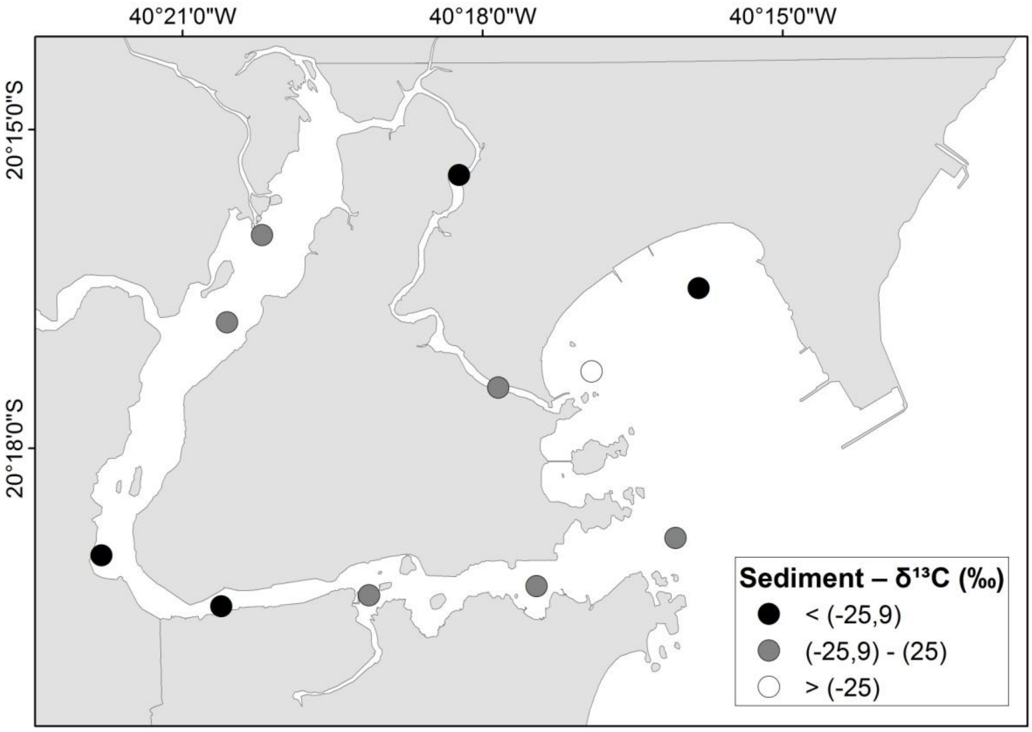

Figure 4. Carbon isotopic values for the sediment samples at study sites.

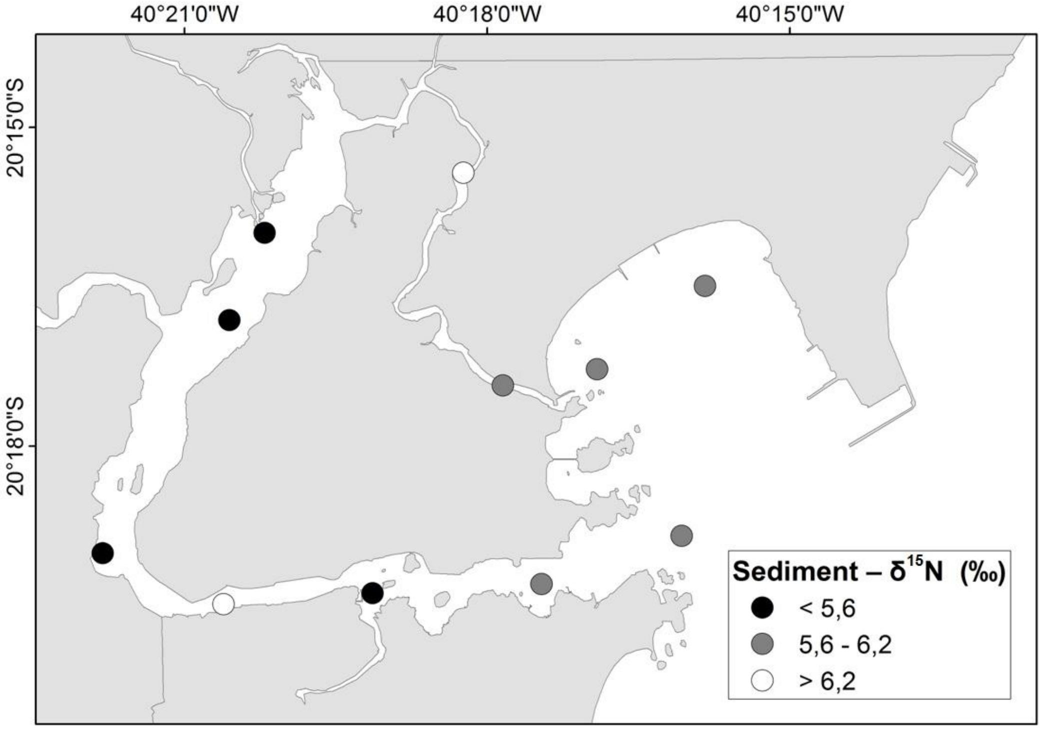

Figure 5. Nitrogen isotopic values for sediment samples at study sites.

2.3.2.2 Macrofaunal assemblages

The $\delta^{13}C$ and $\delta^{15}N$ signatures of fauna ranged from -27 to -14.7 ‰ (Table 5) and from 3.1 to 13.2 ‰ (Table 4), respectively.

Carnivores presented $\delta^{13}C$ signatures between -21.6 to -17.2 ‰ (Table 6), with no spatial changes from the inner to the oceanic portion along estuarine complex. The most depleted $\delta^{13}C$ signature (-21.6 ‰) was observed at a non-contaminated site (BV 30) whereas the most enriched (-17.2 ‰) was observed at a contaminated site (BV 19). The carnivores polychaeta Goniadidae presented heavier $\delta^{15}N$ signatures (13.2 ‰) when compared to all other families (3.1 to 12.4 ‰). $\delta^{15}N$ trophic fractionation is higher than at $\delta^{13}C$, changing about 3 ‰ per each trophic link (Cabana & Rasmussen, 1994; Minagawa & Wada, 1984). When comparing Goniadidae nitrogen signatures (from 8.6 to 9.4 ‰) in sites contaminated and non-contaminated (from 8.8 to 13.2 ‰), depletion at contaminated results is observed.

At three higher coprostanol concentrations sites, depositivores presented $\delta^{15}N$ averages 5.9 ‰ ± 1.59 (CP 02), 5.3 ‰ ± 1.48 (BV 15) and 4.7 ‰ ± 1.06 (BV 19). At lower coprostanol concentration sites, Depositivores $\delta^{15}N$ averages were 6.2 ‰ ± 0.83 (BV 09), 8.4 ‰ ± 1.45 (BV 30) and value of 10.2 ‰ (BV 34), where no replication was possible. Omnivores $\delta^{15}N$ average at most contaminated sites were 4.6 ‰ (CP 02, where replication was not possible), 8.7 ‰ ± 1.0 (BV 15) and 6.2 ‰ ± 0.6 (BV 19). At lower contaminated sites, omnivore $\delta^{15}N$ average 9.7 ‰ ± 1.8 (BV 09), 11.1 ‰ (BV 30) and 10.7 ‰ (BV 34), two last values are from sites without replication. Suspensivores presented 3.6 ‰ ± 0.45 at CP 02, 4.0 ‰ ± 1.3 at BV 15 and 4.8 ‰ (no replication) at BV 19. Suspensivores $\delta^{15}N$ average at BV 09 was 4.6 ‰ ± 0.3 and at BV 30 and BV 34 there were no exemplars of this feeding group. So, it is possible to observe that depositivores and omnivores presented depletion of $\delta^{15}N$ averages when comparing signatures between the three sites with higher coprostanol concentrations (CP 02, BV 15 and BV 19) and the three lower coprostanol concentrations (BV 09, BV 30 and BV 34).

Deposit-feeders average $\delta^{13}C$ signatures ranged from -22.6 ‰ ± 0.5 to -16.7 ‰ ± 1.2, similar to what Gearing (1991) observed at laboratorial experiments under sewage conditions (-19.5 ‰ ± 1.6 to -17.7 ‰ ± 0.5). Similar $\delta^{13}C$ signatures and slightly depleted in relation to ours, were observed in depositivores by Sampaio (2010), at an estuarine ecosystem under organic enrichment ($\delta^{13}C$ -22.6 ‰ ± 1.5 to -18.3 ‰ ± 1.1). Enriched $\delta^{13}C$ (-15.4 ‰) signatures of depositivores were observed at a non-contaminated site (BV 30) and the most depleted (-27.1 ‰), at a contaminated site (BV 15). At food chains, $\delta^{13}C$ presents little enrichment from food source to consumer (0–1 ‰) (DeNiro & Epstein, 1978) but can vary between different producers and, due to this, it is more commonly used as a source indicator (Abrantes et al., 2014). Deposit-feeders ingest surface or subsurface food particles deposited in sediments (Fauchald & Jumars, 1979), and these observations may reflect that assimilation of carbon is different under different conditions of organic enrichment at study area. Omnivores $\delta^{13}C$ average ranged from -19.9 ± 0.7 ‰ to -18.1 ± 0.9 ‰ (Table 8). Benthic omnivores may simultaneously and sequentially feed on more than one type of food (Jumars et al. 2015). In relation to sediment (-25.7 ‰ ± 0.77), group average results (-18.9 ‰ ±1.7) are 6.8 ‰ enriched, indicating assimilation of food at sediment and other possible sources. Suspension-feeders $\delta^{13}C$ signatures ranged from -22 ‰ ± 0.69 to -21‰ ± 0.35 (Table 8). These values are depleted in comparison to Gearing (1991) laboratory experiments of sewage assimilation in suspension feeders (-20.1 ‰ ± 0.7 to -19.8 ‰ ± 0.4).

The most depleted of all $\delta^{13}C$ signatures (-27.1‰) were observed at a contaminated site (BV 15) and the most enriched (-14.7 ‰), at a non-contaminated

(BV 34). The enriched $\delta^{13}C$ signatures observed from carnivore (-18.6 ‰), deposit-feeder (-15.4 ‰) and omnivores (-14.7 ‰) at non contaminated sites (BV 34, BV 30 and BV 34, respectively) suggests assimilation from different food sources than other members of same families. Suspension-feeders had enriched values (-20.4 and -20.8 ‰) at contaminated sites (BV 15 and CP 02, respectively) (Table 5). Differences observed to contaminated and non-contaminated sites were identify by an Analysis of Similarity (ANOSIM) between trophic groups considering the factor impact and illustrated with a two-dimensional non-metric scaling (MDS) (Fig. 6).

Table 6. $\delta^{13}C$ and $\delta^{15}N$ signatures of Carnivore group, represented by Goniadidae taxa and means ± standard deviation of feeding guilts groups per site. Number of replication in brackets. Empty spaces correspond to sites were there was none member of certain feeding guilt.

Site	Carnivore		Depositivores		Omnivores		Suspensivores	
	δ13C	δ15N	δ13C	δ15N	δ13C	δ15N	δ13C	δ15N
BV07		13.2	-19.3 ± 2.09 (5)	9.1 ± 1,36 (6)		12,4	-22 ± 0.69 (2)	6.2 ± 0.5 (2)
BV 09	-20.0	9.9	-21.9 ± 3,1 (3)	6.2 ± 0.83 (2)	-19,9 ± 0,7 (2)	9,7 ± 1,8 (2)	-21.4 ± 0.4 (3)	4.6 ± 0.3 (3)
BV 15		9.4	-22.5 ± 4 (3)	5.3 ± 1.48 (4)	-20.5	8,7 ± 1,0 (2)	-20.4	4.0 ± 1.3 (2)
BV 17	-17.9	10.5	-19,5 ± 0.3 (3)	6.4 ± 0.95 (4)				
BV 19	-17.2	8.6	-20.1 ± 2.1 (4)	4.7 ± 1.06 (4)	-18,7 ± 0 (2)	6,2 ± 0,6 (2)	-27	4.8
BV 21			-18.9 ± 0,5 (5)	6.8 ± 0.9 (5)	-18,1 ± 0,9 (2)	8,8 ± 2,2 (2)		
BV 24			-17.6 ± 1.25 (2)	8.5 ± 2.7 (2)	-18,7	10.1		
BV 30	-21.6	12.9	-16.7 ± 1.2 (3)	8.4 ± 1.45 (3)	-20.2	11.1		
BV 34	-18.6	8.8	-20.4 ± 0.6	10.2	-14.7	10.7		
CP 02			-20.7 ± 0.7 (3)	5.9 ± 1.59 (4)	-18.5	4.6	-21 ± 0.35 (2)	3.6 ± 0.45 (2)
CP 06	-20.3	9.3	-22.6 ± 0.5 (3)	5.4 ± 1.5 (3)	-21.0	7.5		

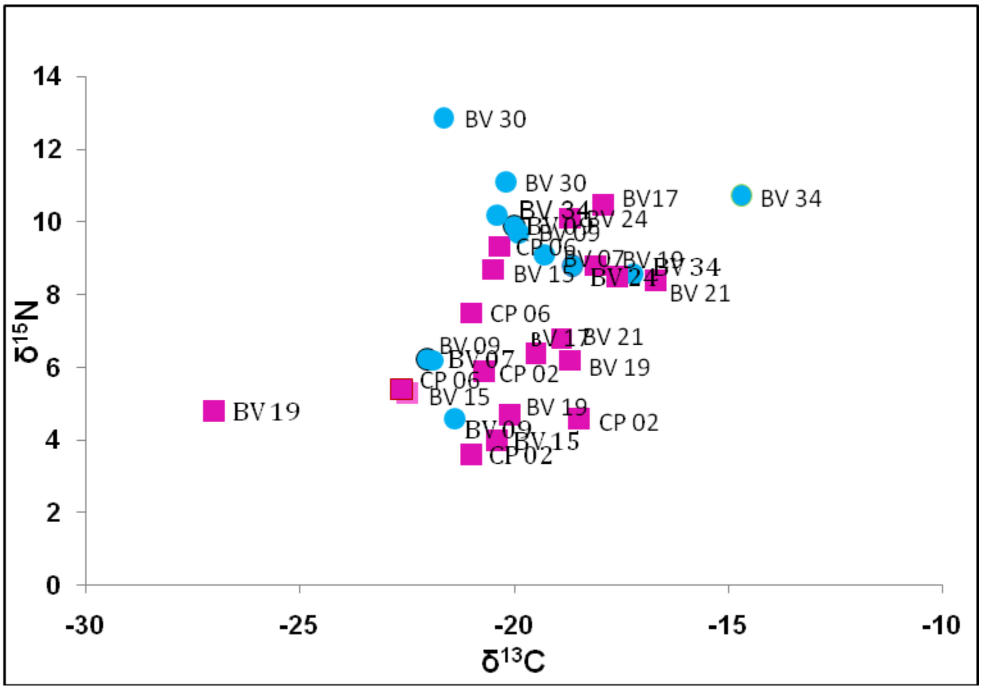

Figure 6. Bi-plot of isotopic signatures of $\delta^{13}C$ and $\delta^{15}N$ from all feeding guilds (see table 5). Standard deviation axes were suppressed for better visualization. Sites are identified by description next to correspondant symbols.Blue dots correspond to non contaminated sites and purple rectangles correspond to contaminated sites.

2.3.3. *Multivariate analysis*

Analysis of similarity (ANOSIM) was performed to test spatial correlation between isotopic signatures of fauna and impact conditions. The high variability on stable isotope signatures between trophic groups did not indicate impact effect between sites (Global R: 0,086, p value: 5.6 %). However, when considering only spatial effects (contaminated and non-contaminated sites) there were significant differences on carbon and nitrogen isotopic signatures (Figure 7; Global R: 0,147, p value: 2.8 %). Values of $\delta^{15}N$ were different between two trophic groups: deposit-feeders and omnivores (ANOVA, F=3.7769, p=0.0252), what suggests that these groups are developing different ecological roles in term of assimilation

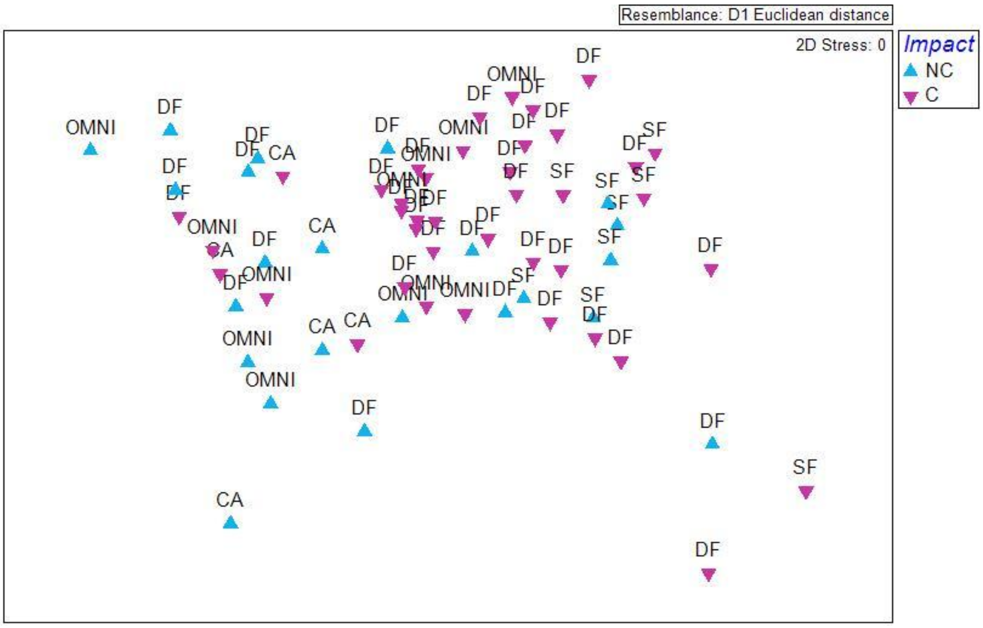

Figure 7. MDS between trophic groups across impact groups, where CA: Carnivore, SF: Suspension-feeder, OMNI: Omnivore, DF: detritive-feeder, C: Contaminated and NC: non-contaminated.

SIBER analysis results indicate clearly a bigger gamma of niche possibilities to be explored by macrofauna at non-contaminated sites in relation to contaminated. The $\delta^{13}C$ range at non-contaminated sites (2.90625) suggests that more types of basal resources are available. The $\delta^{15}N$ range indicated a higher trophic length at non-contaminated sites (2.8488149), if compared to contaminated sites (1.4139166), with more niche diversification at unpolluted sites (Fig. 8 and Fig. 9). These ranges indicate that the fauna could explore more resources at non-contaminated sites than at contaminated. Total area (TA) of the convex hull including all isotopic signatures of the sampled macrofauna is influenced by extremes of signatures on either or both of $\delta^{15}N$ and $\delta^{13}C$ axes. Total Area (TA) is a metric that measure a surface indicatin the trophic niche width or space, is highly sensitive to variations in $\delta^{13}C$ and $\delta^{15}N$ ranges (Brind'Amour & Dubois, 2013) and at our study exhibited wider niche width at non-contaminated sites (0.3841821) (Fig. 10).

Table 7. Layman metrics results for SIBER (Stable Isotope Bayesian Ellipses in R), applied to all families signatures of $\delta^{13}C$ and $\delta^{15}N$ for contaminated condition (sites BV 15, BV 19 and CP 02) and non-contaminated condition (BV 09, BV 30 and BV 34).

	Contaminated	Non-contaminated
$\delta^{13}C$ range	0.92075	2.90625
$\delta^{15}N$ Range	1.4139166	2.8488149

TA	0.3033069	0.3841821
CD	0.7161979	1.4140589
MNND	0.7755691	1.9863103
SDNND	0.6313734	0.1985516

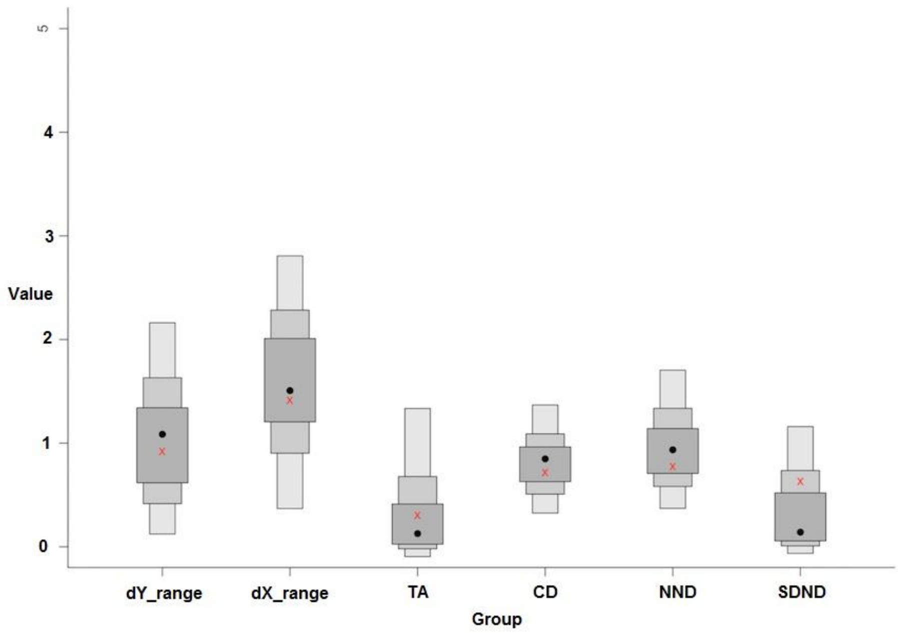

Figure 8. The six Layman metrics applied to all macrofaunal data to contaminated sites. Black dots represent means and red letter “x”, the corrected mean. Shaded boxes represent the 50, 75 and 95% credible intervals from dark to light grey.

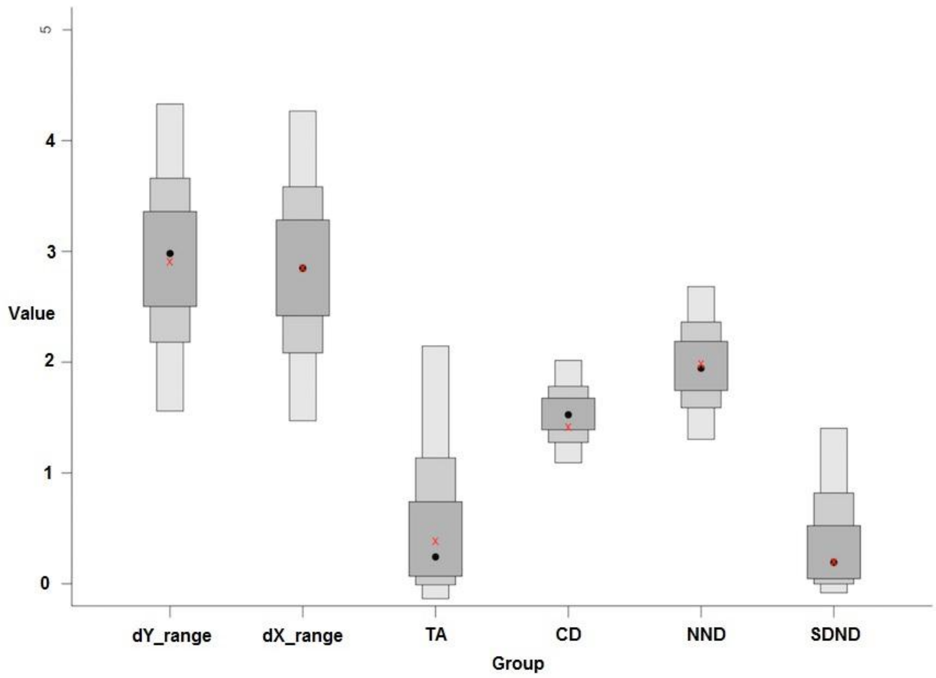

Figure 9. The six Layman metrics applied to all macrofaunal data to non-contaminated sites. Black dots represent means and red letter “x”, the corrected mean. Shaded boxes represent the 50, 75 and 95% credible intervals from dark to light grey.

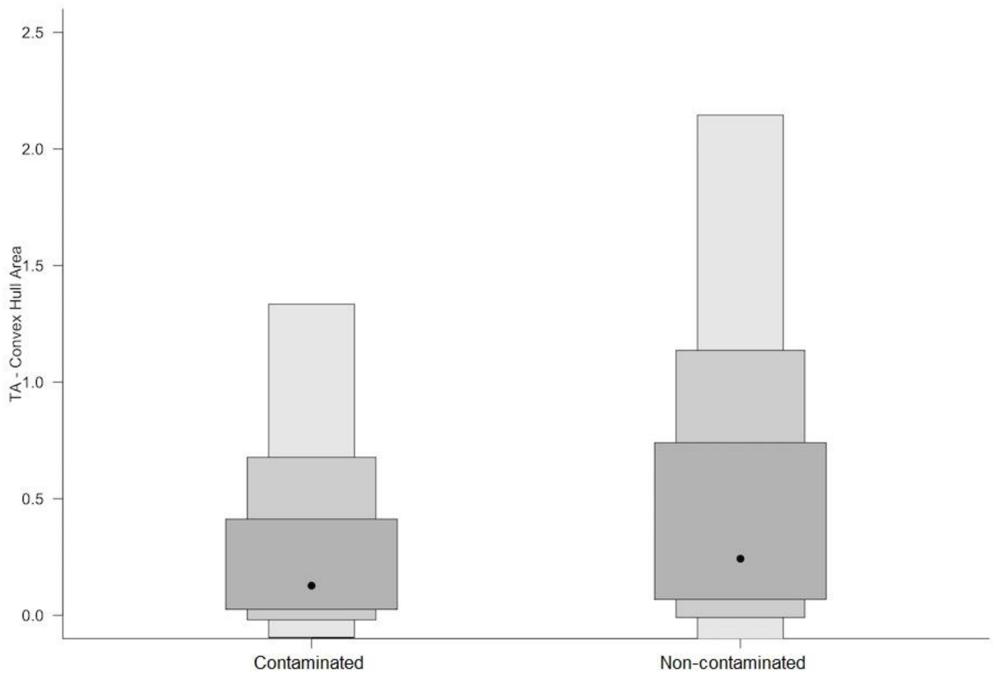

Figure 10. TA (Total Area) calculated. Contaminated and non-contaminated results are shown. Shaded boxes represent the 50%, 75% and 95% credible intervals from dark to light grey.

2.4. Conclusion

The analysis of distribution of trophic niches between broad pollution conditions indicated a restricted use of resources by the fauna.

Carbon isotope pattern at this study was similar to found for other author at contaminated areas by sewage.

Nitrogen isotope stable was capable to indicate change in fauna structure attributable to the sewage contamination.

Stable Isotopes are an important indicator of organic enrichment that should be combined with other descriptors in the evaluation of impacts in the estuarine environment to assure assertive conclusions.

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