UNIVERSIDADE FEDERAL DO ESPÍRITO SANTO
CENTRO DE CIÊNCIAS HUMANAS E NATURAIS
PROGRAMA DE PÓS-GRADUAÇÃO EM OCEANOGRAFIA AMBIENTAL

CÍNTIA DA SILVA VARZIM

USO DE ISÓTOPOS ESTÁVEIS NA DETERMINAÇÃO DE IMPACTOS POR ENRIQUECIMENTO ORGÂNICO EM UM ESTUÁRIO TROPICAL

VITÓRIA
2016

CÍNTIA DA SILVA VARZIM

USO DE ISÓTOPOS ESTÁVEIS NA DETERMINAÇÃO DE IMPACTOS POR ENRIQUECIMENTO ORGÂNICO EM UM ESTUÁRIO TROPICAL

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.

VITÓRIA

2016

CÍNTIA DA SILVA VARZIM

USO DE ISÓTOPOS ESTÁVEIS NA DETERMINAÇÃO DE IMPACTOS POR ENRIQUECIMENTO ORGÂNICO EM UM ESTUÁRIO TROPICAL

Dissertação 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.

Vitória, ___ de_______de____

COMISSÃO EXAMINADORA

____
Prof. Dr. Angelo Fraga Bernardino
Presidente da comissão
Universidade Federal do Espírito Santo/UFES

____
Prof. Dr. Renato Rodrigues Neto
Examinador interno
Universidade Federal do Espírito Santo/UFES

____
Prof. Dr. Raphael Mariano Macieira
Examinador externo
Universidade de Vila Velha/UVV

AGRADECIMENTOS

À Gaia, por me fazer sensível às suas dores e, ao mesmo tempo, forte para combater o que as causa.

Aos que guiaram meus primeiros passos no budismo: Monge Daiju, Tayo e demais ordenados. (*Gassho*).

Aos amados, estimados, admirados, senhor e senhora: meus pais! Nilda e Gilberto, pelo incentivo, paciência e compreensão. Criaram uma filha com asas mais fortes do que as raízes, mas de coração ainda maior que os dois mil quilômetros que nos separaram nesta jornada. Amo vocês.

Aos colegas de pesquisa do “Lab Bentos” por todos os momentos partilhados e, pontualmente, à Heliatrice, por me convidar a compor este time e ensinar sobre identificação de bentos; à Hanieh, pelo suporte acadêmico e pessoal e, ao Gushtavo, por ter se importado e me encorajado. Selva!

Às “estagiárias”, que bem mais que isso, viraram amigas, Luísa e Lívia. E que se tornaram dedicadas oceanógrafas, também.

Aos companheiros de morada e de vida, High e Paris, pela compreensão, afeto e companhia em noites não dormidas.

Às amigas que também deixaram nosso amado pampa em busca do algo mais a que viemos nesta vida. Obrigada pelo apoio e incentivo.

À UFES, Departamento de Oceanografia e todas as pessoas envolvidas que possibilitaram realizar esta pesquisa. Aos professores com quem tive aulas, obrigada pelo empenho de cada um de vocês.

Ao meu orientador, Angelo, o “Boss”, a quem devo o início, meio e fim de tudo isto. Dedico meus maiores agradecimentos, pela acolhida e credibilidade. Pela importância dada ao meu trabalho. Muito obrigada.

RESUMO

Os estuários são importantes áreas de transição caracterizadas pela alta produtividade biológica, vital para as áreas costeiras adjacentes. Encontram-se sob intensa exploração humana, tanto urbana quanto industrial, através do despejo de efluentes não tratados, com impactos no ecossistema. Considerando a ampla quantidade de esgoto lançado nesses ecossistemas e a necessidade de monitorar os impactos causados, é importante utilizar diferentes metodologias para traçar 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 da área contaminada foram enriquecidas 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 as 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 with high biological productivity and are vital for the adjacent coastal areas. They are under intense human exploitation, 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 track the presence of pollutants and their effects on the estuarine food chain. In this project, 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 non-contaminated areas. Macrofaunal trophic niche amplitude at contaminated sites was smaller if compared to non-contaminated areas. 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.

LISTA DE FIGURAS

LISTA DE TABELAS

SUMÁRIO

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 das modificações físicas de modo a adaptar os estuários às necessidades humanas (Schettini et al., 2000; Cooper, 2002; Elliot & Quintino, 2007).

Ambientes estuarinos tropicais provêm importante estrutura e recursos para comunidades diversas de organismos bentônicos (Alfaro, 2005) e são compostas 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 de organismos (Carreira et al., 2001).

As comunidades macrobentônicas são compostas por espécies que podem integrar um conjunto de condições ambientais ao decorrer do seu tempo de vida (Nalesso et al., 2005) e que permanecem junto ao substrato pelo menos durante uma parte do seu ciclo de vida, associados aos diversos 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 universal 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 sobre a fauna presente (Pearson & Rosenberg, 1978). As descargas de águas residuais municipais constituem fonte potencial de grandes montantes de compostos orgânicos de origem antropogênica ao meio marinho e as partículas destes compostos podem ressuspender e serem transportadas ou sofrerem transformações biogeoquímicas 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 em que se têm o coprostanol como indicador de contaminação de causa antrópica, já que ele é o principal esterol de origem fecal humana (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). 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 $\delta^{15}N$ nos 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, o tráfego marítimo aumentou e, junto a isso, 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). Como resultado da crescente urbanização na cidade de Vitória, 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.

1.2. Referências

Alfaro, A. C., 2005. Benthic macro-invertebrate community composition within a mangrove/seagrass estuary in northern New Zealand. Estuarine, Coastal and Shelf Science 66, 97-110.

Alongi, D.M., 1998. Coastal ecosystem processes. CRC Press, Boca Raton, FL, USA.

Barbour, M. T., Gerritsen, J., Snyder, B. D., Stribling, J. B. 1999. Rapid Bioassessment Protocols for Use in Streams and Wadeable Rivers: Periphyton, Benthic Macroinvertebrates and Fish, Second Edition, EPA 841-8-99-002.

Barros, F., Hatje, V., Figueiredo, M. B., Magalhães, W. F., Dórea, H. S., Emídio, E. S., 2008. The structure of the benthic macrofaunal assemblages and sediments characteristics of the Paraguaçu estuarine system, NE, Brazil. Estuarine, Coastal and Shelf Science 78(30), 753-762.

Bouillon, S., Borges, A. V., Castañeda-Moya, E., Diele, K., Dittmar, T., Dujke N. C., Kristense, E., Lee S. Y., Marchand, C., Middleburg, J. J., Rivera-

Monroy, V. H.,Smith III, T. J., Twilley, R. 2008. Mangrove production and carbon sinks: A revision of global budget estimates, Global Biogeochemical Cycles 22, GB 2013.

Carreira, R., Wagener, A.L.R., Fileman, T.; Readman, J.W. 2001.Distribuição de coprostanol (5β(h)-colestan-3β-ol) em sedimentos superficiais da Baía de Guanabara: indicador da poluição recente por esgotos domésticos. Química Nova24 (1), 37-42.

Carvalho, M. C. 2008. Uso dos isótopos estáveis de carbono, nitrogênio e enxofre em estudos de ecologia costeira. Oecologia Brasiliensis 12 (4), 694-705.

Cooper, J. A. G. 2002. Anthropogenic Impacts on Estuaries. Encyclopedia of Life Support Systems (EOLSS).

Del-Pilar-Ruso, Y., De-La-Ossa-Carretero, J. A., Loya-Fernández, A., Ferrero-Vicente, L. M., Giménez-Casalduero, F., Sánchez-Lizaso, J. L. 2009. Assessment of soft-bottom Polychaeta assemblage affected by a spatial confluence of impacts: Sewage and brine discharges. Marine Pollution Bulletin 58, 765–786.

Elliot, M., Quintino, V. 2007. The Estuarine Quality Paradox, Environmental Homeostasis and the difficulty of detecting anthropogenic stress in naturally stressed areas. Marine Pollution Bulletin 54, 640–645.

Fry, B., 2006. Stable Isotope Ecology.XII, 308 p. Springer, New York.

Leeming, R., Ball, A.; Jhones, G., Ashbolt, N., Nichols, P. 1994. Distinguishing Between Human and Animal Sources of Faecal Pollution. Water Research 30 (12), 2893-2900.

López-Gappa, J. J., Tablado, A., Magaldi, N. H. 1990. Influence of sewage pollution on a rocky intertidal community dominated by the mytilid *Brachidontes rodriguezi*. Marine Ecology Progress Series 63, 163-175.

Lotze, H. K., Lenihan, H. S., Bourque, B. J., Bradbury R. H., Cooke, R. G., Kay, M. C., Kidwell, S. M., Kirby, M. X., Peterson, C. H., Jackson, J. B. C. 2006. Depletion, degradation, and recovery potential of estuaries and coastal seas. Science. Reports 312, 1806-1809.

Maldonado, C., Venkatesan, M. I., Phillips, C. R., Bayona, J. M.2000. Distribution of Trialkylamines and Coprostanol in San Pedro shelf sediments adjacent to a sewage outfall. Marine Pollution Bulletin 40(8), 680-687.

Martins, C. C., Montone, R. C., Gambá, R. C., Pellizari, V. H. 2005. Sterols and fecal indicator microorganisms in sediments from admiralty Bay, Antarctica.Brazilian Journal Of Oceanography 53 (1/2), 1-12.

Mazumder D., Saintilan N., Alderson B. 2015. Hollins Inputs of anthropogenic nitrogen influence isotopic composition and trophic structure in SE Australian estuaries. Marine Pollution Bulletin 100(1), 217-23.

Nalesso, R.C., Joyeux, J-C., Quintana C. O., Torezanil E., Otegui, A.C.P. 2005. Soft-bottom macrobenthic communities of the Vitória bay estuarine system, south-eastern Brazil. Brazilian Journal Of Oceanography 53(1/2), 23-38.

Obade, P. T., Koedam, N., Soetaert, K., Neukermans, G., Bogaert, j., Nysse, E., Van Nedervelde, F., Berger, U., Dahdouh-Guebas, F. 2009. International Journal of Design & Nature and Ecodynamics 3(4), 296–320.

Pearson, T. H., Rosenberg, R. 1978. Macrobenthic succession in relation to organic enrichment and pollution of the marine environment. Oceanograph and Marine Biology, An Annual Review 16, 229-31.

Pereira, A. L., 2007. Stable isotopes in ecological studies: methods, applications and perspectives. Revista Biociências, Taubaté 13(1-2),16-27.

Rogers, M. K. 1999. Effects of sewage contamination on macro-algae and shellfish at Moa Point, New Zealand using stable carbon and nitrogen isotopes. New Zealand Journal of Marine and Freshwater Research 33, 181-188.

Schettini, C. A. F., Pereira, Fo., J., Spillere, L. 2000. Notas Técnicas Facimar 4, 11-28.

Venkatesan, M. I., Mirsadeghi, F. H. 1992. Coprostanol as sewage tracer in McMurdo Sound, Antarctica.Marine Pollution Bulletin 25, 328-333.

Zalmon I. R., Krohling W., Ferreira, C. E. L. 2011. Abundance and diversity patterns of the sessile macrobenthic community associated with environmental gradients in Vitória Harbor, southeastern Brazil. Zoologia 28(5), 641–652.

CAPÍTULO 2

Use of Stable Isotopes in Determining Impacts of Organic Enrichment at a Tropical Estuary

Cíntia da Silva Varzim[1, 2], Heliatrice L. Hadlich[2], Angelo Fraga Bernardino[2]

1. Programa de Pós-Graduação em Oceanografia Ambiental, CCHN, UFES
2. Grupo de Ecologia Bêntica, Departamento de Oceanografia, CCHN, Av. Fernando Ferrari, 514, Goiabeiras, Vitória, ES, Brazil

Abstract

Estuaries are important transitional ecosystems that are 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 use different methods to track the presence of pollutants and their effects on the estuarine food chain. In this project we evaluated the organic enrichment impacts on the benthic communities in the Bay of Vitória, by contrasting spatial patterns of stable isotopes signatures ($\delta^{13}C$ and $\delta^{15}N$), with geochemical markers indicating sewage contamination (e.g. coprostanol). Coprostanol concentrations ranged from 0.14 to 13.8μg.g^{-1} (2.95 ± 3.98), at study area. Nitrogen average sediment signatures presented a pattern of distribution along the estuarine complex. Deposit-feeders and omnivores presented significant differences on $\delta^{15}N$, suggesting different trophic positions for these groups at the study area. Carbon isotopic signatures of carnivore, deposit-feeders and omnivores showed depletion at heavily impacted sites in relation to less impacted. Our results indicated that groups at contaminated sites had

less niche amplitudes when compared to the macrofauna at non-contaminated areas. Organic contamination showed to be a limiting factor of resources that alters environmental and benthic trophic chain and using stable isotopes enable us to detect these environmental alterations.

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

2.1. Introduction

Estuaries are ecologically and economically important ecosystems that support many environmental services to humans, but they are at the same time seriously endangered globally (Obade et al., 2009; Bouillon et al., 2008). Human impacts in estuaries and coastal ecosystems altered biodiversity and productivity of these ecosystems (Lotze et al., 2006). The intense anthropogenic impacts on estuaries, originate from pollution 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). In this context, the polychaeta group has the role of indicator of organic pollution (Del-Pilar-Ruso et al., 2009) and 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).

Organisms that belong to benthic communities naturally respond to spatial and temporal changes quickly and are able to show signs of degradation (Warwick, 1992). In general, the ratio of sensitivity to tolerance of benthic species was used to developthe 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 many 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, results in changing factors chemical, physical and biological that has 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). 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). Thus, coprostanol concentrations are useful to track domestic inputs close to source due to the affinity with organic matter on sediments (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 inthe 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 metabolic 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, analyzing 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 for 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). Due to expanding the petroleum industry at region of Vitória, the marine traffic has increasedand thechanges on the input of fresh water became one of the many anthropic impacts that affect the Vitoria Bay duringthe last half century (Zalmon et. al., 2011). 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 an eutrophic environment (Jesus et al., 2004). The aim of this study was to test $\delta^{13}C$ and $\delta^{15}N$ isotopic signatures from estuarine sediments and macrobenthic assemblages offer a good proxy to identify sewage impacts in Vitória Bay. We compared the isotopic signatures with geochemical sewage markers and tested their spatial agreement within several areas with broad range of impact conditions in the estuarine system.

2.2. Materials and methods

2.2.1. *Study area and sampling*

Vitoria Bay is at the metropolitan area of Vitória city, Espírito Santo State, Brazil and comprehends 2051 hectares of mangrove, extending along approximately 25 km, compounding a system with two coastal water entrances, the Vitória Bay itself and the Passage Channel (Sterza & Fernandes, 2006), that communicates the northwest Vitória Bay to the sea, through the Espírito Santo bay (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).

Figure 1.City of Vitória and the Vitória bay estuarine complex.Google Earth.

Local depth varies from 1.5 to 10m outside the main port channel.The average salinity within Vitória Bay ranged between 20 and 36 and the average temperature, ranged between 22 to 27°C (Bernardino, unpublished data). The changes in both parameters indicate the presence of saline gradient (Fig. 2).

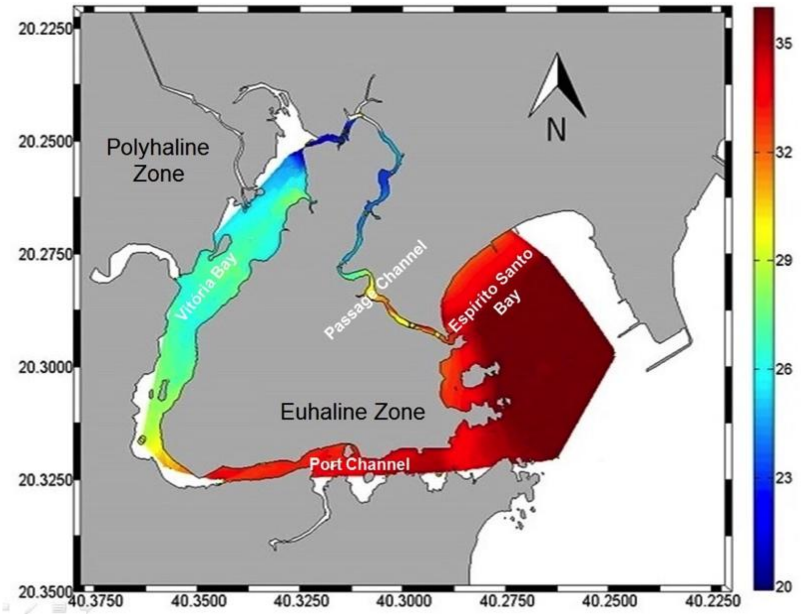

Figure 2. Salinity gradient at the Vitória bay estuarine complex and Espirito Santo bay.

Benthic macroinvertebrates (>0.5 mm) were sampled with two replicates with a Day Grab (about 15L of capacity) at 11 sites (Fig.3). Replicate contents were summed. The samples were then sieved on board 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 submission for analysis (Hadlich et al., in prep).

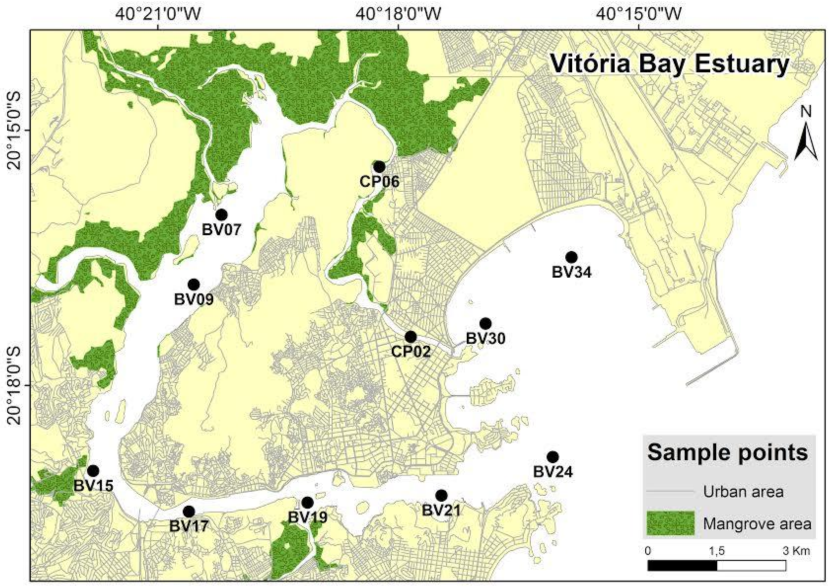

Figure 3. Distribution ofsites at the Vitória Bay estuary and Espirito Santo Bay.

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 level (Table 2).

Table 1. Number of benthic organisms sampled from dominant macrofaunal groups at study area sites and compartments.

Sites	BV 07, BV 09, BV 15 Vitória Bay	BV 17, BV 19, BV 21 Port Channel	BV 24, BV 30, BV 34 Espírito Santo Bay	CP 02, CP 06 Passage Channel	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, deposit-feeder; Goniadidae, carnivore; Spionidae, deposit-feeder; Onuphidae, omnivore and Nereididae, omnivore (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	Taxa	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 dropwise addition of 1M HCl; reducing until fine powder using a mortar and pestle (Bernardino et al., 2010). After acidification, macrofaunal organisms were dried 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 (Bernardino et al., 2010). The isotopic compositions ($\delta^{13}C$ and $\delta^{15}N$) are measured on animal samples to a final dry weight from 0.5 to 2mg, 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, as follow.

$\delta X = [(Rsample / Rstandard)-1] \times 10^3$, 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. As the amount of the heavy isotope components increases, value increase. As the light isotope component increase, value decrease. 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. 6 and 7), 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. *Geochemical analysis*

Samples for geochemical analysis 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). At contaminated sediments, we found sterols as colesterol, campesterol, β-sitosterol, βsitostanol, colestanol, coprostanol (5β-cholestan-3β-ol) and epicoprostanol (5β-cholestan-3α-ol), this last two are originate by human fecal material (Venkatesan & Kaplan, 1990; Hadlich et al., in prep). The principal human faecal sterol is coprostanol, which constitutes ~60% of the total of sterols at human faeces (Leeming et al., 1994). Due to its unique characterization, the use of this sterol constitutes an absolute indicator of fecal material presence and is justifiable employing it as and chemical indicator to water quality, because it is biodegradable and can be removed when there is a suitable sewage treatment (Walker, 1982).

Some authors wonder that using fecal sterol, only, should not be consider an unambiguously attributed to fecal matter inputs, so it does not provide an accurate assessment of the contamination and thus they suggest usingratios involving coprostanol with different sterols (Grimalt et al., 1990; Martins et al., 2010). Considering this, we used the coprostanol concentration obtained as an indicator of pollution and the ratio II (epicoprostanol/ coprostanol), proposed by Grimalti et al. (1990), that provide the status of treated or not treated sewage, where smaller values than 0.2 $\mu g.g^{-1}$ indicates not treated sewage (Table 3).

Table 3. Evaluation parameters of sewage contamination (Grimalti et al. 1990).

Evaluation Parameters	Criteria
Coprostanol	> 1,00 $\mu g.g^{-1}$ → contaminated
Ratio II: Epicoprostanol/Coprostanol	< 0,20 → not treatened

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 or not 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. 2012). 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 analysis.

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 13.8µg.g $^{-1}$) and lower (0.04, 0.2, and 0.14µg.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.

2.3. Results and Discussion

2.3.1 *Geochemical analysis*

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 sites BV 15, BV 17, BV 19, BV 21, BV 24, CP 02 and CP 06 as contaminated (Fig. 4, Table 4).

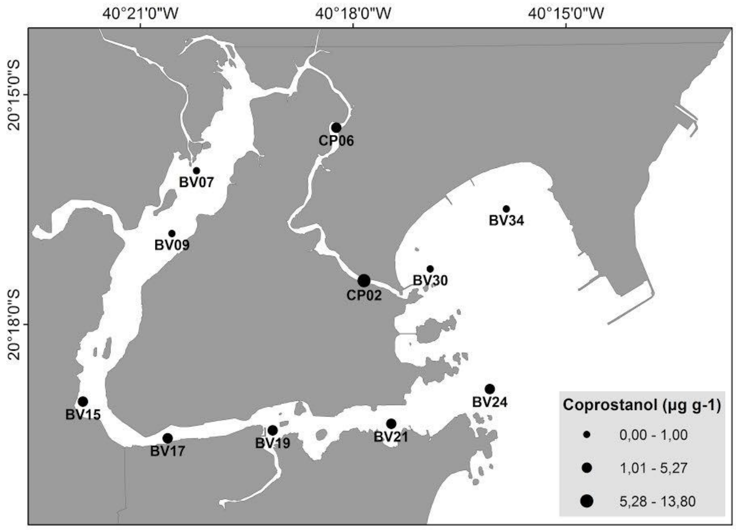

Figure 4. Coprostanol concentration (µg.g^{-1}) at study area sampled points. Values >1.0 µg g^{1} indicate sewage contamination (Grimalti, 1990).

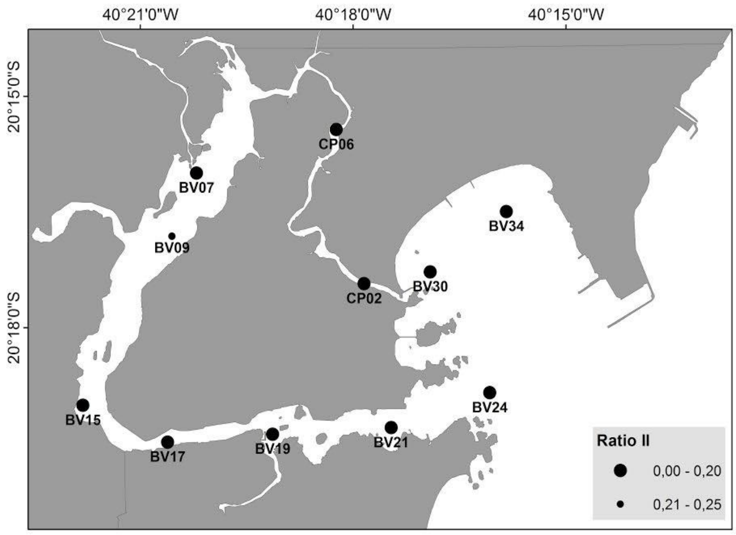

Figure 5. Ratio II (epicoprostanol/coprostanol) at study area sampled points. Values <0.20 indicate not treated sewage (Grimalti, 1990).

Coprostanol concentrations varied from 0.14 to 13.8μg.g^{-1} (2.95 ± 3.98) at study area (Table 4). The two sites less impacted by sewage contamination, BV 30 (0.14μg.g^{-1}) and BV 34 (0.08μg.g^{-1}), are on the marine area near the estuary, at the Tubarão Port, where is situated an industrial complex. BV 07 (0.38μg.g^{-1}) and BV 09 (0.20μg.g^{-1}), not heavily impacted sites, are located at the inner areas of Vitória Bay, where receive contribution of freshwaters from St. Maria da Vitoria and Bubu Rivers, respectively. The higher impacted site, CP 02 (13.8μg.g^{-1}), is at the communication channel between Vitória Bay and Espírito Santo Bay, the Passage Channel, 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 μg.g^{-1} coprostanol concentrations, respectively, at the same area, Channel Port. 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 μg.g^{-1}, as demonstrate for Carreira et al. (2014) studying the Guanabara Bay, at Rio de Janeiro, Brazil (Table 5).

Results for Ratio II (epicoprostanol/coprostanol) indicates only one site where we cannot claim that sewage is absolutely not treated, BV 09 (0.25 μg.g^{-1}), so all the study area except this site indicated presence of not treated sewage (Fig. 5, Table 4). The lowest value for ratio II (0.01μg.g^{-1}) 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 Port Channel 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.

Table 4. Coprostanol concentrations (μg.g^{-1}) and Ratio II (epicoprostanol/coprostanol) values at study area.

Area	Vitória Bay			Port Channel			Espírito Santo Bay			Passage Channel	
Site	BV 07	BV 09	BV 15	BV 17	BV 19	BV 21	BV 24	BV 30	BV 34	CP 02	CP 06
Coprostanol (μg.g-1)	0,38	0,20	4,00	2,17	5,27	1,40	3,02	0,14	0,08	13,8	1,95
Ratio II	0,18	0,25	0,1	0,07	0,07	0,09	0,09	0,14	0,13	0,01	0,11

Table 5. 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‰ (Fig. 7 and 8, Table 7), with an average of 5.8 ± 0.5‰, 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 -25.8‰ (Vitória Bay), < -25.5‰ (Port Channel), < -25.5‰ (Espírito Santo bay) and < -26.2‰ (Passage Channel, with mixed salinity), thus this trend was not observed to $\delta^{13}C$ averages at the present study. The Port Channel and Espírito Santo bay 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‰ (Vitoria Bay), <5.8‰ (Port Channel), <6.0‰ (Espírito Santo Bay), <6.2‰ (Passage Channel, with mixed salinity).

The sediment $\delta^{13}C$ signatures varied from -27 to -24.2‰ (-25.7 ± 0.77‰) (Fig. 6 and 8, Table 6), 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) studied an estuary under sewage impacts that presented $\delta^{13}C$ sediment ranging 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 Passage Channel had depleted $\delta^{13}C$ average (-26.2 ± 0.78‰) when compared to other areas: Vitória Bay (-25.7 ± 0.77‰), Port Channel (-25.5 ± 0.49‰) and Espírito Santo Bay (-25.5 ± 1.42‰), and enriched $\delta^{15}N$ (6.2 ± 0.85‰) when compared to other areas at study site: Vitória Bay (5.3 ± 0.2‰), Port Channel (5.8 ± 0.8‰), Espírito Santo Bay (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 (0.08μg.g^{-1}), a non-contaminated site, have high $\delta^{15}N$ signature (6.2‰), same as Passage Channel media and, the most depleted $\delta^{13}C$ value (-27‰) of all sites. BV 30, which presented low coprostanol concentration (0.14μg.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 ± 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 et al., 2016). Our $\delta^{15}N$ 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 6.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				

Table 7. 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				

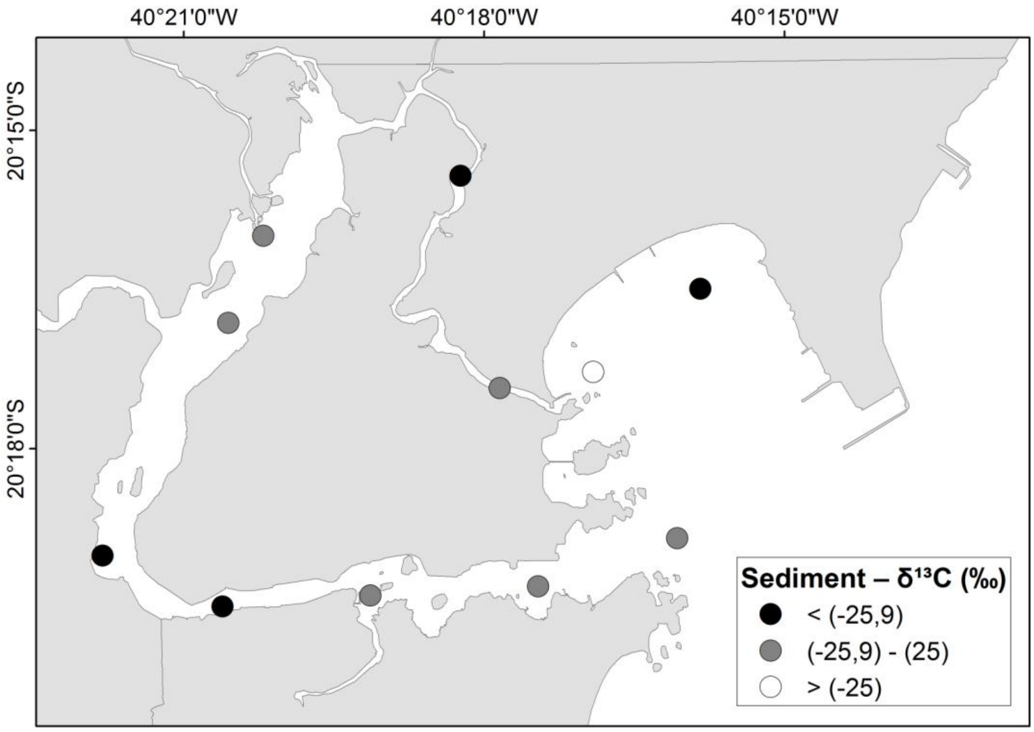

Figure 6. Carbon isotopic values for the sediment samples at study sites.

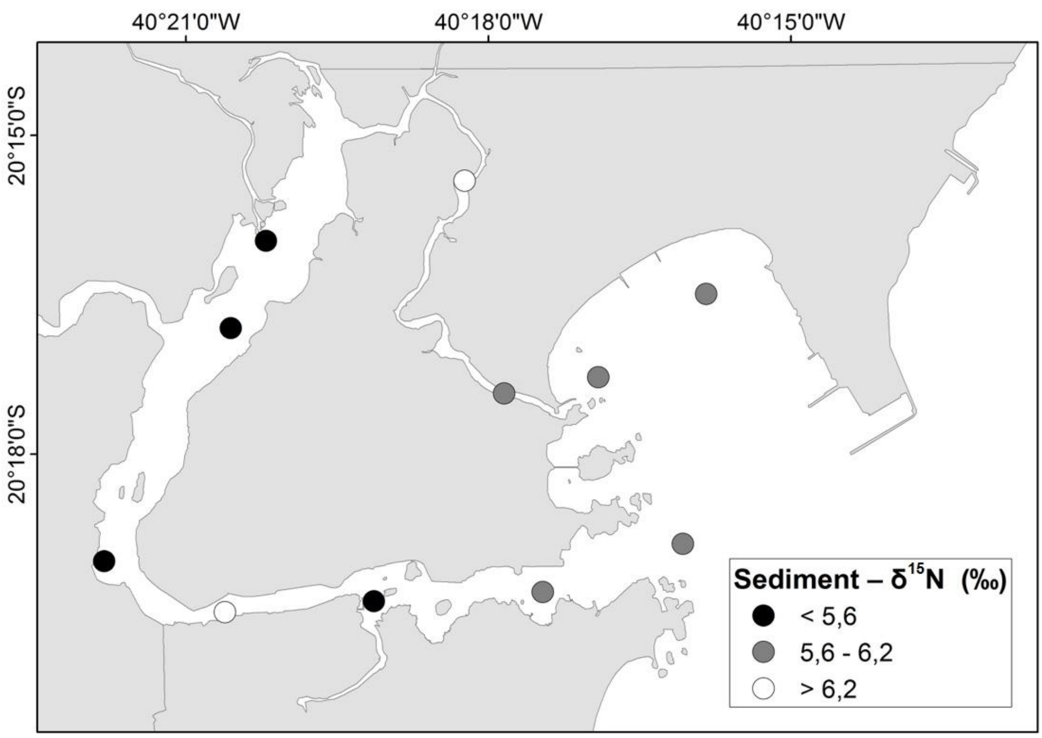

Figure 7. Nitrogen isotopic values for sediment samples at study sites.

2.3.3.2 Macrofaunal assemblages

The $\delta^{13}C$ and $\delta^{15}N$ signatures of fauna ranged from -27 to -14.7‰ (Table 6) and from 3.1 to 13.2‰ (Table 7), respectively.

Carnivores presented $\delta^{13}C$ signatures between -21.6 to -17.2‰ (Table 8), 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) (Table 8).

Deposit-feeders average $\delta^{13}C$ signatures ranged from -22.6 ± 0.5‰ to -16.7 ± 1.2‰, similar to what Gearing et al. (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 et al. (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 6). 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. 9).

Table 8. $\delta^{13}C$ 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		

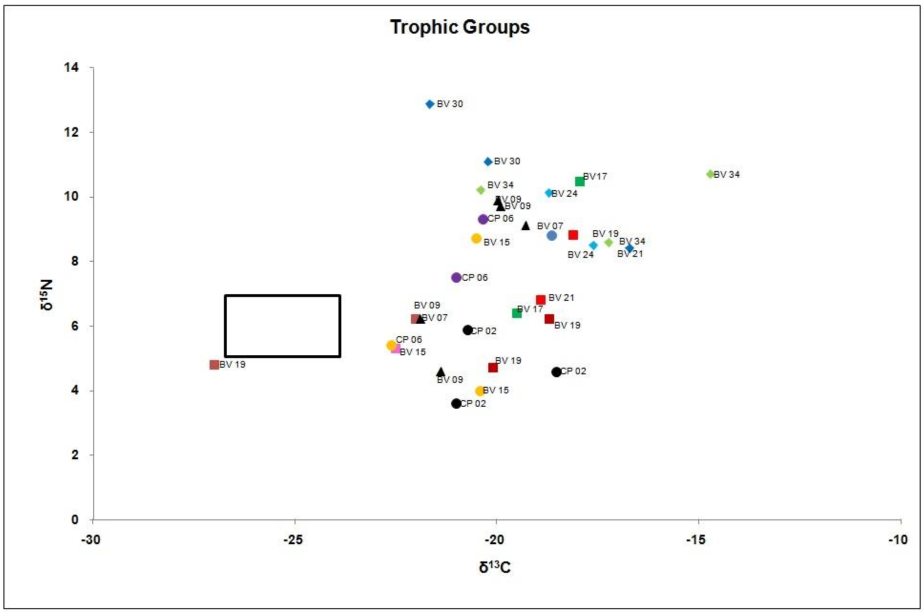

Figure 8. Bi-plot of isotopic signatures ofδ^{13}C and δ^{15}N from all feeding guilds (see table 8). Standard deviation axes were suppressed for better visualization. Sites are identified by description next to correspondant symbols and different colors. Rectangle delimit signature distribution of sediment at study area (δ^{13}C -27 to -24.2‰ and δ^{15}N 5.0 to 6.8‰).

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 9; 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.

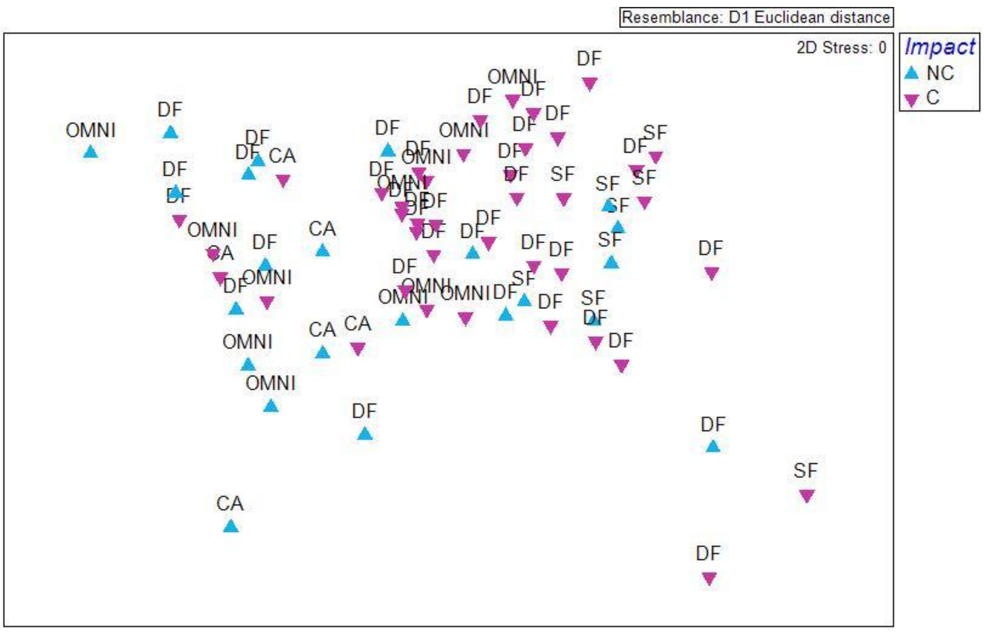

Figure 9. MDS between trophic groups across impact groups, where CA: Carnivore, SF: Suspension-feeder, OMNI: Omnivore, DF: detritive-feeder, C: Contaminatedand NC: non-contaminated.

SIBER (Stable Isotope Bayesian Ellipses in R) was performed considering all macrofauna $\delta^{13}C$ and $\delta^{15}N$ signatures at study área. 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 (Table 9). 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. 10 and Fig. 11). 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. 12). SIBER analysis results indicate clearly a bigger gamma of niche possibilities to be explored by macrofauna at non-contaminated sites in relation to contaminated.

Table 9. 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

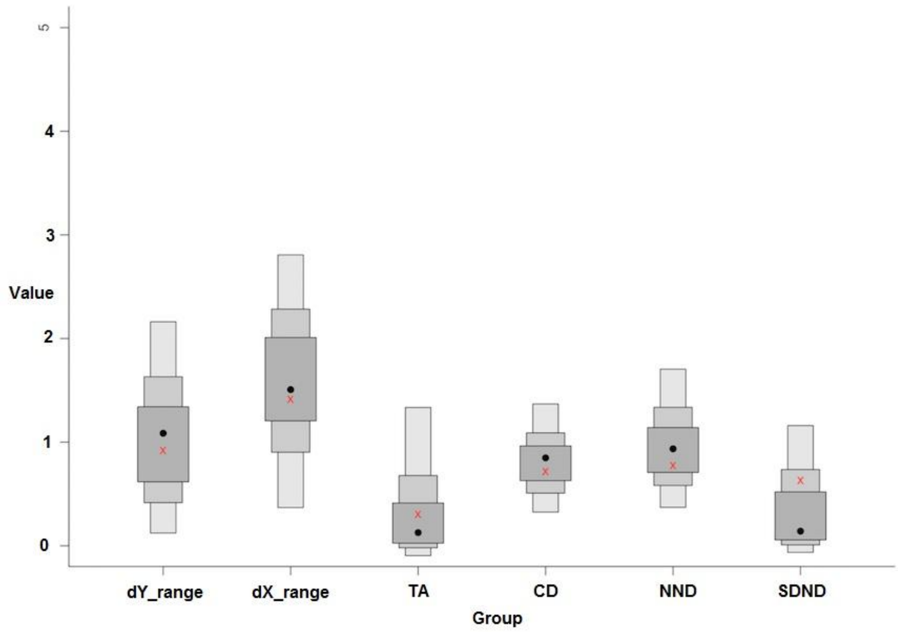

Figure 10. The six Layman metrics applied to all macrofaunal data to contaminated sites. Black dots represent their mean, and red letter “x”, the corrected mean. Shaded boxes represent the 50, 75 and 95% credible intervals from dark to light grey.

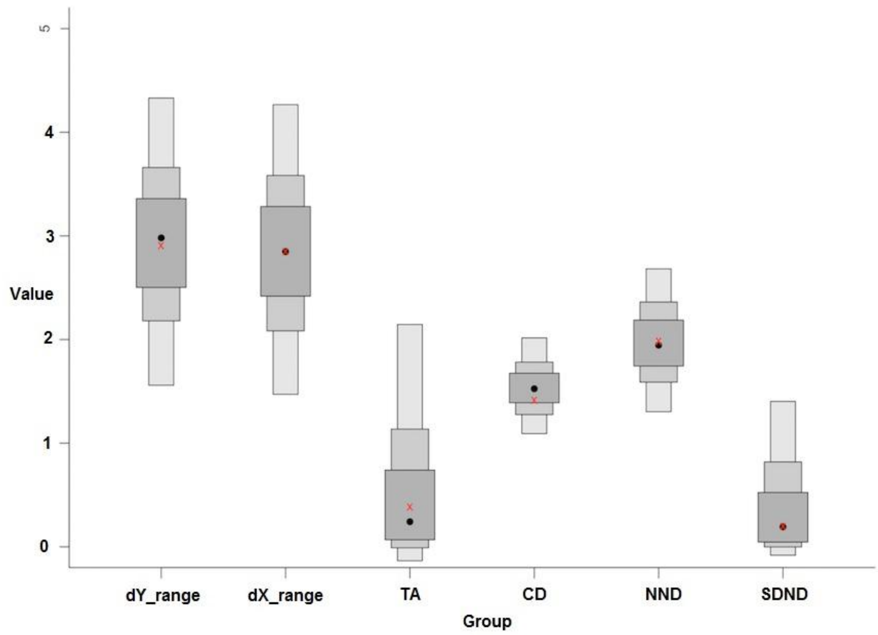

Figure 11. The six Layman metrics applied to all macrofaunal data to non-contaminated sites. Black dots represent their mean, and red letter “x”, the corrected mean. Shadedboxes represent the 50, 75 and 95% credible intervals from dark to light grey.

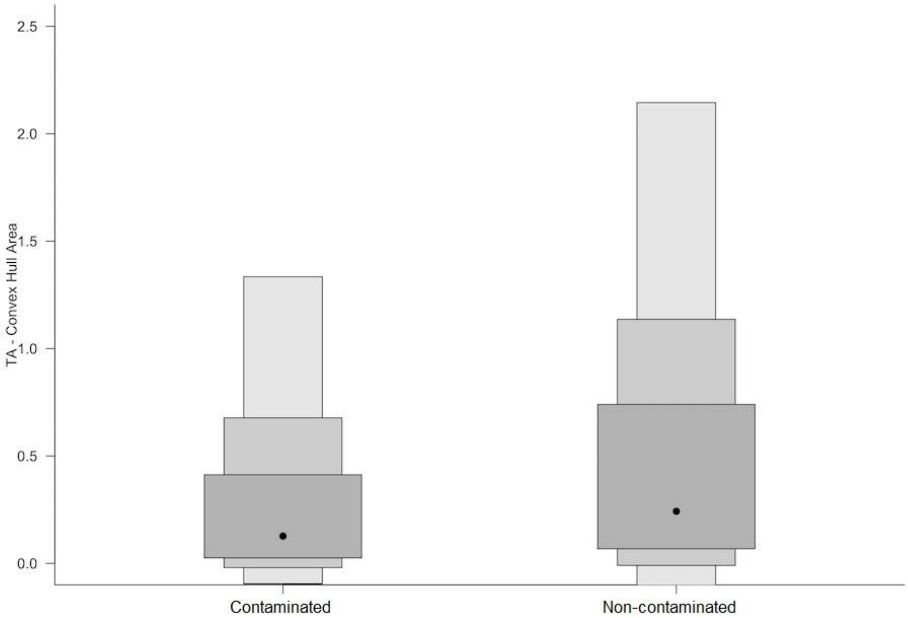

Figure 12. TA (Total Area) calculated. Contaminated and non-contaminated results are shown. Black dots representtheir mean, and read letter “x”, the corrected mean. Shaded boxes represent the 50, 75 and 95% credible intervals from dark to light grey.

2.4. Conclusion

Carbon isotope patterns at sediment in this study have similar signatures than observed by other authors at environment under sewage contamination, which indicates that $\delta^{13}C$ sediment provides trustable information about polluted condition at environment. Although ANOSIM indicates differences between feeding groups under contaminated and non-contaminated conditions, there was no general pattern at $\delta^{13}C$ macrofaunal that could be relate directly to sewage contamination, but to mixed sources of feeding. Nitrogen stable isotope show to be able to indicate change in fauna structure directly attributable to sewage contamination, considering the depletion observed in depositivores and omnivores feeding habits groups, at contaminated sites in comparison to non-contaminated. Furthermore, SIBER analysis evidenced different width between trophic niche under different conditions of contamination. Stable isotopes demonstrated to be an important indicator of organic enrichment that should be combined with other descriptors, como os geoquímicos, in the evaluation of estuarine environments to assure better assertive conclusions.

2.5. References

Abrantes, K. G., Barnett, A., Bouillon, S. 2014. Stable isotope-based community metrics as a tool to identify patterns in food web structure in east African estuaries. Functional Ecology 28, 270–280.

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

Alfaro, A. C., 2005. Benthic macro-invertebrate community composition within a mangrove/seagrass estuary in northern New Zealand. Estuarine, Coastal and Shelf Science 66, 97-110.

Alongi, D.M., 1998. Coastal ecosystem processes. CRC Press, Boca Raton, FL, USA.

Amorim, M. A., Moreira-Turcq, P. F., Turcq, B. J., Cordeiro, R. C. 2009. Origem e dinâmica da deposição dos sedimentos superficiais na Várzea do Lago Grande de Curuai, Pará - Brasil. Acta Amazonica 39 (1), 165-171.

Arruda, E. P., Domaneschi, O., Amaral, A. C. Z. 2003. Mollusc feeding guilds on sandy beaches in São Paulo State, Brazil. Marine Biology, 143, 691-701.

Barbour, M. T., Gerritsen, J., Snyder, B. D., Stribling, J. B. 1999. Rapid bioassessment protocols for use in Streams and wadeable rivers: Periphyton, Benthic Macroinvertebrates and Fish, Second Edition, EPA 841-8-99-002.

Barcellos, R.L., Flores-Montes, M.J., Alves, T.M.F., Camargo, P.B. 2016. Modern sedimentary processes and seasonal variatios of organic matter in an urban tropical estuary, Jaboatão River (PE) Brazil. Journal of Coastal Research 75, 38-42.

Barros, F., Hatje, V., Figueiredo, M. B., Magalhães, W. F., Dórea, H. S., Emídio, E. S., 2008. The structure of the benthic macrofaunal assemblages and sediments characteristics of the Paraguaçu estuarine system, NE, Brazil. Estuarine, Coastal and Shelf Science 78(30), 753-762.

Bernardino, A. F., Smith, C. R., Baco, A., Altamira, I., Sumida, P. Y. G., 2010. Macrofaunal succession in sediments around kelp and wood falls in the deep NE Pacific and community overlap with other reducing habitats. Deep-Sea Research Part I 57, 708-723.

Bouillon, S., Borges, A. V., Castañeda-Moya, E.,Diele, K., Dittmar, T., Dujke N. C., Kristense, E., Lee S. Y., Marchand, C., Middleburg, J. J., Rivera-Monroy, V. H.,Smith III, T. J., Twilley, R. 2008. Mangrove production and carbon sinks: A revision of global budget estimates, Global Biogeochemical Cycles, 22.

Brind'Amour, A., Dubois, S. F. 2013. Isotopic Diversity Indices: How Sensitive to Food Web Structure? PLoS ONE *8*(12), e84198.

Cabana,G, Rasmussen, J. B. 1994. Modelling food chain structure and contaminant bioaccumulation using nitrogen isotopes. Nature 372, 255-257.

Carreira, R., Wagener, A.L.R., Fileman, T.; Readman, J.W. 2001.Distribuição de coprostanol (5β(h)-colestan-3β-ol) em sedimentos superficiais da Baía de Guanabara: indicador da poluição recente por esgotos domésticos. Química Nova 24(1), 37-42.

Carreira, R. S.; Wagener, A. L. R.; Readman, J. W. Sterols as markers of sewage contamination in a tropical urban estuary (Gauanabara Bay, Rio): space-time variations. Estuarine and Coastal Shelf Science 60, 587-598.

Carvalho, M. C. 2008. Uso dos isótopos estáveis de carbono, nitrogênio e enxofre em estudos de ecologia costeira. Oecologia Brasiliensis 12 (4), 694-705.

Clarke, K. R., Gorley, R. N. 2006. PRIMER v6: User Manual/Tutorial. PRIMER-E, Plymouth, 192pp.

Cooper, J. A. G. 2002.Anthropogenic Impacts on Estuaries. Encyclopedia of Life Support Systems (EOLSS).

Couch, C. A. 1989. Carbon and Nitrogen Stable Isotopes of Meiobenthos and their Food Resources. Estuarine, Coastal and Shelf Science 28, 433-441.

Dean, H. K., Blake J. A. 2015. The Orbiniidae (Annelida: Polychaeta) of Pacific Costa Rica. Zootaxa 3956 (2).

Demopoulos, A. W. J., Fry. B, Smith, C.R. 2007. Food web structure in exotic and native mangroves: a Hawaii–Puerto Rico comparison. Oecologia 153, 675–686.

Del-Pilar-Ruso, Y., De-La-Ossa-Carretero, J. A., Loya-Fernández, A., Ferrero-Vicente, L. M., Giménez-Casalduero, F., Sánchez-Lizaso, J. L. 2009. Assessment of soft-bottom Polychaeta assemblage affected by a spatial confluence of impacts: Sewage and brine discharges. Marine Pollution Bulletin 58, 765–786.

DeNiro, M. J., Epstein, S. Influence of diet on the distribution of carbon isotopes in animals. 1978. Geochimica et Cosmochimica Acta 42, 495-506.

Elliot, M., Quintino, V. 2007. The Estuarine Quality Paradox, Environmental homeostasis and the difficulty of detecting anthropogenic stress in naturally stressed areas. Marine Pollution Bulletin 54, 640–645.

Fauchald, K., Jumars, P. A. 1979. The diet of worms: a study of polychaete feeding guilds. Oceanography and Marine Biology Annual Review 17, 193–284.

Fry, B., 2006. Stable Isotope Ecology.XII, 308 p. Springer, New York.

Gao, X.; Yang, Y.; Wang, C. 2012. Geochemistry of organic carbon and nitrogen in surface sediments of coastal Bohai Bay inferred from their ratios and stable isotopic signatures. Marine Pollution Bulletin 64 (6) 1148–1155.

Gearing, P.J., Gearing, J.N., Maughan, J.T., Oviatt, C.A. 1991. Isotopic distribution of carbon from sewage sludge and eutrophication in the sediments and food web of estuarine ecosystems. Environmental Science and Technology 25, 295–301.

Grimalt, J. O., Fernández, P., Bayona, J. M., Albaigés, J., 1990. Assessment of fecla sterols and ketones as indicators of urban sewage inputs to coastal waters. Environmental Science & Technology 24, 357-363.

Hu,L., Guo, Z., Feng, J.,Yang, Z., Fang, M. 2009. Distributions and sources of bulk organic matter and aliphatic hydrocarbons in surface sediments of the Bohai Sea, China. Marine Chemistry 113, 197–211.

Iken , K., Brey T., Wand, U., Voigt, J., Junghan, P. 2001. Food web structure of the benthic community at the Porcupine Abyssal Plain (NE Atlantic): a stable isotope analysis. Progress in Oceanography 50, 383-405.

Jackson, A.L., Inger, R., Parnell, A.C. & Bearhop, S. 2011. Comparing isotopic niche widths among and within communities: SIBER – Stable Isotope Bayesian Ellipses in R. Journal of Animal Ecology 80, 595–602.

Jackson, M.C., Donohue, I., Jackson, A.L., Britton, J.R., Harper, D.M. & Grey, J. 2012. Population-level metrics of trophic structure based on stable isotopes and their application to invasion ecology. PLoS One 7, e31757.

Jesus, H. C, Costa, E. A., Mendonça, A. S. F., Zandonade, E., 2004. Distribuição de metais pesados em sedimentos do sistema estuarino da Ilha de Vitória - ES. Química Nova 27(3), 378-386.

Jumars, P. A., Dorgan, K. M., Lindsay, S. M. 2015. Diet of Worms Emended: An Update of Polychaete Feeding Guilds. Annual Review of Marine Science 7, 20.1-20.24.

Kabat, A. R., Hershler, R. 1993. The Prosobranch snail family Hydrobiidae (Gastropoda: Rissooidea): Review of classification and supraspecific Taxa. Smithsonian Contributions to Zoology, 547.

Layman, C.A., Arrington, D.A., Montaña, C.G. & Post, D.M. 2007. Can stable isotope ratios provide for community-wide measures of trophic structure? Ecology 88, 42-48.

Leeming, R., Ball, A.; Jhones, G., Ashbolt, N., Nichols, P. 1994. Distinguishing Between Human and Animal Sources of Faecal Pollution. Water Research 30 (12), 2893-2900.

Levin, L. A., Carolyn, C. 2012. Stable Isotope Protocols: Sampling and Sample Processing. Scripps Institution of Oceanography Technical Report.

López-Gappa, J. J., Tablado, A., Magaldi, N. H. 1990. Influence of sewage pollution on a rocky intertidal community dominated by the mytilid Brachidontes rodriguezi. Marine Ecology Progress Series 63, 163-175.

Lotze, H. K., Lenihan, H. S., Bourque, B. J., Bradbury R. H., Cooke, R. G., Kay, M. C., Kidwell, S. M., Kirby, M. X., Peterson, C. H., Jackson, J. B. C. 2006. Depletion, degradation, and recovery potential of estuaries and coastal seas. Science Reports 312, 1806-1809.

McClelland, J. W., Valiela, I., Michener, R. H. 1997. Nitrogen stable isotope signatures in estuarine food webs: A record of increasing urbanization in coastal watersheds. Limnology and Oceanography 42, 930-937.

McClelland, J. W.,Valiela, I. 1998. Linking nitrogen in estuarine producers to land-derived sources. Limnology and Oceanography 43(4), 577-585.

Maldonado, C., Venkatesan, M. I., Phillips, C. R., Bayona, J. M.2000.Distribution of Trialkylamines and Coprostanol in San Pedro shelf sediments adjacent to a sewage outfall.Marine Pollution Bulletin 40(8), 680-687.

Manetta, G. I., Benedito, E., Ducatti C. 2011. Effect of alcohol and formaldehyde on the $\delta^{13}C$ and $\delta^{15}N$ isotopic composition of Plagioscion squamosissimus and Hypophthalmus edentatus (Pisces, Osteichthyes). Acta Scientiarum. Biological Sciences 33 (4), 393-397.

Martins, C. C., Montone, R. C., Gambá, R. C.,Pellizari, V. H. 2005. Sterols and fecal indicator microorganisms in sediments from admiralty Bay, Antarctica.Brazilian Journal Of Oceanography 53 (1/2), 1-12.

Martins, C. C., Ferreira, J. A., Taniguchi, S., Mahiques, M. M., Bícego, M. C., Montone, R. C. 2008. Spatial distribution of sedimentary linear alkylbenzenes and faecal steroids of Santos Bay and adjoining continental shelf, SW Atlantic, Brazil: Origin and fate of sewage contamination in the shallow coastal environment. Marine Pollution Bulletin 56, 1353-1376.

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

Mazumder D., Saintilan N., Alderson B. 2015. Hollins Inputs of anthropogenic nitrogen influence isotopic composition and trophic structure in SE Australian estuaries.Marine Pollution Bulletin100(1), 217-23.

Mayr, C. C., Försterra, G., Häussermann, V., Wunderlich, A., Grau, J., Zieringer, M., Altenbach, A. V. 2011. Stable isotope variability in a Chilean fjord food web: implications for N- and C-cycles. Marine Ecology Progress Series 428, 89-104.

Minagawa, M., Wada, E. 1984. Stepwise enrichment of ^{15}N along food chains: further evidence and the relation between $\delta^{15}N$ and animal age. Geochimica et Cosmochimica Acta 48, 1135–1140.

Muniz, C. D., Nieto, P. J. G., Fernández, A. J. R., Torres, J. M., Taboada, J. 2012. Detction of outliers in water quality monitoring samples using functional data analysis in San Esteban estuary (Northern Spain). Science of the Total Environment 439, 54-61.

Nalesso, R.C.,Joyeux, J-C.,Quintanal, C.O.,Torezanil E.,Oteguil, A.C.P. 2005.Soft-bottom macrobenthic communities of the Vitória bay estuarine system, south-eastern Brazil. Brazilian Journal Of Oceanography 53 (1/2), 23-38.

Narchi, W. 1972. Comparative study of the functional morphology of Anomalocardia brasiliana (Gmelin, 1791) and Tivela Mactroides (Born, 1778) (Bivalvia, Veneridae). Bulletin of Marine Science 22(3).

Obade, P. T., Koedam, N., Soetaert, K., Neukermans, G., Bogaert, j., Nysse, E., Van Nedervelde, F., Berger, U., Dahdouh-Guebas, F. 2009. Impactc of anthropogenic disturbance on a mangrove forest assessed by a 1D cellular automaton model using lotka-volterra-type competition. International Journal of Design & Nature and Ecodynamics 3 (4), 296–320.

Ogrinc, N., Fontolan, G., Faganeli, J., Coveli, S. 2005. Carbon and Nitrogen Isotopic Compositions of organic matter in coastal sediments (the Gulf of Trieste, N Adriatic Sea): indicators of sources and preservation. Marine Chemistry 95, 163-181.

Pardo, E. V., Amaral, A. C. Z. 2004. Feeding behavior of the Cirratulid Cirriformia filigera (Delle Chiaje, 1825) (Annelida: Polychaeta). Brazilian Journal of Biology 64(2), 283-288.

Pearson, T. H., Rosenberg, R. 1978. Macrobenthic succession in relation to organic enrichment and pollution of the marine environment. Oceanography and Marine Biology, An Annual Review 16, 229-31.

Pereira, A. L., 2007. Stable isotopes in ecological studies: methods, applications and perspectives. Revista Biociências, Taubaté 13(1-2),16-27.

Peterson, B. J., Fry, B., 1987. Stable isotopes in ecosystem studies. Annual Review of Ecology and Systematics 18, 293-320.

R Development Core Team (2007) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org.

Ramaswamy, V., Gaye, B., Shirodkar, P.V., Rao, P.S., Chivas, A.R., Wheeler, D., Thwin, S. 2008. Distribution and sources of organic carbon, nitrogen and their isotopic signatures in sediments from the Ayeyarwady (Irrawaddy) continental shelf, northern Andaman Sea. Marine Chemistry 111, 137-150.

Ramos, R. J., Travassos, M. P., Leite, G., R., 2010. Characterization of macrofauna associated with articulated calcareous algae (Corallinaceae, Rhodophyta) occurring in hydrodynamic gradient on the Espírito Santo State Coast, Brazil. Brazilian Journal of Oceanography 58(4), 275-285.

Rogers, M. K. 1999. Effects of sewage contamination on macro-algae and shellfish at Moa Point, New Zealand using stable carbon and nitrogen isotopes. New Zealand Journal of Marine and Freshwater Research 33, 181-188.

Sampaio, L., Rodrigues, A. M., Quintino, V. 2010. Carbon and nitrogen stable isotopes in coastal benthic populations under multiple organic enrichment sources. Marine Pollution Bulletin 60, 1790-1802.

Schettini, C. A. F., Pereira, Fo., J., Spillere, L. 2000. Notas Técnicas Facimar 4, 11-28.

Sterza, J. M., Fernandes, L. L. 2006. Zooplankton community of the Vitória Bay estuarine system (Southeastern Brazil): Characterization during a three-year study. Brazilian Journal of Oceanography 54(2-3), 95-105.

Syväranta, J., Martino, A., Kopp, D., C´er´eghino, R., Santoul, F. 2011. Freezing and chemical preservatives alter the stable isotope values of carbon and nitrogen of the Asiatic clam (Corbicula fluminea). Hydrobiologia, Kluwer Academic Publishers, 658, p 383-388.

Tucker, J., Sheats, N., Giblin, A. E., Hopkinson, C. S., Montoy, J. P. 1999. Using stable isotopes to trace sewage-derived material through Boston Harbor and Massachusetts Bay. Marine Environmental Research 48, 353-375.

Venkatesan, M. I.; Kaplan, I. R. 1990. Assessment of fecal sterols and ketones as indicators of urban sewage inputs to coastal waters. Environmental Sciences and Technology 24(2), 208-214.

Venkatesan, M. I., Mirsadeghi, F. H. 1992. Coprostanol as sewage tracer in McMurdo Sound, Antarctica.Marine Pollution Bulletin 25, 328-333.

Veronez-Junior,P., Bastos, A. C., Quaresma, V. S. 2009. Morfologia e distribuição sedimentar em um sistema estuarino tropical: Baía de Vitória, ES. Revista Brasileira de Geofísica 27(4), 609-624.

Waldron, S., Tatnerb, P., Jackc, I., Arnotta, C. 2001. The impact of sewage discharge in a marine embayment: A stable isotope reconnaissance. Estuarine, Coastal and Shelf Science 52, 111–115.

Walker, R. W., Wun, C. K., Litsky, W., Dutka, B. J. 1982. Coprostanol as an indicator of fecal pollution. C R C Critical Reviews in Environmental Control 12(2), 91-112.

Warwick, R.M.1992. A new method for detecting pollution effects on marine macrobenthic communities. Marine Biology 92, 557-562.

West, J. B.; Bowen, G. J.; Cerling, T. E. and Ehleringer, J. R. 2006. Stable isotopes as one of nature's ecological recorders. Trends in Ecology and Evolution 21, 408-414.

Yu, F., Zng, W., Lloyd J. L., Huang, G., Leng, M. J., Kendrick, C., Lamb, A. L., Yim, W. W. 2010. Bulk organic $\delta^{13}C$ and C/N as indicators for sediment sources in the Pearl River delta and estuary, southern China. Estuarine, Coastal and Shelf Science 87, 618-630.

Zalmon I. R., Krohling W., Ferreira, C. E. L. 2011. Abundance and diversity patterns of the sessile macrobenthic community associated with environmental gradients in Vitória Harbor, southeastern Brazil. Zoologia 28 (5), 641–652.