UNIVERSIDADE DO SUL DE SANTA CATARINA
PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS AMBIENTAIS

THÁBATA FERNANDES CÂNDIDO

MULTIPLE BENTHIC INDICATORS SUGGEST LOW SEWAGE IMPACT FROM AN OCEAN OUTFALL IN A HIGH-ENERGY SANDY SHORE (SOUTH BRAZIL)

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Dissertação apresentada ao Programa de Pós-Graduação em Ciências Ambientais, como quesito parcial à obtenção do título de Mestre em Ciências Ambientais

Orientador: Dr. Sérgio Antônio Netto

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PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS AMBIENTAIS – MESTRADO

ATA Nº 07/2019 DE DEFESA PÚBLICA DE DISSERTAÇÃO

Aos doze dias do mês de julho de dois mil e dezenove, às catorze horas, na sala de videoconferência do Campus Unisul Virtual da Universidade do Sul de Santa Catarina, foi realizada a sessão pública de apresentação e defesa de Dissertação de Mestrado de Thábata Fernandes Cândido, como requisito para obtenção do título de Mestre em Ciências Ambientais, de acordo com o Regimento Interno do Programa de Pós-Graduação em Ciências Ambientais – PPGCA. A comissão avaliadora foi composta pelos seguintes professores: - Dr. Sérgio Antonio Netto, orientador e presidente da banca; - Dra. Natália Venturini, avaliadora externa da Facultad de Ciencias, Universidad de la República, presente por videoconferência; - Dr. Tiago José Pereira, avaliador externo da University of California, presente por videoconferência. A dissertação tem como título: "Multiple benthic indicators suggest low sewage impact from an ocean outfall in a high-energy sandy shore (South Brazil)". Área de concentração: Tecnologia, Ambiente e Sociedade e linha de pesquisa: Tecnologia & Ambiente. Após a apresentação, a mestranda foi arguida pelos professores da banca. Feito os questionamentos e ouvidas as explicações, a banca avaliadora emitiu o seguinte parecer:

(X) Aprovado

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Obs:____

Nada mais havendo a tratar, foram encerrados os trabalhos e, após lida, foi a presente ata assinada pela Mestranda, pelo membro da Comissão Avaliadora presente e pelo presidente da sessão em nome dos avaliadores presentes por videoconferência.

Dr. Sergio Antonio Netto: ____________________,

Dra. Natalia Venturini: ____________________,

Dr. Tiago José Pereira: ____________________,

Discente Thábata Fernandes Cândido: Thábata F. Cândido.

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Resumo

A urbanização de áreas costeiras não tem sido acompanhada de tratamento adequado de esgoto, e a sobrecarga de matéria orgânica compromete importantes valores econômicos, ecológicos e sociais suportados por esses ecossistemas. Os emissários oceânicos podem ser uma alternativa eficaz para o destino final das águas residuárias nas regiões costeiras, desde que devidamente instalados e monitorados. Neste estudo nós investigamos os efeitos de um pequeno emissário em uma costa arenosa e de alta energia, utilizando como indicadores esteroides sedimentares, composição bioquímica e qualidade nutricional da matéria orgânica e biodiversidade da meiofauna. Os resultados mostraram que perturbações físicas predominaram nas áreas próximas ao emissário, afetando as densidades da fauna e a concentração de fitopigmentos. As maiores concentrações de esteróis sedimentares, detectados a 1000 m à NE do emissário, indicaram a influência da hidrodinâmica local no processo de transporte e dispersão do esgoto. Nesta área, a contaminação do esgoto alterou o estado trófico bêntico e levou a uma forte diminuição na diversidade trófica e aumento de formas oportunistas. No geral, os resultados mostraram um impacto espacial relativamente baixo e espacialmente limitado do emissário. O uso combinado de esteróis fecais, estado trófico bêntico e biodiversidade da meiofauna foram muito eficazes na detecção da contaminação de esgoto por um pequeno emissário de águas residuárias em uma costa arenosa e de alta energia.

Palavras-chave: Esgoto; Esteroides fecais; Biopolímeros orgânicos; Nematoda; Estado trófico bêntico

Abstract

Increasing urbanization in coastal areas has not paralleled with adequate sewage treatment, and the overload of organic matter compromise important economic, ecological and social values supported by these ecosystems. Ocean outfalls may be an effective alternative to the destination of wastewater in coastal regions, provided they are properly installed and monitored. In this study, we investigated the effects of a small outfall on a high-energy sandy shore, southern Brazil, using sedimentary steroids, biochemical composition and nutritional quality of organic matter and meiofauna biodiversity as indicators. The results showed that physical disturbances prevail near the outfall, affecting the densities of the fauna and phytopigments. The higher concentrations of sedimentary sterols detected at 1000 m NE from the outfall indicated the influence of the local hydrodynamic conditions in the transport and dispersion process of the sewage. In this area, the sewage contamination changed the benthic trophic state and lead to a strong decrease in the trophic diversity and increase of opportunistic nematode taxa. Overall the results showed a relatively low and limited spatial impact from the outfall. The integrated use of fecal sterols, benthic trophic state and meiofauna biodiversity were highly effective in detecting sewage contamination of low input of wastewater in a high-energy sandy shore.

Keywords: Sewage; Fecal sterols; Organic biopolymers; Nematodes; Benthic trophic state

1. Introduction

Coastal areas have always been considered a central asset for humankind, supporting a range of natural and built environments, all of which containing important economic, ecological and societal values. The attractiveness as a potential working and living site has transformed coastal areas with innumerous homes to support a large and still growing proportion of the world population (Sarkar, 2018; Schlacher and Thompson 2012). As coastal population continues to grow, the need for proper sewage treatment becomes of paramount importance. However, in many countries, particularly developing ones, adequate sewage treatment is largely ignored during coastal development and occupation. In Brazil, for example, only around 40% of the coastal cities have some sort of sewage services and treatment (SNIS, 2017). In such circumstances, well-designed ocean outfalls are a fast, safe and affordable solution to improve the sanitation system, with minimal environmental impacts (Sharp, 1990; Puente and Diaz, 2015; Feitosa, 2017).

Worldwide, ocean outfalls are often used in coastal communities for the disposal of domestic wastes, ranging from relatively small (i.e., serving only a few thousand people) to very large ones (i.e., serving millions of people; Roberts et al., 2010). An ocean outfall is a simple technological solution of pretreatment (or treatment, depending local regulations) and the final destination for sewage. The mechanics of the outfall is based on turbulent dispersion, transport (both by diffusion and advection), and depuration by the ocean (Mukhtasor et al., 1999; Roberts et al., 2010). When effective, an outfall system should not simply dilute the sewage in the adjacent ocean, but also allow natural processes to stabilize the waste without causing significant environmental impact.

Overall, the response of the marine environment to the discharge of outfall wastewater depends on: i) discharge mass and flow rate and effluent composition; (ii) the characteristics of the outfall flow (length and number of diffusers, orientation, jet spacing); iii) the dynamics and biodiversity of the receiving area (Jirka and Harleman, 1979; Puente and Diaz, 2015). If improperly designed, excessive discharges by the outfall and consequently the accumulation of organic material in the receiving ocean area can lead to eutrophication affecting local and potentially region biodiversity.

Most of the natural and human-derived organic matter released in coastal areas does not remain in suspension for long periods and tends to settle, altering biochemical conditions of the sediments and their associated biodiversity (e.g. Bertocci et al., 2019; Silva et al., 2017; Egleton and Thomas, 2004). Sewage inputs increase the organic load of sediments and may strongly affect the nature and fate of the organic matter, changing the benthic trophic state and the biopolymeric composition of the organic matter (Hadlich et al., 2018). Thus, organic markers, compounds of natural or anthropogenic origin, are ideal be to determine events or processes of organic contamination due to their specific nature, high chemical stability and resistance to degradation due (e.g. Bianchelli et al., 2018 and references therein).

Besides, molecular markers such as sterols have been used to characterize the source of the organic matter, to identify anthropogenic fecal contamination (Abreu-Mota et al., 2014; Cabral et al., 2018). Sterols are persistent in sediments, easily associate with particulate material and have resistance to anaerobic degradation (Muniz et al., 2015). Fecal sterols, including coprostanol, are used as tracers for human waste because they are largely present in human fecal and sewage effluents (Montone et al., 2010; Venturini et al., 2015).

For the benthic fauna, the known effects of marine outfalls are those derived from studies with macrofauna (see review by Puente and Diaz, 2015; and references therein). Effective responses to human activities have been widely reported for the meiofauna (Egres et al., 2019; Baldrighi et al., 2018; Semprucci et al., 2017) due to the characteristics of their life cycles (small size, high turnover, and lack of larval dispersion), and because free living nematodes represent the most abundant metazoans in Earth (Heip et al., 1985; Vanreusel et al., 2010). However, to our current knowledge, there are no studies evaluating the response of benthic meiofaunal communities to ocean outfalls. In that regard, only Santos et al. (2018) conducted an indoor experiment exposing meiofauna to sea water collected near an outfall, whereas Fraschetti et al. (2006) assessed the effects of outfalls on the nearby hard substrate meiofauna.

The balance between the total organic mass discharged by the outfall and the energy of the environment seems to be the key factors in determining the impacts on the benthic system (Puente and Diaz, 2015). According to the predictive model of Puente and Diaz (2015), minor impacts are expected in low to moderate discharge (e.g., 12.000 to 75.000 m^3day^{-1}) outfalls associated to very exposed environments. In this study, we investigate the potential effects of a marine outfall of low organic matter discharge (i.e., up to 5.184 m^3day^{-1}) in a high energy sandy shore in southern Brazil, using as indicators fecal sterols, the biochemical composition and nutritional quality of sedimentary organic matter and benthic meiofauna.

2. Material and Methods

2.1 Study area

This study was carried out in the inner continental shelf adjacent to the Mar Grosso beach, located in the eastern portion of the city of Laguna, Santa Catarina state, Southern Brazil (Fig. 1). The Mar Grosso is a high-energy dissipative sandy beach, northwards of the only inlet of the Laguna Estuarine System, a chocked coastal lagoon of 184 km^2 (Netto et al., 2018). The most frequent swell wave direction along this coast is from the south, with average heights of 2.5 m; the coast has a micro-tidal regime with mean astronomic tide of less than 1 m; the general alongshore littoral drift is from S-SE to ENE-NE, but local reversals might take place during strong NE wind conditions (Hesp et al., 2009). According to Eichler et al. (2012) the inner shelf has sandy bottoms and spots of sandy mud, possibly originated from the coastal lagoons.

The sewage collected by the network goes to a preconditioning station, where it passes through a railing system for large solids to then be discharged (around 3.456 to 5.184 m^3day^{-1})

at 12 m depth, 1.500 m off the shoreline. The Mar Grosso outfall, functioning since 1986, was originally built to support a population around 14,461 habitants. Currently, the population linked in the outfall network is around 3,500, increasing during summer (IBGE, 2017). Along these 33 years, no monitoring program was conducted, and the only study was by Eichler et al. (2012) using foraminiferans and fecal coliforms, and suggested the fine material derived from the outfall was accumulating on the southwestern and northwestern parts of the beach.

2.2. Sampling and sample processing

Sediment sampling was carried out by scuba diving by end-summer (March 2018) in depths ranging from 10 m to 15 m. Forty concentric sites were sampled around the outfall considering two aspects for detection of the area putatively affected (Fig. 1): - the distance, divided into 50 m, 100 m, 250 m, 500 m and 1000 m from of the outfall, with 8 sampling sites in each distance; - the direction, with 5 sampling sites in each (East, Northeast, North, Northwest, West, Southwest, South and Southeast).

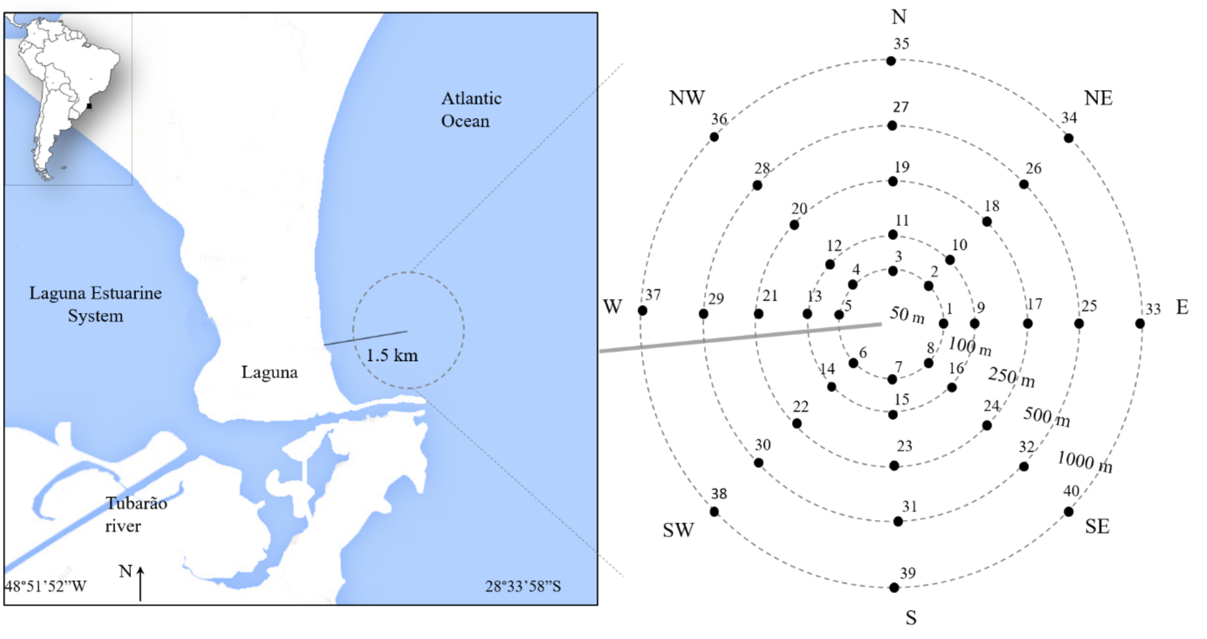

Fig. 1. Map showing the location of the city of Laguna and the Mar Grosso outfall, Southern Brazil, and in detail the schematic representation of sampling sites.

At each site, one sample for sterols, biopolymers, sediment granulometry and meiofauna were taken, and bottom water salinity and temperature data were recorded *in situ* using a handheld Multiparameter instrument (YSI 556). For the analysis of sterols, biopolymers and microphytobenthos biomass, superficial sediment was collected using sterile 150 g glass jars; for granulometry superficial sediment samples were collected using 200 g plastic containers; meiofauna samples were collected with a plexiglass manual corer (internal diameter 4 cm pushed to a depth of 10 cm). Samples for sterols and microphytobenthos were covered with pre-cleaned aluminum foil still in the field. All samples for organic matter and biomass determinations were stored at −20°C until used for analyses in the laboratory. Samples for the study of meiofaunal communities were immediately fixed in 4% formalin in the field.

The sediments for sterols were freeze-dried, carefully homogenized with a mortar and pestle and stored in sterile glass bottles at room temperature until analysis. Sedimentary sterols coprostanol, cholesterol and cholestanol were determined according to Morelli (2019) using ultrasonic assisted extraction (UAE) followed by pre-concentration with the disposable pipette extraction (DPX) technique (Chemat et al., 2017). Gas chromatography coupled to mass spectrometry (GC-MS) was used for the separation and detection of the previously extracted compounds. The optimized DPX parameters were 5 cycles of extraction with 700 μL of sample, of 10 s each, and 2 cycles of desorption of 200 μL, of 20 s each; the optimized desorption solvent was a mixture composed of ethyl acetate:acetonitrile (60:40 v/v; Morelli, 2019).

Microphytobenthic pigments (chlorophyll-*a* and phaeopigments) were analyzed photometrically. Pigments were extracted with 90% acetone (24 h in the dark at 4°C). After centrifugation (800 g for 20 min), the supernatant was used to determine the functional chlorophyll-*a* and acidified with 0.1 N HCl to estimate the amount of phaeopigments, following the procedure of Plante-Cuny (1978). Chlorophyll-*a* (Chl-*a*) and phaeopigments (Phaeo) values were estimated following Lorenzen's method (1967).

Analysis of sedimentary organic biopolymers (i.e., proteins, carbohydrates and lipids) followed the procedures described in Danovaro (2010). Total proteins (PRT) analysis was conducted following extraction with NaOH (0.5 M, 4 h) and determined according to Hartree (1972) as modified by Rice (1982) to compensate for phenol interference. Total carbohydrates (CHO) were analyzed according to Gerchacov and Hatcher (1972). Total lipids (LIP) were extracted from 1 g of freeze-dried homogenized sediment by ultrasonication (20 min) in 10 ml chloroform:methanol (2:1 v/v) and analyzed following the protocol described in Marsh and Weinstein (1966). Blanks for each analysis were performed with pre-combusted sediments at 450 and 480ºC for 4 h. Concentrations of PRT, CHO and LIP were expressed as bovine serum albumin (BSA), glucose and tripalmitine equivalents, respectively. All analyses were carried out in triplicate. Concentrations of PRT, CHO and LIP were converted to carbon equivalents assuming a conversion factor of 0.49, 0.40 and 0.75, respectively (Fabiano and Danovaro, 1994). The sum of protein, lipid and carbohydrate carbon equivalents was reported as the biopolymeric carbon (BPC) and used as a reliable estimate of the labile fraction of organic carbon (Fabiano et al., 1995). Also, the protein to carbohydrate (PRT:CHO) and carbohydrate to lipids (CHO: LIP) ratios were used to evaluate the status of biochemical degradation processes (Galois et al., 2000).

The sedimentary contents of phytopigments and biopolymeric C pools reflect the overall trophic conditions of marine coastal sediments, whereas the algal fraction of biopolymeric C pools reflects the food quality of sedimentary detritus (Pusceddu et al., 2009). The percentage of chlorophyll-*a* and phaeopigments to BPC concentrations, after transformation into C equivalents using 30 as a conversion factor (Danovaro, 2010; Pusccedu et al., 2014), were used as an estimate of the organic material of algal origin including either the living (Chl-*a*) and senescent/detrital (Phaeo). Although the C to microalgae pigments ratio may vary from 10 to higher than 100 and the use of a constant conversion factor may involve errors (De Jonge, 1980), using this conversion factor (30 in this study) allowed us to compare our results with other studies focused on coastal areas (e.g. Pusceddu et al., 1999, 2009, 2011).

Meiofaunal samples were processed following Somerfield et al. (2005). Samples were washed through sieves with mesh openings of 500 μm and 63 μm, and meiofaunal organisms retained on the smaller mesh were extracted by flotation with Ludox TM-50 (specific gravity of 1.15). Samples were then evaporated to anhydrous glycerol, and permanent slides were made for meiofauna identification. Meiofaunal organisms were identified to the lowest possible taxonomic level, and free-living marine nematodes to genus level. Grain size analysis was performed following the sieving methods of Suguio (1973).

2.3 Data Analysis

For sterols, the concentrations of coprostanol, cholesterol and cholestanol were used as a marker of domestic sewage input. Several steroid ratios were established and used for the detection and source assignment of fecal inputs into the environment, especially regarding human fecal matter (e.g. Leeming et al., 1996; Bull et al., 2002). Besides, we also use the ratios coprostanol/(coprostanol+cholestanol) and coprostanol/(coprostanol+cholesterol) as markers of sewage input. (Bull et al., 2002; Takada et al., 1994). Descriptors of organic matter biochemical composition were the concentrations of CHO, PRT, LIP and BPC, whereas for the microphytobenthos we use the concentration of Chl-*a*, Phaeo and the percentage of the algal contribution to the BPC concentrations.

To visualize the similarity of nematode assemblages surrounding the outfall, similarity matrices of log-transformed nematode abundances were constructed based on the Bray-Curtis similarity measure. Ordination was done by nMDS and Goodness-of-fit given by the stress value (Clarke, 1993). In addition to the structural number of genera and density, functional attributes of nematode assemblages were analyzed using the index of trophic diversity (ITD) and the maturity index (MI). The ITD (Heip et al., 1985) is based on the proportion of each of the four feeding types (i.e., selective deposit feeders, nonselective deposit feeders, epi-growth feeders and predators/omnivores; *sensu* Wieser, 1953). 1-ITD was used to better visualize the changes in functional diversity (highest value of the index of trophic diversity is 0.75 and the lowest trophic diversity is 0). The maturity index (MI), which is derived from life history features of marine nematode genera, was calculated for each sample according to Bongers (1990) and Bongers et al. (1991; 1995). Nematodes were classified along a scale of 1–5, with colonizers (e.g., inter alia short life cycle, high reproduction rates, high colonization ability and tolerant to disturbance) weighted as 1 and persisters (e.g., inter alia, long life cycles, low colonization ability, few offspring and sensitive to disturbance) weighted as 5.

Contour maps of abiotic and biotic indicators (i.e., silt+clay percentages, sedimentary fecal sterols, biochemical composition of organic matter, and functional attributes of meiofauna) were used to enhance spatial visualization of the data. We used kriging (Ordinary Kriging; Englund and Sparks, 1991) based on field measures as a spatial interpolation technique to make inferences of the spatial variability. This method is highly flexible allowing users to investigate spatial autocorrelation, prediction, prediction standard error, and probability maps while minimizing the prediction error (Goovaerts, 1997).

The trophic state of meiofaunal communities at the sites was classified according to Dell'Anno et al. (2002) and Pusceddu et al. (2011). Dell'Anno et al. (2002) classification is based on protein and carbohydrate trophic thresholds: -hyper-trophic systems (PRT > 4 mg.g^{-1}; CHO > 7 mg.g^{-1}), -eutrophic systems (PRT 1.5–4 mg.g^{-1}; CHO 5–7 mg.g^{-1}), -meso-oligotrophic systems (PRT < 1 mg.g^{-1}; CHO < 5 mg.g^{-1}). Pusceddu et al. (2011) classification is based on the biopolymeric carbon concentrations and algal contribution to BPC: - eutrophic systems (BPC > 3 mg.g^{-1}; algal fraction < 12%); in mesotrophic systems (BPC 1–3 mg.g^{-1}; algal fraction 12–25% mg.g^{-1}); oligotrophic systems (BPC < 1 mg.g^{-1}; algal fraction > 25% mg.g^{-1}).

To identify potential drivers explaining the variability in nematode assemblages, a distance-based linear model (DistLM; Legendre and Anderson, 1999) was applied on similarity matrices of fauna abundance data (based on Bray-Curtis similarity). The BEST selection procedure, combined with the Akaike Information Criterion (AIC), was used to ascertain the optimal combination of environmental variables that explained the majority of the variation in assemblage structure and for the distance-based redundancy analysis (dbRDA) models (McArdle and Anderson, 2001). Predictor variables comprised the sedimentary fecal sterols, biochemical composition of organic matter (PRT, CHO, LIP), biopolymeric carbon, PRT to CHO ratios, CHO to LIP ratios, algal contribution to BPC (total, Chl-*a* and Phaeo), sediment grain size, silt+clay percentages and sorting.

3. Results

3.1 Sediment proprieties and sterols

Granulometry was relatively homogeneous among the sites, with sediments composed of well sorted fine sands in the southern portion, and moderately sorted northwards of the outfall. The percentages of fines (silt+clay; Fig. 2A) ranged between 0 and 2.7% (mean of 2.5%) and were concentrated northwards, with higher values in the NE direction, specifically at 500 m and 1000 m from the outfall.

The concentration of total sterols (i.e., coprostanol, cholestanol and cholesterol) ranged from 0 to 4.89 $\mu g.g^{-1}$. However, in most of the samples (>95%), sterol values were below the detection limit (0.075 $\mu g.g^{-1}$ for coprostanol and cholestanol, and 0.0075 $\mu g.g^{-1}$ for cholesterol). Both coprostanol and cholestanol were not detected in distances up to 100 m from the outfall (< detection limit). The highest values of coprostanol and cholestanol (2.7 $\mu g.g^{-1}$ and 2.12 $\mu g.g^{-1}$, respectively), were registered at the 1000 m (site 34) in the NE direction (Fig. 2B). In four other sites, the coprostanol and cholestanol were detected but in concentrations lower than the limit of quantification (<0.25 $\mu g.g^{-1}$): - at 250 m (site 19); - at 500 m (site 29); - at 1000 m (site 33 and site 35; Fig. 2B). The sterol ratios, determined only for site 34, were 0.56 for coprostanol/(coprostanol+cholestanol) and 0.4 for coprostanol/(coprostanol+cholesterol).

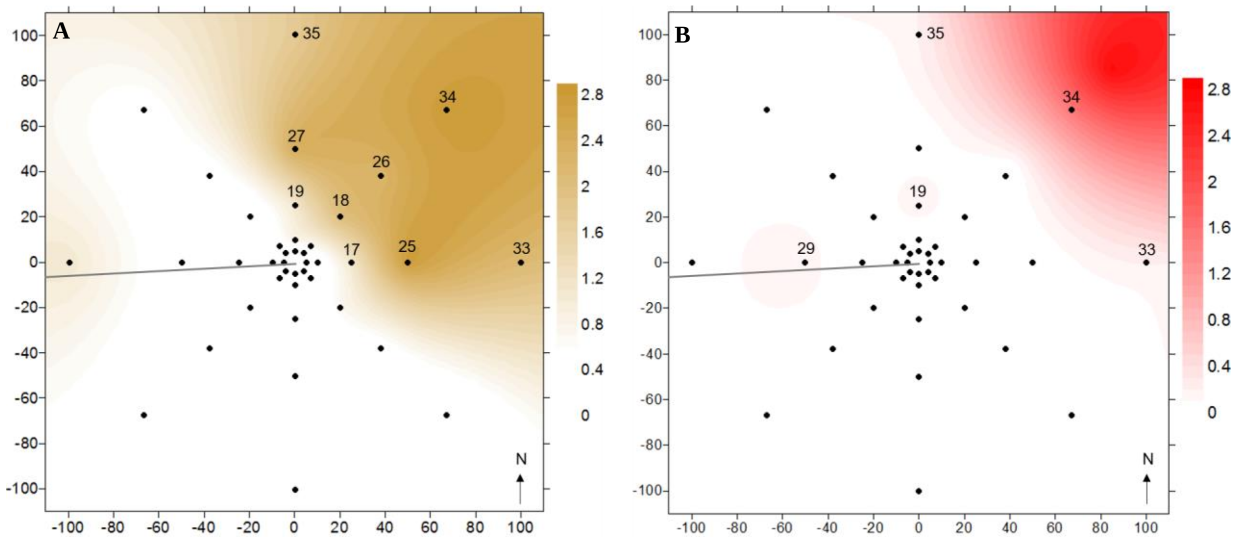

Fig. 2. Spatial variation of (A) silt+clay percentages and (B) the concentrations of fecal sterol coprostanol around the Mar Grosso outfall.

3.2 Biochemical composition and nutritional quality of sedimentary organic matter

The spatial variation of PRT, CHO, LIP, PRT:CHO ratio, CHO:LIP ratio and BPC are shown on Fig. 3. Concentrations of PRT (ranging from 0.075 to 0.828 mg.g^{-1}), CHO (from 0.076 to 0.334 mg.g^{-1}) and LIP (between 0.0308 and 0.636 mg.g^{-1}) showed similar spatial configurations, with increasing values towards the NE. Higher concentrations of these components associated to organic matter were detected at 1000 m in the NE direction from the outfall (site 34; Appendix Table S.1). The PRT:CHO ratio was relatively homogeneous over the area, with most sites displaying values ≥ 1 (total mean 1.1, Fig. 3D). The highest PRT:CHO ratio (2.48) was detected at 1000 m in the NE direction from the outfall (site 34; Appendix Table S.1). On the other hand, the CHO:LIP ratio was more variable (ranged from 0.52 to 4.24) with the highest value at 50 m in the S direction (site 7) and the lowest at 1000 m in the NE direction (site 34; Appendix Table S.1, Fig. 3E). Similarly, BPC (mean value of 0.22 mg.C.g^{-1}; Appendix Table S.1) followed the same spatial trend as shown by the biochemical components of the organic matter, with highest concentrations (1.01 mg.C.g^{-1}) at 1000 m in the NE direction from the outfall (site 34; Fig. 3F).

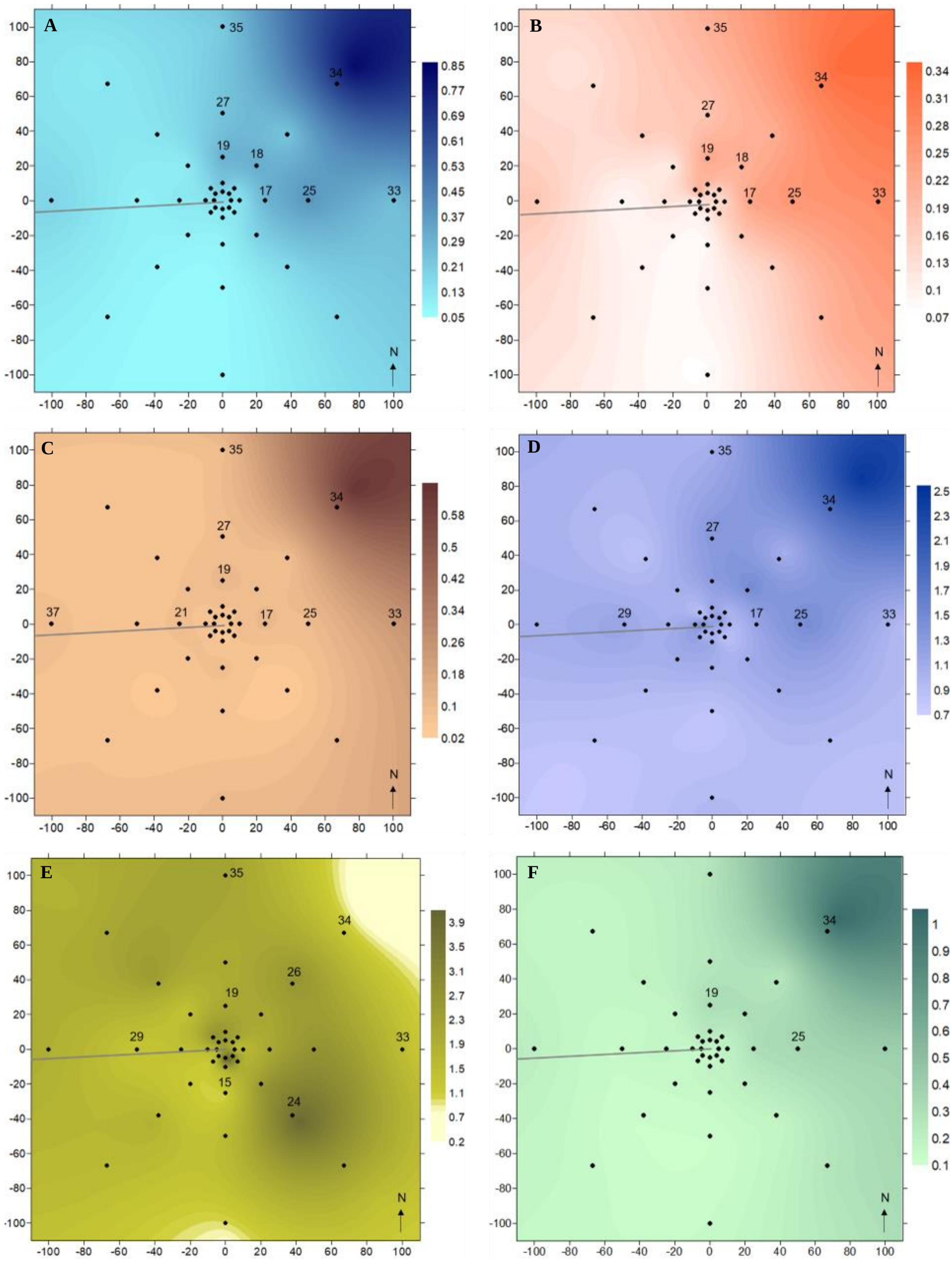

Fig. 3. Spatial variation of (A) proteins, (B) carbohydrates, (C) lipids, (D) PRT:CHO ratio, (E) CHO:LIP ratio, and (F) biopolymeric carbon concentrations (BPC) around the Mar Grosso outfall.

Microphytobenthic pigment concentrations in the sediments were generally higher in sites near the outfall (Appendix Table S.1). For example, Chl-*a* was higher up to 500 m (mean of 0.55 $\mu g.g^{-1}$) and lower at 1000 m (mean 0.25 $\mu g.g^{-1}$) distances from the outfall. In the case of Phaeo, higher values were detected near the outfall (50 m; mean of 0.77 $\mu g.g^{-1}$), decreasing in intermediate distances (from 100 to 500 m; mean of 0.46 $\mu g.g^{-1}$) and relatively low at 1000 m (mean 0.11 $\mu g.g^{-1}$).

Contribution of carbon derived from algal fractions varied drastically among the sites (Fig. 4). The algal contribution to the BPC (total mean-13.4 %) ranged from 46.6% in sites near the outfall to less than 5% at 1000 m (Fig. 4). Near the outfall, the contribution of the algal fractions was mainly due to Phaeo. For example, in site 2 (50 m) the Phaeo contribution to BPC was as high as 32%. In intermediate distances, Phaeo contributions decreased (around 6%), then reaching the lowest values at 1000 m (i.e., less than 1%). Overall, the contribution of Chl-*a* to BPC was less variable among sites (ranged from 3.9% to 14%). Nevertheless, and as observed for Phaeo, Chl-*a* contributions drastically decreased at 1000 m from the outfall (average of 2%).

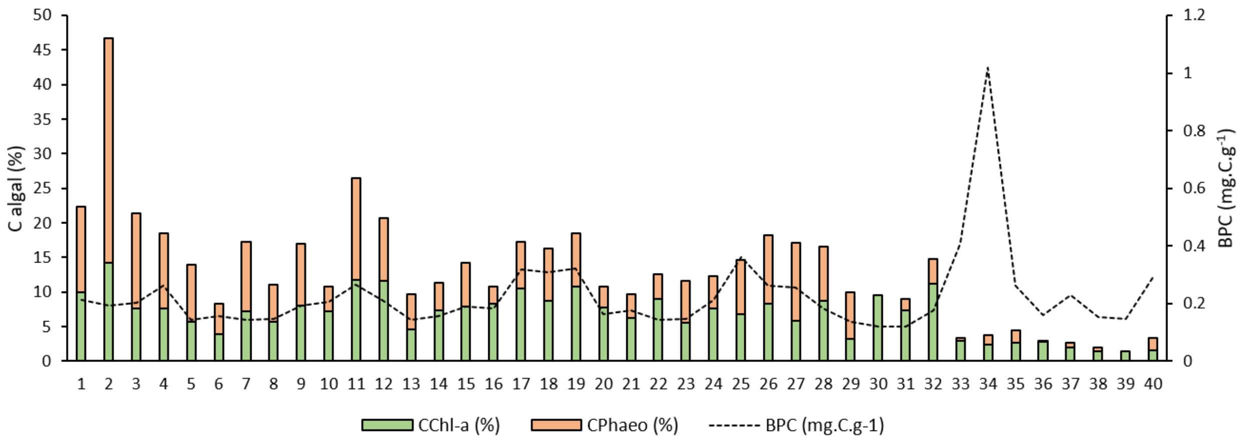

Fig. 4. Contribution of carbon derived from the algal fractions (C algal) to the biopolymeric carbon concentrations (BPC). CChl-*a*- Chlorophyll-*a* contribution to BPC; CPhaeo- Phaeopigments contribution to BPC.

According to the trophic state classification of Dell'Anno et al. (2002) and based on the concentrations of PRT and CHO, all the sampling sites were considered meso-oligotrophic, except for the site 34, classified as eutrophic. Yet, following Pusceddu et al. (2011), the trophic state based on BPC and algal contributions to BPC suggested an oligotrophic state for all sites.

3.3 Nematode biodiversity

A total of 5,437 nematodes belonging to 85 genera (25 families), with densities ranging from 10 to 371 inds.10cm^{-2}, were recorded in the present study. Genera *Sabatieria* (Comesomatidae), *Pseudosteineria* (Xyalidae) and *Microlaimus* (Microlaimidae) were the numerically dominant taxa, accounting for 34% of all identified nematodes. Mean values of nematode descriptors (density, number of genera, diversity, ITD, MI) are shown on Appendix Table S.2.

The nMDS ordination derived from nematodes abundances (Fig. 5A) showed a clear separation of nematode assemblages from 50 m to 1000 m from the outfall. This variability partially reflected the differences in nematode density, lower at 50 m (mean of 57 inds.10cm^{-2}, especially site 2 with only 10 inds.10cm^{-2}), and higher at 1000 m from the outfall (mean of 159 inds.10cm^{-2}), reaching the highest density at site 34 with 371 inds.10cm^{-2}. When superimposing the densities of the two most abundant nematode genera on the nMDS plot (Fig. 5B), we observed differential spatial distributions, with *Sabatieria* being more abundant on sites distant from the outfall, and with *Pseudosteineria* being more abundant in intermediate and closer distances from the outfall.

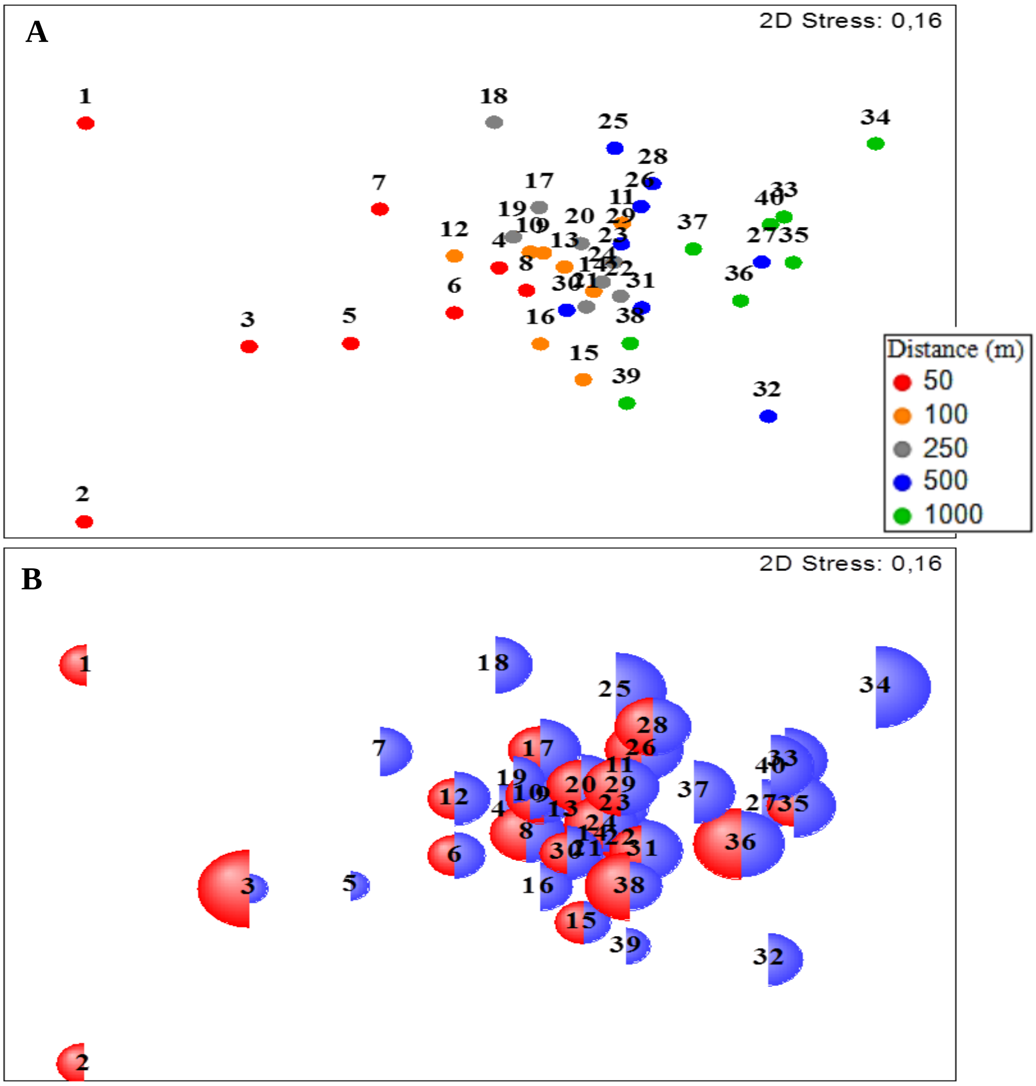

Fig. 5. nMDS ordinations for log-transformed nematode abundances among distances from the outfall (A) and superimposed abundances (inds.10cm^{-2}) for the two most abundant genera *Sabatieria* (blue) and *Pseudosteineria* (red).

The analyses of nematode feeding types showed that non-selective deposit feeders (48% of all nematodes) and epi-growth feeders (32%) were the most abundant trophic guilds. Selective deposit feeders accounted for 12.3% while predators/omnivores represented 7.3% of the nematode community. The ITD was relatively homogeneous across all sites (Fig. 6A), and with most values higher than 0.63. The lowest ITD value (0.12) was detected at site 34, 1000 m in the NE from the outfall. Different from the ITD, the spatial variation of MI, with values ranging from 2 to 3.1, was more noticeable (Fig. 6B). Overall, higher values of MI were detected up to 250 m from the outfall (2.66), decreasing towards the NE direction, where at 1000 m (site 34) was recorded the lowest value.

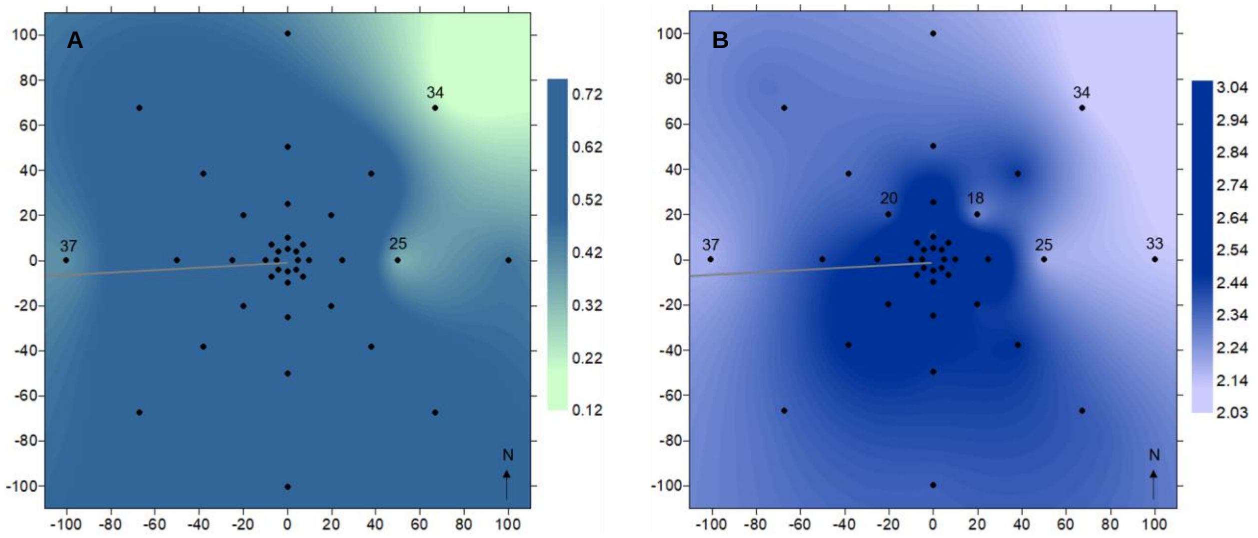

Fig. 6. Spatial variation of the (A) index of trophic diversity and (B) maturity around the Mar Grosso outfall.

3.4 Relationships between sterols, sedimentary organic matter and nematode biodiversity

The DistLM analysis detected significant correlations between nematode assemblages and most of the predictor variables, and the results of the BEST distance-based linear model (R^2=0.69) included all variables (Table 1). The dbRDA ordination (Fig. 7) showed that the first two axes explained 25.4% of the variability in the faunal dataset and 65.3% of the relationship between nematode genera and environmental variables. The first axis, represented by the distance from the outfall and responsible for 47.3% of the fitted model, showed that assemblages near the outfall (positive portion of the axis) were mainly related to higher concentrations of microphytobenthos pigments and sedimentary organic matter with higher contribution of the algal fractions. On the other hand, nematode assemblages at 1000 m (sites 33, 34 and 35; negative portion of the axis) were related to higher values of fine sediments (silt+clay), proteins, lipids, biopolymeric carbon concentrations and fecal steroids (coprostanol) (Fig. 7). The second axis, responsible for 18% of the fitted model, represented mostly the environmental variability, particularly the energy in the study area; nematode assemblages in the southern portion of the outfall related to well sorted sands (negative values of the axis), and those in the north of the outfall associated to presence of fine sediments.

Table 1
Distance-based linear model of nematode assemblages against environmental drivers around a marine outfall in Laguna, Southern Brazil. Proportion of variance in nematode assemblages explained by environmental variables in stepwise sequential tests following AICc selection criterion. AIC= 289.9; Best model included all variables R^2=0.69

Variable	SS (trace)	Pseudo-F	P	Var%
Calgal	6810.8	5.6658	**0.001**	12.975
CPheo	6677.8	5.5391	**0.001**	12.722
Fines	5076.1	4.0682	**0.002**	9.6706
Phaeo	4962.3	3.9676	**0.001**	9.4539
CChl-*a*	4884.1	3.8986	**0.001**	9.3049
CHO	4190.7	3.2971	**0.002**	7.9838
BPC	4118.1	3.2351	**0.014**	7.8456
LIP	3920.7	3.0675	**0.035**	7.4695
PRT	3675.2	2.861	**0.016**	7.0018
Coprostanol	2867.7	2.196	**0.046**	5.4633
Chl-*a*	2860.6	2.1903	**0.016**	5.4499
CHO:LIP	2291.3	1.7345	0.051	4.3652
Sorting	2084.7	1.5716	0.134	3.9716
PRT:CHO	1984.1	1.4928	0.128	3.78
Grain size	1882	1.4131	0.156	3.5854

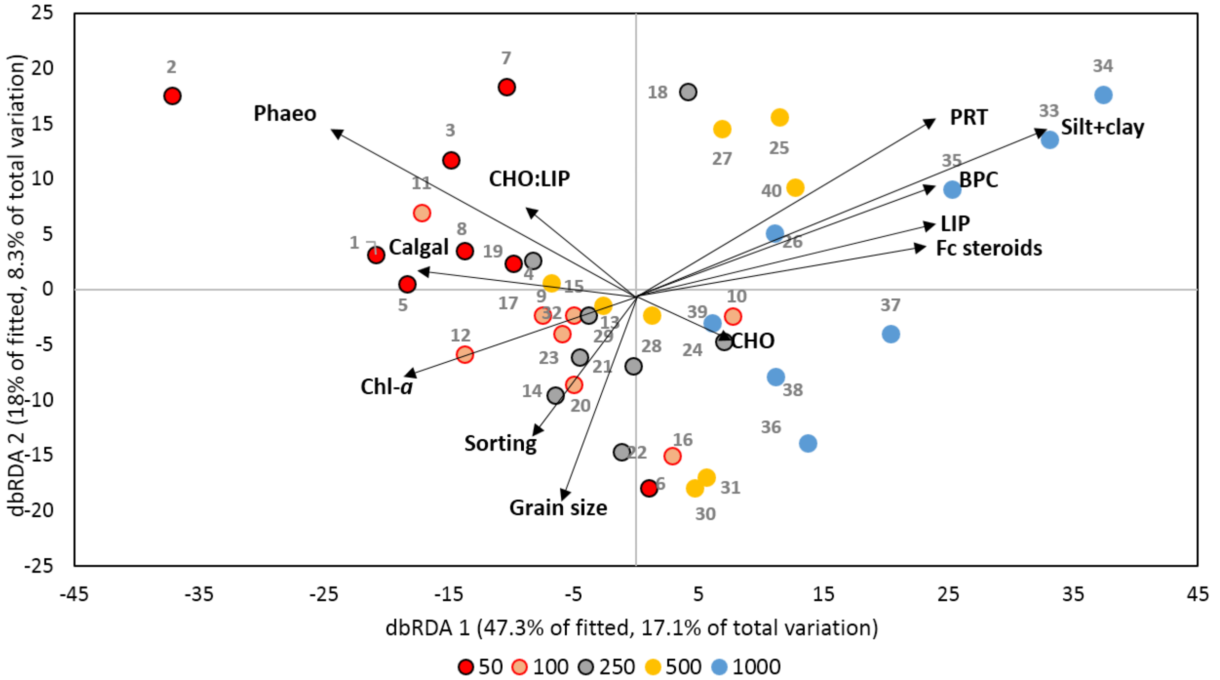

Fig 7. Distance-based RDA ordination of fecal sterols, biochemical descriptors of sedimentary organic matter, granulometry and nematodes.

4. Discussion

More than one-third of the world's population lives near coastal ecosystems (e.g. beaches, bays, estuaries). However, the great majority of coastal populations lack access to safe sanitation (WHO and UNICEF, 2017). Extensive work around the world has shown that disposing domestic wastewater through effective ocean outfalls is an economical and reliable strategy for wastewater disposal with minimal environmental effects (Roberts et al., 2010). Our results, using fecal sterols, biochemical composition and nutritional quality of organic matter, and marine nematodes as indicators, showed a relatively low impact in the surrounding areas of a marine outfall of low wastewater discharge in a high energy sandy shore. These results support Puente and Diaz (2015) contention that balance between the total organic mass discharged by the outfall and the energy of the adjacent environment are the key factors in determining the impacts on the benthic system.

Sedimentary sterols, particularly coprostanol, have successfully been used as a marker of fecal contamination, since this sterol is not natural in the marine environment and it is directly associated with fecal material (e.g., Cabral et al., 2018; Harrault et al., 2019). Coprostanol is formed from cholesterol bacterial transformation in the vertebrate guts and is the most abundant sterol in human feces (around 60% of the total sterols; Leeming et al., 1996; Takada and Eganhouse, 1998). Concentrations > 0.10 $\mu g.g^{-1}$ (Writer et al., 1995), > 0.50 $\mu g.g^{-1}$ (Gonzalez-Oreja and Saiz-Salinas, 1998) or >1 $\mu g.g^{-1}$ (Martins et al., 2007) are indicative of sewage contamination. Moreover, ratio coprostanol/(coprostanol+cholestanol) > 0.5 (Leeming et al., 1996) or > 0.7 (Bull et al., 2002), and coprostanol/(coprostanol+cholesterol) > 0.5 (Takada et al., 1994) are often used as complementary good to indicate sewage contamination. Our results showed sewage contamination only in the site 34 (coprostanol- 2.74 $\mu g.g^{-1}$) at 1000 m in the NE direction of the outfall, where the sterol ratios with cholestanol and cholesterol were 0.56 and 0.4, respectively. Coprostanol was also detected in four other sites, but in concentrations lower than the limit of quantification, at 250 m (site 19), 500 m (site 29), and 1000 m (site 33 and site 35) suggesting some influence of the outfall.

The highest concentrations of sedimentary sterols at 1000 m NE from the outfall indicated the influence of local hydrodynamic conditions in the transport and dispersion process of sewage. Coprostanol is preferentially adsorbed to the fine particles, both in suspension and in the sediments and their concentrations may be affected by the variation of sediment grain size (Bull et al., 2002; Cabral et al., 2018). During transport, the concentrations of coprostanol may decrease as the distance from the source of sewage increases (Abreu-Mota et al., 2014; Brauko et al., 2015; Speranza et al., 2018). Thus, primary source of sterols was also probably the primary source of silt and clay sized sediments and the observed differences in the biochemical composition and nutritional quality of sedimentary organic matter which distributions were likely to be modulated by tidal currents and waves.

In marine environments, the concentrations of proteins, carbohydrates and lipids are typically lower than in other aquatic ecosystems such as estuaries and rivers (Pusceddu et al., 2009, Misic and Harriague, 2013). In fact, in our study the concentrations of proteins, carbohydrates and lipids were generally low, with high values only detected at 1000 m NE (site 34) of the outfall. This result indicates a punctual accumulation of sewage within the study area, that also contributed to the prevalence of proteins in the sediments, resulting in higher proportions of PRT:CHO (Venturini et al., 2012), as observed in site 34 (PRT:CHO 2.4). In addition, higher lipid concentrations as observed in the site 34, are also associated with anthropogenic sources of oil and sewage (Cotano and Villate, 2006; Venturini et al., 2012). The PRT:CHO ratio was ≤ 1 in most of the other sites indicted that the study area may be classified as meso-oligotrophic, except for site 34 considered as eutrophic.

The biopolymeric fraction of sediment organic carbon, measured as the sum of protein, carbohydrate and lipid carbon (BPC) represents the labile fraction of organic matter available to benthic consumers through remineralization (Aguiar et al., 2013; Pusceddu et al., 2003, 2009). Only a fraction (5 to 30%) of these biopolymers is enzymatically digestible by consumers (Pusceddu et al., 2003). The BPC are highly sensitive to spatial and temporal changes in the benthic trophic status associated to both natural and human-induced environmental alterations (Bianchelli et al., 2018; Pusceddu et al., 2009; Hadlich et al., 2018). In our study, values of BPC were relatively homogeneous and low and ranged from 0.11 to 0.44 $mg.C.g^{-1}$, except for the site 34 (1.01 $mg.C.g^{-1}$). Similarly, algal contribution to BPC was higher near the outfall (indicating higher contribution autochthonous organic matter) and lower at 1000 m distances (higher contribution of the outfall-derived organic matter). These results support Pusceddu et al. (2009; 2010) contention that increasing BPC concentrations in the sediment might also prompt a progressive decrease on the percentage contribution of bioavailable carbon to BPC. This would indicate that the accumulation of organic carbon by the outfall (e.g., on site 34) affected the benthic trophic status and changed the relative importance of the labile and refractory fractions of sediment organic carbon.

The Mar Grosso (in Portuguese meaning "rough sea") is a high-energy dissipative sandy beach. The high variability of productivity in these environments, associated with sediment instability caused by the high hydrodynamic conditions, strongly influence the benthic associations. The structural and functional attributes of free-living nematodes showed different signals reflecting the main modulating factors in the area surrounding the outfall. In the nearest sites (within a 50 m radius), where no contamination from the outfall was detected, nematode communities were mainly distinguished by their low densities (one third of the total mean density) and the dominance of the genus *Pseudosteineria*, suggesting that local physical processes affected the fauna. Outfalls may change the seabed topography in their vicinity, producing depressions and resulting in waves deformation, turbulent flows and secondary currents (Shand et al., 2005). Moreover, nematode density near the outfall was positively correlated to phaeopigments, which concentrations is also known to increase to higher levels in sandy sediments subjected to a reduced stability (Widdows et al., 2004).

In the area affected by sewage contamination, a 1000 m NE of the outfall, the decrease in the bioavailable carbon to BPC and the change of benthic trophic state diminish the trophic diversity (6 times lower than total average) as a result of dominant opportunistic taxa. *Sabatieria* and *Theristus*, the most abundant genera in this area, are indicators of organic enrichment (Wilson and Kakouli-Duarte, 2009; and references therein). *Sabatieria* in particular, is known to migrate to spots of decaying organic matter (Gerlach, 1977; Heip, 1995) as nematodes in this genus may directly use refractory components and microbial community of the organic matter during feeding (Riemann and Hekmke, 2002).

In summary, this study showed a relatively low and limited spatial scale impact from an outfall of low input of wastewater in a high-energy sandy shore. While the physical disturbance prevails near the outfall, affecting the densities of the fauna and phytopigments, the sewage contamination, at 1000 m, changed the benthic trophic state and lead to a strong decrease in the trophic diversity and increase of opportunistic nematodes. Finally, our integrative approach (i.e., use of fecal sterols, benthic trophic state and meiofauna biodiversity) shown to be highly effective in detecting both the physical disturbance and the sewage contamination of a small outfall in a high-energy sandy shore.

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Appendix A. Supplementary data

The following are the Supplementary data to this article:

Cândido and Netto.doc

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Multiple benthic indicators suggest low sewage impact from an ocean outfall in a high-energy sandy shore (South Brazil)

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Supplementary material

Table S.1
Mean values (of the three replicates) of the biochemical variables for the sediments of Mar Grosso beach. PRT= total proteins; CHO= total carbohydrates; LIP= total lipids; PRT:CHO= proteins to carbohydrates ratio; CHO:LIP= carbohydrates to lipids ratio; BPC= biopolymeric carbon; CChl-a= chlorophyll-a carbon contribution to BPC; Cphaeo= phaeopigments carbon contribution to BPC; C algal= total algal carbon contribution to BPC.

Site	Copro stanol $ug.g^{-1}$	Chole sterol $ug.g^{-1}$	Chole stanol $ug.g^{-1}$	Chl-a $ug.g^{-1}$	Phaeo $ug.g^{-1}$	PRT $mg.g^{-1}$	CHO $mg.g^{-1}$	LIP $mg.g^{-1}$	PRO: CHO -	CHO: LIP -	BPC $mg.C.g^{-1}$	CChl-a (%)	CPhaeo (%)	C Algal (%)
1	ND	0,14	ND	0,71	0,89	0,17	0,15	0,10	1,18	1,54	0,21	9,99	12,39	22,39
2	ND	0,05	ND	0,91	2,07	0,18	0,14	0,07	1,28	2,09	0,19	14,29	32,38	46,67
3	ND	0,10	ND	0,51	0,92	0,19	0,16	0,06	1,20	2,45	0,20	7,62	13,71	21,32
4	ND	0,29	ND	0,67	0,94	0,21	0,23	0,09	0,92	2,53	0,26	7,66	10,80	18,46
5	ND	ND	ND	0,27	0,39	0,14	0,09	0,05	1,65	1,78	0,14	5,70	8,27	13,97
6	ND	0,45	ND	0,21	0,23	0,11	0,12	0,07	0,93	1,71	0,16	3,94	4,33	8,27
7	ND	0,09	ND	0,35	0,48	0,14	0,13	0,03	1,05	4,24	0,14	7,27	10,04	17,31
8	ND	0,05	ND	0,28	0,26	0,13	0,13	0,04	0,97	3,41	0,15	5,73	5,30	11,03
9	ND	0,09	ND	0,52	0,57	0,14	0,14	0,09	0,96	1,65	0,19	8,11	8,92	17,03
10	ND	ND	ND	0,50	0,25	0,16	0,17	0,08	0,98	2,17	0,21	7,21	3,61	10,82
11	ND	0,12	ND	1,03	1,30	0,25	0,20	0,08	1,20	2,49	0,26	11,73	14,79	26,52
12	ND	0,11	ND	0,81	0,64	0,16	0,19	0,07	0,86	2,69	0,21	11,60	9,07	20,66
13	ND	0,08	ND	0,22	0,24	0,12	0,10	0,05	1,17	1,90	0,14	4,62	5,08	9,70
14	ND	ND	ND	0,39	0,21	0,12	0,10	0,08	1,21	1,25	0,16	7,34	3,96	11,30
15	ND	ND	ND	0,50	0,40	0,15	0,15	0,07	1,00	2,12	0,19	7,87	6,30	14,17
16	ND	0,03	ND	0,50	0,15	0,13	0,15	0,08	0,86	1,98	0,18	8,27	2,48	10,75
17	ND	0,04	ND	1,12	0,71	0,29	0,24	0,11	1,21	2,20	0,32	10,58	6,70	17,27
18	ND	ND	ND	0,89	0,77	0,31	0,21	0,10	1,49	2,13	0,31	8,70	7,54	16,23
19	<LOQ	0,44	<LOQ	1,16	0,82	0,30	0,23	0,11	1,30	2,04	0,32	10,82	7,64	18,47
20	ND	0,03	ND	0,42	0,17	0,12	0,11	0,08	1,06	1,33	0,16	7,70	3,08	10,79
21	ND	0,06	ND	0,37	0,20	0,13	0,12	0,09	1,05	1,41	0,18	6,25	3,38	9,63
22	ND	0,05	ND	0,43	0,17	0,10	0,09	0,08	1,08	1,20	0,14	8,99	3,59	12,58
23	ND	ND	ND	0,28	0,30	0,09	0,09	0,09	1,03	0,95	0,15	5,56	6,12	11,68
24	ND	ND	ND	0,54	0,32	0,19	0,17	0,06	1,12	2,71	0,21	7,68	4,61	12,29
25	ND	0,09	ND	0,81	0,95	0,38	0,26	0,09	1,47	2,74	0,36	6,77	7,88	14,65
26	ND	0,19	ND	0,73	0,85	0,23	0,22	0,08	1,06	2,67	0,26	8,34	9,82	18,15
27	ND	1,00	0,13	0,50	0,95	0,24	0,18	0,09	1,35	2,03	0,25	5,85	11,21	17,06
28	ND	0,04	ND	0,54	0,48	0,14	0,16	0,07	0,85	2,51	0,18	8,74	7,86	16,60
29	<LOQ	0,69	<LOQ	0,15	0,31	0,10	0,09	0,07	1,20	1,28	0,14	3,18	6,84	10,02
30	ND	0,36	ND	0,38	0,00	0,09	0,09	0,05	0,93	1,84	0,12	9,58	0,00	9,58

Site	Copro stanol ug.g-1	Chole sterol ug.g-1	Chole stanol ug.g-1	Chl-a ug.g-1	Phaeo ug.g-1	PRT mg.g-1	CHO mg.g-1	LIP mg.g-1	PRO: CHO -	CHO: LIP -	BPC mg.C.g-1	CChl-a (%)	CPhaeo (%)	C Algal (%)
31	ND	0,22	ND	0,29	0,07	0,08	0,09	0,06	0,80	1,59	0,12	7,37	1,66	9,02
32	ND	0,25	ND	0,65	0,21	0,17	0,16	0,04	1,09	3,92	0,18	11,14	3,59	14,73
33	<LOQ	0,59	<LOQ	0,41	0,05	0,26	0,27	0,23	0,94	1,18	0,41	2,97	0,36	3,33
34	2,74	4,05	2,13	0,81	0,47	0,83	0,33	0,64	2,48	0,52	1,02	2,40	1,38	3,78
35	<LOQ	1,12	<LOQ	0,24	0,15	0,22	0,21	0,09	1,03	2,29	0,26	2,74	1,74	4,48
36	ND	0,72	ND	0,15	0,01	0,13	0,12	0,06	1,04	1,87	0,16	2,81	0,14	2,95
37	ND	0,69	<LOQ	0,15	0,06	0,19	0,18	0,09	1,09	2,00	0,23	1,96	0,78	2,74
38	ND	0,22	ND	0,07	0,03	0,09	0,12	0,08	0,78	1,53	0,15	1,40	0,56	1,96
39	ND	0,39	ND	0,07	0,00	0,08	0,08	0,10	0,99	0,73	0,15	1,45	0,00	1,45
40	ND	0,48	<LOQ	0,16	0,17	0,19	0,22	0,14	0,86	1,51	0,29	1,64	1,80	3,44

Coprostanol and Cholestanol: LOD (limit detection) = 0,075 ug.g-1 LOQ (limit quantification) = 0,25 ug.g-1; Cholesterol: LOD = 0,0075 ug.g-1 LOQ = 0,025 ug.g-1; ND= lower than the detection limit.

Table S.2

Mean values of the fauna descriptors. N= inds.10cm²; S= richness; ES51= diversity; ITD= index of trophic diversity; MI= maturity index.

Site	Direction	Distance (m)	N	S	ES51	ITD	MI
1	East	50	24,68	14	14,00	0,69	2,81
2	Northeast	50	10,35	7	7,00	0,70	3,15
3	North	50	35,83	16	16,00	0,56	2,49
4	Northwest	50	82,80	23	17,75	0,66	2,83
5	West	50	50,16	20	18,14	0,66	2,81
6	Southwest	50	81,21	27	20,18	0,65	2,76
7	South	50	44,59	20	19,60	0,71	2,76
8	Southeast	50	126,59	28	18,08	0,62	2,50
9	East	100	141,72	25	16,97	0,65	2,62
10	Northeast	100	119,43	25	17,88	0,73	2,81
11	North	100	177,55	21	16,26	0,64	2,37
12	Northwest	100	80,41	20	15,90	0,67	2,72
13	West	100	143,31	28	20,58	0,70	2,57
14	Southwest	100	107,48	29	21,40	0,70	2,68
15	South	100	130,57	26	18,44	0,71	2,68
16	Southeast	100	93,15	24	18,31	0,68	2,84
17	East	250	149,68	27	18,71	0,72	2,72
18	Northeast	250	54,94	21	18,92	0,69	2,27
19	North	250	75,64	26	20,69	0,65	2,63
20	Northwest	250	109,87	26	19,57	0,64	2,39
21	West	250	132,96	28	18,70	0,69	2,53
22	Southwest	250	89,17	28	21,54	0,63	2,61
23	South	250	99,52	30	22,84	0,67	2,55
24	Southeast	250	76,43	23	18,73	0,58	2,44
25	East	500	147,29	17	9,47	0,34	2,18
26	Northeast	500	136,94	30	20,64	0,61	2,45
27	North	500	56,53	22	20,26	0,58	2,34
28	Northwest	500	84,39	24	18,13	0,65	2,33

Site	Direction	Distance (m)	N	S	ES51	ITD	MI
29	West	500	83,60	26	20,54	0,63	2,42
30	Southwest	500	82,01	23	18,67	0,63	2,49
31	South	500	195,06	23	16,91	0,59	2,45
32	Southeast	500	31,05	13	13,00	0,62	2,47
33	East	1000	122,61	18	13,48	0,46	2,13
34	Northeast	1000	371,82	14	7,16	0,13	2,05
35	North	1000	74,04	18	14,09	0,44	2,24
36	Northwest	1000	258,76	28	18,12	0,52	2,31
37	West	1000	204,62	20	14,02	0,40	2,18
38	Southwest	1000	87,58	29	21,52	0,51	2,32
39	South	1000	50,96	21	19,34	0,58	2,36
40	Southeast	1000	103,50	20	14,51	0,53	2,36