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Article

Domestic Sewage Outfall Severely Altered Environmental Conditions, Foraminiferal Communities, and Ecological Quality Statuses in Front of the Nearshore Beach of Cigarras (SE Brazil)

Luciana Saraiva Filippos 1,2, Wâ**nia Duleba ³ , Johann Hohenegger ⁴ , Leonardo Ant**ô**nio Pregnolato [3](https://orcid.org/0000-0002-4140-9326) , Vincent M. P. Bouchet 5,[*](https://orcid.org/0000-0001-5458-1638) and Maria Virginia Alves Martins 6,7**

- 1 Instituto de Geociências, Universidade de São Paulo (USP), Rua do Lago, 562—Butantã, São Paulo 05508‑080, SP, Brazil
- ² Laboratório de Química Orgânica Marinha, Instituto Oceanográfico, Universidade de São Paulo, Praça do Oceanográfico 191, São Paulo 05508‑120, SP, Brazil
- ³ Escola de Artes, Ciências e Humanidades, Universidade de São Paulo, São Paulo 05508‑120, SP, Brazil
- 4 Institut für Paläontologie, Universität Wien, Althanstrasse 17, A 1090 Wien, Austria
- ⁵ Laboratoire d'Océanologie et de Géosciences, Univ. Lille, CNRS, Univ. Littoral Côte d'Opale, IRD, UMR8187, LOG, Station Marine de Wimereux, F 59000 Lille, France
- ⁶ Departamento de Estratigrafia e Paleontologia, Faculdade de Geologia, Universidade do Estado do Rio de Janeiro, Av. São Francisco Xavier, 524, Sala 2020A, Maracanã, Rio de Janeiro 20550‑013, RJ, Brazil
- ⁷ Departamento de Geociências, Campus de Santiago, Universidade de Aveiro, GeoBioTec, 3810‑193 Aveiro, Portugal
- ***** Correspondence: vincent.bouchet@univ‑lille.fr

Abstract: This study aims to analyses the response of meiofaunal organisms (foraminifera) to dis‑ turbances caused by the diffusers of domestic sewage outfall at Cigarras beach, SE Brazil. Hydro‑ graphical, sedimentological (grain size and geochemical), and living benthic foraminiferal recorded in 2006 and 2007 analyzed in ten stations were compared with the same results analyzed in two control/reference stations (sampled in 2008). The results of this work show that, in the benthic environment of the Cigarras region, moderated hydrodynamic conditions, relatively high total organic carbon, total nitrogen, total sulfur contents, oxic water column and anoxic sediments, organic matter supplied by marine productivity and from mixed sources prevail. Living foraminiferal assemblages denote that the Cigarras region is undergoing environmental degradation due to progressive organic enrichment directly influenced by the domestic sewage outfall. The effluents discharged by the domestic sewage constrained the composition of foraminiferal communities (which include mainly stress tolerant species) with probable impacts on the entire marine trophic chain. Noticeably, the tolerant species *Ammonia tepida*, *Bolivina striatula* and *Buliminella elegantissima* dominated at the stations under the influence of the sewage outfall. In addition, *Ammonia parkinsoniana* was found in moderate abundances, and the moderate level of TOC enrichment by the sewage outfall did not prevent the survival of this sensitive species. The ecological quality status inferred from the diversity index $Exp(H'_{bc})$ calculated on foraminifera showed the poor ecological status of benthic habitats in the area. Overall, this work highlighted the adverse effects of the sewage outfall on the benthic ecosystem in front of the Cigarras beach in Brazil. Future works should investigate the current ecological quality of the area to figure out if any change occurred since the present study sampling.

Keywords: sewage pollution; biomonitoring; benthic foraminifera; biotic index; diversity

1. Introduction

Many coastal areas experience high population densities, especially in summer, which induces in the discharge of large amounts of nutrients and pollutants into the seawater [1]. The dumping of urban wastewater into the ocean has been considered a safe way to remove

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many contaminants from the coastal region due to the high dispersion capacity of pollutants by currents. However, this procedure has been causing the pollution of the oceans and produces negative effects on these natural ecosystems. However, in many regions of the world, sewage submarine outfalls combined with water treatments prior to the release in coastal areas are being used [2,3], mitigating the damage caused on the quality of the marine environment. Therefore, it is essential to study the costs and benefit and possible impacts before installing a submarine outfall [4]. Notably, if not well planned, the marine disposal can contribute to the degradation of marine environments and cause negative im‑ pacts, such as alterations in the local fauna [4], eutrophication [5], contamination of water, sediments and organisms by chemical compounds [6,7], and may, lastly, also affect human health [8–10]. Furthermore, dispersion may be limited in sheltered transitional waters [11], leading to the accumulation of sewage water in shallow coastal areas.

In SE Brazil, the São Paulo State has a coastal population of over two million people that can be doubled during the summer season [12]. Most Brazilian coastal towns have a poor sewage collection system and wastewater treatment plant [6,13]. Therefore, some coastal municipalities use submarine outfalls to disperse sewage water. In the São Sebastião channel, for example, there are three submarine outfalls located on the continental side, with Cigarras and Araçá being employed for domestic disposal and Tebar being used as an oil terminal [14]. Specifically, the Cigarras beach outfall (23*◦*43*′*5 *′′* S–45*◦*24*′*1 *′′* W) is close to the northern entrance of the São Sebastião Channel (CCS). The area is a shallow coastal environment with a maximum depth of 10 m $[15]$ and a tendency for fine-grained sediment accumulation [16]. However, no studies have considered the potential effect of the Cigarras outfall on benthic habitats.

To assess the negative impact of pollutants on the environment, many scientists have recognized the importance of using the response of living benthic organisms [17,18]. For example, benthic foraminifera are among the most important benthic organisms that can be used as bioindicators to assess anthropogenic effects on the environment [19–21], particularly in the case of water sewage [22–24]. Benthic foraminifera are abundant [25] and an important component of modern benthic communities, representing up to 50% of the benthic eukaryotic biomass [26], being a key link between microalgae and bacteria to the higher trophic levels [27,28] and playing a key role in bioturbation processes in soft-bottom sediments [29,30]. These organisms are sensitive to either natural or anthropogenic impacts (see review [31]), which constrain the assemblages composition, altering the abundance and diversity of organisms [22,32,33], leading to the formation of test anomalies [19,34,35], and favoring the development of opportunistic species due to their tolerance to pollutants and adverse environmental conditions [20,36,37]. Hence, foraminifera have been used to evaluate environmental impacts as sewage outfall [20,23,38,39], heavy metal [35,40], aquaculture [41,42], and petroleum hydrocarbon [43,44]. This led to the development of biotic indices based on foraminifera to be implemented in studies evaluating the health of benthic habitats [18,45–49], such as the $Exp(H'_{bc})$ index based on the diversity of living benthic foraminifera [18], or the TSI-med and Foram-AMBI based on the species sensitivity to pollution [45,47].

In this context, the present study intends to analyze the response of benthic foraminifera to environmental disturbance in the area near the domestic sewage outfall of Cigarras beach, located in CCS and under the influence of a sewage submarine outfall (Figure 1; São Paulo State, SE Brazil). Results of hydrographic and sedimentological (grain size and geochemical) and living benthic foraminiferal data, acquired during two consecutive years (2006 and 2007), were statistically compared. In these comparisons, the results obtained in two control stations located in São Sebastião Channel, in the same region (Figure 1; São Paulo State, SE Brazil), were also taken into account. In addition, the ecological quality status (EQS) was, for the first time, evaluated in the sewage outfall of Cigarras beach, in both years, using the index $Exp(H'_{bc})$ according to Bouchet et al. [18].

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Figure 1. Study area and the analyzed station's location near Cigarras Beach. The position of the control stations in São Sebastião Channel (CTL1 and CTL2) is also presented. Legend: S.S.O.—sewage age submarine outfall. submarine outfall.

2. Study Area

2. Study Area São Sebastião city is located in the northeast coast of São Paulo State (SE, Brazil). The 2004). São Sebastião Channel (CCS) separates the continent and the Ilha Bela Island (Figure 1). The CCS channel is about 25 km long and variable in width, 2 km in the central area to 6–7 km at the north and south entrances [50] (Figure 1). The water depth is about
as a separate in the Indian of the Indian of the Indian Island (Figure 1). The water depth is about economy of the region is based on seaport, petroleum, and tourism activities (CETESB, 20–25 m at the inlets and about 40 m along the channel axis [51].

 $25-25$ in at the finers and about 40 in along the enannel axis $[31]$.
Sediment distribution in the CCS is heterogeneous due to the irregular bottom topography and local hydrodynamic conditions [51]. Sediment deposition occurs in the continental portion, while erosion usually occurs in the near-island region [51]. Although coastal water (CW) is the primary water mass in the CCS, the channel water is a mixture of CW , Sauth, Atlantic control water (CACM), and transical water (TM) ISM. Device a very see or late spring, the SACW flows into the channel, while the TW tends to flow mainly in the t_1 autumn [52]. CW, South Atlantic central water (SACW), and tropical water (TW) [52]. During summer autumn [52].

This area has currents that move northeastward with a velocity of 0.2 m/s [15], but more often, currents continuously change in direction and intensity. This submarine outfall has been in operation since 1985 [14]. The pipeline is 1090 m long [53] with an internal diameter of 16 cm. During the low season, 1.5 l/s of domestic sewage are disposed of, with a maximum of 11.6 l/s in the high season [54]. Before the effluent is discharged into the ocean, a pre‑treatment is performed, consisting only of sieving and chlorination [54].

3. Materials and Methods

Ten sites distributed in a growing circle grid around the end of the Cigarras outfall (State of São Paulo, SE Brazil) were sampled in September 2006 and 2007. A total of twenty bottom surface sediment samples were collected at both sampling events for textural, geochemical, and meiofaunal (foraminifera) analyses. Two other samples were collected away from the Cigarras outfall during a cruise realized in 2008 in the São Sebastian Channel (Figure 1). There are situated in a similar environmental setting as stations under the in‑ fluence of the sewage outfall. The geographic positions of the sampling stations were determined using the global positioning system (GPS), with the UTM SAT 69 datum (Table S1). The stations around the Cigarras Beach pipeline are identified throughout the text as "Cig", followed by their respective number (e.g., Cig1-Cig10), and the control stations located in the São Sebastião Channel were referenced as CTL1 and CTL2.

In each station, a CTD Seacat was used to assess hydrographic data (water depth, temperature, salinity, pH, and dissolved oxygen) (Table S1). A modified stainless-steel Petersen grab sampler, with an upper opening, was used to collect sediments for sedimentological and foraminifera analyses. Only the uppermost (0–2 cm) undisturbed bottom, sediments were collected for foraminifera samples (*≈*500 mL per station). Mixtures of black (anoxic) and brown (oxic) sediments were avoided. The sediment samples were immediately preserved with alcohol 70*◦* and stained with Rose Bengal (1.5–2 g l*−*¹) to distinguish stained (living) from unstained (dead) foraminifera [55,56].

3.1. Grain Size and Geochemical Analyses

Grain size analysis was performed using standard sieve and pipette methods [57]. Textural typology was determined with Wentworth's classification [58], and sediments were described as proposed by Shepard [59]. Calcium carbonate content was evaluated by difference in weight of dry sediment after acid dissolution [60]; carbonate contents were clas‑ sified according to Larsonneur [61]. Total organic carbon (TOC) and total nitrogen (N) analyses were performed by LECO[®] CHN-1000 analyzer. For total sulfur (S), a LECO[®] SC‑432 equipment was used. For TOC evaluation, the sediment samples were previously submitted to acid treatment with 10% HCl. The C/N and C/S ratios were estimated to discriminate the origin of the organic matter and the redox conditions of the sediments.

3.2. Foraminiferal Analysis

In the laboratory, sediment aliquots were washed on a 63 μ m mesh sieve [62] and dried in an oven at 50 *◦*C. After that, foraminifera were separated from the sediment frac‑ tion >63 μ m by flotation in trichloroethylene (CCl₄) [63]. For living foraminifera studies, successive aliquots of 10 cm³ were analyzed $[23,39]$ until at least 100 specimens were obtained [64]. The picked living specimens were identified with Zeiss Stemi SV6 Stereo Microscope and mounted on microslides. Taxonomic identification was based on, for example, Cushman [65–68], Loeblich, Tappan [69,70], and Boltovskoy et al. [71]. The species name followed the World Register of Marine Species—WoRMS [72].

3.3. Statistical Analysis

Species richness in sampling stations was defined by the 'Chao‑1*′* index, allowing an approximation of the universal number of species to be presented at the station [73]. Heterogeneity was evaluated using the 'Shannon Index' [74] and 'Evenness' [75].

To obtain comparable frequency data necessary for linear statistical analyses, densi‑ ties of 10 cm³ sampling quadrats (= confined investigation areas [76]; standing crop [77]) were recalculated from the original data given in percentages by:

〖density 〗_ij = 〖percentage 〗_ij/100*·* 〖frequency 〗_(j, 〖10 cm 〗^3)

where i indicates the species and j the sampling station.

Ecological quality status were determined with the diversity index $Exp(H'_{bc})$ based on living benthic foraminifera diversity [18]. The criteria for transitional areas were retained for the present study, i.e., 0–3: bad, 3–7: poor, 7–11: moderate, 11–15: good, >15: high [78].

For the species community data, correspondence analysis (CA) was applied to the foraminiferal data from 2006 and 2007. Community data (relative abundances) were $log(x + 1)$ transformed prior to analysis. Procrustes analysis [79] was used to compare unconstrained ordinations of the foraminiferal (CA) community data from 2006 and 2007.

Correlation between the main species relative abundances (>4%) and environmental parameters in the sediment was investigated using Kendall's coefficient of rank correlation (*τ*). Kendall's coefficient of correlation was used in preference to Spearman's coefficient of correlation (*ρ*) because Spearman's *ρ* gives greater weight to pairs of ranks that are further apart, while Kendall's *τ* weights each disagreement in rank equally [80].

4. Results

4.1. Hydrographic Data, Grain Size and Geochemical Analysis

Geographical positions, hydrographic data, grain size, and geochemical results are presented in Tables S1 and S2, both for 2006 and 2007 sampling events and for control stations. The characteristics of the analyzed variables are summarized in the following items.

4.1.1. Water Column

During the sampling event of 2006, the mean values of temperature and salinity in the water column were, respectively: 22.18 *◦*C/33.69 at the surface; 22.24 *◦*C/33.64 in the middle; and 22.23 *◦*C/33.64 near the bottom. The water turbidity oscillated from 16 NTU (at the surface) to 63 NTU (near the bottom). The water column of most stations exhibited oxygen concentrations <6 mg l*−*¹ and pH values from 8.11 to 8.18 (mean 8.13).

In 2007, the highest temperatures were recorded at the surface, and a decreasing trend with depth was observed, as in in 2006 (Table S1). The salinity followed an opposed pattern (Table S1). The recorded averaged values of temperature and salinity were similar to 2006, respectively: 21.4 *◦*C/31.51 at the surface; 20.92 *◦*C/31.82 in the middle water column; and 20.65 *◦*C/31.92 near the bottom (Table S1). The water turbidity varied from 1.2 NTU (at the surface) to 25 NTU (near the bottom) (Table S1). Better oxygen concentrations were observed in 2007 (>7.2 mg l*−*¹) in the water column in all the stations and pH values varied from 7.75 to 8.21 (mean 8.06) (Table S1).

4.1.2. Sediment Parameters

Sediment mean grain size (SMGS) was similar in 2006 and 2007. According to the gran‑ ulometric analyzes, the studied stations are composed by silty sand or sand silty sediments (Figure 2). In the sampling network around Cigarras outfall, there is a predominance of silt fraction, while in the control stations, very fine sand fraction predominates (Figure S1).

Figure 2. Grain size distribution of sediment samples near the Cigarras submarine outfall. Legend: **Figure 2.** Grain size distribution of sediment samples near the Cigarras submarine outfall. Legend: S.S.O.—sewage submarine outfall. The numbers inside the rectangle represent the stations and the S.S.O.—sewage submarine outfall. The numbers inside the rectangle represent the stations and the numbers in the graphics are the percentage of granules, sand, and mud fractions. numbers in the graphics are the percentage of granules, sand, and mud fractions.

Carbonate contents were similar during both years with the lowest values observed C/N were similar in 2006 and 2007. The lowest values of TOC, N, S, C/S, and C/N were found near the sewage outfall and the highest values in the north, west, and east directions of the outfall (Figures S2 and S3). at Cig4 (about 11%) and the highest at Cig10 (about 23%). Patterns of TOC, S, N, C/S, and

4.1.3. Control Stations Environmental Features and Comparison to the Impacted Stations

The average values recorded for temperature and salinity were, respectively: 23.3 °C/ 36.70 at the surface; 23.2 °C/ 36.85 in the middle water column; and 22.5 °C/36.95 near the bottom. In station CTL1, lower values of salinity and turbidity were recorded than in CTL2 (Table S1). The water turbidity oscillated from 2 NTU (at the surface) to 2.8 NTU (near the surface) of $\frac{1}{2}$. Bottom). The station CTLI presented oxygen contentiations so mg/r in the middle and hear the bottom of the water column. The oxygen contents were higher in all depths of the water column in the station CTL2. The pH values oscillated from $\frac{1}{2}$, $\frac{1}{2}$ to 8.08 (mean 8.04) in both stations (Table S1). According to the Shepard classification, sand bottom). The station CTL1 presented oxygen concentrations <6 mg/l in the middle and

Sediment mean grain size was 4.47 Φ in CTL1 and 4.10 Φ in CTL2 (Table S2). The presence of very poorly sorted sediments was found in both stations: 1.70 σ in CTL1; and
1.56 give CTL2 (Table S2), According to the Shapend (1954) sodiment classification, and aily and sand were found in CTL1 and CTL2 stations, respectively (Figure S1). The CLT1 pre-In CTL1 and CTL2, CaCO3 contents were 11.97% and 8.02% (Table S2). Values of 1.56 σ in CTL2 (Table S2). According to the Shepard (1954) sediment classification, sand silt

sented intermediate SMGS, and percentage of mud and TOC similar to the other stations was sampled in 2006 and 2007 (Table S2).

In CTL1 and CTL2, CaCO3 contents were 11.97% and 8.02% (Table S2). Values of TOC, S, N, C/S, and C/N acquired in 2008, in the control stations, are presented in Figure S4 and Table S2. TOC and S contents reached higher values in CTL1 (1.18% and 0.23, respectively) than in CTL2 (0.58% and 0.16%, respectively). A slightly higher N content was recorded in CTL2 (0.17%) than in CTL1 (0.13%). The C/N and C/S were respectively: 9.04 and 5.11 in CTL1; and 3.48 and 3.59 in CTL2. The control stations had a lower percentage of S, N, and TOC compared to the impacted stations (Figure 3).

Figure 3. Comparison of sediment mean grain size (SMGS), mud (fine) sediment content and TOC, in the cigarras near the Cigarras ne S, N, C/S, and C/N values in 2006 (blue) and 2007 (green) in the analyzed stations near the Cigarras submarine outfall and in 2008 (red, see TOC figure for details) in the control stations from São Sebastião Channel.

4.2. Living Foraminiferal Assemblages

4.2.1. Comparison between Foraminiferal Communities in 2006, 2007 and at Control Stations

The number of species at the stations varied over the sampled periods (Tables S3 and S4). The number of species varied in 2006 between nine and 18 (mean = 9.7) and in 2007 between 11 and 23 species (mean = 11.3). The control stations (sampled in 2008) presented 20 species. Considering all stations and sampling years (Table S3), the following values were recorded (Table S4): density (n[°] per 10 cm³), ranging between 65–250; species richness (Chao‑1) between 9–60; Shannon index (H'; diversity) between 1.0 and 2.1; and evenness (Pielou) between 0.2–0.5. The living foraminiferal assemblages were dominated by two species (Figure S5) at all sampling stations, which is expressed by the low "evenness" measures at all stations (Tables S3 and S4).

The number of species at the stations varied over the sampled periods (Tables S3 and S4). The number of species varied in 2006 between nine and 18 (mean = 9.7) and in 2007 between 11 and 23 species (mean = 11.3). The control stations (sam‑ pled in 2008) presented 20 species. Considering all stations and sampling years (Table S3), the following values were recorded (Table S4): density (n[°] per 10 cm³), ranging between 65–250; species richness (Chao‑1) between 9–60; Shannon index (H'; diversity) between 1.0 and 2.1; and evenness (Pielou) between 0.2–0.5. The living foraminiferal assemblages were dominated by two species (Figure S5) at all sampling stations, which is expressed by the low "evenness" measures at all stations (Tables S3 and S4).

Regarding the species composition (Figure 4), *Ammonia tepida* (47–76% in 2006, 44–54% in 2007), followed by *Ammonia parkinsoniana* (9–33% in 2006, 21–35% in 2007), dom‑ inated in 2006 and 2007 and also at the control stations (control stations; Figures 4 and S5). The presence of *Bolivina striatula* (0.7–10.2%) was observed in 2006 and 2007 and only in 2006 for *Buliminella elegantissima* (0–4.6%). At control station 2 in 2008, *Pseudononion japon‑ icum* (5.8%) and *Pararotalia cananeianensis* (3.8%) also followed the two dominant species (Figure 4).

Foraminiferal communities from 2006 and 2007 were significantly correlated (Pro‑ crustes analysis, *p* < 0.001).

4.2.2. Main Species Relative Abundance Correlation with Environmental Parameters

Ammonia parkinsoniana was significantly positively correlated with SMGS (*p* < 0.05), silt, TOC, S, and C:N ratio $(p < 0.01)$ in 2006 (Table 1, Figure S6) and negatively with sand (*p* < 0.05) in 2007 (Table 1, Figure S7). In 2006, *Ammonia* sp. was significantly positively correlated with C:S ratio (*p* < 0.001) in 2006 (Table 1). *Ammonia tepida* did not correlate with environmental parameters.

Table 1. Correlation between the main species relative abundances (>4%) and environmental parameters in the sediment (Kendall's coefficient of rank correlation, *τ*). Significant correlations are highlighted in bold (*: *p* < 0.05, **: *p* < 0.01 and ***: *p* < 0.001).

2006	SMGS	Sand	Silt	Clav	TOC	N	S	C: N	C: S
Ammonia parkinsoniana	$0.54*$	$-0.56*$	$0.60*$	-0.31	$0.69**$	-0.045	$0.67**$	$0.64**$	0.38
Ammonia sp.	0.18	-0.16	0.2	-0.31	0.38	-0.09	0.22	0.33	$0.78***$
Ammonia tepida	-0.22	0.24	-0.29	0.22	-0.38	0.36	-0.31	-0.42	-0.16
Bolivina striatula	$-0.63 *$	$0.64**$	$-0.69**$	0.45	$-0.51*$	-0.4	$-0.54*$	-0.2	-0.2
Buliminella elegantissima	-0.22	0.24	-0.29	0.36	-0.2	0.18	-0.27	-0.33	-0.16
2007									
Ammonia parkinsoniana	-0.11	0.067	-0.11	0.29	-0.067	-0.4	-0.067	0.29	-0.24
Ammonia tepida	-0.39	0.33	-0.38	-0.16	0.29	θ	-0.27	0.47	0.47
Bolivina striatula	-0.16	0.24	-0.2	-0.16	-0.33	θ	-0.31	$-0.60*$	-0.16
Buliminella elegantissima	$0.75**$	$-0.78**$	$0.60*$	0.14	0.32	0.44	$0.77**$	0.18	0.14

Figure 4. Heatmap showing the spatial and temporal distribution patterns of the main species (relative abundances >4%).

The species *Bolivina striatula* significantly correlated sand ($p < 0.01$) and negatively with SMGS, silt, TOC and S ($p < 0.05$) in 2006 (Figure S6). In 2007, it correlated negatively with silt ($p < 0.05$), SMGS and S ($p < 0.01$) and negatively with sand ($p < 0.05$, Table 1). with C:N ratio (*p* < 0.05, Table 1). *Buliminella elegantissima* significantly correlated in 2007

Ammonia parkinsoniana was significantly positively correlated with SMGS (*p* < 0.05), silt, TOC, S, and C:N ratio (*p* < 0.01) in 2006 (Table 1, Figure S6) and negatively with sand 4.2.3. Ecological Quality Status

The index $\exp(H'_{bc})$ varies between 3.3 and 7.4 at the stations near the sewage water outfall (Figure 5). Most stations were classified as having a poor EcoQS. An increase in the index was observed from 2006 to 2007 at all stations except stations 7 and 9. At the control stations, the index values are 6.3 and 10.5 (Figure 5). Station 11 has a poor EcoQS, and the control stations have a moderate, almost good EcoQS.

cording to the criteria defined by Bouchet et al. [78]. Figure 5. Ecological Quality Status (EcoQS) derived from exp(H'_{bc}). The EcoQS was evaluated ac-

5. Discussion

5.1. Degraded Environmental Conditions Due to the Sewage Outfall

cording to the criteria defined by Bouchet et al. [78].

The results of the present study showed that there is a strong impact on benthic habitats from the Cigarras water sewage outfall, as previously reported for other urban sewers [22,24,81], and currents disperse the outfall to the west and south.

In detail, higher turbidity values were recorded at the bottom in 2006 and in 2007 in the Cigarras area, and, in most cases, discharges were released through outfalls into shallow subtidal habitats [81]. Grain size and distribution pattern of TOC are related to the sewage discharged by the outfall, similar to what has been observed in other studies [22,24,81] and conditioned by hydrodynamics, which act heterogeneously over time in the region. According to Furtado [51], the bottom currents of Cigarras Beach are characterized by a continuous change in direction and intensity. Indeed, currents can play a crucial positive role in the dispersal of organic matter waste [82]. In turn, control station 2 is composed of sandy sediments, which is typical of a non-impacted area.

When compared with other submarine outfall areas (e.g., Teodoro et al., [23]; Duleba et al., [39]; Pregnolato [83]), we can consider that the TOC levels in Cigarras region (from 0.76% to 2.75%) are intermediate and in São Sebastião Channel are intermediate to low. This is probably due to the absence of significant river discharges in the region [50] and the small volume of effluent disposal in Cigarra region when compared to the Dutos e Terminais do Centro Sul (South Central Oil Pipeline and Outfall—DTCS [39]). In addi‑ tion, active local water circulation limits the accumulation and preservation of organic matter [16,23,84].

Furthermore, reduced sediments were observed at most of the studied stations with C/S ratios between 1.5 and 5.0 [39], similar to the distribution pattern observed for the TOC content. The C/S ratios observed in the study area (between 1.5 and 5.0) are common in anaerobic marine sediments that are subjected to sulfate reduction under an oxygenated water column [85]. Thus, the stations sampled in the Cigarras region in 2006 and 2007 showed oxic water column conditions and anoxic sediments, as in other regions affected by urban sewage [81,86].

5.2. Cigarras Sewage Outfall Induced Low Diversity and Favored Tolerante Species

Diversity was quite low at study sites impacted by the sewage outfall. Similarly, in the Saguenay fjord (Canada), paper mill discharges induced a significant decrease in benthic foraminiferal diversity [87]. In the Firth of Clyde (Scotland), foraminiferal densities and diversity were also low in the vicinity of a sewage sludge [22].

The dominant species that reached higher abundance were *Ammonia tepida* and *A. parkinsoniana*. *Ammonia tepida* did not show any strong correlation with environmental parameters, which may suggest a high ecological plasticity. *Ammonia tepida* is a quite com‑ mon species in transitional waters and is generally dominant in environments with high organic matter enrichment in inner and eutrophic areas of bays and lagoons (e.g., Martins et al., [88–91]; Raposo et al., [92]; Bouchet et al. [46]). It is known to be an opportunis‑ tic species, able to tolerate a broad range of salinity, temperature, pH, oxygen level, and other parameters, and it can survive in low oxic transitional environments [93–95]. It has been reported as a dominant species in areas close to sewage discharges [24] and in sediments impacted by heavy metals, chemical and thermal pollution, fertilizers, caustic soda, organo‑chlorates, and hydrocarbons (e. g. Cearreta et al., [96]; Vilela et al., [97]; Le Cadre and Debenay, [98]; Bouchet et al., [46]).

Ammonia parkinsoniana is often found in shallow coastal habitats (e.g., Martins et al., [88–91]; Raposo et al., [92]; Bouchet et al., [46]) but is more abundant in areas under high marine influence as reflected by its distribution patterns in the study area [99] and seems to be more sensitive to environmental degradation than *A. tepida* [100]. In the study area, *A. parkinsoniana* tends to rise its relative abundance in muddy sediments with moderate TOC concentrations, which is reflected by its presence close to the area disturbed by the water sewage. It suggests that *A. parkinsoniana* abundances may be not negatively affected by the presence of the sewage outfall. It tends to co‑occur with other species, such as *A. rolshauseni*, *Cribostomoides* sp., *Hopkinsina pacifica*, *Neoconorbina terquemi*, *Bolivina striatula*, *B. doniezi*, *B. ordinaria*, *B. compacta*, *Pseudononion japonicum*, and *P. cananeiaensis*.

The present study results suggest that *Bolivina striatula* may be more sensitive than the other main species to the sewage outputs. Noticeably, this species is found in varied marine settings [101,102], although it occurs in transitional waters where it reaches relatively high abundances [90,103]. However, it is typically an oceanic species and has preference for organic matter resulting from marine productivity [104]. Similar observations were made in Bizerte Lagoon (Tunisia), where this species is more correlated with organic matter of high quality, especially enriched in proteins, carbohydrates, and chlorophyll a than with the amount of organic matter itself [90].

Buliminella elegantissima seems to be adapted to the sewage pollution in the Cigarras area. It is a common species in transitional waters [90,103] and can occur in impacted and polluted sediments by metals [97,105].

The abundance of *P. cananeianensis*is low, and its distribution is patchy in the region of Cigarras. Its occurrence is slightly different in the years 2006 and 2007. This species only was found living in CTL2, the most external station. *P. cananeiaensis* is a shallow water marine species, common in phytal environments, which allow one to infer the input of marine waters in estuarine areas [106,107].

5.3. Foraminiferal Diversity Index Shows That the Health of Benthic Habitat Are Altered

Ecological quality assessment studies around sewage outfalls, conducted based on macrofauna [81,108] and macroalgae [109], have generally revealed moderate to poor EcoQS.

The diversity index $\exp(H_{bc})$ showed that the health of benthic habitats around the Cigarras Sewage Outfall was severely altered. A slight improvement in the EcoQS was ob‑ served in 2007. No change was observed from 2006 to 2007, which would be expected since recovery of benthic communities around sewage outfalls occurs over longer periods [108]. The present study results are in accordance with previous ones based on foraminifera, showing that water sewage leads to environmental degradation of benthic habitats [22,24]. Furthermore, this confirms that benthic foraminifera are good indicators of organic matter pollution induced by sewage outfalls [22,87]. Finally, the present study confirmed that benthic foraminifera are important bioindicators of EcoQS in sewage-polluted benthic habitats [24,110].

In the case of the study area, only control station 2 exhibited environmental conditions typical of an area not impacted by sewage effluent (sandy sediments and low TOC). At this station, the EcoQS shows a quality of near good. In future monitoring surveys of the Cigaras Outfall, only this station should be considered as control.

6. Conclusions

The environmental parameters analyzed in this work showed the strong impact of the water sewage outfall which led to organic matter enrichment and anoxic conditions in the sediment. Furthermore, the results also evidence that the water currents in the area largely disperse the sewage outfall far from the source point.

The analyses of grain size‑geochemistry and the biocoenoses show that the area of the Cigarras outfall diffusers is undergoing organic enrichment from domestic sewage outfall. Meiofaunal organisms (foraminifera) are responding to this effect, reducing in abundance and diversity of their living assemblages, changing their composition, and including mainly opportunistic species tolerant to excessive organic enrichment. The living foraminiferal assemblages clearly show that the meiobenthos are being affected by the Cigarras outfall diffusers. The poor to moderate EcoQS clearly highlighted the effect of the sewage outfall, confirming the high potential of benthic foraminifera as bio-indicators.

Supplementary Materials: The following supporting information can be downloaded at: [https:](https://www.mdpi.com/article/10.3390/w15030405/s1) [//www.mdpi.com/article/10.3390/w15030405/s1](https://www.mdpi.com/article/10.3390/w15030405/s1), Figure S1. Shepard Diagram (1954) of sediments near the Cigarras outfall and control points in São Sebastião Channel, Figure S2. Values of TOC, S, N, C/S and C/N acquired in 2006, in the analyzed stations near the Cigarras submarine outfall. Legend: S.S.O.—sewage submarine outfall. The meaning of C/S (modified from <http://www.ozcoasts.gov.au>, accessed on 15 December 2022) and C/N values is also presented, Figure S3. Values of TOC, S, N, C/S and C/N acquired in 2007, in the analyzed stations near the Cigarras submarine outfall. Legend: S.S.O.—sewage submarine outfall. The meaning of C/S (modified from <http://www.ozcoasts.gov.au>, accessed on 15 December 2022) and C/N values is also presented, Figure S4. Values of TOC, S, N, C/S and C/N acquired in 2008, in the control stations in São Sebastião Channel. Legend: S.S.O. sewage submarine outfall. The meaning of C/S (modified from <http://www.ozcoasts.gov.au>, accessed on 15 December 2022) and C/N values is also presented, Figure S5. Ordered densities of living foraminifera in the years 2006, 2007 and 2008, Figure S6. Correlation of environmental parameters and the main foraminiferal species in 2006. Figure S7. Correlation of environmental parameters and the main foraminiferal species in 2007, Table S1: Geographical coordinates, hydrological data, Table S2: Geographical coordinates, grain size and geochemical results. Table S3: Cigarras 2006— Living Foraminifera—number of specimens, Table S4: Living assemblages density standardized for 10 mL and biotic parameters.

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