Environ Monit Assess (2022) 194:908
https://doi.org/10.1007/s10661-022-10636-1

Ecotoxicological assessment of estuarine surface waters receiving treated and untreated sanitary wastewater

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Received: 13 September 2021 / Accepted: 7 October 2022

Abstract Pollution from sewage discharge is one of the most critical environmental problems worldwide, e.g., in Brazil, where basic sanitation is still scarce. As pollution can affect biomes, especially estuaries where intensive ecological and human activities occur, has caused widespread concern. This work aimed to study the water quality of the Jundiaí/Potengi Estuary (JPE) in an area close to the discharge of treated and untreated wastewater for 18 months. Physicochemical and microbiological parameters were measured and integrated using the Water Quality Index of the Canadian Council of Ministers of the Environment. Ecotoxicological tests were performed with Brazilian endemic organisms to assess the impact of water pollution on biota. A generalized linear regression model was applied to understand the effects of water quality on ecotoxicological responses. Concentrations of metals, dissolved oxygen, total ammonia nitrogen, nitrate, and thermotolerant coliforms did not comply with Brazilian environmental regulations. A significant increase in the mortality rate of *Mysidopsis juniae* and *Nitocra* sp. and a significant decrease in the reproductive rate of *Nitocra* sp. indicated the most affected areas related to the discharge of treated and untreated wastewater. Only 10% of the samples from sites without direct wastewater impact showed a toxic response in at least one organism. Both water quality and sampling sites were statistical predictors of ecotoxicological response, describing not only the pollutant load but also the type of effluent. This study demonstrated the degradation of the environmental quality of the JPE, particularly due to the discharge of sanitary wastewater, and highlights the importance of protection and remediation measures to preserve this protected area.

Keywords Ecotoxicology · *Mysidopsis juniae* · *Nitocra* sp. · Wastewater treatment plants

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Published online: 17 October 2022

Introduction

Estuaries play an important role in the life cycle of many organisms, being a crucial site for feeding, reproduction, and migration of several species (Savenije, 2012). Brazil has large estuarine areas, more than 30% of which are located in the northeastern region (Lessa et al., 2018). One of them is the Jundiaí-Potengi Estuary (JPE), where the city of Natal, Rio Grande do Norte (RN) is located.

Despite their importance to the environment, estuaries around the world are targets of pollution, primarily from the discharges of sanitary and industrial wastewaters, which has significant negative impacts on these ecosystems (Kalloul et al., 2012; Wittmann et al., 2015). The increasing accumulation of exogenous substances in estuarine areas has negatively affected their biota and led to ecosystem imbalance (Pimentel et al., 2016). In addition, toxic compounds can bioaccumulate and be transferred through the food chain (Souza et al., 2014), posing a risk to human health (Rabaoui et al., 2017).

In Brazil, the wastewater network covers 61.9% of the population in urban areas, but only 49.1% of the total wastewater is treated (SNIS, 2020). In addition, existing wastewater treatment plants (WWTP) are inefficient in removing some substances such as metals, pesticides, and pharmaceuticals, which are used in large quantities but not monitored. The JPE is not immune to this reality, as it receives various wastewater types from the Natal/RN urban area. The sanitary sewage system collects only 20 to 30 percent of the wastewater produced by a population of 1.6 million residents (IBGE, 2016), but only 70 percent of the collected wastewaters are effectively treated (SEHARPE, 2015).

Water quality indices (WQI) are developed and used to easily monitor and classify water bodies integrating various physical, chemical, and microbiological parameters (Lopes et al., 2021; Zhao et al., 2020). The WQI proposed by the *Canadian Council of Ministers of the Environment* (CCME) presents the flexibility to include or exclude parameters and use local reference values. It becomes an efficient tool, easy to calculate, and adaptable to different data types (Lopes et al., 2021), reliably representing the water quality (Menezes et al., 2013). Nevertheless, interpreting the characteristics of the water body in ecological and/or sanitary terms requires the use of sensitive indicators that represent, in a comprehensive way, the potential biological impacts.

Ecotoxicological tests have been reported in many studies, as an approach to analyze the deterioration of water quality in rivers and coastal regions and, even, in estuarine ecosystems (Pereira et al., 2015; Pimentel et al., 2016). Yet, there are scarce ecotoxicological studies in these environments, particularly in the northeast region of Brazil (Nilin et al., 2013, 2019; Oliveira et al., 2014), and most of these studies have only assessed sediment quality. Regarding JPE, the existing studies are still incipient (Buruaem et al., 2013; Gurgel et al., 2016; Souza et al., 2016; Lopes et al., 2018).

Thus, this study aimed to analyze the impact of human activities developed in the region of the lower estuary of the JPE, focusing on the influence of the emission of treated and untreated sanitary wastewater on water quality. For this purpose, monitoring of physicochemical and microbiological parameters was carried out, together with ecotoxicological assessment using *M. juniae* e *Nitocra* sp. as test organisms, as they are endemic to Brazil.

Materials and methods

Study area

The Jundiaí-Potengi estuarine complex (JPE) has, as its main tributary, the Potengi River, which springs in the vicinity of Serra de Santana (Cerro Corá/RN), nearly 500 m altitude. The studied area includes the lower JPE site where the city of Natal/RN is located (Tavares et al., 2014). This region is greatly anthropized due to urban growth and shrimp farms on the banks of the river; mangrove stripes, natural vegetation of these areas, are reduced or absent (Souza & Silva, 2011). The Baldo WWTP, implemented in 2011, was the first in the city with an up-flow anaerobic sludge blanket (UASB) system, including tertiary treatment (nutrient removal and disinfection of the final effluent through ultraviolet radiation).

The region's climate is hot and humid with rainy summers (Jankovic et al., 2019). In the rainy season (RS), the average monthly rainfall rate was 316.5 mm in 2015 and 156.6 mm in 2016, while in the dry season (DS), it was 6.6 mm in 2015 and 22.7 mm in 2016 (EMPARN, 2016).

Water sample collection

Surface water samples from the lower JPE were collected monthly, at five sampling points (Fig. 1), between June 2015 and December 2016. Ninety samples were analyzed, 18 samples per point. All these points are close to the Baldo WWTP on the right bank of the estuary: P01 (5°46′54.05″S, 35°12′58.10″O) is an untreated effluent source, P02 (5°47′10.41″S, 35°12′74.07″O) downstream the WWTP, P03 (5°47′20.49″S, 35°12′88. 93″W) in front of the WWTP outlet, and P04 (5°47′24.05″S, 35°12′99.51″W) and P05 (5°47′21.70″S, 35°13′11.18″W) are upstream the WWTP.

Samples were collected at low tide (0.0–0.7 m) to minimize the influence of seawater entering the estuary (Gurgel et al., 2016; Nilin et al., 2019; Ribeiro et al., 2018).

Surface water samples were collected in 4 L polyethylene containers for physicochemical and ecotoxicological analyses. Aliquots of 100 mL for microbiological analyses were collected in glass flasks previously sterilized in an autoclave. All samples were kept properly refrigerated until use. The collection of surface water samples took place approximately 15 m from the right bank at each point of the JPE and required the use of a small motorized boat. The collection, conservation, and preparation of the samples followed the NBR 15,469:2007 standard (ABNT, 2007).

Physicochemical and microbiological analyses

The following physicochemical and microbiological parameters were analyzed in surface water samples, according to *Standard Methods for the Examination of Water and Wastewater* (APHA, 2012): pH, salinity, oils and grease (OG), suspended solids (SS), settleable solids (SSe), total solids (TS), ammoniacal nitrogen (NH_3-N), organic nitrogen (ON), total ammoniacal

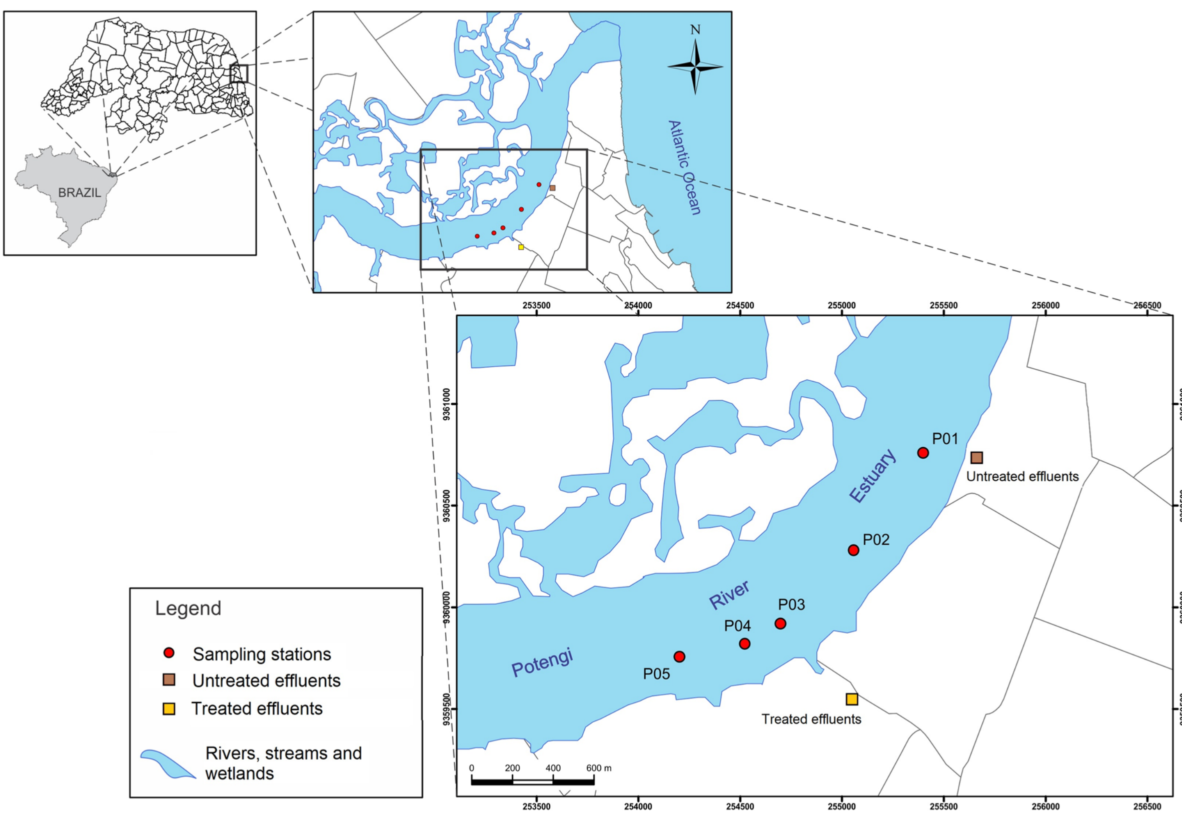

Fig. 1 Geolocation of the Jundiaí-Potengi estuarine complex (JPE), Natal/RN, and sampling points flagging: P01, discharge site of untreated wastewater; P02 downstream the WWTP; P03, at the WWTP effluent discharge; P04 and P05 upstream the WWTP

nitrogen (TAN), nitrite (NO_2^-), nitrate (NO_3^-), nitrogen (N), phosphorus (P), potassium (K), dissolved oxygen (DO), and biochemical oxygen demand (BOD_5), in addition to quantification of total coliforms (TC) and thermotolerant coliforms (TtC).

Furthermore, the contents of the metals, cadmium (Cd), copper (Cu), lead (Pb), chromium (Cr), iron (Fe), manganese (Mn), nickel (Ni), zinc (Zn), aluminum (Al), cobalt (Co), and silver (Ag) were quantified by atomic absorption spectrophotometry with electrothermal atomization by means of a varian atomic absorption spectrometer model 50B. The water samples (100 mL each) were treated by adding 5 mL of concentrated nitric acid. After evaporation on a hot plate to a final volume of 10 mL, 2.5 mL of hydrochloric acid was added to the samples, and the mixture was heated for 30 min. Samples were then, dried, filtered, and reconstituted to 100 mL with ultrapure water (APHA, 2012). The quantification of metals used calibration curves from the absorbance reading of six standards prepared for each element. Each sample replica was analyzed three times and the mean values were calculated.

Canadian Council of Ministers of the Environment—Water Quality Index

Water Quality Index (WQI) was determined according to *Water Quality Guidelines for the Protection of Aquatic Life* by *the Canadian Council of Ministers of the Environment* (CCME, 2001). The CCME WQI is calculated considering 3 factors: the scope/target—F1 (Eq. 1), which involves the number of parameters that did not meet the legal standard; frequency—F2 (Eq. 2), which represents the number of times these standards were not met (failed tests); and amplitude—F3 (Eq. 3), which involves the amplitude of the test failures, that is, the times that the parameter exceeded its limit.

$$F1 = \left(\frac{Number\ of\ failed\ parameters}{Total\ number\ of\ parameters}\right)x100 \tag{1}$$

$$F2 = \left(\frac{Number\ of\ failed\ tests}{Total\ number\ of\ tests}\right)x100 \tag{2}$$

$$F3 = \left(\frac{Normalised\ sum\ of\ variation - nsv}{0.01nsv + 0.01}\right) \tag{3}$$

The normalized sum of variation (nsv) is calculated according to Eq. 4, when the value cannot be superior to the limit, or by Eq. 5, when the value cannot be inferior to the limit (only DO is on this type of calculation).

$$Varition = \left(\frac{Amount\ exceeding\ the\ limit\ value}{Limit\ value}\right) - 1 \tag{4}$$

$$Varition = \left(\frac{Limite\ value}{Amount\ exceeding\ the\ limit\ value}\right) - 1 \tag{5}$$

Subsequently, nsv is calculated using Eq. 6.

$$nsv = \frac{\sum_{i=1}^{n} variation}{Total\ number\ of\ essays} \tag{6}$$

Finally, CCME WQI is calculated according to Eq. 7, where the divisor 1.732 normalizes the resulting values to a range between 0 and 100.

$$WQI\ CCME = 100 - \left(\frac{\sqrt{F1^2 + F2^2 + F3^2}}{1.732}\right) \tag{7}$$

The parameters used to calculate the CCME WQI were those established in the Brazilian legislation for saline waters, CONAMA nº357/2005 (CONAMA, 2005): DO, pH, NH_3-N, NO_2^-, NO_3^-, P, TtC, and the metals: Cd, Cu, Pb, Cr, Fe, Mn, Ni, and Zn.

Ecotoxicological tests

The organisms used in the ecotoxicological tests were selected because they are native to Brazilian estuarine regions, becoming representative organisms of the studied environmental scenario (Stringer et al., 2014), and they have characteristics that make them well established as test organisms (Artal et al., 2019; Silva et al., 2018).

Acute ecotoxicological tests with *Mysidopsis juniae* were performed following the standardized protocol described in standard 15.308/2011—Acute Toxicity (AT)—Method of test with misidaceans (Crustacea) of the Brazilian Association of Technical Standards (ABNT, 2011), at the Ecotoxicology Laboratory ECOTOX-Lab/ NUPRAR/ UFRN. Ten young *M. juniae* specimens (with 5 to 7 days) were put into 3 glass flasks (500 mL) and exposed to the following environmental samples' concentrations: 100, 50, 25, and 12.5%. The experimental conditions were salinity 34 ± 2 ppm; incubation temperature 25 ± 2 °C for 96 h with 12:12 h (light: dark) photoperiod. After the experimental period, dead organisms were counted.

Acute and chronic tests using *Nitocra* sp. as test organisms were performed following the protocol described by Lotufo and Abessa (2002). Ten ovigerous females of *Nitocra* sp. were exposed to 10 mL of previously diluted environmental samples, on polyethylene flasks (30 mL). The tests were performed in triplicate and the environmental samples were diluted to 50, 25, 12.5, and 6.25%. The whole test system was at 25 ± 2 °C incubation temperature for 96 h and photoperiod of 12:12 h (light: dark), and sample salinity was corrected to 17 ± 2 ppm. After the experimental period, the contents of each replica were fixed with formaldehyde (10%) and rose bengal dye (0.1%); dead females (acute toxicity) and *nauplii* (chronic toxicity) were then counted using a stereomicroscope (Coleman, model XTB-2B).

Acute toxicity (AT) is expressed as the concentration that leads to a mortality of 50% of the organisms (LC50-96 h). The pollution concentration effect on *Nitocra* sp. reproduction is expressed as no observed effect concentration (NOEC), and the lowest concentration (LOEC) on organisms. The viability of the test organisms was confirmed by performing a negative control with potassium dichromate ($K_2Cr_2O_7$) as a reference substance for *Nitocra* sp. and zinc sulfate heptahydrate ($ZnSO_4.7H_2O$) for *M. juniae*. The LC50—96 h was 9.12 mg L^{-1} for *Nitocra* sp. in $K_2Cr_2O_7$ (min: 6.70; max: 15.09 mg L^{-1}), and 0.36 mg L^{-1} for *M. juniae* in $ZnSO_4.7H_2O$ (min: 0.3–max: 0.45 mg L^{-1}). A triplicate positive control (0%) was performed as determined in standard 15.308 (ABNT, 2011) and in the protocol described by Lotufo and Abessa (2002).

Data analysis

The physicochemical and microbiological parameters determined were compared with the reference values for class 2 saline waters present in Brazilian resolution no. 357/2005 (CONAMA, 2005). Inferential statistical analysis involved data comparison by sampling points during the experimental period using the Kruskal–Wallis test. Post hoc analysis was performed using the Dwass-Steel-Critchlow-Fligner (DSCF) test. Significant differences were considered when $p < 0.05$. The effect size was described using epsilon square. Principal component analysis (PCA) with varimax rotation was performed, to analyze the variance explained through the physicochemical and microbiological parameters. The adequacy of the data set was defined by the Kaiser–Meyer–Olkin test (> 0.6) and Bartlett's test of sphericity ($p < 0.05$). The selection criterion for the components was carried out through the sedimentation graph, and eigenvalues were greater than 1. The software used was IBM-SPSS 26.

The determined CCME WQI were qualitatively classified into five levels (CCME, 2001): poor (0–44), marginal (45–64), regular (65–79), good (80–94), and optimal (95–100) as described in the protocol.

The trimmed Spearman-Karber statistical method (Hamilton et al., 1977) was used to calculate the LC_{50}-96 h using the TOXTAT 3.5 Software. ANOVA one-way analysis of variance was used to obtain LOEC and NOEC values for parametric data, and the Kruskal–Wallis on ranks test was used for nonparametric data. The post hoc comparison between groups was performed using Dunnett's test for parametric data, and Dunn's method for nonparametric ones. The statistical program used was Statistic 7.0.

A generalized linear model (GzLM) analyzed the influence of environmental quality on the observed ecotoxicological effects. For this, ecotoxicological data are represented by the survival or reproduction rates (*nauplii* hatching). These rates were estimated by dividing the organisms counted at sample 50% concentration by the survives and *nauplii* at the control group.

Two types of data distribution were considered: normal and gamma. The adherence of the chosen models was assessed based on the Akaike Information Criterion (AIC) estimator. For each ecotoxicological assessed endpoint, sampling points were included as the independent variable, and CCME WQI was considered a covariate. The model fit was verified by analyzing the normality of the residuals. Pairwise analysis was performed using the Bonferroni test. Statistically significant differences were set at $p < 0.05$. Statistical analysis was performed using SPSS version 26 software.

Results and discussion

Characterization of water quality through physicochemical and microbiological parameters

Monitoring of physicochemical and microbiological parameters is used to understand the pollution level of a water body, as well as to evaluate the effectiveness of control measures for contaminated areas and the impact of pollutants on public health (Wu et al., 2016; Zhao et al., 2020). Data on physicochemical and microbiological parameters of surface waters of JPE (Table 1) were compared with the reference values for the classification of saline water bodies from Decision No. 357/05 (CONAMA, 2005).

Dissolved oxygen (DO) is essential for the survival of aquatic life (Munna et al., 2013). More than 70% of all analyzed samples presented DO values lower than 5 mg L^{-1}, the required limit for class 2 water (CONAMA, 2005), confirming the values described by Lopes et al. (2018) in the same region. The lowest mean values of DO were measured at the discharge points of untreated wastewater (P01—3.79 mg L^{-1}) and treated wastewater (P03—3.35 mg L^{-1}), with no significant differences from the sampling points upstream of these discharge sites. These results described an imbalance in the oxygen production/consumption throughout the studied area. However, biochemical oxygen demand (BOD_5) showed significant differences when the values obtained in P01 and P03 were compared to those obtained in other sampling sites; BOD_5 values were approximately five times higher in P01 and P03 than in the other sampling sites ($p < 0.001$). High BOD_5 values have been associated with the mortality of organisms (Matos et al., 2017). Considering the determined values of DO and BOD_5, the wastewater emission points could influence the survival of test organisms. The normalization of BOD_5 values at P02, P04, and P05 indicated rapid recovery; however, oxygen values did not reach reference levels considered adequate for aquatic life.

Parameters such as settleable solids (SSe), ammoniacal nitrogen (NH_3-N), total ammoniacal nitrogen (TAN), nitrogen (N), phosphorus (P), total coliforms (TC), and thermotolerant coliforms (TtC) are considered indicators of urban pollution from sanitary wastewater emissions (Barletta et al., 2019; Marins et al., 2007; Nilin et al., 2019). Comparing the higher values of these parameters obtained at sampling points P01 and P03, with those obtained at sampling points that do not receive wastewater, statistically significant differences were found. Therefore, wastewater discharge contributes to an excess of organic matter, nutrients, and bacteria of fecal origin in estuarine waters.

The lowest salinity concentrations were found in the surface water samples from P01 and P03 in the lower part of the JPE, which can be attributed to the constant wastewater flow at these sites. Only at P03, the salinity of the water samples was significantly lower compared to the other sampling points ($p < 0.001$), possibly due to the dilution effect caused by the greater flow of fresh water at this point, originating from the Baldo channel and the wastewater treatment plants (WWTP).

The levels of TAN in water samples of P01, P02, P03, and P04 exceeded the limit established in resolution no. 357, class 2, of CONAMA (2005). TAN and NH_3-N concentrations were also higher in samples P03 and P01, with statistically significant differences between samples P03 *versus* P02, P04, and P05 ($p < 0.001$) and from P01 *versus* P05 ($p < 0.001$). The relationship between pH and NH_3-N was not significant (Spearman's correlation coefficient Rho $= -0.095$; $p > 0, 05$). The low variability of pH values (7.57–7.86), always below pH 8, did not promote the conversion of NH_3-N to a more toxic gaseous form (Kinidi et al., 2018). Studies by Nascimento et al. (2018) in surface waters of a bay in southeastern Brazil, which is highly urbanized and receives domestic and industrial wastewater, found TAN and NH_3-N levels like those reported in this study at sites that do not receive wastewater, indicating environmental degradation of the studied area.

According to the Brazilian legislation saline waters of class 2, the estimated TtC value in the surface waters of the studied area exceeded the permissible limits at all sampling points. In particular, in P03, the detected TtC values were statistically higher than in the upstream sampling points (P04 and P05), confirming the pollution of this water body by the emission of treated wastewater ($p < 0.001$).

The analysis of oils and grease (OG) was performed by visual inspection (presence or absence), according to the established standards. However, the quantitative analyses performed by the gravimetric method revealed OG, even if not detected during visual inspection. Throughout the experimental

Table 1 Physicalchemical and microbiological characterization of surface water of the lower Jundiaí-Potengi Estuary (JPE) per sampling point, during the monitoring period

	Physicochemical and microbiological parameters (average ± SD)					CONAMA 357/05 (class 2)	*P*-value	ε^2
	P01	P02	P03	P04	P05			
DO (mg L^{-1})	**3.79 ± 1.39**	**4.66 ± 1.44**	**3.35 ± 1.73**	**4.80 ± 1.57**	**4.79 ± 1.52**	5.00	N.S	0.092
pH	7.81 ± 0.46	7.86 ± 0.35	7.57 ± 0.58	7.80 ± 0.33	7.69 ± 0.62	6.50 a 8.50	N.S	0.099
Sal (ppm)	30.41 ± 7.85	32.35 ± 4.25	16.69 ± 8.45	33.29 ± 3.98	35.47 ± 3.50	-	< 0.001	0.357
BOD_5 (mg L^{-1})	20.68 ± 27.26	4.40 ± 4.02	20.42 ± 22.72	3.44 ± 2.18	2.93 ± 1.96	-	< 0.001	0.324
ON (mg L^{-1})	0.46 ± 0.35	0.40 ± 0.25	0.81 ± 0.64	0.28 ± 0.16	0.24 ± 0.13	-	< 0.001	0.273
TAN (mg L^{-1})	**4.08 ± 4.36**	**1.12 ± 0.99**	**8.44 ± 5.49**	**0.74 ± 0.37**	0.56 ± 0.36	0.70	< 0.001	0.441
NH_3—N (mg L^{-1})	3.47 ± 3.32	0.55 ± 0.49	7.38 ± 5.05	0.47 ± 1.38	0.34 ± 0.26	-	< 0.001	0.440
OG (mg L^{-1})	2.84 ± 1.63	2.83 ± 1.87	3.55 ± 2.52	3.84 ± 3.18	3.01 ± 2.58	-	N.S	0.053
SS (mg L^{-1})	156.60 ± 245.13	144.74 ± 198.56	96.35 ± 79.02	70.79 ± 54.68	94.48 ± 75.63	-	N.S	0.020
SSe (ml L^{-1})	0.43 ± 0.74	0.06 ± 0.08	1.03 ± 1.96	0.06 ± 0.09	0.10 ± 0.11	-	0.011	0.177
TS (g L^{-1})	44.62 ± 11.03	46.48 ± 11.80	25.23 ± 13.19	47.46 ± 15.98	52.31 ± 14.53	-	< 0.001	0.342
TC (MPN/100 mL)*	457E ± 779E	129E ± 426E	485E ± 1350E	18.5E ± 57.5E	1.46E ± 3.97E	-	0.002	0.233
TtC (MPN/100 mL)*	**135E ± 199E**	**6.65E ± 14E**	**160 E ± 302.53E**	**1.86E ± 3.72E**	**1.21E ± 3.96E**	0.025E	0.001	0.247
N (mg L^{-1})	5.14 ± 4.60	2.00 ± 0.92	10.01 ± 5.50	1.91 ± 0.79	1.56 ± 0.56	-	0.002	0.232
P (mg L^{-1})	0.71 ± 1.19	0.10 ± 0.86	1.55 ± 1.41	0.11 ± 0.12	0.08 ± 0.08	-	< .001	0.350
K (g L^{-1})	0.435 ± 0.203	0.474 ± 0.174	0.283 ± 0.207	0.479 ± 0.164	0.494 ± 0.129	-	0.037	0.148
NO_2^- (mg L^{-1})	0.03 ± 0.07	0.03 ± 0.03	0.24 ± 0.49	0.03 ± 0.03	0.02 ± 0.02	-	< 0.001	0.411
NO_3^- (mg L^{-1})	**1.15** ± 1.18	**1.00** ± 0.51	**1.27** ± 0.07	**1.17** ± 0.77	**0.97** ± 0.48	0.70	N.S	0.010
Cd (mg L^{-1})	**0.086** ± 0.053	**0.110** ± 0.063	**0.058** ± 0.036	**0.099** ± 0.093	**0.074** ± 0.038	0.040	N.S	0.098
Cu (mg L^{-1})	**0.084** ± 0.052	**0.082** ± 0.049	**0.061** ± 0.037	**0.079** ± 0.051	**0.070** ± 0.029	0.0078	N.S	0.027
Pb (mg L^{-1})	**0.530** ± 0.220	**0.618** ± 0.320	**0.387** ± 0.231	**0.447** ± 0.263	**0.518** ± 0.219	0.210	N.S	0.121
Cr (mg L^{-1})	0.046 ± 0.020	0.048 ± 0.020	0.030 ± 0.027	0.042 ± 0.022	0.051 ± 0.020	1.100	N.S	0.114
Fe (mg L^{-1})	**1.013** ± 0.531	**1.062** ± 0.635	**0.814** ± 0.511	**1.083** ± 0.610	**0.938** ± 0.499	0.300	N.S	0.046
Mn (mg L^{-1})	**0.234** ± 0.363	**0.354** ± 0.673	**0.168** ± 0.228	**0.123** ± 0.087	0.095 ± 0.045	0.100	N.S	0.026
Ni (mg L^{-1})	**0.544** ± 0.497	**0.630** ± 0.513	**0.407** ± 0.533	**0.654** ± 0.644	**0.601** ± 0.414	0.074	N.S	0.050
Zn (mg L^{-1})	**0.127** ± 0.122	0.078 ± 0.071	0.098 ± 0.091	0.070 ± 0.061	**0.188** ± 0.337	0.120	N.S	0.030
Al (mg L^{-1})	0.583 ± 0.104	0.750 ± 0.350	0.398 ± 0.303	0.533 ± 0.333	0.350 ± 0.350	1.500	N.S	0.199
Co (mg L^{-1})	1.048 ± 0.124	1.095 ± 0.095	0.858 ± 0.292	1.135 ± 0.088	0.720 ± 0.499	-	N.S	0.300
Ag (mg L^{-1})	**0.172** ± 0.006	**0.178** ± 0.006	**0.142** ± 0.033	**0.178** ± 0.003	**0.173** ± 0.008	0.005	N.S	0.274

Analyses were performed according to APHA (2012). pH was measured by potentiometric method and salinity (Sal) by refraction. Oils and grease (OG), suspended solids (SS), and total solids (TS) were determined by gravimetry. Sedimentable solids (SSe) were quantified by sedimentation. Phosphorus (P), nitrite (NO^{2-}), and nitrate (NO^{3-}) were measured by colorimetric methods. Total nitrogen (N), ammoniacal nitrogen (NH_3-N), organic nitrogen (ON), total ammoniacal nitrogen (TAN), biochemical oxygen demand (BOD_5), and dissolved oxygen (DO) were estimated by titrimetry. Potassium (K) was determined by flame photometry. Total coliforms (TC) and thermotolerant coliforms (TtC) were determined by membrane filtration. The metals cadmium (Cd), copper (Cu), lead (Pb), chromium (Cr), iron (Fe), manganese (Mg), nickel (Ni), zinc (Zn), aluminum (Al), cobalt (Co), and silver (Ag) were determined by atomic absorption spectrophotometry

Bold: values exceeding the limits established by resolution CONAMA 357/2005 for class 2 saline waters

*Scientific notation: 10^5

period, the mean values of OG reached a maximum of 3.84 mg L^{-1} at P04 and lower values at all other points. The results of this study were similar to those recently described in the harbor area of JPE near point P01 (Souza & Neto, 2019).

Regarding the profile of metallic impurities, the contents of Cr and Al were within limits established by Brazilian legislation. For Co, no reference value is established in Brazilian legislation for saline waters. However, the mean values of Co were 16 times lower than in another study conducted on the Jundiaí River, a tributary of the Potengi with industrial activities such as textile production (Gurgel et al., 2016). The Cd, Cu, Pb, Fe, Ni, and Ag contents were higher than the values established by CONAMA 357/2005 for class 2 brackish water at all sampling sites (Table 1). In samples P01 and P02, the values for Mn and Ni exceeded 10 and 20 times the reference value, respectively. Cu had maximum values in P01 that exceeded 24 times the limit. Silver concentrations exceeded the limit by up to 36 times in P02, P03, P04, and P05, with no statistically significant differences observed among sampling sites, possibly due to high variability. Maximum values occurred in the dry season, while minimum values occurred in the rainy season.

According to Gurgel et al. (2016), the Jundiaí River location with the highest pollution was a treated industrial wastewater emission point. However, the metal contents were lower than the assessed in this study at P01 and P03. On average, depending on the considered metal, the values varied from once for Zn to 89 times for Ag. Furthermore, lower JPE sediment analyses carried out by Mendonça et al. (2021) showed enrichment in Cd, Cu, Pb, Cr, Ni, and Zn, confirming the estimated values in surface water analyses.

The relationship between physicochemical and microbiological parameters was integrated by a principal component analysis—PCA (Table 2). Nine components accounted for nearly 80% of the variance explained. Principal component 1 (PC1) accounts for 18.83% of the total explained variance. Variables involved included salinity (−0.751); BOD_5 (0.674); TAN (0.958); NH_3-N (0.951); total solids—TS (−0.636); P (0.675); N (0.958); and potassium—K (−0.615). PC1 describes the behavior of the variables related to the richness of the effluent in organic matter and a possible dilution effect due, at least to some extent, to the freshwater input.

PC2 and PC3 account for 11.25% and 10.56% of the total variance, respectively, and are mainly metallic elements such as Pb (0.737), Cr (0.862), Fe (0.892), and Ni (0.672), and PC3 is positively associated to Cd (0.890), Cu (0.922), Mn (0.783), and Ni (0.506). PC5, accounting for 7.69% of the total variance, is related to the metals Al (0.769), Co (0.802), and Ag (0.774). Al and Fe, as well Co, are natural soil components that enter water bodies through surface runoff processes (Gurgel et al., 2016; Hu et al., 2013). The fact that these natural elements are distributed to the above principal components indicates the influence of anthropogenic sources of Al, Fe, and Co.

The presence of Zn, Cr, and Cu in rivers has been attributed to surface runoff in heavily trafficked areas, where fuel is burnt and car tires and brake pads wear out (Adachi & Tainosho, 2004; Rule et al., 2006). The town of Natal is located on the banks of the JPE, which may contribute to the metal levels identified during this monitoring. Another source of pollution to consider is shipping traffic at the harbor of Natal, which is located near P01. The fuels and lubricating oils used in this port have high concentrations of metals, namely Cd, Cr, Cu, Mn, Ni, Pb, and Zn (Pulles et al., 2012; Coufalík et al., 2019). Studies on the presence of these metals in domestic wastewater (Souza et al., 2014; Thewes et al., 2011) support the role of

Table 2 Principal components analyses (PCA) of physicochemical and microbiological profile of lower Jundiaí-Potengi Estuary

Total explained variance						
Main component	Initial eigenvalues			Sum of rotated squares		
	Total	% of variance	% accumulated	Total	% of variance	% accumulated
1	6.51	22.44	22.44	5.46	18.83	18.83
2	4.56	15.71	38.15	3.26	11.25	30.08
3	2.42	8.33	46.48	3.06	10.56	40.65
4	2.18	7.51	53.99	2.61	8.99	49.63
5	1.97	6.80	60.79	2.23	7.68	57.32
6	1.74	6.00	66.79	1.77	6.10	63.41
7	1.37	4.71	71.50	1.67	5.75	69.16
8	1.12	3.87	75.37	1.54	5.31	74.47
9	1.03	3.55	78.92	1.29	4.44	78.92

wastewater emissions in metal pollution in the aquatic environment.

Another activity that may affect metal content is shrimp farming, which is widespread, especially in the study area. Their effluents and sludges contain high levels of Cu, Zn, and Hg (León-Cañedo et al., 2017), which would contribute to the profile of metals found (Buruaem et al., 2013). In addition, Cr and other metals were detected in existing textile effluents prior to the JPE (Costa-Böddeker et al., 2017; Gurgel et al., 2016). The distribution and fate of heavy metals in estuarine environments where there is an interaction between different stressors such as land use and hydrodynamics is a complex issue (Costa-Böddeker et al., 2017).

Variables related to microbiological characteristics (BOD_5, TC, and TtC) appear in water bodies receiving wastewater in association with other variables indicative of organic matter and nutrients (Shin et al., 2013). PC4 explained 8.99% of the observed total variance and was positively related to microbiological parameters such as total coliforms (0.956), thermotolerant coliforms (0.895), and nitrite (0.859). Thus, described domestic sewage pollution due to wastewater discharged in P01 and P03.

The remaining principal components (PC6, PC7, PC8, and PC9) explained 21.6% of the total observed variance. PC6 was positively related to DO (0.656) and nitrate (0.744), contrary to what usually happens (Arafat et al., 2022). This suggested DO recovery in water due to mixing with seawater. In Natal/RN, water consumption has high nitrate contents (Costa et al., 2017). Nevertheless, the major contribution of this element comes from the oxidation of ammonia and nitrite by microbiological activity during the decomposition of organic matter, as described in PC4.

PC7 was positively related to organic nitrogen—ON (0.695) and SSe (0.825), variables that may be associated with sanitary sewage or surface runoff, and PC8 was related to pH (0.694) and suspended solids—SS (0.843). PC9, on the other hand, was related to Zn (0.836), suggesting a source of Zn other than other metals, which may not be related to anthropogenic pollution.

As mentioned above, the Canadian Water Quality Index (CCME WQI) has the advantage of being versatile, as it allows the inclusion of different variables and takes into account the variability of the parameters involved, as well as the limits imposed by environmental legislation. The sampling sites associated with untreated and treated wastewater discharge had the lowest CCME WQI values. The mean and standard deviation of these CCME WQI values were 48.04 ± 11.57 (poor—for P01) and 34.60 ± 13.00 (marginal—for P03). Sampling sites P02 and P04 were considered marginal with CCME WQI values of 51.91 ± 6.15 and 59.29 ± 4.76, respectively. The best situation was recorded upstream of the sewage disposal area P05 (CCME WQI $= 64.15 \pm 4.41$) classified as regular. However, all selected points were outside the limits of adequacy described in the water quality classification indicator (good or optimal), indicating significant environmental degradation in the studied area.

In Brazil, few studies are using CCME WQI to assess estuarine water quality. However, Araújo et al. (2017) determined CCME WQI values in some Brazilian rivers and estuaries: Bracuí River was classified as good (CCME WQI = 86), while São João River Estuary was classified as marginal (CCME WQI = 54), Macaé (CCME WQI = 30), and Perequê-Açu (CCME WQI = 34) as poor. According to the authors, the deterioration of this water was caused primarily by the influence of nutrient increase and heterotrophic bacteria resulting from anthropogenic pollution, including oil extraction, urbanization, and domestic sewage.

Several water quality indices (WQIs) have been used worldwide to assess surface water quality in different types of water bodies, including estuaries. A review of 107 studies conducted in different countries (Uddin et al., 2021) showed that 91.65% of the studies assessed water quality in rivers and lakes, while the remaining studies examined estuaries and marine waters. Regarding the choice of index, 50.47% of these studies used the CCME WQI and the National Sanitation Foundation (NSF) WQI, which can be used in estuaries. The NSF WQI, adapted by the Environmental Agency of the State of São Paulo (CETESB), is the most widely used in Brazil (Souza et al., 2020), with modifications to classify waters intended for public supply. The CETESB WQI uses a fixed number of 9 parameters but does not include toxic substances such as metals.

Studies on estuarine water quality in northeastern Brazil have been conducted with other types of indicators, mainly those aimed at assessing the eutrophication status of aquatic ecosystems (Chagas et al., 2020). Using the trophic index (TRIX), Tavares et al. (2014) described the JPE as a eutrophic water body that depends on the tidal regime and precipitation, mainly due to the input of pollutants from urban runoff and through surface runoff. However, TRIX does not include toxic parameters, as is the case with CCME WQI, but takes into account primary production through chlorophyll-a, DO, and nutrients such as nitrogen and phosphorus (Filho et al., 2020; Tavares et al., 2014).

Ecotoxicological characterization

Ecotoxicological testing is considered essential to assess the effects of total contaminant exposure on biota (Santana et al., 2015). In addition to determining the effects of isolated compounds, exposure to complex mixtures, such as effluents and environmental samples, offers the advantage that ecotoxicological tests consider the effects resulting from interactions between compounds and represent a real environmental scenario of exposure (Magalhães & Filho, 2008). In this sense, ecotoxicological tests provide a holistic and comprehensive overview of the observed effects on a biological system and, consequently, on the affected biotope.

Few ecotoxicological studies have been conducted in northeastern Brazilian estuaries (Souza et al., 2016); most of them focus on the analysis of sediments as the main pollutant reservoir and neglect the biological effects of water pollution on resident biota (Nilin et al., 2019).

Ecotoxicological response profiles were associated with sampling sites and test organisms (Table 3). *M. juniae* showed acute effects in the water samples of P01 and P03 (sampling sites where the effluents are discharged). The other samples did not affect the survival of *M. juniae*, except for the samples collected in September and October 2016 from P02 (LC50-96 h = 24.34% and < 12.50%) and in October 2016 from P04 (LC50-96 h = < 12.50%). *Nitocra* sp. showed a different acute response pattern and lower sensitivity than *M. juniae* (Table 3). However, samples P01 and P03 caused chronic toxicity to *Nitocra* sp. consistent with the acute toxicity these samples caused to *M. juniae*.

Chronic tests are particularly important for environmental monitoring and species protection and conservation because risks to biota can be detected when sublethal effects are considered (Silva & Abessa, 2019). Biological effects of pollutants from wastewater emissions have been reported to cause biochemical and physiological perturbations under sublethal exposure conditions (Karrasch et al., 2019; Wigh et al., 2017).

In an area of the Jundiaí River, approximately 30 km upstream of the current study area, where a textile industry is located, Gurgel et al. (2016) reported a mortality rate of *M. juniae* six times higher than the control group, and a reproductive rate 53% lower than that observed in the control group, suggesting the potential ecological impact on ecosystem biota. Other work reported more than 50% reduced survival of *M. juniae* in surface water samples from estuaries in northeastern Brazil (Nilin et al., 2019) and chronic toxicity in *L. variegatus* associated with the discharge of domestic and industrial effluents (Souza-Santos & Araújo, 2013).

A generalized linear model (GzLM) was used to study the ecotoxicological effects of water quality and sanitary effluents' influence. This model considered the CCME WQI as the water quality descriptor, the seasonality as an environmental factor of aquifer loading, and sampling points as pollution sources. Within the model, two distributions were tested: the normal distribution and the gamma distribution. The normal distribution showed the best fit (the Akaike Information Criterion lowest value).

The parameters of the estimated model are described below, using upstream point P05 as a reference because it is the point least affected by wastewater discharge (Table 4).

Lower survival rates of *M. juniae* were recorded on P01 and P03, discharge sites of untreated and treated sewage, respectively (Fig. 3). Both sampling point (Wald's X_2: 48.262; df: 4; $p < 0.001$) and water quality (Wald's X_2: 5.225; df: 1; $p < 0.05$) significantly affected the survival rate, while seasonality did not significantly influence any of the considered endpoints.

Considering P05 as a reference site, the viability of *M. juniae* was reduced by 38.72% at P01 (untreated wastewater discharged site), and by 52.15% at P03 (treated wastewater discharged) with no significant difference in ecotoxicological effect between the two sites (Fig. 2). Likewise, there were also no significant differences in survival rates, between P05 and the downstream sites P04 and P02.

Table 3 Lethal concentration (LC_{50}-96 h, %), concentration that causes no effect on organisms (CENO, %), and concentration effect observed (CEO, %) of the acute and chronic toxicity tests, using as test organisms *Mysidopsis juniae* and *Nitocra* sp. in surface water samples from the lower Potengi River Estuary

Year/month		Test organism	P01		P02		P03		P04		P05	
			LC_{50}	CENO/CEO	LC_{50}	CENO/CEO	LC_{50}	CENO/CEO	LC_{50}	CENO/CEO	LC_{50}	CENO/CEO
2015	Jun	Mys	NT	*	NT	*	< 12.5	*	NT	*	NT	*
		Nit	NT	NT	NT	6/12	50	25/50	NT	NT	NT	NT
	Jul	Mys	< 12.5	*	NT	*	NT	*	NT	*	NT	*
		Nit	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
	Ago	Mys	< 12.5	*	NT	*	< 12.5	*	NT	*	NT	*
		Nit	NT	25/50	NT	NT	85.00	6/12	NT	6/12	NT	NT
	Set	Mys	NT	*	NT	*	34.67	-	NT	*	NT	*
		Nit	NT	NT	NT	NT	56.00	NT	NT	NT	NT	NT
	Out	Mys	NT	*	NT	*	NT	*	NT	*	NT	*
		Nit	NT	NT	NT	NT	-	-	NT	NT	NT	NT
	Nov	Mys	93.89	*	NT	*	31.93	*	NT	*	NT	*
		Nit	NT	NT	NT	NT	48	NT	NT	NT	NT	NT
	Dez	Mys	43.53	*	NT	*	< 12.5	*	NT	*	NT	*
		Nit	15.00	6/12	NT	NT	NT	25/50	NT	NT	NT	NT
2016	Jan	Mys	34.93	*	NT	*	82.92	*	NT	*	NT	*
		Nit	NT	x/6	NT	NT	NT	50/100	NT	NT	NT	25/50
	Feb	Mys	70.71	*	NT	*	12.50	*	NT	*	NT	*
		Nit	NT	12/25	NT	NT	NT	NT	NT	NT	NT	NT
	Mar	Mys	< 12.5	*	NT	*	28.65	*	NT	*	NT	*
		Nit	NT	12/25	NT	NT	NT	NT	NT	NT	NT	NT
	May	Mys	< 12.5	*	NT	*	< 12.5	*	NT	*	NT	*
		Nit	NT	12/25	NT	NT	NT	50/100	NT	12/25	NT	NT
	Jun	Mys	< 12.5	*	NT	*	< 12.5	*	NT	*	NT	*
		Nit	40.00	50/100	NT	NT	NT	NT	NT	NT	NT	NT
	Jul	Mys	< 12.5	*	NT	*	NT	*	NT	*	NT	*
		Nit	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT

Table 3 (continued)

Year/month	Test organism	P01		P02		P03		P04		P05	
		LC_{50}	CENO/CEO	LC_{50}	CENO/CEO	LC_{50}	CENO/CEO	LC_{50}	CENO/CEO	LC_{50}	CENO/CEO
Aug	Mys	NT	*	NT	*	29.10	*	NT	*	NT	*
	Nit	NT	NT	NT	NT	61.00	50/100	NT	NT	NT	NT
Sep	Mys	NT	*	24.34	*	39.08	*	NT	*	NT	*
	Nit	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
Oct	Mys	< 12.5	*	NT	*	14.18	*	< 12.5	*	NT	*
	Nit	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
Nov	Mys	NT	*	NT	*	32.42	*	NT	*	NT	*
	Nit	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
Dec	Mys	NT	*	< 12.5	*	< 12.5	*	NT	*	NT	*
	Nit	NT	NT	NT	NT	NT	25/50	NT	NT	-	-

NT, sample did not show toxicity in the concentrations tested

(-) Test not performed

(x) Value not calculated

(*) Does not apply to acute toxicity tests

Table 4 Generalized linear model (GzLM) parameter estimates using a normal distribution

	M. juniae survival rate		*Nitocra* sp. survival rate		*Nitocra* sp. reproduction rate	
	Estimated coefficient	*p*-value	Estimated coefficient	*p*-value	Estimated coefficient	*p*-value
P01	− 38.719	**0.000**	− 17.041	**0.000**	− 40.064	**0.000**
P02	− 8.113	0.209	− 4.159	0.188	− 1.162	0.908
P03	− 52.152	**0.000**	− 27.484	**0.000**	− 55.208	**0.000**
P04	− 4.688	0.437	1.254	0.669	14.688	0.115
P05	–		–		–	
Rain season	− 4.586	0,227	2.247	0.224	6,882	0.240
Dry season	–		–		–	
WQI	− 0.706	**0.022**	− 0.719	**0.000**	− 1.451	**0.002**

P01, downstream point of WWTP, near the sea and the port of Natal; *P02*, between port and WWTP; *P03*, on WWTP outlet; *P04*, between P03 and P05; *P05*, upstream sampling point

Ecotoxicological tests concerning *Nitocra* sp. survival showed a statistically significant relationship with the sampling site (Wald's X_2: 52.435; df: 4; $p < 0.0001$) and water quality assessed by CCME WQI (Wald's X_2: 23.014; df: 1; $p < 0.0001$). The ecotoxicological pattern of *M. juniae* versus *Nitocra* sp. revealed a higher resistance of *Nitocra* sp. to water samples, expressed by its higher survival rate (Fig. 3). The mean survival rate of *M. juniae* was lower than that of *Nitocra* sp.: 27.45% lower at P03 (treated wastewater emission site) and 17.09% lower at P01. Thus, *M. juniae* showed an ecotoxicological response twice as high as that of *Nitocra* sp.

The reproduction rate of *Nitocra* sp. was also statistically significant concerning sampling points (Wald's X_2: 35.608; df: 4; $p < 0.001$) and water quality (Wald's X_2: 9.35; df: 1; $p < 0.002$) (Fig. 4). *Nitocra* sp. reproduction rate decreased by 55.10% in P03 and 40.21% in P01. When comparing the ecotoxicological pattern of *M. juniae* with that of *Nitocra* sp., the latter revealed a higher resistance to the water samples, expressed by its higher survival rate.

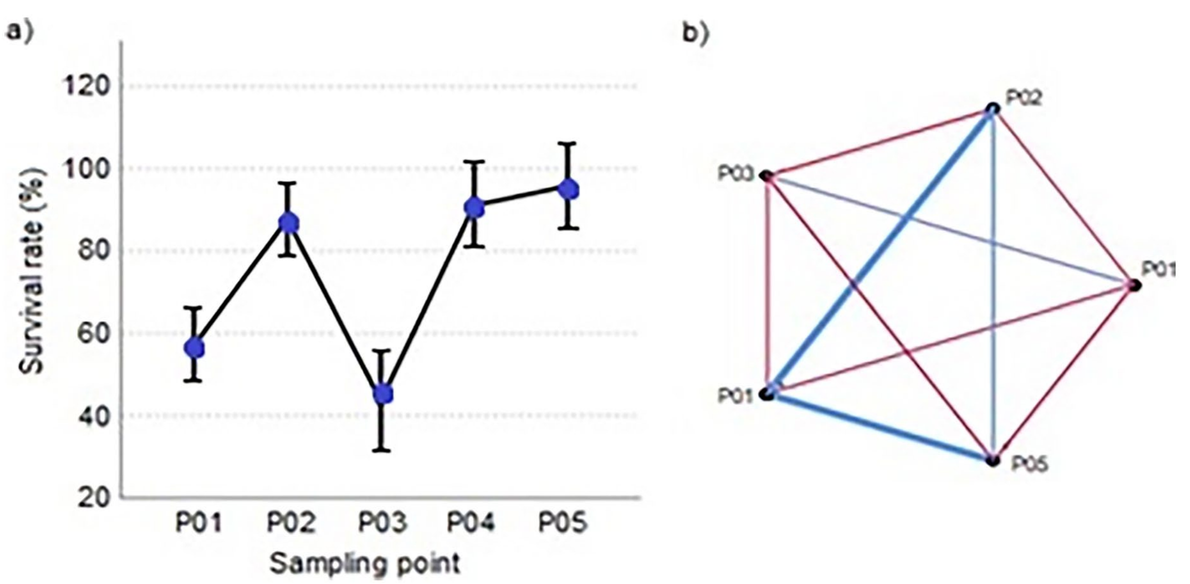

Fig. 2 Generalized linear model presenting sampling points and water quality (WQI) related with **a** *M. juniae* survival rates (mean ± 95% confidence interval); **b** post hoc analysis using Bonferroni's test. Red lines describe significant differences ($p < 0.05$) between sampling points

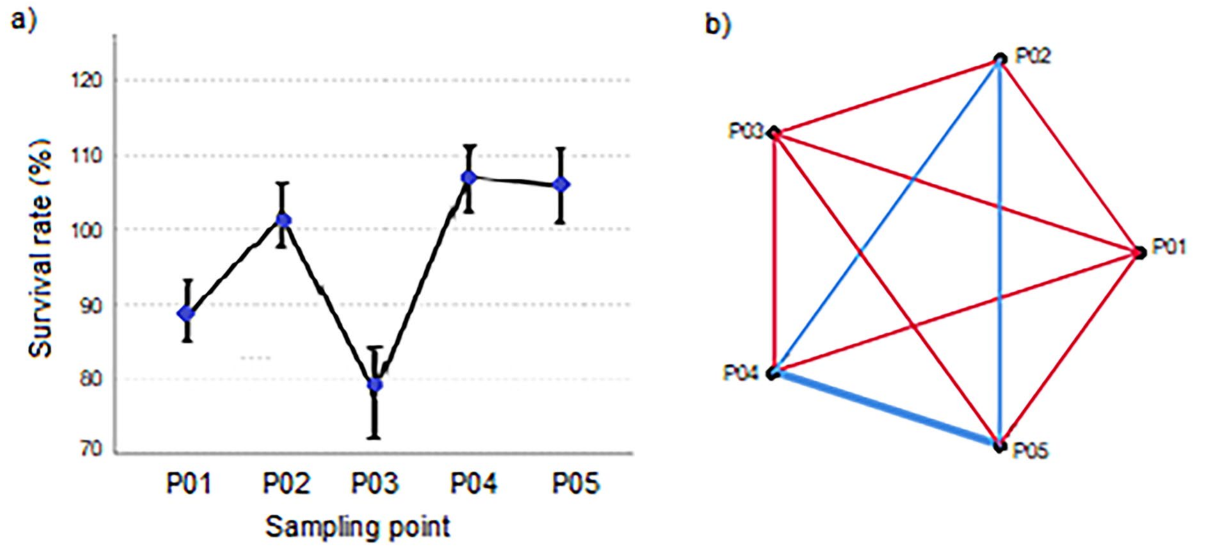

Fig. 3 Generalized linear model presenting sampling points and water quality (WQI) related with **a** *Nitocra* sp. survival rates (mean ± 95% confidence interval); **b** post hoc analysis using Bonferroni's test. Red lines describe significant differences ($p < 0.05$) between sampling points

Despite physicochemical, microbiological, and ecotoxicological parameters being similar at P01 and P03, the visual appearance of the untreated wastewater at P01 appeared less polluted than at P03 affected by the discharge of treated wastewater from Baldo's WWTP. The lower amount of untreated wastewater (on average, 368 L s^{-1} during the experimental period) had an impact on the surface water of the JPE similar to that caused by a higher flow of treated wastewater. The negative ecotoxicological effect of treated wastewater has been widely reported as relevant, although less important than the effect of raw wastewater (García et al., 2014; Gargosova & Urminska, 2017; Palli et al., 2019). Toxic effects

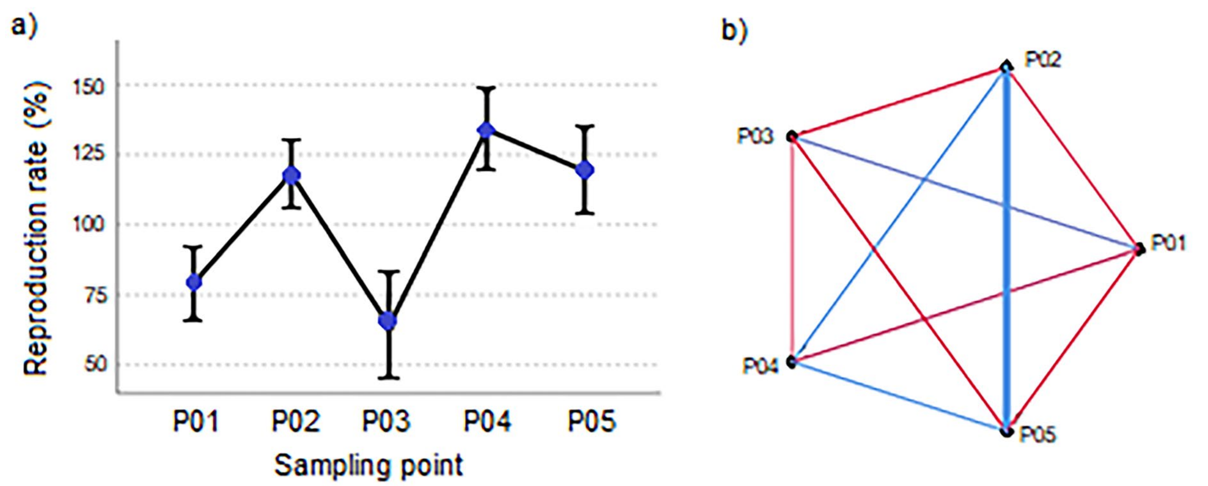

Fig. 4 Generalized linear model presenting sampling points and water quality (WQI) related with **a** *Nitocra* sp. reproduction rates (mean ± 95% confidence interval); **b** post hoc analysis using Bonferroni's test. The red lines describe significant differences ($p < 0.05$) between sampling points

on reproduction, feeding activity, hormonal disturbances (estrogenic activity, thyroid), and embryonic development of aquatic organisms of different trophic levels have been related to sanitary effluent discharge (García et al., 2014; Välitalo et al., 2017; Kienle et al., 2019).

The effects of metallic elements on organisms are linked to their bioavailability. The bioavailability depends on physicochemical factors, such as pH and salinity (Chapman & Wang, 2001; Kumar et al., 2015). If the treatment process is inefficient, other toxic substances can remain in the treated wastewater and may be released into the environment (Magdeburg et al., 2014). Organic micropollutants such as pesticides, pharmaceuticals, and personal care products (PPCPs) have been associated with treated wastewater ecotoxicity (García et al., 2014; Kienle et al., 2019; Palli et al., 2019). Ribeiro et al. (2020) analyzed treated effluents from a WWTP in Goiás (Brazil), and detected the presence of a mixture of several pollutants responsible (even at low concentrations) for delayed hatching and high mortality of *Danio rerio* embryos, and morphometric, physiological, sensory, skeletal, and muscular alterations of the tested fishes.

Besides the pollution sources mentioned above, other sources present along the water bodies need to be into account. For instance, the upstream banks of the JPE are affected by wastewater from other treatment plants, industrial effluents (e.g., from shrimp farms), and agricultural and livestock activities. Thus, future research should consider the occurrence and analysis of these substances not covered by conventional (traditional) monitoring, as well as the contribution of these substances to the ecological damage reported in this study.

Native species when used as test organisms make ecotoxicological results more representative. However, there are few Brazilian species used in ecotoxicology, mainly from marine and estuarine environments (Artal et al., 2019). The two test organisms used in this study are endemic to Brazil and showed differences in ecotoxicological response. *M. juniae* was more sensitive than *Nitocra* sp. The latter, proposed by Lotufo and Abessa (2002) as a test organism for sediment and interstitial water analysis, showed high potential for ecotoxicological analysis of surface water.

Thus, even if the ecotoxicological test effects (mortality or reproduction) manifest differently at various scales and test organisms, the same pattern was observed, providing an additional dimension to understand the real environmental scenario of the study area affected by the discharge of sanitary effluents.

Conclusions

The influence of sewage emission on the water quality of an estuarine area of the Brazilian Northeast was analyzed, highlighting the polluting effect on biota. These findings describe a significant degradation in JPE surface water, especially, in sampling point recipients of untreated and treated sewage. Although the emission of untreated sewage is lower than that of the treated one, both have a negative impact on the quality of receiving water bodies. The CCME WQI summarized the information on the main parameters analyzed, with the classification of the stretch studied always below good or excellent, with the worst scenario in P01 and P03. Furthermore, it proved to be adequate in the statistical evaluation considering the generalized linear model, which confirmed the influence of water quality on the survival and reproduction of test organisms. Monitoring programs based on WQI and ecotoxicology provide a more comprehensive assessment, being particularly important in estuarine regions such as the JPE, which is classified as an environmental protection area. In addition, analyses of other parameters with high potential for toxicity even at low concentrations, such as drugs, pesticides, and petroleum derivatives, should be performed and even included in the CCME WQI. Given this, it is of paramount importance to implement public policies aimed at minimizing the emission of untreated effluents, as well as to evaluate the efficiency of the existing WWTPs.

Acknowledgements The authors would like to thank Professor Denis Abessa, from UNESP (State University of São Paulo), and collaborating researcher at the USP Oceanographic Institute (University of São Paulo), for donating the *Nitocra* sp. organisms. To CNPq (National Council for Scientific and Technological Development) for their support regarding the project [430383/2018-5].

Author contribution Jaísa M. dos S. Mendonça: conceptualization; methodology; validation; formal analysis; investigation; resources; writing—original draft; writing—review and editing; visualization; project administration. Júlio A. Navoni: validation; formal analysis; resources; writing—original draft; writing—review and editing; visualization; supervision. Guilherme F. Medeiros: conceptualization, methodology, investigation, resources, supervision. Isabel A. P. Mina: conceptualization; methodology; writing—review and editing; visualization; supervision.

Data availability Data will be provided when requested.

Declarations

Conflict of interest The authors declare no competing interests.

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