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River Flows Influence on Nutrients (Si, N and P) and Fecal Coliforms (*E. coli*) in Two Tributaries of the Estuarine Channel of Bertioga (Santos Estuary, São Paulo, Brazil)

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Abstract

Sewage introduction into rivers has altered the physical and chemical properties of waters and also the microbial metabolism. This study aimed to evaluate the *Escherichia coli* and nutrient concentrations in the Maratuã and Crumaú rivers (Santos Estuary, Brazil) during two periods with distinct magnitudes of freshwater runoff, verifying possible relation of abiotic changes with the microbial metabolism. Water sampling was performed in October/2012 (dry season) and January/2013 (rainy season) at two points in the Crumaú river (upstream and downstream zone) and one in the Maratuã river (downstream zone). The water subsamples were obtained for *E. coli* and nutrient analyses while the velocity of water flow, water level, temperature, salinity, and dissolved oxygen were measured *in situ*. The *E. coli* concentrations were under the detection limit in the Maratuã downstream during the dry season reaching a maximum value (1.47×10^4 CFU/100mL) in the Crumaú upstream during the rainy season. *E. coli* presented strong positive correlation with nutrients (ammoniacal-N and phosphate), evidencing the sewage source in the Crumaú upstream shown by this association. In both periods, the low oxygen saturation (<50%) and high ammoniacal-N concentrations (>100 $\mu mol \cdot L^{-1}$) indicated considerable predominance of heterotrophic metabolism in the Crumaú upstream. The low dissolved oxygen values in Crumaú River are corroborated to show a low self-depuration capacity in the rainy period due to maintenance of high nutrient and *E. coli* at two points in the Crumaú river (upstream and downstream zone) and one in the Maratuã river (downstream zone). Besides, these results evidenced that the tendency of the metabolism changed from autotrophic to heterotrophic under high river flow events at this studied estuarine sector located at Santos estuarine complex.

Keywords

Eutrophication, Domestic Sewage, Water Quality, Seasonal Period, Estuary

1. Introduction

The atmospheric deposition is one of the major mechanisms of the cycling and redistribution of several chemical elements on the surface of the planet. The water volume from atmospheric deposition leads a high amount of terrestrial material into rivers, where the physical, biological, and chemical processes tend to control its traveled distance in the aquatic system, and consequently, the loads that reach the adjacent sea. Furthermore, the wastewater input from several human activities has altered the environmental controls on river metabolism (*i.e.* heterotrophic microorganism increase) over the last decades, affecting the water quality and biodiversity (Arroita et al., 2018). Nutrients sources to rivers and estuaries range from a diverse group of both diffuse non-point agricultural, urban, and rural point sources (e.g. wastewater and industrial discharge) (Bianchi, 2007).

Nutrients are essential for primary production, the plant growth that forms the base of the food web in all coastal systems. In these waters, the autotrophic organisms assimilate predominantly the dissolved inorganic nutrient forms (N, P, and Si), which are represented by ammonium, nitrate, nitrite, phosphate and silicate ions (Braga, 2002). Due to vital function to the majority of the phytoplankton, nitrogen (N) and phosphorus (P) normally act as limiting factors to the primary production in aquatic systems (Begon et al., 2006). However, this nutrient over-enrichment (eutrophication) can result in toxic algal blooms, shellfish poisoning, and other harmful outcomes (Conley et al., 1993; Howarth, 2008). Conversely, the silicate is assimilated only by a small parcel relative of the phytoplankton (mainly diatoms) (Bell, 1994; Conley & Malone, 1992), however, due to its terrestrial origin and relative abundance, this nutrient normally presents a conservative behavior in estuarine systems (Braga et al., 2000). In general, nutrients constitute an important pressure driver used in the evaluation of the anthropogenic impacts (Turner, 2000) in the coastal systems helping the environmental managers.

The estuarine circulation presents a relationship between the size of their basins and the tidal range (Kjerfve, 1987) and also, can be highly influenced by river flows (Miranda et al., 2002). According to Bianchi (2007), the estuarine circulation often leads to the trapping of particles in the region where the fresh and saline waters meet, being thus a potential site for the removal of nutrients from the water column to sediment. The nutrients may return to the water column as function the resuspension driven by the tidal current friction (mainly in macro tide regions) (Dittmar & Lara, 2001), as well as, by the height variation of the water column in shallow estuarine zones (tidal creek systems) (Ovalle et al., 1990). However, when examining inputs and losses of nutrients from estuaries to the oceans, it has been shown that the net export from estuaries is essentially a function of the residence time of freshwater (Dettmann, 2001; Ferguson et al., 2004; Nixon et al., 1996).

In the case of Brazilian urban estuaries, Piveli & Kato (2006) reported that the majority of the cities do not present tailored sewage treatment plants to remove the excess of nutrients. Besides, the disordered demographic growth in the majority of these regions established great human occupation in mangrove areas, where the sewage is directly dumped in the water body. Due to frequent sewage discharges, these aquatic environments normally present high concentrations of fecal coliforms. Such microorganisms were found with potentially pathogenic loads on salad vegetables, offering risks to human health (Alam et al., 2013).

The majority of coliforms found in feces of warm-blooded animals is *Escherichia coli* (*E. coli*), which accounts for 80% of the thermotolerant coliforms (Hachich et al., 2012). Meanwhile, the high presence of these heterotrophic bacteria in an urban stream (subject to high sewage discharges) was demonstrated to act considerably on the nitrogen metabolism (Medeiros et al., 2016), whereby the nitrate reduction using a nitrate reductase in the respiration process in *E. coli* was evidenced (Berg & Stwart, 1990). Furthermore, the reduction process of nitrogenous compounds can occur effectively into two-stage ($NO_3^- \rightarrow NO_2^-$ and $NO_2^- \rightarrow NH_4^+$) (Harborne et al., 1992).

Due to the historic importance of the economic activities adjacent to the São Vicente and Santos estuarine channels that integrate the Santos Estuary (**Figure 1**), there are a high number of studies about the eutrophication processes and the water quality in practically all sectors of these sub-systems. The nutrient biogeochemical cycles are significantly influenced by the Santos harbor (the largest in Latin America), industrial complex of Cubatão city (one of the industrial complex largest in Brazil) and gross sewage from human communities living on stilts (one of the largest in the world) (Braga et al., 2000; Moser et al., 2005; Berbel et al., 2015). Furthermore, these anthropogenic nutrient inputs were pointed in external areas of the Santos Bay (Braga et al., 2017).

In contrast, the Bertioga Channel presents few studies about its waters, highlighting just Gianesella et al. (2000) and Gianesella et al. (2005). This last study concluded that the lower zone of this estuarine channel is susceptible to the introduction of pollutants from coastal areas. However, environmental quality indexes and hydrological data from tributary rivers are scarce, mainly in the upper zone. This estuarine zone of the Bertioga Channel is drained by the two greatest rivers (Crumaú and Maratuã) of the Santo Amaro Island (**Figure 1**). Sutti et al. (2012) reported low indexes of water quality in the Crumaú upstream next to a

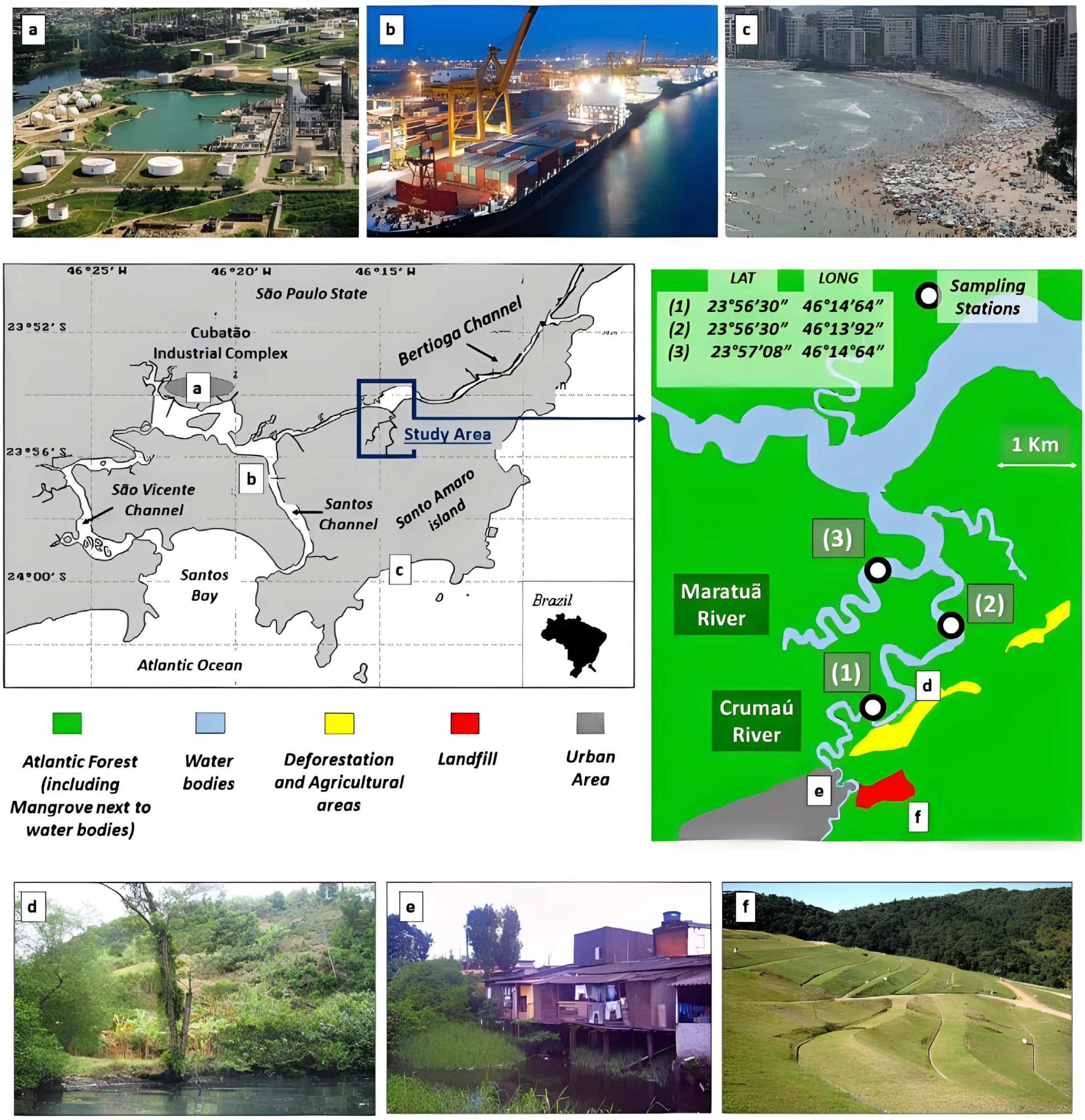


Figure 1. Santos estuarine system (central-left), highlighting the main economic activities: Industrial pole of Cubatao city (a) (Estadão Conteúdo, 2017), Santos harbor (b) (Tribuna, 2019), and touristic beach of Guarujá city (c) (Vejanomapa, 2014). The upper zone of the Bertioga channel (central-right), focusing on the sampling stations (Crumaú 1-2, and Maratuã 3), as well as the main anthropogenic activities surrounding: deforestation and agricultural areas (d) (Authors), stilt houses (e) (Authors), and the *Saco do Funil* landfill (f) (Engeoconsult, 2011).

landfill and a community living on stilts, where the *E. coli* and ammoniacal-N concentrations reached 8.1 10^5 colony forming units (CFU/100mL) and 9.5 $mg \cdot L^{-1}$ (about 12 times higher than the limit of the law), respectively.

Under this context, the present study aimed to evaluate the *E. coli* and nutrient concentration in surface layers of the Maratuã and Crumaú rivers during two periods with distinct magnitudes of freshwater runoff, verifying also the association level of these variables with the microbial metabolism.

2. Material and Methods

2.1 Study Area

The central coast of São Paulo state is under the domain of the Wet Tropical Climate, where the rainfall index is characterized by high rainfall with an average above 200 millimeters during the summer months (December-March) (Nunes, 1997). Considering the hydrological system, the Bertioga Channel presents several tributary rivers that came from the Santo Amaro Island (Santo Amaro mountain) and the continent (Serra do Mar—mountain chain), which present short paths due to the narrow plain. Thus, the majority of these tributaries act as riverine-associated inlet systems, thereby being dominated by freshwater runoffs during rainy periods and tidal currents during drier periods (Alfredini & Arasaki, 2009).

Despite the industrial and harbor activities around the neighboring Santos Channel, visibly affected by the economic activities, the banks of the Bertioga Channel are still dominated by a dense mangrove forests (Schmiegelow & Gianesella, 2014). Santo Amaro Island belongs to Guarujá city that presents a fixed population of about 300 thousand inhabitants (IBGE, 2014), but which can duplicate due to the tourist arrival in the summer months. The city has presented critical problems concerning the disposal of wastes over the last decades, and thus the local authorities took action to amplify the Saco do Funil Landfill (Engeoconsult, 2011) (**Figure 1**).

The Crumaú and Maratuã rivers (located in northern of the Santo Amaro Island) are physically similar, presenting a meandering system conditioned by bidirectional fluxes (tidal and fluvial). The depths range approximately from 1 to 4 meters in most of their extensions (~8 kilometers), and the width are maximum at the mouths (~90 meters). However, the Crumaú River is directly influenced by wastewaters from a small suburban area (neighborhood Morrinhos), agricultural areas (mainly banana crop), and a Landfill (Saco do Funil) (**Figure 1**). On the other sense, the mangrove areas over the Maratuã riverbanks are in a better preservation state (Sutti et al., 2012).

Both rivers reach the central portion of the Bertioga Channel (Largo do Candinho), where the tide currents meet (tidal-wave convergence). From this region up to Bertioga bar (**Figure 1**), Miranda et al. (1998) classified the estuary as a partially mixed type, which changes from highly stratified at neap tides to moderately stratified at spring tides. Meanwhile, the increased influence of river discharge and the shallower areas on the upper zone create sub regions where there is dominance of ebb current that drives the stratification (Seiler et al., 2020).

2.2. Sampling and Analytical Methods

Water samplings were carried out in October/2012 (drier period) and January/2013 (rainy period) in two points of the Crumaú river, *i.e.* upstream (station 1) and downstream (station 2) and one in the Maratuã downstream (station 3) (**Figure 1**) to determine *E. coli* and nutrients. *In situ* data (flow velocity, water levels temperature, salinity and dissolved oxygen) were measured at these points. The rainfall data were obtained in an official meteorological station located in the Santos city (CIIAGRO, 2013). Along the water system, geographic coordinates and the depths were obtained using a Sonar & GPS M52 (Lowrance®).

In the downstream stations (2 and 3) (**Figure 1**), the water flow velocities and the water level were measured every 30 minutes (covering the most of the spring tide cycle), respectively by mechanic fluxmeter (General Oceanics® 2030R6) and a metric ruler. In these stations, the estuarine water samples and *in situ* measures took place in the ebbing tide phase. The fluxmeter was dipped to a depth of 1m over the deepest area (thalweg) of the transverse section. Meanwhile, the estuarine water samples and *in situ* measures in the station 1 took place during the phase of tide reversion (slack waters), thereby covering periods no movement of water.

At the same time of each surface water collection, dissolved oxygen (DO) and temperature were measured directly in the water column through an oximeter MO-910 (Instrutherm®). Furthermore, DO values were used as a basis to calculate the percentage of dissolved oxygen saturation (%DO) following Grasshoff et al. (1983). Onboard, water aliquots were taken for the salinity determination using a refractometer RTS-101ATC (Instrutherm®). The estuarine water was sampled in triplicate by van Dorn bottle and transferred to autoclaved polyethylene bottles (1L) that, in turn, were maintained inside of thermal boxes (~4˚C) until the laboratory analysis.

Under laboratory environment, the pH was determined using the parameter B474 (micronal®). A volume (400 mL) of water sample was used for the ammoniacal-N (NH_3-N + NH_4^+-N) determination, which followed the volumetric method 4500-D (APHA, 1999). As described in this method, the ammonia (NH_3-N) acts as a base in aqueous solution, acquiring hydrogen ions from H_2O to yield ammonium (NH_4^+-N) and hydroxide ions ($NH_{3(aq)} + H_2O_{(l)} \leftrightarrow NH_{4\ (aq)}^+ + OH^-_{(aq)}$).

Another water fractions were filtered in cellulose acetate membranes (AP40—Millipore®) with porosity 0.45 μm to attend the determination of nitrate, nitrite, silicate, and phosphate. These nutrients were quantitated by the colorimetric method using spectrophotometric (E-225-D—CELM®) following APHA (2005) method. The Dissolved Inorganic Nitrogen (DIN) was obtained by the sum of ammoniacal-N (NH_4^+ + NH_3), nitrite (NO_2^-), and nitrate (NO_3^-). Due to the low concentrations of NO_2^-, the NO_2^- + NO_3^- concentrations were expressed as NO_3^-.

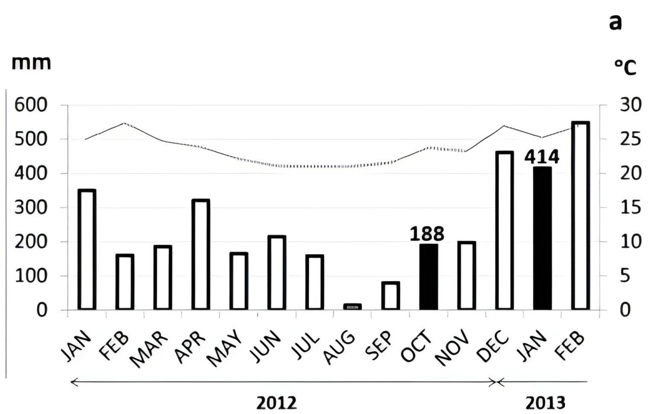
The *Escherichia coli* concentration was determined by the membrane filtration technique (APHA, 2005), which consisted of the filtration of a known volume of sample (after successive dilutions) through sterile membranes (porosity 0.45 μm). After this step, the membranes were put in Petri dishes containing a selective and differential chromogenic crop (Agar Biochrome Coliform—Biolog®). The results were expressed by units of bacterial colonies to each 100 mL of samples (CFU/100mL). The results obtained in triplicate were expressed in column graphics as average (±standard deviation) using the GraphPad Prism® for Windows (version 5.03). The same software was used to generate the Pearson correlation (significance degree at $p \leq 0.05$), which included all parameters except the flow velocities. Each sampling period was represented by one Pearson correlation with 9 samples and 10 parameters.

3. Results

3.1. Meteorological, Hydrodynamic and Physicochemical Variables

In January (2013), the rainfall volume was more than twice the October (2012) rainfall one, reaching values similar to the historical average under data computed since 1960 (CII AGRO, 2013). Also, it is important to highlight the expressive rainfall that occurred before the sampling days of January/2013 (Figure 2). Considering it, the sampling days of October (2012) represented a period moderately dry of springtime, and January (2013) ones, a common wet period of summertime. In general, the flow velocities in the ebb tide were higher than the observed in flood tide in the downstream stations (2 and 3). In these stations, the highest river flow was indicated by the higher ebbing currents during the rainy period (Figure 3).

The surface water temperature reached the lowest values in the sampling days of the rainy period. The salinity values ranged from 5.0 (station 1—rainy period) to 23.0 (station 3—drier period). During the rainy period, the surface water of the three stations presented pH, dissolved oxygen (DO) and saturation (%DO) values lower than the observed in the drier period, showing the influence of freshwater input by continental drainage in this system. Spatially, these values increased toward the lower estuary (from station 1 to stations 2 and 3).



b

Days	October (2012)		January (2013)	
	T (ºC)	P (mm)	T (ºC)	P (mm)
25	23.1	0.0	28.5	1.0
26	23.7	0.3	25.5	1.0
27	22.5	0.0	23.5	44.7
28*	26.0	0.0	24.5	2.0
29**	28.1	0.3	24.0	1.0

Figure 2. Climatological and meteorological data: (a) air temperature (gray line) and monthly rainfall, highlighting in black columns the values computed for the two sampling months; (b) daily rainfall: (*) sampling day in the Maratuã River; (**) sampling day in the Crumaú River. Central coast of São Paulo, Brazil.

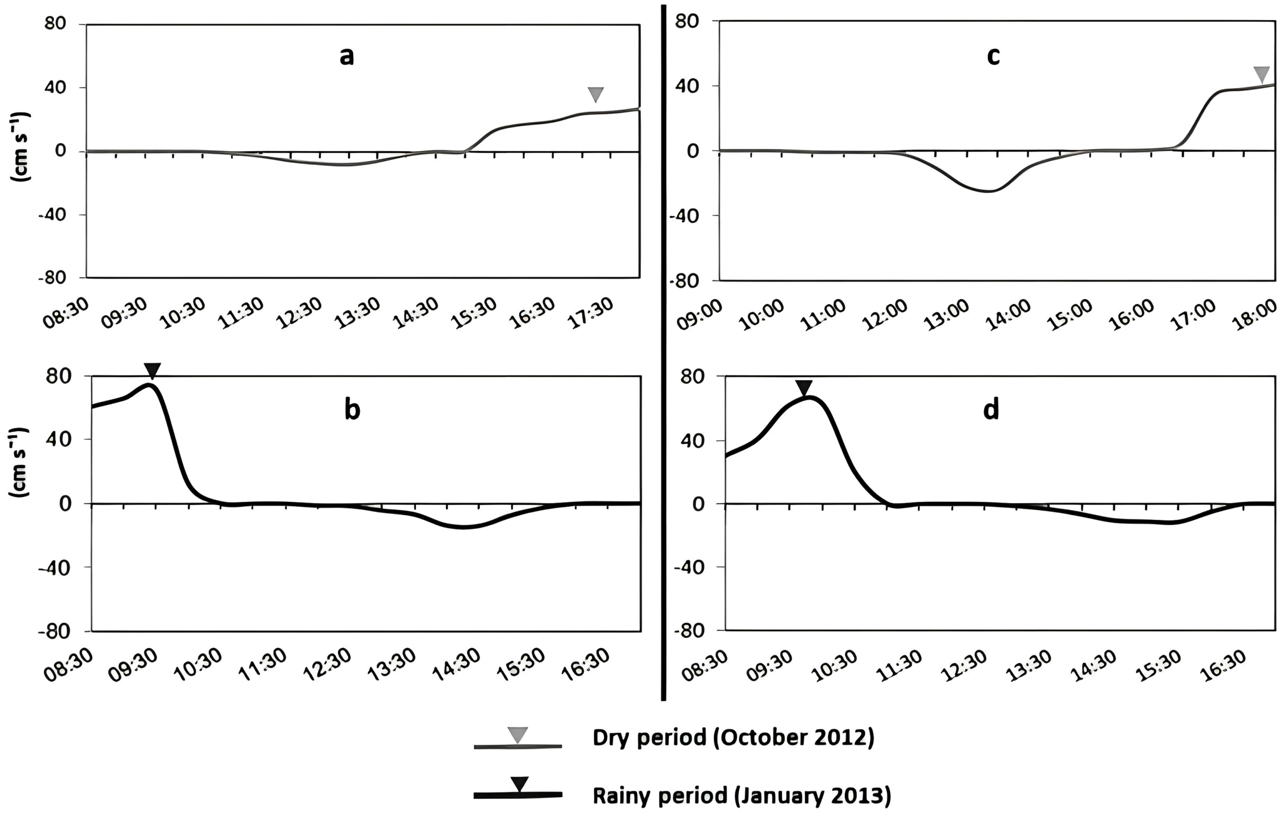


Figure 3. The line graphs represent the flow velocity variability at spring tides in the downstream zone of the rivers: Maratuã (station 3) in dry (a) and rany (b) periods; and Crumaú (station 2) in dry (c) and rany (d) period. The Inverted triangles indicate the moment of water collection. Crumaú and Maratuã tributary rivers of the estuarine channel (São Paulo, Brazil).

3.2. Nutrient Concentrations and *E. coli* Data

Overall, considering the variation of salinity as a function of the tidal range and freshwater input, the nutrients presented a great range of variation. The nutrient concentration ranged from 0.65 $\mu mol \cdot L^{-1}$ (nitrate in the station 2) to 114.05 $\mu mol \cdot L^{-1}$ (ammoniacal-N in the station 1) in the drier sampling, and from 5.82 $\mu mol \cdot L^{-1}$ (ammoniacal-N in the station 2) to 112.27 $\mu mol \cdot L^{-1}$ (ammoniacal-N in the station 2) in the rainy period (**Figure 4**). In comparison to the drier period, the rainy period revealed a nitrate and silicate increase and an ammoniacal-N decrease in the downstream stations (2 and 3), whereas in the Crumaú upstream (station 1) was observed a phosphate decrease and a nitrate increase.

In the drier period, the *E. coli* concentration ranged from 0.00 (<Detection Limit) in the station 3 (Maratuã downstream) to 2.03×10^3 colony forming units (CFU/100mL) in the station 1 (Crumaú upstream). Meanwhile, in the rainy period, the lowest value (3.0×10^1 CFU/100mL) was found in the station 3, and the highest value (1.47×10^4 CFU/100mL) in the station 1 (**Figure 4**).

3.3. Data Correlation

The Pearson correlation matrix showed important correlations between nutrients and *E. coli* contributing to infer the sewage influence on the eutrophication and sanitary risks in this system. The correlations established between the physicochemical variables (T, S, DO, and pH) and the nutrient and *E. coli* concentrations (**Figure 5**), in turn, can be useful to understand the influence of

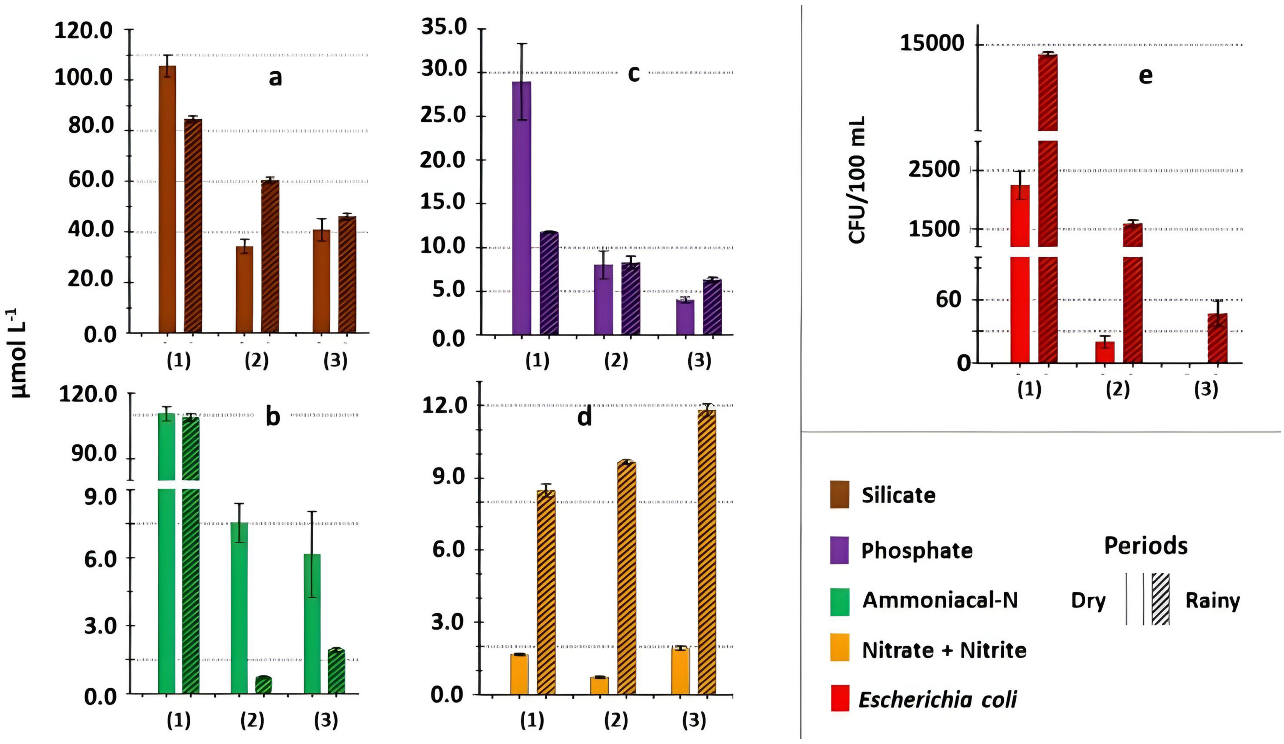


Figure 4. Concentrations (±standard deviation) of silicate (a), ammoniacal-N (b), phosphate (c), nitrate (d) and *Escherichia coli* (e), at surface waters of the rivers, Maratuã (station 3) and Crumaú (stations 1 and 2). Upper zone of the Bertioga estuarine channel (São Paulo, Brazil).

Dry period (October 2012)

	NH_4^+	NO_2^-	NO_3^-	PO_4^{3-}	Si	*E. coli*	OD	pH	Sol	Temp
NH_4^-										
NO_2^-	-0.580									
NO_3^-	0.314	-0.579								
PO_4^{3-}	**0.972**	-0.567	0.167							
Si	**0.985**	-0.560	0.415	**0.937**						
E. coli	**0.994**	-0.573	0.322	**0.959**	**0.975**					
OD	**-0.720**	0.095	0.420	**-0.790**	-0.644	**-0.715**				
pH	**-0.963**	**0.715**	-0.264	**-0.964**	**-0.924**	**-0.961**	**0.718**			
Sol	**-0.977**	0.463	-0.114	**-0.973**	**-0.943**	**-0.972**	**0.850**	**0.948**		
Temp	**0.701**	-0.075	-0.444	**0.774**	0.623	**0.695**	**0.994**	**-0.700**	**-0.835**	

Rainy period (January 2013)

	NH_4^+	NO_2^-	NO_3^-	PO_4^{3-}	Si	*E. coli*	OD	pH	Sol	Temp
NH_4^-										
NO_2^-	**-0.983**									
NO_3^-	**-0.73**	**0.824**								
PO_4^{3-}	**0.896**	**-0.945**	**-0.872**							
Si	**0.921**	**-0.966**	**-0.938**	**0.964**						
E. coli	**0.994**	**-0.993**	**-0.808**	**0.929**	**0.955**					
OD	-0.535	0.640	**0.930**	**-0.769**	**0.807**	**-0.619**				
pH	**-0.940**	**0.892**	0.645	**-0.835**	**0.824**	**-0.929**	**0.468**			
Sol	**-0.863**	**0.917**	**0.961**	**-0.947**	**-0.983**	**-0.911**	**0.888**	**0.790**		
Temp	**0.697**	-0.590	-0.070	0.380	0.376	0.620	0.232	**-0.684**	-0.240	

Variables: Nutrients - *Escherichia coli* - Physicochemical

Figure 5. Pearson correlation matrix ($p < 0.05$).

these abiotic members on the microbial metabolism in this system. In general, *E. coli* presented a significant negative correlation with S, pH, and DO (−0.97, −0.96 and −0.71 respectively) in dry sampling, maintaining this behavior for S and pH (−0.91, −093 respectively) in the rainy period. In general, it is possible to verify stronger ($p < 0.05$) correlations between *E. coli* and all nutrients in the rainy period (i.e. NH_4^+, NO_2^-, NO_3^-, PO_4^{3-} and Si of 0.99, −0.99, −0.81, 0.93 and 0.96, respectively). On the other hand, in the drier period, just the phosphate and silicate presented strong correlations ($r > 0.96$) with *E. coli*.

Regarding the nitrogen metabolism, it is important to mention that the ammoniacal-N was the most positively correlated to *E. coli* (0.99 in both periods), whereas the oxidized forms (nitrite and nitrate) presented strong negative correlations (−0.99 and −0.81, respectively) with *E. coli*, just in the rainy period. These results suggest a higher influence of fresh sewage on the whole aquatic system during the rainy period and a more intense N-oxidation in inner areas of the Crumaú River during the drier period.

4. Discussion

4.1. Influence of Hydrological and Hydrodynamic Factors on the Nutrient and *E. coli* Variation

First, it is important to mention that station 1 (Crumaú upstream) is located near to the point of reversal current where the local circulation is less intense. Highest values of salinity and weaker ebbing currents observed during the October sampling (2012) indicated a low river flow and hence a high influence of tidal creek dynamic (e. g. a higher efficiency of vertical transport during the tidal cycles) on the Crumaú and Maratuã mouths (stations 2 and 3). Also, the long period under rainfall absence before the sampling days in this period probably allowed a high local nutrient regeneration in the Crumaú upstream (station 1), where the water movement is effective just under high river flows.

On the other hand, the sampling period of January (2013) presented a high river discharge driven by the increase of precipitation, which was indicated by the low salinity variation observed between stations 1 and 2 (5 and 9, respectively) and the intense ebbing current in the Crumaú downstream (station 2). This hydrodynamic condition, in turn, reflected a period of water renewal in the Crumaú upstream and hence, favoring a transport of regenerated nutrients toward the lower zone. Besides, it is important to reinforce that the rainfall intensification normally increases the nutrient leaching from watersheds to the aquatic systems.

The strong significant negative correlation between salinity and silicate reflects the silicate input from terrestrial sources (e.g. leaching of sediments and sedimentary rocks) and its dilution by seawater toward the lower estuary. This longitudinal distribution of silicate was documented in several estuaries worldwide (Bell, 1994; Braga et al., 2000; Conley & Malone, 1992). The present study evidenced distinct silicate inputs to the aquatic system considering the evaluated sampling periods. On the one hand, the high rainfall index reported before the January (2013) sampling days (rainy period) probably unleashed diffuse terrestrial inputs from banks located between the stations 1 (Crumaú upstream) and 2 (Crumaú downstream), thereby explaining the silicate increasing observed in the downstream stations (2 and 3). In this case, this increment can be attributed to erosion processes that usually are intensified in deforestation areas during events of high rainfalls.

In contrast, the saline intrusion was evidenced as an important hydrodynamic factor to lead the dissolved nutrients from sediment pore water to the water column during the dry period. This vertical dynamic of nutrients was observed in several estuaries (Ovalle et al., 1990; Paudel et al., 2015; Li et al., 2017). In the phosphate case, the input from sediment can occur with the salinity increase during the saline water intrusion, since the sulfate from sea salt tends to promote the complexation between sulfide and iron, disfavoring the phosphate sequestration from the water column and maintaining it in the dissolved form (Hartzell & Jordan, 2012). In the Pearl River Estuary (Subtropical eutrophic estuary in China), Li et al. (2017) reported that, during a low river flow, a strong vertical mixing increased the levels of dissolved phosphate (1.44 ± 0.57 $\mu mol \cdot L^{-1}$) in the surface waters, whereas a river discharge input created a stratification and enhanced the phosphorous transport (associated to the suspended particulate matter) toward the lower zone.

In the Crumaú river, Ferreira (2002) verified at surface sediments, a gradient of sewage contamination indicated by phosphorus concentration, which ranged from 31 (upstream) to 16 $mg \cdot kg^{-1}$ (downstream). This suggests that the surface sediment in practically all the Crumaú river can represent important phosphate source to the water column, mainly when the saline intrusion (salt-wedge) reaches great distance toward the upper zone. As previously exposed, this dynamic was observed in the dry period under low river flow and high salinities. Meanwhile, the high river flow during the rainy period probably increased the transport of anthropogenic phosphorus from upstream to downstream, thereby explaining the phosphate increment in surface waters of the stations (2 and 3) and the strong correlation performed between this nutrient and *E. coli*.

The nitrogen transformation in subtropical and tropical estuaries also is highly driven by the magnitude of river discharges (Bianchi, 2007). Souza et al. (2006) reported considerable nitrate (12.1 $\mu mol \cdot L^{-1}$) and ammonium (2.8 $\mu mol \cdot L^{-1}$) values in the rainwater composition in the Ilha Grande region (about 300 km from Santos estuary), thereby demonstrating that the wet deposition also can be an important nitrogen input to regional estuaries. In turn, the organic matter decomposition in mangrove floodplains is an important input of DIN to the estuarine waters (Dittmar & Lara, 2006), which normally is intensified during the rainfall periods. In the present study, these nitrogen inputs probably were important contributions of nitrate to surface waters, as pointed out by the high concentrations observed in the samples collected in the rainy period. Besides, this period presented a considerable nitrate increment from the station (1) to stations (2) and (3), which probably responded to the leaching increase in agricultural areas over the eastern banks of the Crumaú river and the N-oxidation associated to sewage input in Crumau upstream.

E. coli presented significant strong positive correlations with ammoniacal-N and phosphate, pointing out the sewage sources around the Crumaú upstream as important inputs of these nutrients during the rainy period. The phosphate also is originated from minerals (e.g. apatite mineral), but its source into the terrestrial crust is considerably lower than the silicate one (e.g. aluminosilicate minerals) (Troeh & Thompson, 2007). However, the nitrogen remineralization from the organic matter decomposition can represent an expressive ammoniacal-N input to the water column of polluted estuaries due to the high presence of labile organic matter (e.g. sewage) (Braga et al., 2000; Azevedo & Braga, 2006; Berbel et al., 2015).

The relative high nitrate concentration in the station 3 (Maratuã downstream) observed in the rainy period can be related to the influence of the Crumau River plume during the flood tide, since the Maratuã upstream is not impacted by any significant human activity. The high *E. coli* presence in the station (3) observed in the rainy period reinforces this hypothesis. The fecal coliforms do not survive for a long time in marine environments, especially due to the action of salinity, high solar radiation, high water temperatures, and ecological factors (such as predation and competition) (Davies-Colley et al., 2008; Hughes, 2003; Jovanovic et al., 2017). Thus, the high river flow characterized in the rainy period probably minimized these inhibitory effects on the development of this bacterial group, thereby allowing a higher traveled distance alive toward the lower estuary. On the other hand, the phytoplankton assimilation (evidenced by the highest %OD values) can have contributed to the lower DIN concentration observed in the downstream stations (2 and 3) during the drier period. According to Ferguson et al. (2004), the DIN uptake by algal blooms and sediments accounted for most of the uptake DIN loading in the Brunswick estuary during low river flows.

According to Piveli and Kato (2006), the high ammoniacal-N percentual within the DIN total can be an indication of recent sewage inputs in urban aquatic environments due to the intense decomposition of organic nitrogen (ammonification process). In typical domestic wastewater, ammonia nitrogen represents about 55% - 60%, organic nitrogen about 40% - 45%, and nitrates plus nitrites together about 0 - 5% of the total nitrogen (Huang & Shang, 2006). These theoretical aspects can explain the high ammoniacal-N concentration found in the Crumaú upstream (station 1), as well as the strong positive correlation that the ammoniacal-N obtained with the *E. coli*. Also, it is important to report that the leachate from the Saco do Funil landfill can be a DIN input even more expressive than the releases of raw sewage to the Crumaú upstream. The bacterial decomposition makes intensified inside landfills due to the drainage process across the older leachate, from upper to deeper layers, leading to a decrease of organic matter concentration, and consequently contributing to an accumulation of the nitrogen reduced forms (Kalyuzhnyi & Gladchenko, 2004).

Multidisciplinary research has been carried out for years on the environmental status of the Santos estuary, which is one of the most important polluted estuaries in Brazil. The comparatively clean Cananéia estuary nearby (southern coast of São Paulo) is not impacted by any significant human activity and thus has been considered a suitable reference site (Azevedo & Braga, 2006). In comparison to average values obtained by the authors (op. cit.) in the Cananéia estuary, the present study revealed DIN and phosphate values around nine times higher. Meanwhile, similar average values of phosphate (5.5 $\mu mol \cdot L^{-1}$) and DIN (150 $\mu mol \cdot L^{-1}$) were observed in a tributary river of the Guanabara Bay (about 450 km from Santos estuary) (Brandini et al., 2016).

4.2. Microbial Metabolism Analysis Based on Physicochemical Parameters, Dissolved Inorganic Nitrogen Species, and Escherichia Coli Bacteria Group

The highest values of salinity observed in the downstream stations (2 and 3) responded to the greater marine influence on these estuarine sites, explaining the strong correlations performed between salinity and pH values. Generally, river waters present lower pH than marine waters, and a linear increase in the values occur with the salinity increase. Within a tidal cycle, the lower zones (mouths) of tributary rivers usually present a higher water renewal than in upper zones. In the mixture zone, the carbonate-bicarbonate-CO_2 system influenced by the buffer capacity of the seawater acts on the neutralization of H^+ ions, and consequently, promotes the pH increase. Besides, the pH also can increase with the primary productivity, since the phytoplankton community absorbs CO_2 from water for the photosynthesis process. Under ideal sunlight conditions, Zhang et al. (2019) reported that the estuarine eutrophication resulted in high biomass of phytoplankton and elevated the pH in surface waters. This biological process can have contributed to the pH and DO values observed in the downstream stations (2 and 3), mainly during the dry period due to higher oxygen saturation (% DO).

On the other hand, the organic matter decomposition process in aquatic environments normally produces CO_2 and consumes the DO. This process likely contributed to the lowest pH and %DO (less than 50%) values observed in the Crumaú upstream (station 1), since this local is under low hydrodynamic and is closer to the sewage sources. According to Breitburg (2002), hypoxic conditions in coastal waters are favored under %DO values less than 50%. Diaz & Rosenberg (2008), in turn, reported that DO values lower than 2.5 $mg \cdot L^{-1}$ is considered one of the most threats to coastal waters worldwide. Moreover, the eutrophic estuarine zones may provide bacterial biomass higher than phytoplankton biomass (Abreu et al., 1992), since the respiration rates in bacteria may exceed phytoplankton production (Giorgio et al., 1997). In both periods, this scenario of heterotrophic metabolism was strongly evidenced in the station 1 (Crumaú upstream).

The concentration ranges of ammoniacal-N observed in the station 1 were similar to reported in bioreactor environments by Anthonisen et al. (1976). These authors, concerning the two steps of the nitrification process in bioreactors, observed that free ammonia inhibited the Ammonia-Oxidizing Bacteria (AOB) at concentrations as high as 10 $mg \cdot L^{-1}$ (~110 $\mu mol \cdot L^{-1}$) and the Nitrite Oxidizing

Bacteria (NOB) at concentrations as low as 0.1 mg·L^{-1} (~1.1 μmol·L^{-1}). Besides, under temperatures higher than 20˚C and alkaline conditions, a considerable amount of ammonium (NH_4^+) cations is converted to ammonia gas (NH_3) in the aqueous phase (Huang & Shang, 2006). Despite the pH values next to 7, the high temperatures (26˚C - 28˚C) associated with the high ammoniacal-N concentration (~110 μmol·L^{-1}) likely disfavored the nitrification process in the Crumaú upstream (station 1).

For a long time, Denitrification (Dettmann, 2001; Burgin & Hamilton, 2007) and Ananmox (Trimmer et al., 2003) have been pointed out as the main processes in the transformation of dissolved inorganic nitrogen (DIN) into gaseous products in eutrophic estuaries worldwide. In subtropical estuaries, Fernandes et al. (2012) showed that denitrification is more important than anammox in sediments, whereas Zhu et al. (2018) showed that the water column can present a high denitrifying activity mediated by suspended particulate matter. However, recent reports on the contribution of dissimilatory nitrate reduction to ammonium (DNRA) to nitrogen removal in these systems indicated a similar or higher importance (Koop-Jakobsen & Giblin, 2010; Dong et al., 2011; Giblin et al., 2013). Moreover, the NO_3^- affinity manifested of nitrate ammonifier bacterias is higher than that by denitrifying bacteria at temperatures above 10˚C (King & Nedwell, 1984; Oglivie et al., 1997), and the heterotrophic DNRA is stimulated in environments with high availability of organic carbon (Tiedje, 1988; Yin et al., 2002). These theories suggest that the Crumaú upstream zone, due to the high temperatures and the constant labile organic matter ‘inputs (e. g. sewage), is an environment propitious to this microbiological pathway on the nitrogen transformation.

According to Einsle et al. (1999), DNRA is a facultative, two-step anaerobic process involving nitrate (NO_3^-) reduction to nitrite (NO_2^-) followed by the 6-electron reduction of nitrite to ammonium (NH_4^+). Regarding the last step, the nitrite reduction to ammonium can be catalyzed by the cytoplasmic NADH-dependent nitrite reductase NirB or its to two-subunit variant NirBD and/or the periplasmic pentaheme cytochrome *c* nitrite reductase NrfA (Harborne et al., 1992). In this case, it is important to know that *Escherichia coli* was shown to harbor and express genes for both enzymes (Cole, 1996; Wang & Gunsalus, 2000). In addition, Bonin (1996) observed in strains (isolated from coastal marine sediment) of *Escherichia coli* specie that the accumulation of acetate and formate occurred with NO_2^- reduction to NH_4^+.

The present study has not an analytical structure to point out which was the most important microbiological pathway (Anammox, Denitrification, or DNRA) on the nitrogen transformation at each sampling station. However, we raise the hypothesis that the DNRA obtains importance when *E. coli* that is enough representative in a group more extended of bacteria in upper zones of subtropical eutrophic estuaries, in this case, submitted to high sewage discharges under periods of high river drainage. The rainy period established a strong negative correlation between the oxidized forms of nitrogen (nitrite and nitrate) and *E. coli* colonies, thereby evidencing a considerable nitrate reduction associated to this bacterial group. This evidence is reinforced in the Crumaú upstream (station 1) during the rainy period since the heterotrophic metabolism of this area was associated with high values of ammoniacal-N/Nitrate ratio and colony-forming units of *E. coli*.

5. Conclusion

The estuarine systems are submitted to the terrestrial and anthropogenic influence and the nutrients as N and P reveal the terrestrial inputs from land to the estuary as well as the *E. coli* demonstrate the human influence on the sanitary condition of the hydrological system. Besides it, the aquatic system presents strategies to metabolize the nutrient inputs and the microbial conditions as the case of dissolved oxygen and salinity that act on the *E. coli* surviving and in availability of nutrients.

In this study, the results involving concentrations of dissolved inorganic nutrients and *E. coli* colonies in the environmental context considering river flow and physical and chemical distribution revealed distinct surface water biogeochemical behavior observed between dry and rainy weather conditions. In general, this study showed that the freshwater runoffs drive considerably the availability of nutrients, N-forms, and *E. coli* input, which together act on the microbial metabolism of this upper zone of the Bertioga estuarine Channel. The Crumaú River presented a lower self-depuration during the rainy period due to the fresher sewage (recently introduced), which was pointed out by the high concentrations of nutrients and *E. coli* observed in the surface waters of the downstream stations.

In both periods, the low oxygen saturation and high ammoniacal-N concentration demonstrated a domain of heterotrophic metabolism in the Crumaú upstream. On the other hand, in the downstream stations, the higher nutrient concentration and lower oxygen saturation observed in the rainy period responded to intensifying the river flow and the leaching of their banks. In summary, it is possible to attribute evident alternation in metabolism predominance (from autotrophic to heterotrophic) in the downstream zones of these tributary rivers of the Bertioga channel during events of high river flows. So, the influence of the river flow was evaluated and it is recommended a continuity of this kind of observation to better understand the metabolic processes that occur in the coastal zone with intense hydrodynamic and in different rain regimes.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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