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Correlation Between Total Bacteria and Inorganic Nutrients in Water of Camboriú River and Beach (Brazil)

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ABSTRACT

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Balneário Camboriú is one of the most important resort beaches in the south of Brazil. During summertime, the increase in population amount causes an increase of sewage that came to treatment plant. This change leads to higher numbers of coliforms bacteria and nutrients in this same environment. The objective of this study was to verify the total bacteria biomass and its relationship with chemical parameters in Balneário Camboriú (Brazil). The water samples were collected between June-2000 and July-2001 every two weeks, in five sites. The PO_4^{3-} and NH_4^+ were determined by colorimetric methods and the estimative of bacteria biomass was determined by fluorescence microscopy method, using orange acridine like color. The results obtained by this work suggest that this estuary is in eutrophic state influencing the water quality of Balneário Camboriú Bight. The levels of PO_4^{3-} and NH_4^+ in the river are higher that levels established by Federal Legislation, with annual mean of 0.14mg-P/L and 12.05mg-N/Lm respectively. The bacteria showed different behavior in river and beach environments. In the river, the bacteria community showed a direct correlation with NH_4^+, PO_4^{3-}, and temperature; and in the beach, this community has a direct correlation just with temperature, characterizing this variable like a main factor controlling bacteria biomass.

ADDITIONAL INDEX WORDS: *Estimative bacteria biomass, inorganic nutrients, wastewater.*

INTRODUCTION

Heterotrophic bacteria have been played in aquatic environment its importance and role in food chain due its capacity to transfer dissolved and particulate organic matter for higher trophic levels. This process is realized by recovery these organic compounds to alive biomass, or by its remineralization to inorganic compounds (CARLSSON and CARON, 2001).

The studies in microorganism chain, showed that bacteria are efficient in assimilate PO_4^{3-} and NH_4^+, competing with phytoplankton (GOLDMAN *et al*., 1987). In marine and fluvial environments, about 60% of PO_4^{3-} and 30% of NH_4^+ present in these environments are assimilating by these bacteria (CESAR,1997).

In whole of the world, main areas heavily occupied, like coastal areas, have been showed a significant increase in a load of nutrients in them waters, they became by direct or indirect discharge of domestic sewage (LEEMING *et al.*, 1998). These excessive discharges could be favorable or limit the growth of bacteria biomass (HASSAN, 1993), observed by POMEROY *et al.* (1995), in laboratory experiments realized by PO_4^{3-}.

Besides these factors, physical parameters (temperature, Dissolved Oxygen-DO, salinity and pH) by each environment can to influence in the bacteria's growth and enzymatic activities, and these factors can be sometimes more relevant than discharge of nutrients.

Balneário Camboriú, located in Santa Catarina, southern of Brazil, is one of the beaches most looked for tourists during summer season. During this time, the nutrients concentrations in Camboriú River and Balneário Camboriú Beach waters show a great increase (KUROSHIMA *et al.*, 2000), which is caused by direct and indirect discharge of domestic sewage. These discharges of organic and inorganic matters to the water environment can increase, probably, a bacteria biomass, too, or they can limit its growth.

In this context, the objective of this paper is to estimate bacteria total biomass and verify its relation with physical (temperature, DO, salinity and pH) and chemical (inorganic nutrients) in Balneário Camboriú Beach and Camboriú River.

Table 1. *Description of samples collection sites*

Sample collection sites	Description of samples collection sites
1	river: dam upstream, water caption to municipal supply
2	river: discharge of municipal sewage treatment plant.
3	beach: Southern area, influenced by river discharge
4	beach: central area, influenced by pluvial discharge
5	beach: northern area, influenced by natural channel

METHODS

The water samples were collected superficially, at 0.5meters depths in Camboriú River and 1.0 meter in Balneário Camboriú Beach, every two weeks, between June-2000 and June-2001, in fixed stations (Table 1, Figure 1). All samples used to chemical determinations were collected in five liters cleaned plastic bottles (pre-cleaned with diluted HCl). Samples to bacterial biomass 5 determination were collected in 0.25liters plastic bottle, cleaned with detergent neutral, and preserved with formalin (40%). Total coliforms determination samples were collected in 0.3liters sterilized glass bottle. All the samples were kept in dark and refrigerated during they transport to university laboratory.

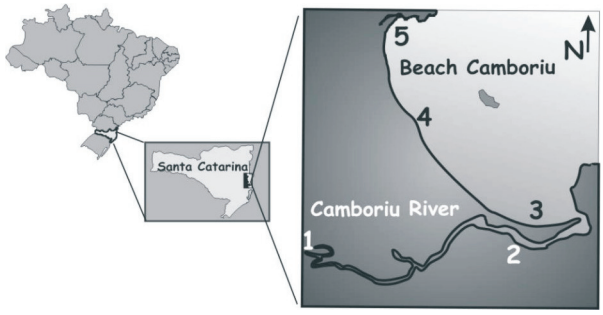

Figure 1. Study area, it shows the samples collection sites in the river and in beach.

Physical-chemical parameters (pH, temperature, salinity and DO) were determined *in situ* using a multianalizer Horiba (model U-10).

Samples collected to inorganic nutrients analysis were filtered (HA Millipore filters, 0.45µm porosity) separated in some aliquots and determined using colorimetric methods.

The phenate method was used to determine NH_4^+, by it reaction with hypochlorite, phenol and sodium nitroprusside forming a intense blue compound. The NO_2^- were determined through formation of reddish purple azo dye (reaction of nitrite with diazotized sulfanilamide followed by *N*-1-naphthyl-ethylenediamine dihydrochloride), NO_3^- was determined by cadmium reduction method. In this method NO_3^- turn into NO_2^-, which was determined according to previous description. PO_4^{3-} was determined by ascorbic acid method producing an intense colored molybdenum blue compound. All procedures were realized according to methods describing in APHA (1998). The chemical analyses were carried out immediately after samples arrived in laboratory in the same day, and were made in triplicate

Biomass bacteria estimative were determined according to methods described by HOBBIE *et al.* (1977). The bacteria were counted directly by epifluorescent microscopy of formalin-preserved samples after staining with acridine orange (AO) and collection on Nuclepore filters (0.2µm porosity, 25mm diameters).

RESULTS AND DISCUSSION

Physical Parameters

The annual mean values obtained to physical parameters are summarized in Table 2. They were separated in river and beach environment due difference physic characteristics.

Temperature temporal distribution registered to river and beach showed a characteristic pattern observed to subtropical regions, like is Balneário Camboriú city. The highest values were observed during summer season and lowest, during winter season (Figure 2A). The annual mean temperature value observed to study area was 21.7 ± 4.01°C without significant difference between these two environment (Table 2). The lowest value registered during all time evaluated was 10.2°C and the highest was 30.8°C, observed respectively in Jul/00 and Feb/01. Spatially, these distributions didn't have differences.

The temporal distribution of DO followed inverse pattern observed to temperature, showing lowest value during summer season (2.08mg-O_2/L) and the highest value in winter-time (9.34mg-O_2/L). These patterns are indicating the occurrence of physical processes controlling the concentration of DO, besides this, biological processes could have occurred, too. In high temperature the bacterial activities are intensified, so, consume of DO by these microorganisms can increase, decreasing they concentration in water. The mean values observed to river were slightly lower than observed to beach, due probably, to more intense physic mixture by waves action in beach area (Figure 2B).

Salinity temporal distribution not showed seasonal differences, oscillating during all the time evaluated (Figure 2C).

Table 2. *Annual Mean and standard deviation (SD) to physical, chemical and microbiological parameters evaluated.*

	River		Beach	
	Mean	SD	Mean	SD
Temperature (C)	21.3	4.3	21.9	3.9
OD (mg/L O_2)	5.30	1.62	7.05	1.50
Salinity (psu)	2.8	2.4	29.7	2.4
pH	6.73	0.27	7.83	0.14
NH_4^+ (mg-N/L)	12.05	6.14	0.18	0.14
NO_2^- (mg-N/L)	0.02	0.01	0.01	0.01
NO_3^- (mg-N/L)	0.08	0.03	0.03	0.01
PO_4^- (mg-P/L)	0.14	0.18	0.02	0.02
Bacteria (cell/ml)*	$1.15x10^{16}$	$2.48x10^{16}$	$1.08x10^{15}$	$3.97x10^{15}$

* geometric mean

SCHETTINI *et al.*, (1996), in previous study realized in the same area, showed a variation between 16 to 25PSU in a fixed sample station, this variation were registered during five hours in a neap tide. KUROSHIMA *et al.* (2000), showed that salinity in this area are influenced by tide and by precipitation, mainly in areas influenced by rivers discharges. During the period evaluated the salinity's highest value was 34.4PSU, observed in site 4 in Jul/00 and lowest value was 0.0, observed in site 1. The annual mean values are summarized in Table 2.

Chemical Parameters

Table 2 summarizes the annual average values and standard deviation registered to chemical and microbiological parameters observed in river and beach samples. The average values used to microorganisms count were geometric mean. Ammonium was the most representative among dissolved inorganic nitrogen (DIN= NH_4^+ + NO_3^- + NO_2^-) nutrients evaluated. In the river, the annual mean value was 67 times higher than observed to beach samples (Table 2).

The highest value to NH_4^+ was 56.7mg-N/L, observed in site 2 during Jan/01, and the lowest was 0.01mg-N/L (site 4 in Jul/00). According to PEREIRA FILHO (2002), these values are extremely high to natural waters, other Brazilian coastal areas influenced by anthropogenic activities showed lowest values (BRAGA *et al.*, 2000).

These high values can be occurred by discharge of municipal sewage treatment plant in this river (site 2). This plant just realizes the treatment of organic matter and microorganism (coliforms), and it not decreases inorganic nutrients. By this reason, when the treated effluents of this plant are discharged in the river, inorganic nutrients concentrations in it are very high due the mineralization or organic matter occurred in sewage treatment plant's facultative lagoons. This increase in inorganic nutrients is higher during summer periods when the population is higher, too, and the sewage treatment is not enough to that demand.

NO_2^- concentrations were the lowest among DIN (Table 2). The highest values was 0.076mg-N/L during all time evaluated, observed in site 2 (Jul/00).

NO_2^- and NH_4^+ spatial distribution's annual mean values (Figure 3) are indicating the same fonts for both. The highest values for both were observed in site 2, in front at sewage treatment plant effluent. So, this discharge could be the main font to both nutrients.

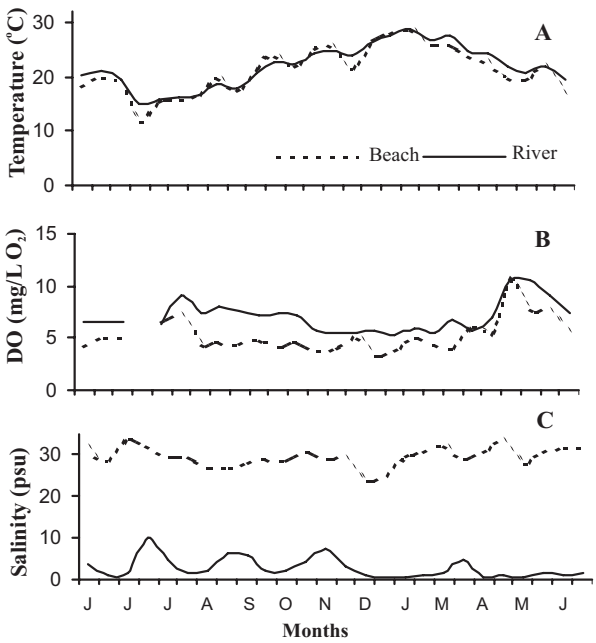

Figure 2. Temporal distribution of mean physics parameters: temperature (A), DO (B) and salinity (C), in river and beach.

Spatial distribution to NO_3^- showed different pattern, the highest values were observed upstream of river, which were decreasing in direction to the beach (Figure 3), suggesting another contribution to this nutrient.

This pattern takes a suggestion that the main contribution to NO_3^- to this environment could be the fertilizers spread used by agricultural activities, river upstream. According to SEMHI *et al.* (2000), river NO_3^- concentrations have positive correlation with municipal population indices, lixiviation processes and with fertilizers spread, and about 85% of total nitrogen amount became from agricultural activities, which of them 60-70% became from fertilizers.

In the beach, the highest values were observed in site 5, influenced by small natural channel that receive a lot of raw domestic sewage. These results show that NO_3^- in this environment is influenced by two processes: contribution of fertilizers used river upstream and oxidization of NH_4^+ discharged by domestic effluent.

The temporal distribution of nitrogenous compounds showed that during all the time monitored occurred oscillations in their concentrations and they don't show a seasonality pattern (Figure 4).

These oscillations may be evidencing the hypothesis that the concentrations of these nitrogenous compounds have great anthropogenic influences, which don't have a seasonal cycle.

Spatial distribution of PO_4^{3-} showed the same pattern observed to NH_4^+, with high values in river environment and low values in beach, this increase was about 7 times the values observed in beach. The highest value observed to PO_4^{3-} was 1.23 mg-P/L observed in site 5 (Dez/00), and the lowest value was 0.01mg-P/L, observed in site 4 (Mar/01).

ARAUJO *et al.*, (1995) studied stabilization lagoons from sewage treatment plants and observed that with decreasing retention time of sewage in each lagoon, followed by increasing in organic matter input in it, occurred a functional disorder in that lagoon. As a result, decreases in the remobilization of phosphorus compounds from the system are observed.

This process could be occurred in this system plant, considering that a increase in population is observed during summer season, and consequently a increase in raw sewage that input to sewage treatment system.

Bacteria Biomass

The bacteria counting methods used in this study can't differentiate the kinds of bacteria that are present. Therefore, the results of this method can just estimate the bacteria biomass.

To evaluate all the chemical and physical variables analyzed in this study with bacteria biomass was realized principal components analyses (PCA). This kind of statistical methods can correlates all the variables in the same time, turn in easily the interpretations.

The PCA was realized to beach and river in separate analysis. The first plan could explain 55.7% of variables dispersion for beach data and 55.1% for river environment.

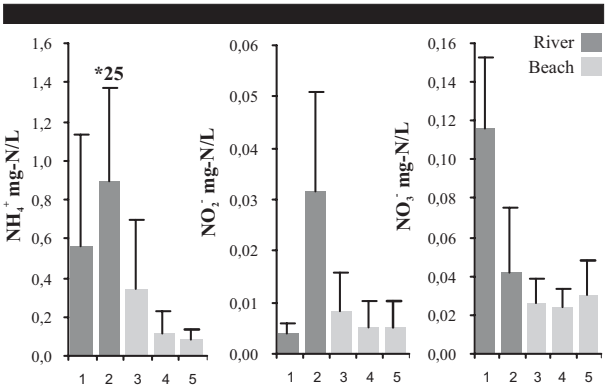

Figure 3. Spatial distribution of mean values with respective standard deviation to NH_4^+, NO_2^- and NO_3^- to all samples sites.

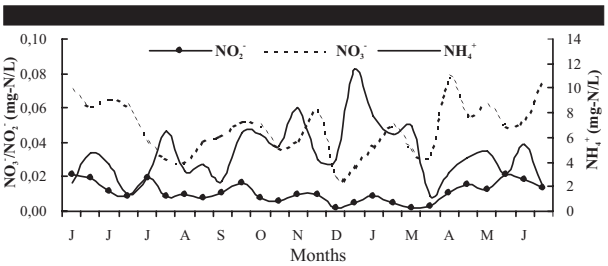

Figure 4. Temporal distribution of mean of nitrogenous compounds during all the time evaluated.

Bacteria and Physical Parameters

The annual mean values observed to number of bacteria in the river were always higher than values observed in the beach. The highest value was $3.1x10^{19}$ cell/ml, observed in February-2001 (site 1) and the lowest was $1.10x10^{6}$ cell/ml, in July-2000 (site 4).

Temporal distributions of bacteria showed a seasonal pattern (Figure 5A), with high value in summer season, decreasing during wintertime. This pattern is indicating a strong influence of temperature in bacteria biomass distribution (Figure 5B), in both environment, river and beach.

The PCA analysis showed the same correlation between temperature and bacteria biomass (Figure 6). LI (1998) suggests that in cold environments, the temperature is the most important factor controlling the bacteria growth, but in higher temperatures environments, besides of this factor, the input of substrate seems to be an important factor too.

DO distribution showed an inverse correlation with bacteria number, mainly in the beach environment (Figure 6). This pattern was suggested since temperature and DO have a indirect correlation.

Some kind bacteria need oxygen to make its metabolic activities and obtain energy. Aerobics and facultative aerobic bacteria have preference to utilize oxygen for their respiration processes, because this mechanism can supply most energy than fermentation process. However, the oxygen distribution is controlled by physical process, too.

Other physic variable that is related in study of bacteria biomass's dynamics is salinity. This parameter has a deleterious effect on bacteria typically river water and could be an important factor to be investigated in estuarine environment strongly influenced by tides, like Camboriú river. In beach environment, bacteria that living there is more tolerant to high salinity and this effect are not observed. In Figure 6, we can notice that this effect is evidence; the salinity has an inverse correlation with bacteria biomass in river environment (Figure 6A). In the beach, this negative correlation is not observed (Figure 6B).

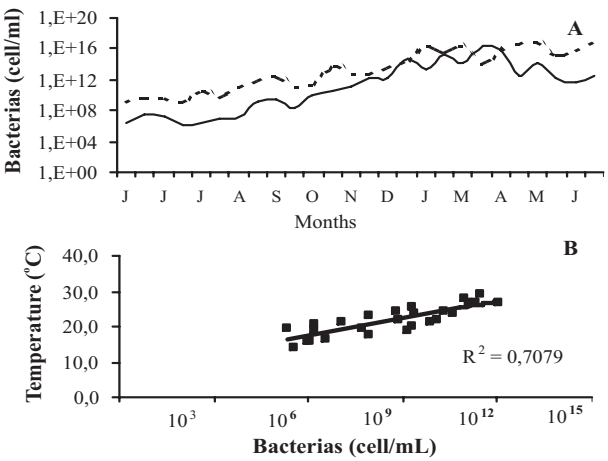

Figure 5. Temporal distribution of number of bacteria in river (----) and beach (—) environment (A) and its correlation with temperature (B).

Bacteria and Chemical Parameters

The bacteria growth can be limited by diverse factors, that which we can select the composition of organic matter that arrives to environments, predator's action, micronutrients and inorganic nutrients (CHURCH *et al.*, 2000). For number of bacteria in an environment, the factors that influence them is growth processes, predation and physical processes of mixture of water column. Each of these factors can limit bacteria growth in different scales, spatially and temporally.

In this work, was evaluated the influence of inorganic nutrients to bacteria biomass due the Camboriú River and adjacent coastal area (Camboriú Bight) receives a great discharges of domestic sewage. Precedent studies realized in this area (MORELLI, 1997, KUROSHIMA *et al.*, 2000, PEREIRA FILHO, 2001), always showed this excessive input of organic compounds to this environment.

The bacteria are considered a great competitor with phytoplankton by nutrients assimilation like NH_4^+ and PO_4^{3-}, and in some systems are the bacteria responsible to control the flux of this nutrients in the environment.

The results obtained by PCA shows that bacteria from river and beach have a different answer to nutrient input to these environments. In the river, we notice a direct correlation between bacteria biomass and NH_4^+ and PO_4^{3-}, this last nutrient shows the best correlation (Figure 6A). The river Camboriú receives a great amount of nitrogenous compounds, from domestic and rural activities, described before.

During a great time, it was believed that bacteria have just assimilated amino acids, and only phytoplankton assimilates NH_4^+ and PO_4^{3-}.

CESAR (1997), realized inorganic nutrients enrichment laboratorial experiments, and noticed that the major density bacteria occurred in estuarine waters enriched by NH_4^+. The experiments realized with PO_4^{3-} showed a good correlation, too. It was observed a quick bacteria growth followed by decrease, indicating utilization of this nutrient during exponential bacterial growth.

In the environment evaluated, we have great concentrations of these nutrients with possibilities the growth bacteria that utilize these nutrients for their metabolic activities, and so, we have a direct correlation between these parameters.

BRION and BILLEN (2000), stand out that impacts made by domestic effluents discharges not affecting just water quality, but microbial dynamic too. These authors verified in Seine River (France), those in locations where occurred sewage treatment with decantation or filters use, was verified a decrease in nitrifying bacteria biomass in the final effluents, resulting in the high concentrations of NH_4^+ along the all the river. Just after the growth of these bacteria, it was observed a decrease on NH_4^+ concentrations, which means, its oxidation. In sewage treatment plant where doesn't have these kind of treatment, the nitrifying bacteria biomass in the river that receives its effluent showed growth up, and their NH_4^+ concentrations were very low.

In Camboriú River, it was verified during all studied period, high NH_4^+concentrations, and spatially, biomass bacteria decaying, occurred in front of Municipal Sewage treatment effluent discharges (Figure 7). This treatment plant doesn't have techniques to remove particles, but is added ClO_2 in final effluent, a strong bactericide used to eliminate bacteria of coliforms genus. This bactericide could be acting over nitrifying bacteria that are present in this sewage effluent and later are discharged in river, so, occurred a reduction in bacteria biomass in specific point in this river, the site 2.

In the beach environment, bacteria showed a inverse correlation with NH_4^+ and PO_4^{3-} nutrients (Figure 6B), and showed a direct strong correlation with temperature. DUCKLOW and CARLSON (1992) noticed in their study the importance of free amino acids for coastal bacteria. These authors verified that in estuarine environments (rich in nutrients) occurred a great absorption of nutrients and amino acid and in coastal areas (low nutrients concentrations), predominate glucose absorptions.

CHURCH *et al.* (2000) studying the bacteria growth limitations in coastal areas, verified that in sub-tropical environments, the experiments that have a great bacteria growth was related in that was added glucose associated with NH_4^+ and PO_4^{3-}. In the experiments where was added just nutrients, the bacteria growth didn't have a significant increase.

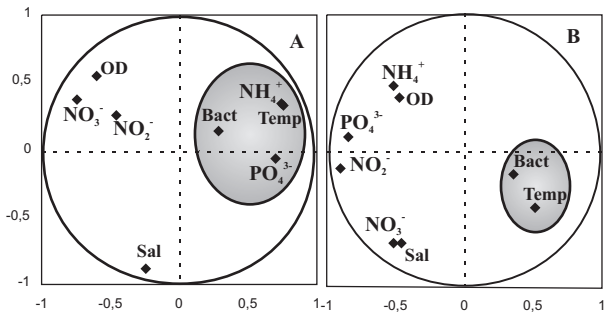

Figure 6. First plan for variables evaluated in river (A) and beach (B) environment, using PCA.

CONCLUSIONS

The Camboriú River estuarine shows high inorganic nutrients concentrations, and it can be classified as eutrophic state conditions when it is compared to another regions, that have impacted conditions. A great part of this nutrient enrichment became from input of municipal sewage treatment effluent, that is located close to river discharge. The resulted of this, the area in bight located closer to this river discharge, showed during all the time, the highest nutrient concentrations.

The biomass bacteria showed distinct behavior between river and beach environment. For river bacteria community, verified a direct correlation between bacteria and NH_4^+, PO_4^{3-} and temperature. In addition, indirect correlation was observed between bacteria an salinity, indicating that these bacteria could be typically from river water.

In the beach environment, was obtained a direct correlation between biomass bacteria with temperature, and inverse with nutrients that predominate in estuarine system. So, we can conclude that in both environment studied, the most important factor that influence biomass bacteria is temperature, since it showed the best correlation with biomass bacteria.

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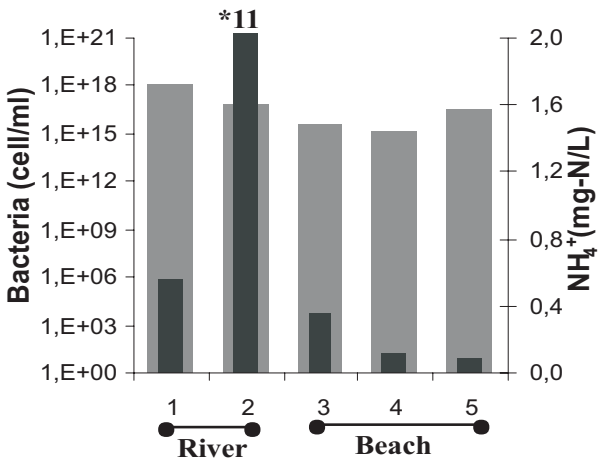

Figure 7: Spatial distribution of bacteria and ammonium mean value, in river and beach environment.

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