



Monitoring of fecal indicator bacteria in two Salvadoran estuaries

JOSÉ ENRIQUE BARRAZA* & VERÓNICA MELARA

Institute of Science, Technology and Innovation, Francisco Gavidia University, El Salvador.

* Corresponding author: jebarraza@ufg.edu.sv

Abstract: Thermotolerant coliform and enterococci levels in surface water as well as physicochemical parameters were measured monthly at estuaries Barra de Santiago and Barra Salada from October 2020 to September 2021. Most of results show that rainfall didn't associate to high bacteriological abundances in both aquatic ecosystems, but for enterococci at Barra Salada where correlation index was high ($r(8) = .96, p < .01$). Ocean inputs and river discharges seem to influence surface water parameters. No strong relationships were found amongst bacteriological densities and dissolved oxygen, pH, salinity, temperature.

Key words: mangrove, pollution, wastewater, water quality.

Monitoreo de bacterias indicadoras fecales en dos estuarios salvadoreños. Resumen: Los niveles de bacterias coliformes termotolerantes y enterococos en agua superficial, así como parámetros fisicoquímicos fueron medidos mensualmente en los estuarios Barra de Santiago y Barra Salada, desde octubre 2020 a septiembre 2021. La mayoría de resultados muestran que la precipitación no se asoció a abundancias bacteriológicas altas en ambos ecosistemas, con la excepción de enterococos en Barra Salada donde el índice de correlación fue alto ($r(8) = .96, p < .01$). No se encontraron relaciones fuertes entre las densidades bacteriológicas y oxígeno disuelto, pH, salinidad, temperatura del agua superficial.

Palabras clave: manglar, contaminación, agua residual, calidad de agua.

Introduction

Several human activities and meteorological factors influence the level of fecal pollution in aquatic environments. A common tool to assess this kind of pollution in water bodies uses the quantification of coliform bacteria as well as enterococci. Their occurrence in aquatic ecosystems reflects the presence of human and warm-blooded fauna feces that are public health concern (Mote *et al.* 2012, Larrea-Murrell *et al.* 2013, Paruch *et al.* 2019).

Important aquatic ecosystems comprise estuaries all over the world, since they provide many environmental services such as: human living, transport, storm barriers, and fishing activities, however their environmental quality is deteriorating worldwide (Day *et al.* 2012, Thrush *et al.* 2013, Yee *et al.* 2019, Angelo & Glass 2021). Mangroves are tropical estuarine ecosystems that encompasses

amphibious forests adapted to tidal and salinity changes (Carugati *et al.* 2018).

Many human settlements are located around estuarine areas in El Salvador, including Barra de Santiago and Barra Salada (Domínguez *et al.* 2018). Despite human intervention including domestic wastewater and manure pollution, both aquatic ecosystems provide services such as fisheries and shellfish extraction. Moreover, the Ministry of the Environment and Natural Resources (MARN in Spanish) manages sustainable policies and law implementation in these areas.

Fecal matter monitoring in estuarine ecosystems in El Salvador encompasses studies using thermotolerant coliform quantification in water or bivalves (Melara-Pérez 2006, Campos-Machado 2007). Also, Guevara-Surio (2015) measured the levels of these bacteria at touristic beaches, and so did Quintanilla-Corena (2020) at gulf of Fonseca. The presence of these bacteria in

water may reflect health hazards due to human exposure to different pathogenic microorganisms. The determination of monthly thermotolerant coliform and enterococci bacteria levels, as well as the influence of aquatic physicochemical parameters could support the management of the involved estuaries by health and environmental authorities. This study reflects the variation of surface water parameters such as dissolved oxygen, pH, salinity, temperature, rainfall as well as distance from ocean inlet that could influence abundances of the mentioned bacteria.

Materials y Methods

Study sites: The two Ramsar Sites are located on the western part of the country. The location of the five sampling stations at Barra de Santiago (T) and Barra Salada (D) are presented in Figure 1. Sample station distances to ocean inlet are included in Table I. Both estuaries present connection to the Pacific Ocean through inlets, however at the latter the sea influx stops during some months of dry season (March and

April), and it is opened again, at different areas using machinery or by the rain that pushes water seawards.

Barra de Santiago (13° 42' 24" N, -90° 0' 59" W) is a Ramsar Site that comprises 11519 hectares of freshwater wetlands, mangrove forests, estuarine channels, rivers, sand bar, human settlements, and agricultural-livestock activities. Sometimes these productive activities alter the different aquatic habitats. It encompasses the mangrove ecoregion of the Northern Pacific of Mesoamerica where many international and national endangered aquatic species inhabit such as: *Cayman crocodylus*, *Crocodylus accutus*, *Hippocampus ingens*, other fishes and invertebrates. About 26000 humans are associated to this wetland, and most of them don't have a sanitary sewer system, so an important fraction of these aquatic residues reaches rivers that join this estuary (Barraza *et al.* 2014). Some of the associated rivers are: El Naranjo, Cuilapa, Guayapa, Aguachapío and Izcanal (Beltrán-Mayorga 2017).

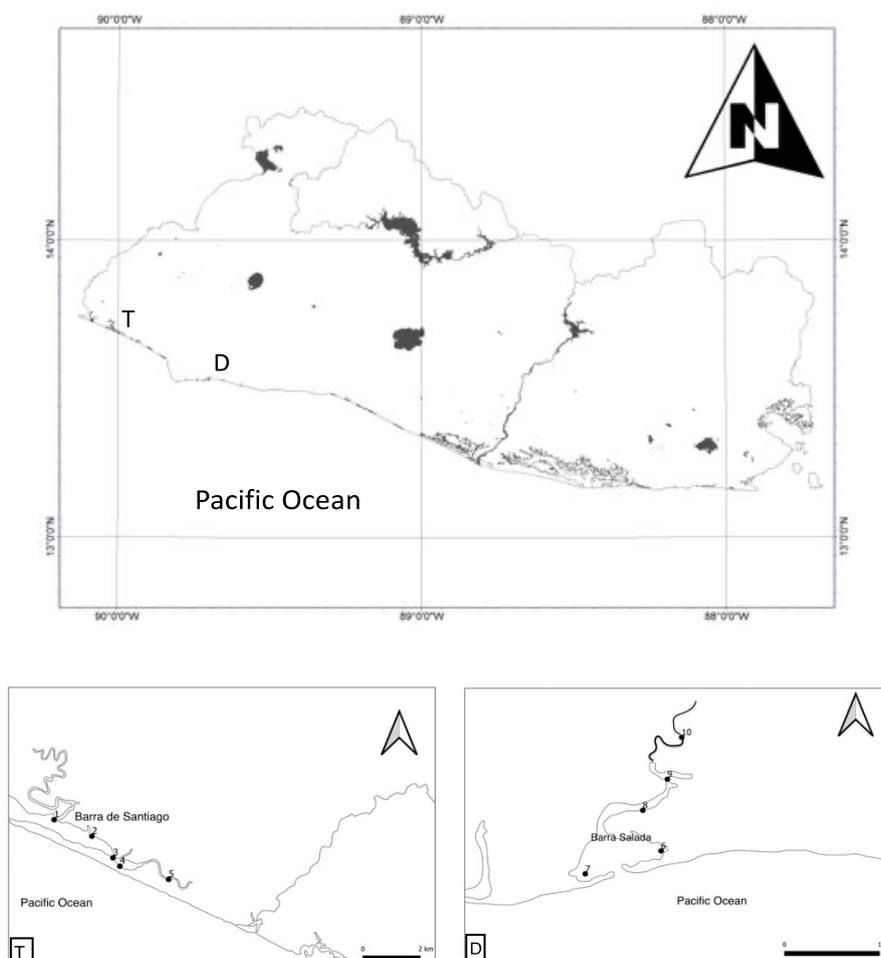


Figure 1. Location of estuaries and sampling stations. D: Barra Salada, T: Barra de Santiago.

Barra Salada estuary (13° 32' 5.7" N, -89° 42' 13.6 W) embraces 414 ha and is included within Complejo Los Cóbano Ramsar Site (21312 ha). This estuary is inhabited by 108 families that live in the mangrove area, and there were alterations caused by agricultural expansion. Also, the rivers that merge to this estuary receive wastewaters from human communities (Méndez-Granados 2019).

Sampling: Two surface water samples (500 ml each) were taken in sterilized dark glass bottles per monitoring station (Figure 1 and Table I). All of water samples were stored at 4 °C within an ice box and delivered to a private laboratory for counting thermotolerant coliform (C) and enterococci (E) (MPN/100 ml) (APHA, AWWA, WEF, 2017) within less than four hours. We measured pH, temperature, salinity (YSI Professional Plus), as well as dissolved oxygen (YSI ProODO) in water surface at each sampling station. The monitoring period ranged from October 2020 to September 2021, however no sampling occurred in January and February 2021. Dry season ranges from November to April, and the rainy period from May through October. Temperature and bacteriological levels were recorded twice per sampling station (5) during the whole study that included 10 months (N = 100) at both estuaries. The statistical comparisons with other parameters that were taken once per station (dissolved oxygen, temperature, salinity, pH, distance of ocean inlet) included the determination of geometric means of paired microbiological data per station (N = 50) for statistical comparisons. Moreover, monthly cumulative rainfalls (10 per estuary) and bacteriological abundances per month that were averaged through geometric mean to determine correlations using ten paired data for each water body.

Table I. Sampling stations and their straight distance to estuary mouth.

Estuary/sampling stations	Distance (m)
Barra de Santiago	
1 El Cajete	3100
2 El Zapatero	5200
3 Centro	5800
4 La Poza	6600
5 Gloria Linda	8600
Barra Salada	
1 Caserío	690
2 Bocana	130
3 Infiernillo	1120
4 Centro	1600
5 Mujeres	2500

Statistical analysis: Levene's and Kolmogorov-Smirnov's tests determined that variances were homoscedastic and presented normal distribution, respectively. To determine the association amongst bacterial abundances and surface water parameters such as dissolved oxygen, pH, salinity, temperature as well as distance from ocean inlet, we used either Pearson or Spearman correlation indexes, according to the results from the tests mentioned before. The authors used the categorization of correlations to determine the strength of indexes according to Hinkle *et al.* (2003) and Yadav (2018). The monthly comparisons for dissolved oxygen and salinity involved post hoc tests Dunn and Tukey, respectively. Comparisons between seasonal data utilized Kruskal-Wallis or T-Student. Geometric mean as a central tendency measurement was chosen because of the mathematical nature of microbiological data as other authors did (Vergaray *et al.* 2007, Elmanama *et al.* 2016, Adeniji *et al.* 2019).

Results

Dissolved oxygen: Surface water dissolved oxygen levels ranged from 0.4 to 7.8 mg/l and 0.3 to 11.7 mg/l at Barra de Santiago and Barra Salada, respectively. Important low oxygen concentrations occurred at the end of dry season (April-May, Figure 2). Oxygen concentration levels were different when the two areas were compared through Kruskal-Wallis test, however when dry and rainy seasons data were contrasted at each place the same test reflected no significant differences (Table II).

Temperature: Surface water temperature presented similar peaks of low and high temperatures in November and May (25.9 – 30.3, 24.7 – 32.9 °C) respectively, for Barra de Santiago and Barra Salada. Correlations between this parameter and bacteria abundances were low or negligible (Table IV). The average temperature for both water bodies was 28.1 ± 1.1 and 28.6 ± 1.9 °C, same order. Kruskal-Wallis test determined significant difference when comparing temperatures from both areas ($H = 7.5$ (1, $N = 200$), $p < .01$, $\alpha = .05$) (Table IV).

Salinity: Lower salinities occurred during rainy season and higher salinities in the dry one, including above 34 o/oo (Figure 3). There were significant differences ($p < .01$) when these data were compared between the two estuaries, and the two seasons locally (Table III). At Barra de Santiago there were not important correlations between this parameter and bacteria abundances, however negative

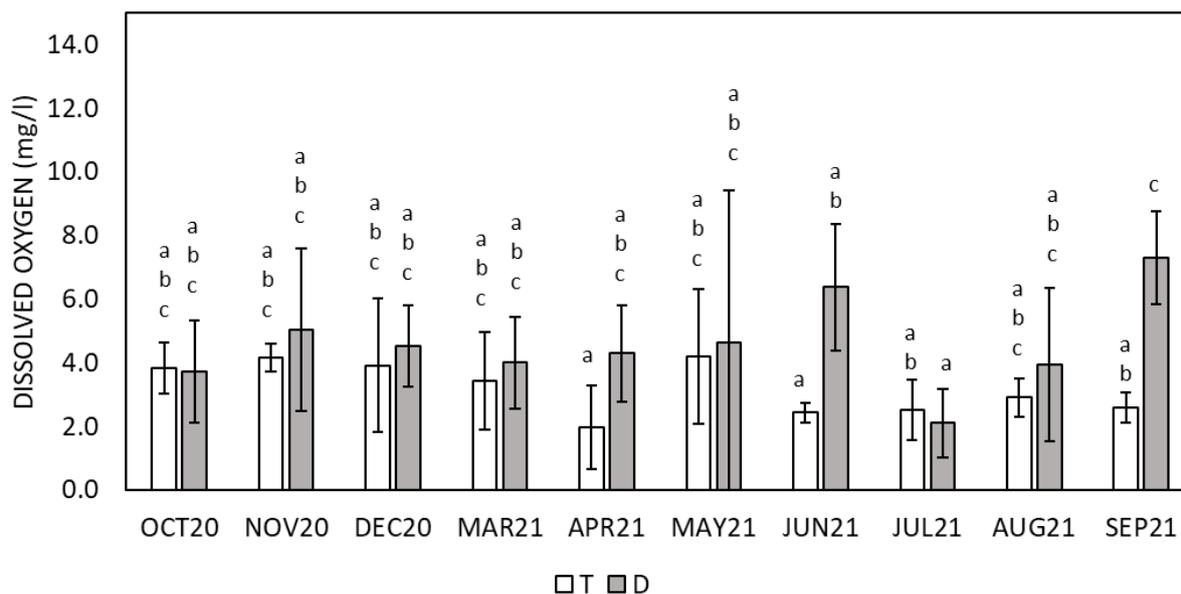


Figure 2. DO concentrations (averages) at both estuaries during the sampling period. D: Barra Salada, T: Barra de Santiago. Standard deviation represented by error bars. Letters above bars present significant differences (Kruskal-Wallis, Post Hoc Dunn tests, $\alpha < 0.05$).

Table II. Comparison of surface water dissolved oxygen levels and temperature between estuaries (T-D) and seasons for each area through Kruskal-Wallis test. (A): comparison of dry and rainy seasons at each place, D: Barra Salada, DO: dissolved oxygen concentration, H: Kruskal-Wallis statistic, N= number of data, NS: no significant ($p > .05$), P: water parameters, S: significant ($p < .05$), T: Barra de Santiago, t: temperature. * $\alpha < .05$.

P	T-D	T (A)	D (A)
DO*	S (H=9.41, $p=0.002$ (1, N =50))	NS (H=2.75, $p = .097$ (1, N=50))	NS (H=.05, $p=.831$ (1, N=50))
t*	S (H=7.46, $p<.006$ (1, N =100))	NS (H=.025, $p=.874$ (1, N =100))	NS(H=1.34, $p=.247$ (1, N =100))

Table III. Comparison of surface water salinity (Sa) between estuaries and seasons for each area through T-Test (independent samples, two tailed). (A): comparison of dry and rainy seasons at each place, D: Barra Salada, F: dry season, G: rainy season, M: media, N: number of data, P: water parameter, S: significant ($p < .05$), SD: standard deviation, T: Barra de Santiago. * $\alpha < .05$.

P	T-D	T (A)	D (A)
Sa*	S(t(98))=-2.20, $p=0.030$ T (M=21.2, SD=13.2) D (M=27.1, SD=13.3)	S (t(48))=6.74, $p<.0001$ F (M=30.30, SD=2.2) G (M=12.1, 8.8)	S (t(48))=-3.46, $p=0.0301$ F (M=21.2, SD=13.2) G (M=32.9, SD=11.2)

moderate and low indexes among salinity and thermotolerant coliform bacteria and enterococci ($r = -.57, -.24$, respectively) were detected (Table IV).

pH: pH values varied above 7.0 mostly. Lowest records occurred at Barra de Santiago in October (4.2 - 4.7) at the five sampling stations reflecting some acidity in water. While the highest record appeared at Barra Salada in September (8.1) when almost all sampling stations reached 8.0-8.1, but station 1 where an important human settlement occurs (Figures 1 and 4, Table I).

Ocean distance to sampling stations: The correlations between distances from the ocean to

sampling stations (Table I) and thermotolerant fecal coliform as well as enterococci were negligible in both estuaries but positive low for Barra Salada ($r (48) = .33, p < .01$) (Table IV).

Rainfall: Relationships amongst accumulated monthly rain data and geometric means for thermotolerant coliform (C) and Enterococci (E) at Barra de Santiago presented positive low and negligible Spearman's correlation indexes ($r (8) = .41, .21$, respectively, $p > .05$). On the contrary, Barra Salada data showed a low and very high Pearson correlation strength for C and E ($r (8) = .30$ and $.96; p > .05, < .01$, respectively).

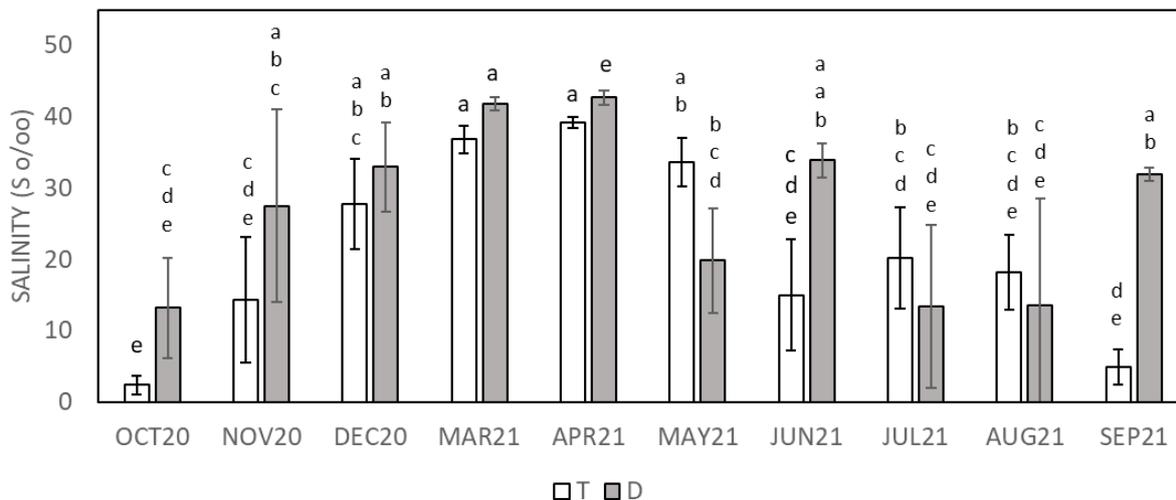


Figure 3. Salinity variations (averages) registered during the sampling period at both estuaries. D: Barra Salada, T: Barra de Santiago. Standard deviation represented by error bars. Letters above bars present significative differences (Student’s T, Post Hoc Tukey tests, $\alpha < 0.05$).

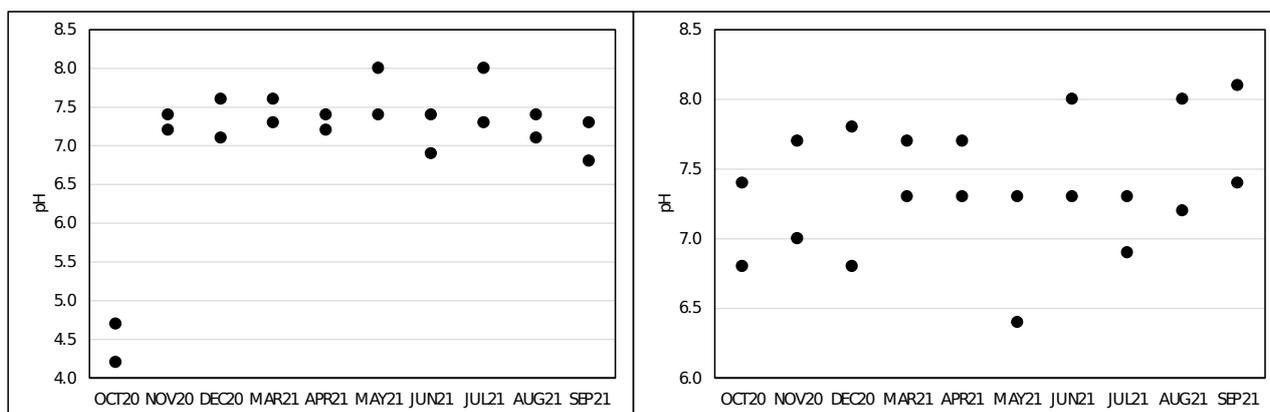


Figure 4. Low and high pH values per station during each sampling period at Barra de Santiago (left) and Barra Salada (right).

Table IV. Spearman’s Correlation coefficients (in parenthesis, N=50) amongst bacteria abundance (MPN/100 ml) and the different surface water parameters as well as ocean distance to sampling stations. C: Thermotolerant coliform, D: Barra Salada, E: Enterococci, T: Barra de Santiago. L: low, M: moderate; N: negligible (Hinkle *et al.*, 2003; Yadav, 2018). * p value < .05, ** p value < .01.

WATER PARAMETER	TC	TE	DC	DE
Temperature (°C)	N (.10)	N (.14)	N (.13)	L (.39)**
Salinity (o/oo)	N (.13)	N (.08)	M (-.57)**	N (-.24)
pH	N (-.09)	N (.01)	N (-.02)	N (.14)
Dissolved oxygen (mg/l)	M (-.60)**	L (-.43)**	N (-.02)	N (-.02)
Distance of sampling station to ocean inlet	L (.33)*	N (.05)	N (.09)	N (.03)

Bacteria monthly variation: The C and E geometric means (Rothenheber & Jones 2018) determined monthly for Barra de Santiago and Barra Salada showed high peaks during the rainy season. There was no clear evidence to detect the most abundant bacteria in this survey. Geometric means were: 102.7

± 410.9 , 53.6 ± 448.6 MPN/100 ml for CF, and 100.9 ± 493.0 , 122.1 ± 644.3 MPN/100 ml for enterococci, respectively. Very wide standard deviations and important geometric means reflected the influence of river inputs during the rainy season (Fig. 5). Spearman index pointed out that the

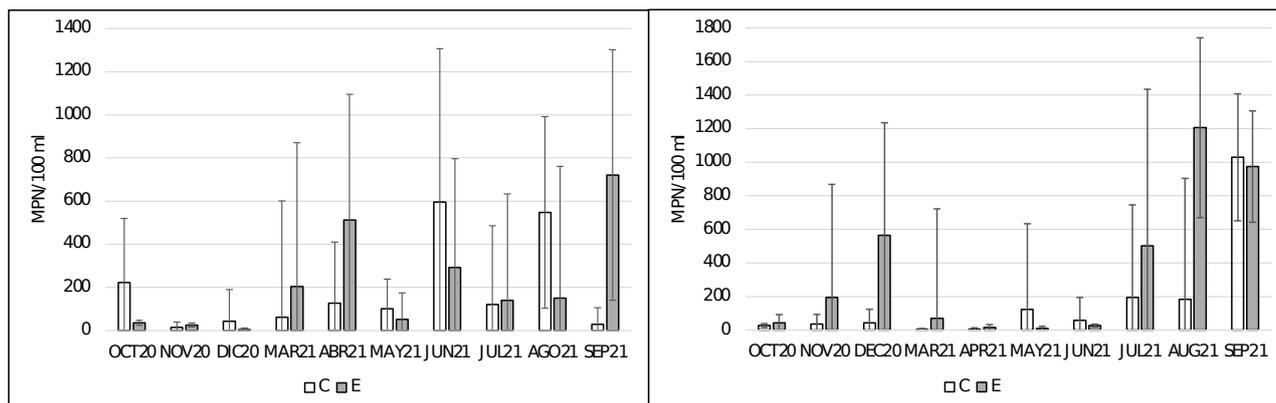


Figure 4. C and E geometric means for the sampling period at both estuaries: Barra de Santiago (left) and Barra Salada (right). Standard deviation represented by error bars.

strength between thermotolerant coliform bacteria and enterococci were positive negligible and low ($r(48) = .17, .39, p > .05, <0.1$, respectively).

Discussion

Sewage discharges generated by urban, farm, touristic infrastructure run off, and other environmental factors influence on the abundance and survival of fecal bacteria in beaches (Vergaray *et al.* 2007) and estuaries (Mote *et al.* 2012, Hassard *et al.* 2017, Rothenheber & Jones 2018).

Dissolved oxygen levels were low at sampling areas relatively far from the estuarine mouth towards the ocean (stations 4 and 5, 1600 and 2500 m, respectively) and close to zones where Mandinga and Pululuya rivers join Barra Salada estuary (Beltrán-Mayorga 2016) (Figure 1, Table I). These levels could be related to rainfall that carries organic matter into the water body and decomposes there. High dissolved oxygen records arose near the mentioned mouth, where oceanic influence is strong, however low values were observed in May 2021 at all sampling stations. Barra de Santiago also exhibited small concentrations at the farthest monitoring sectors in March, April, and July (station 5 mostly, 8600, Figure 1, Table I) and the opposite appeared at station 1, located 3100 m from the sea. During the last four months of dry season, usually northern winds cause many mangrove leaves to litter in the water channels and probably influences hypoxia at both estuaries as Dubuc (2019) observed at New Caledonian mangroves. Apparently, Barra de Santiago presented important inputs from confluent rivers and the Pacific Ocean, and this influenced local dissolved oxygen concentrations. Same trend was also observed by Hsieh *et al.* (2021) in a tropical coastal lagoon at Sri Lanka. The dissolved oxygen concentrations observed at both aquatic

ecosystems were significantly different (Kruskal-Wallis, $H = 9.411, p = .0022$ (1, $N = 50$) $\alpha = .05$), allowing to consider that each place has different hydrodynamics.

Becker *et al.* (2013) explained that cold fronts from higher latitudes caused air temperature to diminish in El Salvador and northern Central America, therefore this parameter decreased at both estuaries from October to December 2020, and March 2021. Moreover, important high peaks arose in May 2021 at the end of the dry season. Field observations let the authors assume that water temperature is also influenced by rainfall, river inputs, sun irradiance, cloud cover, and forest shade. Correlations were negligible ($p > .05$) when comparing this parameter and bacteriological loads, however the authors found a low positive strength ($r = .39$) for enterococci at Barra Salada (Table IV). Despite of these mixed results, some authors have suggested that temperature is an important factor for intestinal bacteria abundance and survival in coastal marine environments at different latitudes (Mote *et al.* 2012, Larrea-Murrel *et al.* 2013, Adeiniji *et al.* 2019, Hsieh *et al.* 2021).

Low salinity values turned out during rainy season, sometimes reaching less than 1.0 o/oo at a sampling station at Barra de Santiago where river inputs are very important in this period of the year. On the contrary, in the last months of dry season (March and April) salinity extended up to values superior to adjacent sea (> 34 o/oo) (Figure 3). This parameter strongly influenced by rainfall and oceanic influx (Hassard *et al.*, 2017), apparently did not impact concentrations of thermotolerant coliform and enterococci at this estuary (Figures 3 and 5). The same important peaks arose at Barra Salada on the latter month in 2017 and was associated to high evaporation rates and blocked estuarine mouth, as

well as low salinities in August 2017 (Méndez-Granados, 2019). The negative moderate correlation determined between salinity and thermotolerant fecal coliform ($r = -.57$, Table IV) at Barra Salada reflected that probably salt concentration affected these bacteria, this coincides with the findings of Rodríguez-Cuitiva (2011) who found a decrescent significative correlation using similar data with higher salinities at bahía Tumaco, Colombia. Similar trend was observed at an estuary in Florida, USA (Ortega *et al.* 2009).

Water pH usually kept around 7.0 up to 8.0 at both estuaries (Figure 4). However, lowest values were found at all sampling stations (4.2 – 4.7) showing some water acidification at Barra de Santiago in October 2020. Highest value (8.1) was observed once at Barra Salada at sampling station close to the ocean in September 2021 which, may be related to strong ocean influence (alkalinity) due to fast and large waves that occurred from September 10th to 14th (MARN, 2021). Hsieh *et al.* (2021) also, found that pH increased in areas close to oceanic inlet in a tropical coastal lagoon at Sri Lanka, suggesting this influence on pH above 7.0.

Bacteriological abundances and distance to sea inlet presented negligible correlation coefficients ($p > .05$), but fecal thermotolerant coliform bacteria at Barra de Santiago pointed out low positive correlation, $p < .05$ (Table IV), pointing out that the bacteria abundance depends upon many factors, including salinity, river input, rainfall (Araujo *et al.* 2015). Another important factor to consider is that all rivers pass through important settlements and carry their wastewater into both estuaries as Méndez-Granados (2019) reported for Barra Salada.

The most important bacteria abundance values and dispersion occurred during rainy season (May – October, Figure 5). Accumulated monthly rain rates and enterococci densities presented negligible correlation index ($r(8) = .21$) and thermotolerant coliform a low strength ($r(8) = .41$) at Barra de Santiago. At the other estuary these indexes presented a different trend with a very high correlation relationship between rain rates and enterococci ($r(8) = .96$) and low strength for thermotolerant coliform ($r(8) = 0.30$). Despite low quantity of data ($N = 10$) due to the use of geometric mean, enterococci seem to reach important abundances during the rainy season, reflecting the importance of rainfall for bacterial dispersion as Connel *et al.* (2012) determined for a temperate estuary.

Moreover, Spearman correlation indexes between thermotolerant fecal coliform as well as enterococci were negligible at Barra de Santiago and positive low for Barra Salada ($r(98) = .17, .39, p > .05, < .01$, respectively). Suggesting that bacteriological abundances are not related at Barra de Santiago, and the relationship is positive low at Barra Salada probably associated to local hydrodynamics. Ortega *et al.* (2009) found higher correlation ($r = .56$) when comparing total coliform (not fecal) and enterococci concentrations at an estuary in Florida, USA.

This study revealed no clear evidence of interactions of temperature, salinity, pH, dissolved oxygen, distance of ocean inlet and rainfall on thermotolerant coliform bacteria and enterococci abundances at both estuaries. However, we found negative moderate and positive very high correlations detected for salinity, as well as rainfall with enterococci, respectively, at Barra Salada, a small wetland. This coincides partly with the findings of Rothenheber & Jones (2018) who determined that rain and river discharges are environmental factors that partly influence the abundance and distribution of these bacteria in a coastal ecosystem. Moreover, a negative moderate correlation was detected between dissolved oxygen in water and thermotolerant coliform bacteria at Barra de Santiago, a large water body.

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Ethical statement

No animals were included in this study.

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