

Microbiological water quality and sources of contamination along the coast of the Department of Atlántico (Caribbean Sea of Colombia). Preliminary results.

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ABSTRACT

Microbiological sea water quality is a public health problem that has serious repercussions in the tourism and economy of Colombia. This study determines the concentrations of *Escherichia coli*, *Enterococcus faecalis* and *Clostridium perfringens* at eleven beach water points and seven streams along the coast of the Department of Atlántico, Colombia. In seawater, total *E. coli*, *E. faecalis* and *C. perfringens* concentrations were found between 16-572 cfu/100 ml, 7-450 cfu/100 ml and 2-125 cfu/100 ml, respectively. The highest counts were observed mainly on urbanized beaches and in correspondence with streams whose waters had a high concentration of faecal origin microorganisms, which represent a serious health risk factor for bathers. Relevant efforts have to be addressed to improve the microbiological quality of these beaches by the establishment of efficient wastewater management programs aimed at enhancing the efficiency of the local treatment plant and the control of illegal sewage pouring onto the coast.

Keywords: *E. coli*, *E. faecalis*, *C. perfringens*, Beach water, stream water, Barranquilla.

According to Klein et al. (2004) and UNWTO (2018), “travel and tourism” is one of the largest growth industries in the world. In 2017, international tourist arrivals recorded the highest growth (7%) since 2010, a value that is well above the annual forecasted increase (3.8%) predicted by UNWTO (2018) for the 2010-2020 time-span. In the same year, the Caribbean Region recorded an increase of tourist arrivals of 4% respect to 2016, showing a clear recovery after the impact of hurricanes Irma and Maria. In 2016, Colombia recorded, with respect to 2015, an 8.9% enhancement in international tourist arrivals with 3,317,000 visitors (corresponding to US\$ 4,773,000 income), and moved up to 8th place in the world rank for number of arrivals (UNWTO, 2017). Tourism represents one of the most important activities for Colombia and its Caribbean coast has a privileged geographic location, with varied ecosystems and a remarkable biological richness. It is projected to be visited by 6.3 million tourists in 2019, of which 4.3 million will be “sun and beach” tourists (Gallardo, 2013; Williams et al., 2016). However, beaches of the Department of Atlántico do not have adequate conditions to provide good tourist services, especially because of their poor environmental condition (Rangel-Buitrago et al., 2017, 2018; Yanoff and Díaz-Solano, 2018). As evidenced at international level by Ergin et al. (2004) and Williams (2011) by means of numerous questionnaires, beach users are especially concerned with five parameters of greatest importance: safety, facilities, no litter, scenery and water quality, and the latter is the focus of this investigation.

On a world scale, epidemiologic studies have consistently established that swimmers at marine water develop gastrointestinal illness more frequently than non-swimmers (Dorevitch et al., 2015). Bathing in contaminated waters produces approximately 120 million annual cases of gastrointestinal diseases and 50 million acute respiratory diseases (Fujioka et al., 2015). In Latin America and the Caribbean, one of the main causes of beach water pollution is the discharge of untreated, or inadequately treated, wastewaters that contain a large load of microorganisms of faecal origin, principally causing gastrointestinal diseases (Larrea-Murrell et al., 2013). Specific microbial indicators have been utilized worldwide to investigate faecal contamination in oceans and seas (Karbadehi et al., 2017; Dorevitch et al., 2015) although their presence does not necessarily cause illness (Haller et al., 2009). Among the most widely used microbial indicators there are *Escherichia coli* and *Enterococcus spp* (Karbadehi et al., 2017). Although *Clostridium perfringens* is not

commonly used as an indicator of faecal contamination in seawater, the ability of its spores to survive adverse conditions makes it a valuable indicator of remote episodes of anthropogenic contamination (Garrido-Pérez et al., 2008; Tomoyuki et al., 2008; Mohammed et al., 2012).

This study constitutes a first preliminary step to determine the counts of faecal indicators such as, *E. coli*, *Enterococcus faecalis* and *Clostridium perfringens* in the seawater of natural and urban beaches of the Department of Atlántico (Colombia) and to establish possible sources of contamination allowing the design of adequate plans for environmental improvement of investigated beaches.

The study area, c. 35 km in length, is located in the Caribbean coast of Colombia, in the Department of Atlántico, between the Magdalena River mouth and Puerto Velero (Fig. 1). It is a tropical environment with two rain periods, or winter seasons, observed in April-May and October-November and two dry periods, or summer seasons, recorded in November-April and July-September. Maximum precipitations are *circa* 2,500 mm/year and mean temperatures are less than 27°C. Tides along the Caribbean coast are of the mixed semi-diurnal type, with maximum amplitudes of 60 cm (Andrade, 2008) and Trade winds (*Alisios*) predominate during summer times. Waves approach the coast from the third and fourth quadrants; significant wave height is about 2 m and the average peak period is 7 seconds (INVEMAR, 2006). Net longshore drift has a dominant south-westward component, minor reversals to the northeast takes place during the rain periods when southerly winds dominate and set up short, high-frequency waves.

The coastline is composed of Quaternary sedimentary deposits shaped in complex and varied coastal features (Fig. 1), such as sand spits (at Puerto Velero), bars, lagoons (at Balboa) and beaches with, at places, cliffed, commonly terraced, sectors (Correa and Morton, 2010; Anfuso et al., 2015). The Magdalena River, 1,600 km in length, is the largest fluvial system in Colombia that drains a basin of 257,438 km² (Restrepo and López, 2008) and has an important pollutant load linked to numerous domestic and industrial activities developed along the river basin (Bayona-Arenas, 2018). Several small streams are observed at Salgar, Pradomar and Puerto Colombia (Fig. 1). Specifically, a stream characterised by a small, continuous flow apparently linked to a natural inland freshwater source, is present at Salgar (Fig. 1). At Pradomar, two streams (herein named Northern and

Southern stream) have a dark water colour and unpleasant smell; they are both linked to natural freshwater flows and illegal pouring of wastewaters (Fig. 1). A stream with similar characteristics is observed at Popular Beach (Fig. 1). At the mangrove swamp area of P. Colombia are observed two streams. The northern one, which is the most important of the investigated coast (Fig. 1), presents a constant flow of 40 L/s proceeding from the local wastewater treatment plant (C.R.A, 2017; Cubillos, 2017). The southern one has a smaller, constant flow (Fig. 1).

Summer houses, bars and restaurants are observed at Salgar, Pradomar, Popular beach, Balboa area (houses are built on the backing bluff) and the sheltered area of P. Velero and, last, at P. Colombia village, the most important urbanised area with 26,932 inhabitants. Pleasant weather conditions turns this coast into an attractive place for local (essentially from the near and very populated town of Barranquilla) and national tourists attracted by the remains of an ancient pier, the oldest port of Colombia (Cubillos, 2017).

Eleven sampling points were selected at fixed locations along the investigated area, especially in correspondence of urban areas where recreational water activities are more common, i.e. at Salgar, Pradomar and P. Colombia, but also at more natural coastal sectors such as Sabanilla and P. Velero (Fig. 1). Sampling was carried out in September 2018 during constant meteorological conditions corresponding to the dry period season characterized by a dominant SW directed littoral drift (Anfuso et al., 2015). At each location, three samples of 100 ml of seawater were taken in the nearshore, at a distance from the shoreline corresponding to one meter depth, using sterile Whirl-pak® bags with closure bands. Sampling bags were introduced into the water column and opened in the opposite direction of the current flow at 30 centimetres depth from the sea surface to avoid sampling of the superficial water layer directly affected by ultraviolet radiation. Water samples were preserved in darkness conditions at a temperature lower than 5°C and analyzed within 4 h of collection. Water physical and chemical parameters, such as, pH, conductivity and salinity were measured in situ using a portable multi-parameter probe (Hanna HI9829).

For microbial analysis, column and stream water were filtered according to Standard Method 9-66 9222D using a Millipore stainless steel equipment with 250 mL polysulfide filtration funnels and 0.45 µm pore size cellulose acetate filters. Afterward, the filters were

placed in the corresponding agar. *Clostridium perfringens* counts were done on a Membrane Clostridium m-CP agar (Oxoid®) and incubated in anaerobic conditions for 21 ± 3 hours at $44 \pm 1^\circ\text{C}$; only opaque yellow colonies were considered positive. For enumeration of *Enterococcus faecalis*, the Slanetz-Bartley agar (Oxoid®) was used and samples were incubated at $44 \pm 1^\circ\text{C}$ for 21 ± 3 hours; the red or pink colonies were taken as positive. *Escherichia coli* and Coliforms were counted on Agar Brilliance (Oxoid®) and incubated at 37°C for 21 ± 3 hours; the purple colonies were counted as positive for *E. coli*. Average abundance and associated standard deviation values were calculated for each microorganism in all samples.

In Colombia, the quality of recreational waters is regulated by the decree No. 1594 of 1984 (and modifications, i.e. decree No. 3930 of 2010), which established the use of old and obsolete techniques of microbiological analysis. These days they have been replaced at international level by the membrane filtration technique (Gronewold and Wolpert, 2008), i.e. the one used in this paper. Results presented herein are not comparable with the limits imposed by the Colombian Degree No. 1594 but can be compared with the standards presented in international literature/directives and/or required by international Beach Awards such as the Blue Flag (Blue Flag, 2018). Both the United State Environmental Protection Agency (USEPA 2012 RWQC) and the European Community Commission directives (DIRECTIVE 2006/7/CE) use *Escherichia coli* and *Enterococcus* spp. to evaluate the quality of recreational waters. However, for coastal waters, the EU directive evaluates the count of both microorganisms while the US directive takes only into account the count of *Enterococcus*. The abundance of *E. coli*, *E. faecalis* and *C. perfringens* in beach waters along the investigated sector are presented in Figure 2 and was compared with the limit established by the EU Directive, since this is the one considered by the Blue Flag to determine beach water quality (Blue Flag, 2018).

Escherichia coli counts were described according to the limit (500 cfu/100 mL) proposed by the European Community Commission (Directive 2006/7/CE), which usually refers to the result of numerous surveys carried out at the same place. This was not the case of this preliminary study that is based on a single campaign according to Garrido-Pérez et al. (2008) and Praveena et al. (2013), amongst others. *E. coli* presented a great variability and exceeded the above mentioned limit at all urbanised areas (Figure 2), which are also the

most frequented beaches. The lowest colony counts of *E. coli* were obtained in natural coastal sectors where no human activities and/or streams were observed, namely at Puerto Mocho (point 1 Fig. 1, 16 ± 6 cfu/100 mL), Sabanilla (point 2, 54 ± 12 cfu/100 mL), Salgar points 1 (54 ± 12 cfu/100 mL) and 2 (51 ± 4 cfu/100 mL), and in the exposed beach of P. Velero point 10, (36 ± 6 cfu/100 mL).

Enterococcus faecalis did not show a clear difference between natural and urbanised areas as previously observed for *E. coli*. The *E. faecalis* count at Pradomar beach (440 ± 16 cfu/100 mL) exceeded the limit (200 cfu/100 mL) established by the European Community Commission (Directive 2006/7/EC), Figure 2 b. P. Velero (point 11, 125 ± 20 cfu/100 mL) and Balboa (85 ± 9 cfu/100 mL) also presented high values and the rest of the beaches showed values that ranged from 36 to 63 cfu/100 mL, except for P. Mocho, which recorded the lowest value (7 ± 2 cfu/100 mL).

The count of *Clostridium perfringens* presented maximum values at P. Velero point 11 (Figure 2 c), that was followed by urbanised areas (e.g. Popular, Salgar point 5, etc.), which showed values only slightly higher than the rest of the beaches. As with *E. coli* and *E. faecalis*, P. Mocho beach presented the lowest number of colonies of *C. perfringens* (2 ± 1 cfu/100 mL).

Both pH and conductivity recorded almost constant values along the investigated coast. Specifically, pH values were around 8, the highest value (8.44) was recorded at Salgar point 3 and conductivity values ranged from 50 to 52 S/cm. Salinity ranged from 30 to 33 ppm.

Identification of the sources of faecal contamination is mandatory (Mattioli et al., 2017); for that, analysis of streams water quality was carried out at mouth of water flows along the coast, which are concentrated in urbanised areas (Fig. 1 and Table I). *E. coli* presented the highest numbers at the Salgar stream Fig. 1 and Table I), at the Northern and Southern streams of Pradomar (Fig. 1), and at the northern stream of Puerto Colombia, i.e. in correspondence with sample 8 (Fig. 1 and Table I). The lowest colony counts of *E. coli* were recorded at the stream located at Popular Beach and at Puerto Colombia Southern stream.

The highest numbers of *E. faecalis* counts were recorded at the southern stream of Pradomar and at Salgar and, secondly, at the northern stream of P. Colombia (Fig. 1 and

Table I). The rest of the streams presented values ranging from 10 to 320 cfu/100 mL, such values being broadly of the same order of magnitude as the water sample values.

Counts of *C. perfringens* in water streams presented highest numbers at Salgar (Fig. 1, Tabla I). High concentrations, as previously observed for *E. faecalis*, were not reflected by beach water analysis, probably because the reduced stream flow and the high energy, which is a characteristic of beach environment and favours rapid freshwater dilution. The rest of the beaches presented values ranging from 10 to 50 cfu/100 mL.

E. coli counts above the limit established by the EU Directive were observed at most frequented and urbanised bathing areas, where summer houses, restaurants and hotels are observed, i.e. at Salgar, Pradomar, Popular Beach, P. Colombia and P. Velero (Williams et al., 2016). *E. coli* survival time in marine water is around 19 h while *Enterococcus* survival time is around 58 h (Noble et al., 2003). At all previously mentioned localities, *E. coli* was much more abundant than *E. faecalis* and *C. perfringens* that are dangerous pathogens since the former gives rise to gastrointestinal, respiratory and skin diseases (Boehm and Sassoubre, 2014; Larrea-Murrell et al., 2013) and the latter is considered a very good indicator of water contamination in tropical countries (Mohammed et al., 2012). Further, *E. faecalis* and *C. perfringens* did not record the same trend as *E. coli*; the former over-passed the EU Directive limit only at Pradomar meanwhile the latter recorded very high concentrations only at P. Velero.

In relation to physical and chemical parameters, salinity values recorded in sea water samples were representative for this area that shows a slight variability due to fresh water supplies from the Magdalena River and the seasonal changes in the evaporation/precipitation ratio (Beier et al., 2017). Conductivity values were very uniform and similar to the ones reported by Tyler et al. (2017). No clear relationships were observed among chemical and physical parameters and microbial counts.

As observed in this paper, coastal water pollution was linked to the insufficient capacity of wastewater treatment plants in tourist cities, as observed at many other Latin American places by Rodrigues da Silva et al. (2013), Condé et al. (2015), Torres-Bejarano et al. (2018), Abdul Aziz et al. (2018) and, especially in this case, to the illegal discharge of not treated wastewaters, a common trend in Colombia (Larrea-Murrell et al., 2013).

At all streams, *E. coli* recorded very high concentrations that were much greater than the ones of *E. faecalis* and *C. perfringens*, confirming local observations by Gallardo (2013). As observed in municipal sewages by Sinton et al. (1993), these differences are due to the fact that the density of *E. coli* in human faeces normally is between 10^6 to 10^7 cells g^{-1} while *E. faecalis* normally varies from 10^5 to 10^6 cells g^{-1} (Lunestad et al., 2015). Further, *E. coli* counts in stream samples were often of many orders of magnitude (until 10^6 times) of counts recorded in beach waters; this was not the case for *E. faecalis* and *C. perfringens* that showed maximum concentrations of *c.* 100 times the values observed at the nearest water sample locations but, at most places, their concentrations were in the same order of magnitude or about 10 times the values recorded in beach water samples.

Hence, the concentrations of bacteria, especially of *E. coli*, in beach water samples always recorded a greater diminution, with respect to values observed at streams samples. This remarkable decrease in concentration is due to the dilution phenomena, but also because when the Gram-negative bacterial are introduced into seawater, they lose their ability to form colonies within a short period, though they may still be detected at fairly high levels by viable counting methods (Davies et al., 1995). The decrease of *Enterococcus* in beach water samples could be linked to the processes of sedimentation, resuspension, particle interactions and predation (Boehm and Sassoubre, 2014). Moreover, temperature variation, sunlight inactivation, pH fluctuation and nutrient depletion in seawater are also important factors in the decay of bacteria (Zhang et al., 2015).

At the residential area of P. Velero, facing sheltered waters between the sand spit and the mainland, high counts were observed of *E. coli*, *C. perfringens* and, secondarily, of *E. faecalis*. No streams are observed at this area and the low water quality is linked to the discharge of local, poor treated wastewaters that gives rise to a fluctuating entry (with higher values during holiday seasons) of contaminants into the seawater column (Torres-Bejarano et al., 2018). At this location, a progressive accumulation of *C. perfringens*, which presented the highest counts recorded along the investigated coast, can be linked to the sheltered conditions of this environment. The genus *Clostridium* is one of the most resistant microorganisms because is able to form endospores able to survive in both terrestrial and aquatic habitats, therefore their presence can be considered as an indicator of remote anthropogenic contamination (Garrido-Pérez et al., 2008). *Clostridium* spores may persist

in sediments for an undetermined time and move into the seawater column due to recreational activities carried out by beachgoers or because natural turbulence (Haller et al., 2009). Last, must be highlighted that all natural beaches recorded some (even low) evidence of contamination, i.e. the presence of *E. coli*, *E. faecalis* and *C. perfringens* that, under normal natural conditions should be nonexistent as observed by Garrido-Pérez et al. (2008) at 18 natural coastal bathing zones on the south-western coast of the Iberian Peninsula. Recorded concentrations of investigated bacteria in natural areas did not show very marked differences (as observed in urbanised areas) and are probably linked to the pollutant load of the Magdalena River (Bayona-Arenas, 2018). This river annually discharges 228 km³ of water and drains a basin of 257,438 km² (Restrepo and Kjerfve, 2000), i.e. 25% of the country, flowing through 724 municipalities representing 80% of the total population of Colombia. Specifically, a microbiological quality monitoring program of the Magdalena River waters has evidenced a high contamination that exceeds the limits established by the Colombian Degree No. 1594 of 1984 (Bayona-Arenas, 2018). Given the high sea water salinity, the survival of faecal bacteria and of the majority of enteric bacteria in the sea and in general in aquatic systems (Jovanovic et al. 2017), is associated with their capacity of adsorption in suspended sediments (Jamieson et al., 2005), i.e. Shibata et al. (2004) evidenced a direct relation between *C. perfringens* and water turbidity. Therefore, further investigation should be focused to fully understand the content of faecal microorganisms in the suspended load of the Magdalena River and the dynamic of the associated sediment plume; this is first transported offshore because of the long jetties at the Magdalena River mouth and then turns south-westward during dry-periods because of the SW directed littoral drift (Anfuso et al., 2015), finally arriving at the investigated coast. Hence, the particularly low counts of colonies of *E. coli*, *E. faecalis* and *C. perfringens* at P. Mocho are associated with the fact that this beach is sheltered by the jetties emplaced at the Magdalena River mouth, which prevent the river water plume to arrive at this beach sector (Fig. 1).

Summing up, this investigation obtained a preliminary approximation to the microbiological water quality of both natural and urban beaches along the coast of the Department of Atlántico. All urban beaches presented high counts of *E. coli* that represent a serious health risk factor for bathers. Further investigations have to be carried out to better

understand spatial and temporal variations of microbiological pathogens. At the local level, relevant efforts have to be addressed to improve the microbiological quality of these beaches by the establishment of efficient wastewater management programs aimed at enhancing the efficiency of the local treatment plant and the control of illegal sewage pouring onto the coast.

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Figure captions

Fig. 1: Location map with beach water sampled areas (numbers) and streams (letters, for details see Table I).

Fig. 2. Counts of *E. coli*, *E. faecalis* and *C. perfringens* at investigated beach water samples. Letters indicate the position of streams. Dashed lines indicate limits established by the EU Directive 2006/7/CE.

Table I. Counts of *E. coli*, *E. faecalis* and *C. perfringens* at streams water samples.

Table I. t

Streams		Bacteria Value \pm stand. dev. (cfu/100 mL)		
Point in Fig. 1	Location	<i>E. coli</i>	<i>Enterococcus</i>	<i>Clostridium</i>
a	Salgar	$5.9 \times 10^7 \pm 1.9 \times 10^7$	$4.3 \times 10^4 \pm 7.5 \times 10^3$	$1.2 \times 10^3 \pm 2.5 \times 10$
b	Pradomar North	$1.5 \times 10^5 \pm 1.2 \times 10^5$	$6.5 \times 10 \pm 2.5 \times 10$	$3.5 \times 10 \pm 5$
c	Pradomar South	$1.9 \times 10^8 \pm 5 \times 10^6$	$5 \times 10^4 \pm 1 \times 10^4$	10
d	Popular Beach	$6.6 \times 10^3 \pm 3.1 \times 10^3$	<10	10
e	P. Colombia	$1.5 \times 10^5 \pm 5 \times 10^4$	$6.8 \times 10^3 \pm 4.5 \times 10^2$	10
f	P. Colombia	$7.2 \times 10^3 \pm 1.5 \times 10^2$	$3.2 \times 10^2 \pm 2 \times 10$	$5.0 \times 10 \pm 1 \times 10$

Figure 1
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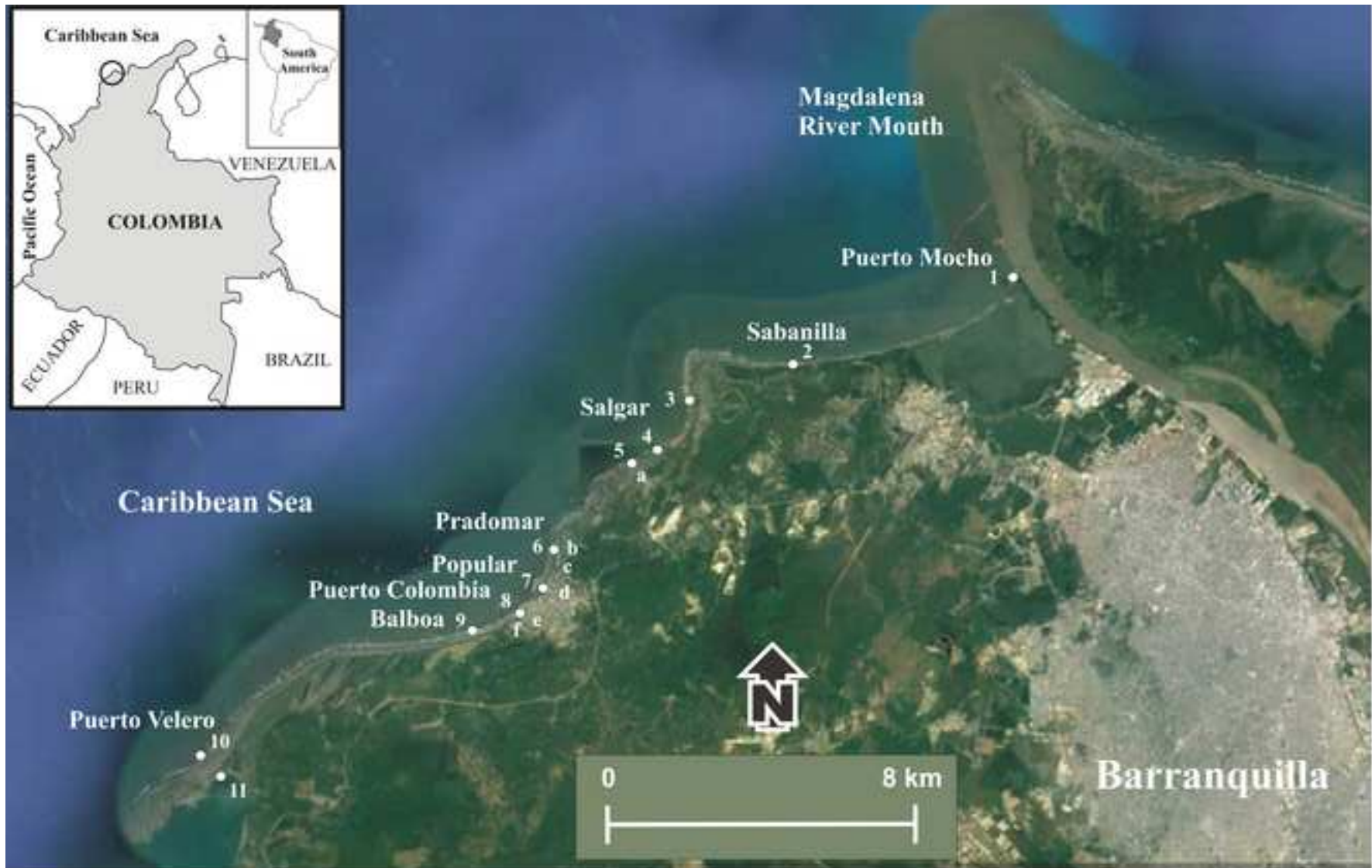


Figure 2

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