

Temporal variability of enterococci and associated sources at three subtropical recreational beaches

by

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Abstract

We have examined enterococci concentrations in water and sand (dry and wet) at three semi-arid subtropical recreational beaches to assess public health risks. To determine the concentration of enterococci, water and sand samples were collected before, during and after the Easter Week (when the largest influx of users occurs), and in the wintertime. The lowest concentrations (< 100 MPN 100 ml^{-1}) were recorded before the Easter Week, the highest concentrations (> 1500 MPN 100 ml^{-1}) during and after the Easter Week, and concentrations < 500 MPN 100 ml^{-1} in the wintertime. Enterococci concentrations in sand were generally < 200 MPN 100 ml^{-1} . Variability in enterococci concentrations can be explained by the influx of users during the Easter Week, rainfall runoff and the increase in water temperature after the Easter Week, as well as by winds and the presence of dogs and birds in the wintertime. The highest health risks occur during and after the Easter Week.

Key words: bacteria, fecal contamination, sand beaches, sanitary quality, enterococci

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Introduction

Recreation is an ecosystem service that best represents beaches. It supports a growing tourism activity that generates jobs and revenue, thus fostering economic development. However, evidence from epidemiological studies and quantitative assessments of the microbial infection risk by recreational exposure to beach water shows an overall high risk of gastrointestinal diseases (Fewtrell & Kay 2015). Tourist exposure to unhealthy beaches has been estimated to cause ~120 million cases of gastrointestinal disorders and ~50 million cases of respiratory diseases per year (Shuval 2003). Thus, the concept of the beach as a microbial habitat and a reservoir of fecal bacteria and pathogens has begun to influence our thinking about the exposure to and recreational use of beach water and sand and their effects on human health (Whitman et al. 2014).

The main sources of fecal contamination on beaches are wastewater discharges, river discharges, and bathers (defecation and/or shedding). Other sources include septic tanks in the vicinity of beaches, animal stools, deposition of airborne pathogens, maritime traffic, as well as transport of fecal bacteria by rain, wind, tides and currents (Praveena et al. 2013; Tilburg et al. 2015). It is usually difficult to relate particular sources to health risks due to the diverse nature of the sources of fecal indicator bacteria, the discharge processes and their destination, as well as the scarce estimates in many watersheds (Huang et al. 2017). Therefore, for a given beach, all potential sources should be examined and more research efforts should be devoted to better understand the variability of fecal indicator bacteria.

The microbiological quality of beach water is commonly assessed using fecal indicator bacteria, including total coliforms, fecal coliforms, *E. coli*, and enterococci (Oliveira et al. 2016). The World Health Organization has established public health risk criteria based on the concentration of enterococci in seawater on beaches (WHO 2003). This is based on the tolerance of enterococcus bacteria to saline environments and the fact that their concentration in water is positively correlated with the incidence of respiratory or gastrointestinal diseases as well as eye and ear infections in bathers (Cabelli et al. 1982; Pruss 1998; Haile et al. 1999). Sand, the other major component of beach recreation, has not been included in the current monitoring and regulation schemes. Both the surface and porous spaces between sand grains provide suitable conditions for the survival, reproduction and viability of microbial populations, and the importance of beach sand for public health has been extensively

documented (Heaney et al. 2014; Whitman et al. 2014; Zhang et al. 2015). Despite the lack of quantitative criteria, it is recommended to monitor the quality of beach sand in order to gather information for standard protocols and to define assessment criteria (Sabino et al. 2014).

It has been observed that an increase in the influx of beach users leads to an increase in enterococci density, indicating fecal contamination, which may be favored by inadequate, insufficient or inefficient sanitation infrastructure (WHO 2003). A better understanding of microbiological quality and the beach surroundings is essential for assessing the implications for human health (Quilliam et al. 2015).

This study aimed at examining the variability of enterococci concentrations in water and sand at three subtropical recreational beaches. Based on this, we have assessed the public health risks during the seasons with a contrasting influx of beach users, taking into account the prevailing wind, rain and water conditions.

Materials and methods

Study Area

The study covered the beaches of Los Algodones, San Francisco and Miramar located in the municipality of Guaymas, Sonora, Mexico (Fig. 1), a subtropical region with dry-warm climate (García 2004). Mean annual precipitation is 230 mm, the highest rainfall occurs in August (~67 mm on average) and the driest month is May (~1 mm on average) (Vega-Granillo et al. 2011).

The surveyed beaches are visited all year round, but the largest influx of users occurs during the Easter Week holiday season, which is the most important during the year in terms of the number of foreign visitors, with the lowest number of visitors in the wintertime. Los Algodones Beach has fine sand and a system of dunes. San Francisco Beach has a gentle slope of quartz sand with several berms that migrate throughout the year, while Miramar is a shallow beach with fine sand (García-Morales et al. 2018). A summary of the features and environment of these beaches is shown in Table 1. Although the three beaches are used by residents, sewage discharges have been observed only on Miramar Beach.

Field Study

Samples of seawater and sand were collected at each of the surveyed beaches to determine

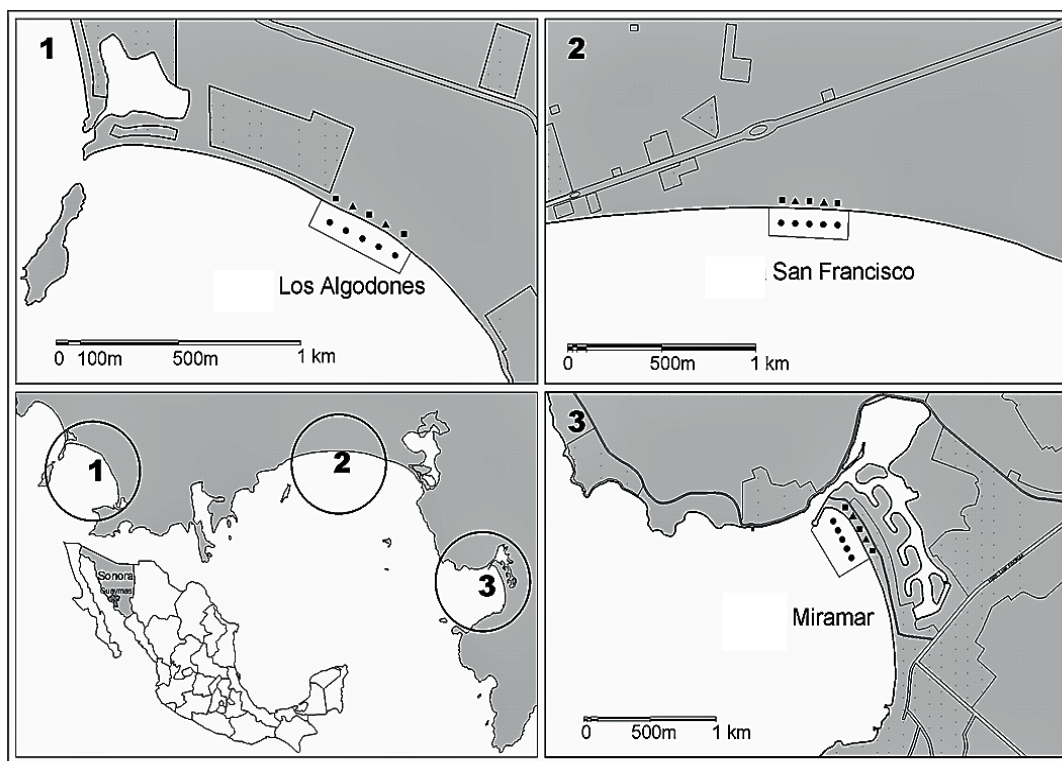


Figure 1

Location of the studied beaches (Los Algodones, San Francisco and Miramar) and sampling sites (dots) at each of them

Table 1

Summary of the main features and environmental characteristics of the studied beaches

Feature	Beaches		
	Los Algodones	San Francisco	Miramar
Type of site	Bay	Open beach	Bay
Beach length used	~1.8 km	~1.5 km	~1 km
Beach area used	~9000 m ²	~7500 m ²	~5000 m ²
Coast type	Micromareal (tidal range < 1 m)	Micromareal (tidal range < 1 m)	Micromareal (tidal range < 1 m)
Wave height	< 1 m	< 1 m	<1 m
Surrounding land	Shrubs Sand dunes	Shrubs Sand dunes	Mangrove forest Urban development
Human activity	Residential Hotel Trails Commerce	Residential Trails	Residential Hotel Streets Commerce
Animals	Domestic animals Sea birds Horses	Domestic animals Sea birds	Domestic animals Sea birds
Prevailing winds	Summertime: Southeast Wintertime: Northwest	Summertime: Southeast Wintertime: Northwest	Summertime: Southeast Wintertime: Northwest
Prevailing coastal currents	Summertime: Southeast Wintertime: Northwest	Summertime: Southeast Wintertime: Northwest	Summertime: Southeast Wintertime: Northwest
Annual rainfall and seasonal distribution	< 300 mm yr ⁻¹ Mostly summertime	< 300 mm yr ⁻¹ Mostly summertime	< 300 mm yr ⁻¹ Mostly summertime
Sewage discharges identified	No	No	Yes
Stormwater mixed with wastewater	Likely influence	Likely influence	Yes
Ships	Occasional	Occasional	Occasional

enterococci concentrations. Seven sampling campaigns were carried out: (1) before the Easter Week, (2) during the Easter Week, (3) one month after the Easter Week, (4) two months after the Easter Week and (5, 6, 7) during three successive weeks in winter. Five sampling sites for water, three sites for dry sand and two sites for wet sand were established on each beach (Fig. 1).

Water samples were collected in sterile 100 ml bottles following the procedure recommended by Mexican Norm NMX-AA-120-SCFI-2016 (SE 2016). Sand samples of 100 g were collected in sealed plastic bags following the procedure recommended by Gonzalez & Emiliani (2005) and Pinto et al. (2012). All samples were kept on ice at 4°C and then transported to the laboratory for analysis. Temperature, salinity, dissolved oxygen and pH were recorded at each water sampling site using a multi-probe YSI (model 556 MPS, Xylem inc., USA). In addition, the number of persons on the beach, either in the water or on the sand was counted during each sampling campaign.

Laboratory Analyses

The technique used for the detection and counting of enterococci in water, dry and wet sand samples was the Enterolert™ Defined Chromogenic Substrate method. This technique is endorsed by the Mexican regulations that establish the requirements and specifications of beach quality sustainability NMX-AA-120-SCFI-2016 (SE 2016). Sand samples were mixed with 900 ml of distilled water under magnetic stirring for 1 min at low speed before analysis (Gonzalez & Emiliani 2005; Pinto et al. 2012).

Analyses of rainfall and wind data

A series of 10-year (2004–2014) precipitation data recorded at the National Water Commission weather station of San Francisco (No. 26292-) was analyzed to study the rain and wind pattern in the region. This station is located in the vicinity of the studied beaches (24.55°N, 110.5°W). Daily rainfall records were pooled by month, and then box and whisker plots were constructed. In addition, wind direction, wind speed and rainfall were recorded during three weeks prior to each of the seven beach sampling campaigns. Wind data were analyzed with an Excel macro available on the Enviroware's website. This macro was extended to produce additional chart options (up to five types) of wind data grouping to calculate frequencies for each cardinal direction. Wind rose graphs were produced using the software GRAPHER V.9.

Statistical analyses

Non-parametric analyses of variance (Kruskal-Wallis) were used to test differences in water and sand quality among the periods studied for each beach; a $p < 0.05$ level was considered as statistically significant. The analyses were conducted using the statistical package Statgraphics plus 4.1.

Assessment of microbiological quality

Microbiological quality of beaches was evaluated based on the concentrations of enterococcus bacteria in water. Values were compared with the reference criteria established by the World Health Organization (WHO 2003) and presented in Table 2. It is worth mentioning that according to the Mexican standard defining the requirements and specifications for beach quality sustainability, the concentration of enterococci in water intended for recreational use should be lower than 100 MPN 100 ml⁻¹ (SE 2016).

Table 2

Water quality criteria for primary contact recreational use established by the World Health Organization

95 th percentile value of intestinal enterococci/100 ml (rounded values)	Estimated risk per exposure
0–40	< 1% GI disease risk/ < 0.3 AFRI risk
41–200	1–5% GI disease risk/ 0.3–1.9% AFRI risk
201–500	5–10% GI disease risk/ 1.9–3.9% AFRI risk
> 500	> 10% GI disease risk/ > 3.9% AFRI risk

GI: Gastrointestinal, AFRI: Acute febrile respiratory illness

Results

Environmental Variables

Minimum, maximum, average and standard deviation values for water-related environmental parameters recorded during the study period at each beach are shown in Table 3.

Rainfall and Winds

The average rainfall pattern over the past 10 years shows a peak in July (~85 mm) and its lowest value in April (< 1 mm) (Fig. 2).

Southeasterly winds with a speed of 8–9 km h⁻¹ prevailed before the Easter Week (Fig. 3a). Winds with

Table 3

Water environmental variables recorded at the three studied beaches: Los Algodones, San Francisco, and Miramar

	Los Algodones			San Francisco			Miramar		
	Min.–Max	Average	SD	Min.–Max	Average	SD	Min.–Max	Average	SD
Temperature (°C)	17.6–31.5	21.50	4.74	18.3–31.5	21.81	4.23	17.2–30.4	21.53	4.41
Salinity (PSU)	30.3–36.8	35.08	1.93	29.7–36.8	34.97	1.84	30.4–36.9	35.13	1.89
Dissolved oxygen (mg l ⁻¹)	3.2–7.5	6.19	1.38	3.4–7.7	6.26	1.26	5.8–8.1	7.04	0.71
pH	7.8–8.6	8.28	0.25	7.8–8.7	8.32	0.30	7.4–8.7	8.31	0.33

SE direction and a speed of 9–10 km h⁻¹ prevailed during the Easter Week (Fig. 3b). SW and S winds with a speed above 10 km h⁻¹ prevailed after the Easter Week (Figs 3c and 3d). WNW winds with a speed greater than 10 km h⁻¹ prevailed in the wintertime (Figs 3e, 3f and 3g).

No perceivable rain was observed prior to our samplings before (1) and during (2) the Easter Week. Precipitation peaks (~80 mm) were recorded just before the samplings conducted after the Easter Week (3 and 4). Rainfall prior to the winter sampling was < 1 mm (Figs 4a and 5a).

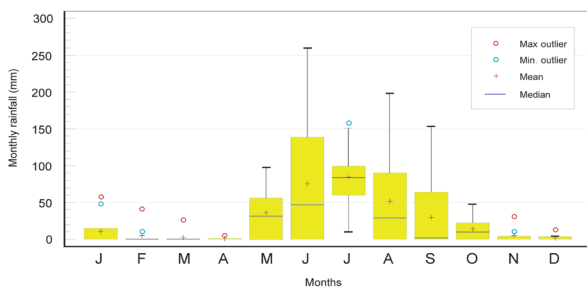


Figure 2
Mean monthly rainfall (2004–2014) in the area where the studied beaches (Los Algodones, San Francisco and Miramar) are located

Influx of Users

The influx of users followed the same pattern on the three surveyed beaches: a high influx during the Easter Week (~5700 users) and lower influx levels in the wintertime and before the Easter Week (48 and 80 users respectively). The influx of visitors after the Easter Week was ~50–83 users (Figs 4b and 5b).

Enterococci Concentrations in Water

In general, the concentration of enterococci in the water at the studied beaches followed a similar pattern. The lowest concentrations of enterococci (< 100 MPN 100 ml⁻¹) were observed before the Easter Week holiday season. The concentrations of

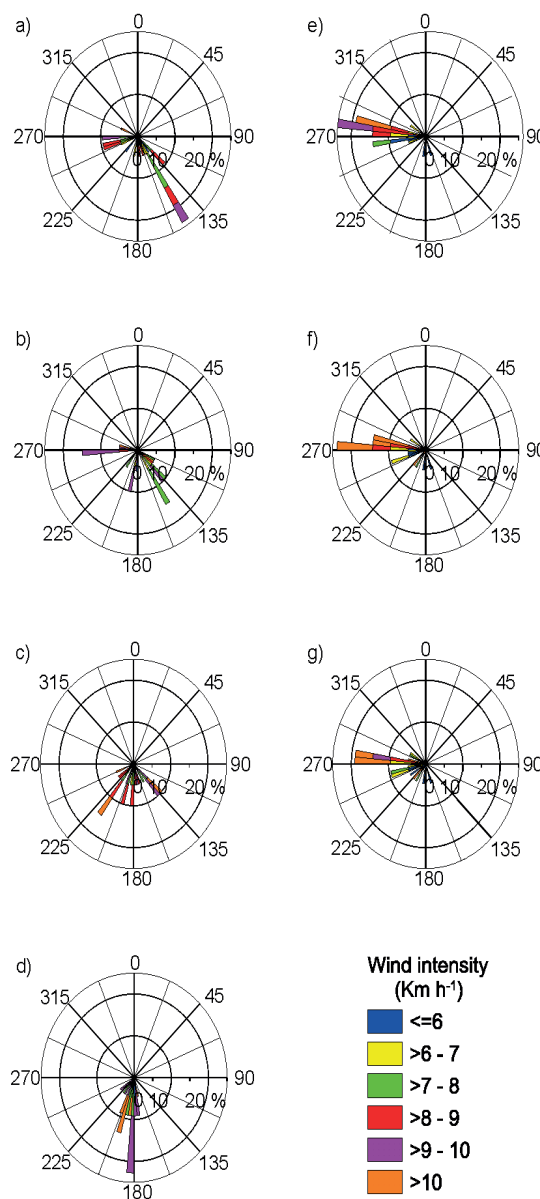


Figure 3
Wind direction and speed during 20 days prior to the sampling campaigns: a) March 16–April 6, b) 1–21 April, c) April 26–May 17, d) May 22–June 14, e) 9–30 January, f) January 15–February 5, g) January 22–February 12

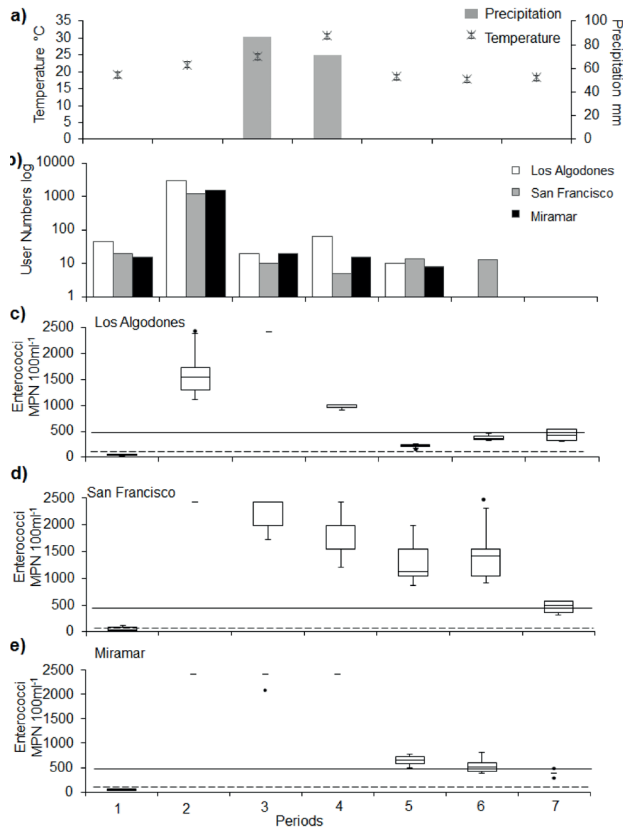


Figure 4

a) Rainfall during the three weeks prior to each sampling campaign and sea surface temperature at the time of sampling, b) Influx of users at the time of sampling, c–e) Concentration of enterococci in water: before (1), during (2), after the Easter Week (3 and 4), and in the wintertime (5, 6 and 7) at the three studied beaches: c) Los Algodones, d) San Francisco, and e) Miramar. The dotted line indicates the maximum concentration of enterococci (100 MPN 100 ml⁻¹) allowed for the sustainability of beaches in Mexico (SE 2016). The continuous line indicates the reference value for public health risks by the World Health Organization (WHO 2003).

enterococci (MPN 100 ml⁻¹) were significantly higher ($p < 0.0001$) during and after the Easter Week (>1000 in Los Algodones, > 1500 in San Francisco and > 2000 in Miramar) compared to other samples from every beach. The concentrations of enterococci in the winter period were in the range of 200–500 for Los Algodones, 1000–1500 for San Francisco and 400–700 for Miramar (Figs 4c, 4d and 4e).

Enterococci Concentrations in Sand

The concentration of enterococci (MPN 100 ml⁻¹) in wet sand showed different patterns on the surveyed beaches. The highest concentrations were determined in winter for Los Algodones (13), during the Easter Week for San Francisco (28) and after the Easter for Miramar (70). However, these values were not significantly different from the values observed in the other periods for each beach. The concentration of enterococci (MPN 100 ml⁻¹) in dry sand followed a similar pattern in Los Algodones and Miramar, where the highest concentrations were detected during the Easter Week (> 100 and 147, respectively). In San Francisco, the concentration in the Easter Week was > 20, while the highest concentration (830) was observed

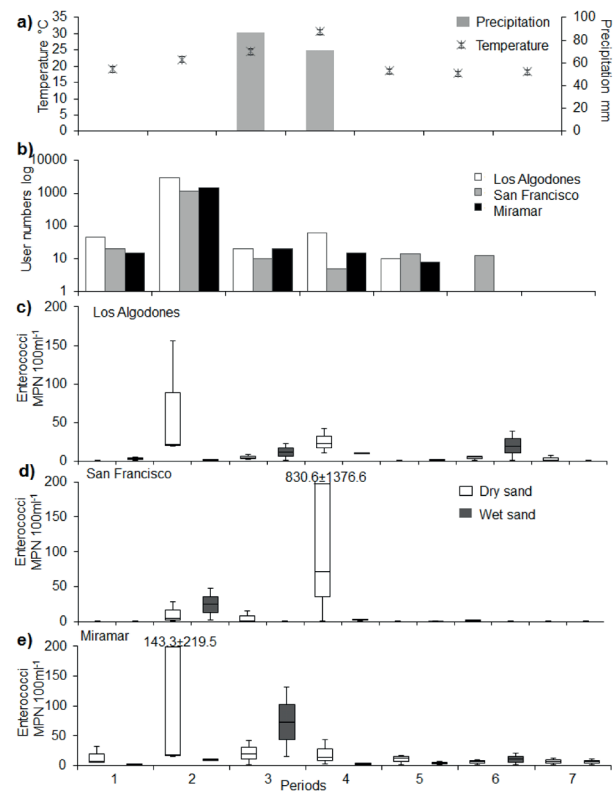


Figure 5

a) Rainfall during the three weeks prior to each sampling campaign and sea surface temperature at the time of sampling, b) Influx of users at the time of sampling, c–e) Concentration of enterococci in dry and wet sand: before (1), during (2), after the Easter Week (3 and 4), and in the wintertime (5, 6 and 7) at the three studied beaches: c) Los Algodones, d) San Francisco, and e) Miramar

two months later. The concentrations of enterococci recorded in the other periods for each beach did not show significant differences (Figs 5c, 5d and 5e).

Discussion

The three studied beaches, Los Algodones, San Francisco and Miramar, showed a similar variability pattern in the concentration of enterococci in water: low concentrations (< 100 MPN 100 ml^{-1}) before the Easter Week, high concentrations (> 1500 MPN 100 ml^{-1}) during and immediately after the Easter Week and decreasing, although always > 200 MPN 100 ml^{-1} , concentrations during the wintertime. Thus, health risks for users of these beaches are higher during and after the Easter Week.

Before the Easter Week, the sanitary quality of water at the three beaches was suitable for primary contact recreational use, the number of users was low < 40 , and there was no rainfall. The highest influx of users at the three beaches ($\sim 96\%$ of the total number of visitors throughout the study period) was observed for a period of approximately 10 days during the Easter Week. This coincided with the time when the highest enterococci concentrations (> 1500 MPN 100 ml^{-1}) were recorded. The lack or insufficiency of sanitary services (García-Morales et al. 2018) on these beaches, combined with the fact that most visitors camp out on the beach during the Easter Week, suggest that the high influx of users is the main source of fecal contamination, reaching levels that pose a threat to public health. This matches with other studies that have shown that the influx of users can be identified as the root cause of fecal contamination in beach water (Fattal et al. 1991; Fleisher et al. 2010). It is therefore important to assess the number and distribution of visitors on the beaches. The analysis of such data provides information useful for environmental and public health management (Dwight et al. 2007), including investment in the construction of sufficient sanitation infrastructure.

The influx of users decreases significantly after the Easter Week, but the level of enterococci in the water remains high. This may be explained as follows: (1) enterococci populations established during the Easter Week still remain in the medium, (2) the effect of runoff from rain that fell prior to sampling at this time, and (3) the observed increase in water temperature ($26\text{--}29^\circ\text{C}$). The populations of enterococci established during the Easter Week may persist as they are able to attach to zooplankton in seawater (Signoretto et al. 2004). The rainfall runoff is an important source of enterococci as it carries materials from land to the

sea (Tilburg et al. 2015; Huang et al. 2017). This effect is more pronounced in the case of urban beaches, as observed at our urban beach (Miramar), where higher concentrations of enterococci were recorded compared to the other two less urbanized beaches. In addition, untreated municipal wastewater is discharged in the Miramar surroundings, which increases the fecal contamination. Furthermore, fecal contamination of beach water at this time of the year is facilitated by the fact that enterococci thrive at temperatures ranging from 10°C and 45°C and their development and persistence on these beaches is thus favored by the water temperature rise (Byappanahalli et al. 2012) that takes place after the Easter Week, reaching $25\text{--}30^\circ\text{C}$. The conditions prevailing on these beaches during the summer, namely the peak of the rainy season (Fig. 2), water temperature $\sim 29^\circ\text{C}$ (Mitchell et al. 2002), and the high influx of users (García-Morales et al. 2018) suggest that the public health risk persists throughout the summer.

The concentrations of enterococci in water during the wintertime were > 200 MPN 100 ml^{-1} , which is indicative of a human health risk. Although enterococcus bacteria grow better at high temperatures, they can withstand adverse conditions in the marine environment, which makes them excellent indicators of fecal contamination on beaches (Hanes & Fragala 1967; Noble et al. 2003). This accounts for their presence in the wintertime when water temperature was $\sim 18^\circ\text{C}$. At this time of the year, when there is a low influx of visitors, the likely sources of fecal contamination are winds, migratory seabirds and domestic animals. The results of the wind analysis showed that the prevailing winds in the area are W–NW with a speed of $18\text{--}22\text{ km h}^{-1}$, which carry dust from land to the sea. It should be noted that in this semi-arid subtropical coastal zone, the surrounding vegetation of shrublands and shrubs favors an effective transport of dust from land. In winter, the presence of migratory birds and pets on these beaches or in their vicinity gives rise to the hypothesis that they represent additional potential sources of fecal contamination. Enterococci brought in by seabirds may play a major role on Miramar and San Francisco beaches due to their location near the estuaries and mangrove forests that provide habitats for thousands of migratory birds in this region of the Gulf of California (Anderson et al. 1976), thus increasing the contribution of excreta. In this regard, Byappanahalli et al. (2015) used molecular markers to show that most fecal contamination on a Chicago beach came from shorebirds (geese and seagulls). In particular, San Francisco Beach is extensively used by visitors to walk dogs (García-Morales et al. 2018), and

there is evidence suggesting that dogs are the major source of enterococci on the beach. A comparison of enterococci sources showed that a single dog fecal event is equivalent to 6940 bird fecal events, while a single human adult contributes approximately the same as a bird fecal event (Wright et al. 2009). The limitation of this study is that the number of dogs and birds on the beaches was not counted and this should be considered in future studies.

On the other hand, the concentration of enterococci in sand was lower compared to beach water. This can be attributed to their transport on bather bodies after contact with water. This is the basis of hygiene rules when using swimming pools, as it is believed that showering before entering the pool removes germs and substances that can be transported on the body. This is not practiced by users of the surveyed beaches, as there is no shower on any of the beaches.

The concentration of enterococci on the three studied beaches was higher in dry compared to wet sand, which coincides with observations for other beaches: Lauderdale Beach, Hollywood Beach, and Hobie Beach, South Florida (Bonilla et al. 2007), and three beaches located in the South Coast region of São Paulo State, Brazil (Pinto et al. 2012). Also Mudryk et al. (2014) observed that dry sand on a recreational beach in the southern Baltic Sea was inhabited by the largest number of potentially human pathogenic bacteria.

The highest concentrations of enterococci were recorded during and after the Easter Week, which was clearly associated with the same sources of enterococci in water for this time of the year (influx of visitors, rainfall and water temperature). In general, enterococci concentrations in sand were < 200 MPN 100 ml^{-1} , except for one sampling at San Francisco Beach where the concentration exceeded 500 MPN 100 ml^{-1} . This finding can be accounted for by runoff. In the wintertime, enterococci concentrations in sand were < 25 MPN 100 ml^{-1} , with the highest concentration recorded at Miramar Beach – the urban beach affected by untreated wastewater. Given the prevailing wind direction and speed, the low enterococci concentrations in dry sand during the winter can be attributed to wind action that transports surface sand toward the sea.

Mexican regulations for the sanitary assessment of beaches only requires the determination of enterococci concentration in water. However, the presence of enterococci in sand may also pose a public health hazard, as this is the part of the beach where users spend most of their time. According to Heaney et al. (2014), it is also important to better understand to what extent people are exposed to fecal bacteria

in sand, the environmental factors that control their occurrence and distribution in sand, and their connection with the concentration of fecal bacteria in water.

Although the three beaches showed a similar variability pattern in enterococci concentrations, Miramar poses higher public health risks as, in addition to the direct impact of users, it is affected by untreated wastewater discharges and urban surroundings. San Francisco Beach lacks sanitation infrastructure, which increases the risk of fecal contamination by users. Although there are some hotels and restaurants in the vicinity of Algodones Beach, the beach itself does not have any public sanitation facilities. The Mexican government has been implementing the National Clean Beaches Program since 2003, which includes 268 beaches, 38 of which are certified according to relevant Mexican regulations and 33 hold the Blue Flag certification. The three surveyed beaches, Algodones, San Francisco and Miramar, are included in the Clean Beaches Program but they lack any certification (CONAGUA 2017).

The results of our study on the seasonality, concentration and variability of enterococci on recreational beaches in the subtropical semi-arid region are useful to raise public awareness about certain periods of the year when greater risks of disease occur. However, according to Byappanahalli et al. (2012), epidemiological studies including the identification of enterococcus bacteria at the species level and the quantification of pathogens are necessary to better understand the public health risks associated with high enterococci concentrations.

Conclusions

The results of this study showed that users of the beaches (Algodones, San Francisco and Miramar) are exposed to different levels of health risk depending on the season of the year. The highest risk occurs during and after the Easter Week when the concentrations of enterococci in water show values > 1500 MPN 100 ml^{-1} . An intermediate health risk is associated with the winter period when concentrations ranged from 200 to 500 MPN 100 ml^{-1} , while the lowest risk is before the Easter Week (< 100 MPN 100 ml^{-1}). As evidenced by our study, the main enterococci sources come from the high influx of users during the Easter Week, the rainfall runoff, and the rise in water temperature particularly after the Easter Week. Other important sources seem associated with the prevailing winds and the presence of dogs and birds in winter. The concentrations of enterococci in sand (both dry and wet) showed similar

patterns to those in water, however, the values were generally < 200 MPN 100 ml^{-1} and they were higher in dry compared to wet sand. This represents an intermediate health risk to users, even those that do not go to the sea for swimming.

The three subtropical beaches studied, Algodones, San Francisco and Miramar, showed a similar variability pattern in the concentration of enterococci in water, with the lowest concentrations (< 100 MPN 100 ml^{-1}) and therefore a low health risk being recorded before the Easter Week. In winter, the concentrations ranged from 200 to 500 MPN 100 ml^{-1} , representing an intermediate health risk level during this period of the year. However, the highest concentrations (> 1500 MPN 100 ml^{-1}) and consequently the high health risk occurred during and after the Easter Week. This study provides evidence that sources of enterococci accounting for this variability pattern are the high influx of users during the Easter Week, rainfall runoff, the rise in water temperature after the Easter Week and, according to our hypothesis, prevailing winds and the presence of dogs and birds in winter. The concentrations of enterococci in sand were generally < 200 MPN 100 ml^{-1} and they were higher in dry sand compared to wet sand. The concentrations were higher during and after the Easter Week; the same pattern was determined for water.

This information on semi-arid subtropical beaches may be a useful reference when comparing the factors that control the abundance of enterococci and its variability on temperate and tropical beaches. It may also support decisions and efforts to prevent public health risks and maintain the recreational and tourism ecosystem services provided by the beaches. The suggested actions to prevent fecal contamination include increasing the number of restrooms, eliminating sewage discharges, controlling access of pets, monitoring sanitary quality of sand and water throughout the year, and informing beachgoers about the sanitary condition of the beaches.

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