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## Temporal variations in fecal indicators bacteria in bathing waters and sediments on a coastal ecosystem (Aytré Bay, Charente-Maritime, France)

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### Abstract

Coastal ecosystems are popular environments for economic and recreational activities that may be exposed to fecal contamination. We present a one-year survey of the fecal indicator bacteria dynamics in the bathing water and beach sediment of Aytré Bay (Charente Maritime, France). First, we quantified the *Escherichia coli* and intestinal enterococci abundance, representing fecal indicator contamination, according to Bathing Water Directive (BWD, European Union 2006/7/CE). Then, we measured the total bacterial abundance and the environmental characteristics of the beach, which included chemical (ammonia, nitrate, nitrite, silicate, phosphate, salinity, pH, dissolved oxygen, and total organic carbon) and climatic (rainfall, temperature at water and sediment surface, air temperature and tidal coefficient) parameters. By sampling the water and the sediment at two sites in the beach, we showed that the fecal indicator bacteria (FIB) were occasionally higher than the regulatory threshold during winter, summer and fall. The “poor quality” of the bathing water at the two sampling sites was mainly due to high intestinal enterococci abundance. The FIB abundances were globally correlated between the two sampling sites and between the water and sediment samples in the North Platin site. We found negative significant correlations between FIB and water temperature and salinity, and positive significant correlations between FIB and rainfall, PO<sub>4</sub>, NO<sub>3</sub>, NO<sub>2</sub> and SiO<sub>2</sub> mainly in the water samples. The relationships between all parameters showed that the main environmental drivers in the water and sediment samples during summer and spring were water temperature and salinity, while in fall and winter; the main environmental drivers were rainfall and dissolved nutrients. These results suggested that FIB occurrence at the two bathing sites of the Aytré Bay had different temporal dynamics according to the seasons.

**Key words:** Bathing water, Coastal beaches, Microbiological quality, Fecal indicator bacteria, Environmental drivers, Seasonal variation.

## 1. Introduction

In the current context of climate change, the urbanization of coastal ecosystems and the anthropic pressure near these ecosystems can emphasize the microbiological quality degradation of these environments (Jeanneau et al., 2012; Kucuksezgin et al., 2020). Fecal contamination in coastal environments increase risk of infections causing diseases such as ear or eye infections, gastroenteritis, or skin irritations (Bonilla et al., 2006; Heaney et al., 2012; Shuval, 2003; World Health Organization, 2003). Fecal pollution can come from various sources, such as industrial and wastewater treatment plants (WWTPs) discharges not connected to the sewer system, sanitary sewer problems, recreational activities (campgrounds) not connected to WWTPs, stormwater and agricultural runoffs (Gourmelon et al., 2010b, 2010a; Jeanneau et al., 2012; Savichtcheva et al., 2007). Presence of warm-blooded animals through agricultural and recreational activities through domestic animals such as poultry, horse and dog, or frequentation of these environments by wildlife such as birds can also contribute to the aquatic ecosystem's fecal contamination (Alderisio and DeLuca, 1999; Benskin et al., 2009; Mieszkin et al., 2009; Nguyen et al., 2018).

Preserving the ecological balance of these environments and ensuring the health and safety of their users are necessary to promote economic development and tourism (Barbier et al., 2011). European bathing water quality is monitored during summer periods following the Bathing Water Directive (BWD, European Union 2006/7/CE). The guidelines define two fecal indicator bacteria (FIB) as a basis for seawater classification: *Escherichia coli* and intestinal enterococci. Thus, bathing sites are classified in four categories depending on the concentration of these FIB in water, which indicates a public health risk related to swimming activities (EC, 2006).

The monitoring of the microbiological quality of recreational waters is generally carried out through water analysis, since health risks are linked to bathing activities (Cheung et al., 1990; Fleisher et al., 1998). However, many studies have demonstrated that coastal marine environments, which are turbid and rich in suspended particles, could promote the persistence of fecal bacteria in these environments by offering physical and chemical protection from biotic and abiotic stresses (Weiskerger et al., 2019). Indeed, mainly sediments constitute favorable habitats for fecal bacteria growth by protecting them from predators (Harwood et al., 2014; Whitman et al., 2014) and are considered as reservoir of FIB for the overlying waters through resuspension by swell, wind or runoff (Beverdors and Mclellan, 2007; Ishii et al., 2006; O'Mullan et al., 2019; Palmer et al., 2020; Whitman et al., 2006). In addition, FIB can associate

with the natural microbial community of these ecosystems through dynamic interactions, becoming part of the endemic microbial community (Byappanahalli et al., 2012; Farhan Mohammad Khan, 2019; Rochelle-Newall et al., 2015). The presence of FIB in seawater and sediment can disrupt the balance of microbial ecological functions through a complex set of activities such as competition for nutrients, predation and viral lysis impacts (Bomo et al., 2004; Feng et al., 2018). In intertidal zones, FIB dynamics are even more complicated by the extensive benthic-pelagic coupling (Nunnally, 2018; O'Mullan et al., 2019; Saint-béat, 2012). Thus, integrating the study of sediment and environmental factors to monitor intertidal bathing seawater is essential to fully understand the dynamics of fecal contaminants.

Ideal fecal indicator bacteria (FIB) should be present when pathogens are present, absent in uncontaminated samples and unable to multiply in the environment (Bitton, 2005). However, in aquatic ecosystems, environmental parameters can directly and indirectly influence FIB concentrations through a variety of mechanisms, including abiotic (salinity, dissolved oxygen, nutrients, marine currents), biotic (interactions with other microorganisms in the environment) and meteorological (precipitation, tidal cycle) parameters (Ackerman and Weisberg, 2003; Boehm et al., 2003; Byappanahalli et al., 2012; Farhan Mohammad Khan, 2019; González-Fernández et al., 2021; Laureano-rosario et al., 2017). Intertidal ecosystems are subject to tidal changes and to seasonal variations (Orvain et al., 2007) that may drive the seasonal dynamics of FIB in these environments. Although, relationships between FIB and environmental conditions are location-specific (Aragónés et al., 2016; Weiskerger et al., 2019). Few studies have analyzed the seasonal dynamics of fecal contaminants by including different physical compartments of these ecosystems as well as the seasonal variations of the abiotic and biotic parameters. To date, the environmental conditions associated with poor microbial water quality and studies are lacking in the intertidal ecosystems.

The aim of this work was to examine the seasonal dynamics of the microbiological quality of a beach located in the bay of Aytré (NE Atlantic Coast, France). The bay is a semi-enclosed beach with two main bathing sites: North Platin and South Platin, where routine analyses of the microbiological bathing water quality are performed. Historical monitoring has shown that the levels of fecal contamination in the two sites can be important, suggesting different sources of pollution (ASCONIT, 2017). In 2012, in accordance with the *Bathing Water Directive BWD*, (European Union 2006/7/CE), the lowering of the thresholds for the regulated parameters has led to a review of the bathing water profile of Aytré beach whose water is then classified as "poor quality" for several successive years leading to its swimming ban since the

summer of 2018. Since then, the pollution has remained and despite the few analyses that were performed, its origin has not yet been determined. Moreover, survey of sediment FIB levels has not been conducted in the bay of Aytré despite the chronic fecal contamination and the associated impact on water quality. Since the bay of Aytré is a very popular zone for various recreational activities, tourism, and shellfish farming, it is essential to understand the dynamic and the distribution of the fecal contamination in the benthic and pelagic compartments to explain and identify the origin of this pollution.

A one-year survey was carried out to characterize the level of the microbiological pollution in the two bathing zones of the bay, focusing on the water column and sediment. This monitoring allowed quantification of FIB abundance over seasons. The measurements of several environmental variables, such as biological (total bacterial abundance, chlorophyll-*a* concentration), physico-chemical (temperature, salinity, pH, dissolved oxygen, nutrients) and meteorological (rainfall, tidal coefficient) parameters, have been performed for a comprehensive and relevant analysis of the seasonal dynamics of the fecal contamination. The main purposes of this study were (1) to describe the annual dynamic of the FIB groups in both compartments (i.e., water column and sediment); (2) to investigate the spatial FIB distribution and the possible connection of FIB in the water and sediment by kinetics analysis; and (3) to identify the relative contributions of environmental variables as possible drivers of FIB dynamics.

## **2. Material and methods**

### **2.1. Study area**

The study was undertaken on Aytré Bay located along the French Atlantic coast in the municipality of Aytré that has 170,000 inhabitants (Figure 1). This municipality is located in the south of La Rochelle city, which has one of the largest marinas in Europe with approximately 5,000 berths and a large wastewater treatment plant. This beach is a bay sheltered by two large islands: the Ré Island in the NW and the Oléron Island in the SW (Figure 1). These islands draw a strait protecting the bay against strong currents and waves. A vast area of marshland borders the bay to the east with outlets for rainwater, marshland, and oyster farming channels that flow into the bay. In addition, oyster farming activities are carried out around this bay with oyster beds located off the bay, oyster beds and feeding stations located north and

south of the beach. Aytré Bay is one of the largest sandy-muddy beaches in this French Atlantic coast with a length of 1.8 km and a foreshore of 2.5 km<sup>2</sup>, which is exposed to the air at low tide up to 1.5 km on a very gentle slope (ASCONIT, 2017). Concerning bathing activities, two main sites located at North Platin and South Platin, have been identified to carry out water quality analysis (Figure 1). The North Platin is very popular for swimming activities while the South Platin is more popular for water sports. Before the bathing prohibition, the beach had an attendance conventionally reaching approximately 500 to 1000 people per day during summer distributed around the two sampling points (L. Vacher, personal communication). Since the permanent closure to bathing, attendance has declined but remains notable, and some people continue to swim despite the ban.

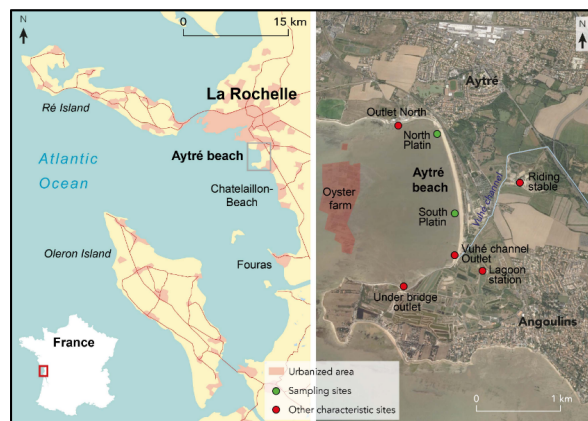


Figure 2: Location of the Bay of Aytré and various sites characteristic of human activities (red dots). The green dots indicate the sampling sites: North Platin and South Platin.

## 2.2. Sampling

### 2.2.1. Sampling frequency

Sampling campaigns were carried out on the two bathing sites defined by the ARS: the North Platin (46°07'30"N, 1°07'38"W) and the South Platin (46°06'58"N, 1°07'28"W) from February 2019 to February 2020 (Figure 1). As recommended by the 2006 European directive, sampling was performed 30 centimeters below the surface water at least one meter deep (Parlement européen, 2006). Sampling was carried out as follows: in winter, there were 2 samples per month and from April to November except for July and August, 3 to 4 samples per month were collected with a biweekly sampling scheme (Supplementary Information Table 1). Sediment sampling was added to the annual monitoring of the water column at low tide before water sampling. The first 5 centimeters of the surface sediment was collected with 15-cm diameter cores. Sampling dates and hours were chosen according to tidal coefficients ranging from 45 to

107. Approximately 5.5 L of water and 1.5 kg of sediment were collected in sterile containers. Each sampling was carried out in triplicate, with approximately 5 minutes between replicates.

### **2.2.2. Abiotic parameters**

During water sampling, *in situ* parameters such as temperature (T°C), salinity (psu), pH and oxygen concentration ([dO<sub>2</sub> (mg/l)]) were measured with multiparametric sensors (Cond 3110, TetraCon 325, WTW, Germany). During sediment sampling, the same parameters were measured in the water at the sediment surface with the same multiparametric sensors used for water samples. The Météo France at La Rochelle - La Pallice meteorological station provided hourly climatic data, including the tide coefficient, seawater temperature, air temperature, wind speed, and rainfall data.

## **2.3. Analyses**

### **2.3.1. Water samples**

#### **2.3.1.1. Enumeration of fecal indicator bacteria**

*E. coli* and intestinal enterococci concentrations were obtained by the standard microplate method: NF EN ISO 9308-3 for *E. coli* and NF EN ISO 7899-1 for enterococci (French Association for Standardization, 1999; International Organisation for Standardisation, 1999) with a detection limit of 15 MPN/100 ml of water.

#### **2.3.1.2. Enumeration of total bacterial abundance**

The total bacterial abundance was quantified by flow cytometry. Water samples were fixed with glutaraldehyde 0.25% and poloxamer 0.01% solutions. Then samples were frozen in liquid nitrogen and kept at -80°C until analysis. The analysis was performed with a FACS CantoII flow cytometer according to the method described in Marie et al. (1999).

#### **2.3.1.3. Nutrients and chlorophyll-*a* analyses**

The concentrations of total organic carbon [TOC (mg/l)] (European Committee for Standardization, 1997), total suspended solids [TSS (mg/l)] and volatile suspended solids [VSS (mg/l)] were quantified by gravimetry as described by the standard method (International Organisation for Standardisation, 2005). The concentrations of dissolved inorganic nutrients such as ammonia [NH<sub>4</sub> (μmol/l)], nitrate [NO<sub>3</sub> (μmol/l)], nitrite [NO<sub>2</sub> (μmol/l)], silicate [SiO<sub>2</sub> (μmol/l)], and phosphate [PO<sub>4</sub> (μmol/l)] were measured by photometric detection as described by the standard method (International Organisation for Standardisation, 2013). The chlorophyll-*a* concentration (μmol/l) was measured by fluorimetry (640 nm, Turner TD 700, Turner Designs, USA) according to Lorenzen's (1966).

## **2.3.2. Sediment samples**

### **2.3.2.1. Enumeration of fecal indicator bacteria**

Ten grams of wet sediment was diluted in 90 ml of tryptone salt and tween solution (2 %). The suspension was homogenized for 15 min at 300-400 tr/mn with a magnetic stirrer (VARIOMAG Electronicrührer Poly 15, No 90750) to dislodge the bacteria from sediment particles. After 1 minute of settling, the supernatant was collected for quantification of fecal bacteria. *E. coli* quantification was performed as water analysis by standard microplate methods: XP X33-019 (French Association for Standardization, 2004). For enterococci, a serial dilution (Stock solution, 1:10, 1:100) was carried out and then the quantification was performed by standard microplate methods NF EN ISO 7899-1 (International Organisation for Standardisation, 1999).

### **2.3.2.2. Enumeration of total bacterial abundance**

Prokaryotic abundance was evaluated by flow cytometry after sample fixation and cell extraction as described by Lavergne et al. (2014).

### **2.3.2.3. Nutrients and chlorophyll-*a* analyses**

The TOC (mg/l) was measured by spectrometry (International Organisation for Standardisation, 1988), and dry matter content was measured by gravimetry as described by the standard method (International Organisation for Standardisation, 1993). The concentrations of dissolved inorganic nutrients were quantified from the pore water of the sediment recovered after centrifugation at 3000 rcf for 20 minutes at 8°C. For silicate analysis, the pore water was stored at 4°C. For phosphates, nitrates, nitrites, and ammonia analysis, the pore water was filtered with a GF/C filter and then stored at -20°C. Nutrient concentrations were quantified with an autoanalyzer (Seal Analytical, Norderstedt, Germany) equipped with an XY-2 sampler according to the method described by Aminot and K  rouel (2007). The chlorophyll-*a* concentration ( $\mu\text{g/l}$ ) was quantified by fluorimetry (640 nm, Turner TD 700, Turner Designs, USA) according to Lorenzen (1966). The mean grain size was measured by laser granulometry (Mastersizer, 2000; Malvern Instruments, U.K.) and evaluated using the GRADISTAT program as outlined by Folk and Ward (1957). Sediment textural groups were defined through the relative abundance (% volume) of mud (for clay and silt with diameter < 60  $\mu\text{m}$ ) and sand (diameter between 63 and 2000  $\mu\text{m}$ ) according to the Udden-Wentworth scale (Blott and Pye, 1986).

## **2.4. Statistical analyses**



In our analysis, we considered a sample to be contaminated when the abundance of at least one of the two fecal indicator contaminants was higher than the European standard regulatory threshold values (*E. coli* > 1000 MPP/100 ml water and intestinal enterococci > 370 MPP/100 ml water). To compare the abundance of FIB between (i) the two sites for each sample type (*i.e.*, water and sediment) and (ii) the water and sediment samples in each site, we performed Pearson correlation tests using the *PerformanceAnalytics* package (Peterson, 2007) in R v4.0.2 (R Core Team, 2020). Similarly, Pearson correlation tests were performed to determine the link between FIB abundance and environmental variables. The analysis of the temporal dynamics of FIB over the survey period, was conducted using a kinetic approach with the R package *Slider* (Ferreira et al., 2018). Here, for both the *E. coli* and intestinal enterococci datasets, Pearson correlations were computed along a sliding window of 5 consecutive sampling dates, between either (i) the abundance in two sites, or (ii) the abundance in water and sediment samples. We chose to analyze sliding windows over five consecutive sampling dates to obtain at least a bimonthly dynamic. All tests were performed with a significance level of 0.05.

To analyze the relationships between the FIB types and environmental data, principal component analysis (PCA) was performed for water and sediment samples at each sampling site using XLSTATS version 2021.1.

### **3. Results**

#### **3.1. Water samples analyses**

##### **3.1.1. *E. coli* and intestinal enterococci abundance in the North Platin and the South Platin sites**

At each site, 46 water samples were collected in triplicates. In the North Platin site, the average abundance of *E. coli* in water samples over the year ranged from 0.0 to  $1.91 \pm 0.92 \times 10^3$  MPN/100 ml (Figure 2A). The abundance of *E. coli* was above the regulatory threshold (1000 MPN/100 ml of water) for three samples, *i.e.*, one sample per season except in spring. The average abundance of intestinal enterococci over the year ranged from 0.0 to  $1.26 \pm 0.41 \times 10^4$  MPN/100 ml (Figure 2C). The abundance of intestinal enterococci was above the regulatory threshold (370 MPN/100 ml of water) for 11 samples out of 46, *i.e.*, 4 in summer, 4 in autumn and 3 in winter. The two FIB types did not exceed the regulatory threshold during spring. In the South Platin site, the average abundance of *E. coli* over the year varied from 0.0 to  $1.36 \pm 0.18 \times 10^4$  MPN/100 ml (Figure 2B). In South Platin, the *E. coli* abundance was above the regulatory threshold for 3 different days compared to the North Platin, *i.e.*, one sample per season except in spring. The average abundance of intestinal enterococci over the year ranged from 0.0 to  $1.63 \pm 0.25 \times 10^4$  MPN/100 ml (Figure 2D). Intestinal enterococci abundance was

above the regulatory threshold for 14 days, *i.e.*, 1 in spring, 5 in summer, 5 in autumn and 3 in winter. On average, for both sites, the total bacterial abundance ranged from  $2.19 \pm 0.03 \times 10^5$  to  $3.73 \pm 0.30 \times 10^6$  cells/ml (Supplementary Information Figure 1).

The global correlation of *E. coli* abundance performed with the Pearson test revealed a significant positive correlation between the North and South Platin sites ( $p < 0.001$ ). The same correlations were noted for intestinal enterococci abundance ( $p < 0.001$ ) at the two sites. In addition to this correlation, the temporal dynamics of FIB abundance were analyzed along a sliding window of 5 consecutive sampling dates during the survey period at the two sites. This correlation (*i.e.*, North and South Platin sites) showed positive correlations with *E. coli* abundances in late winter, spring and autumn. During summer and early winter, *E. coli* abundances followed different trends between the North and the South Platin sites (Figure 3A). In the two sampling sites, the intestinal enterococci abundances were highly correlated in the winter and autumn periods between the two sampling sites, in contrast to the spring and summer periods where opposite trends were observed (Figure 3B).

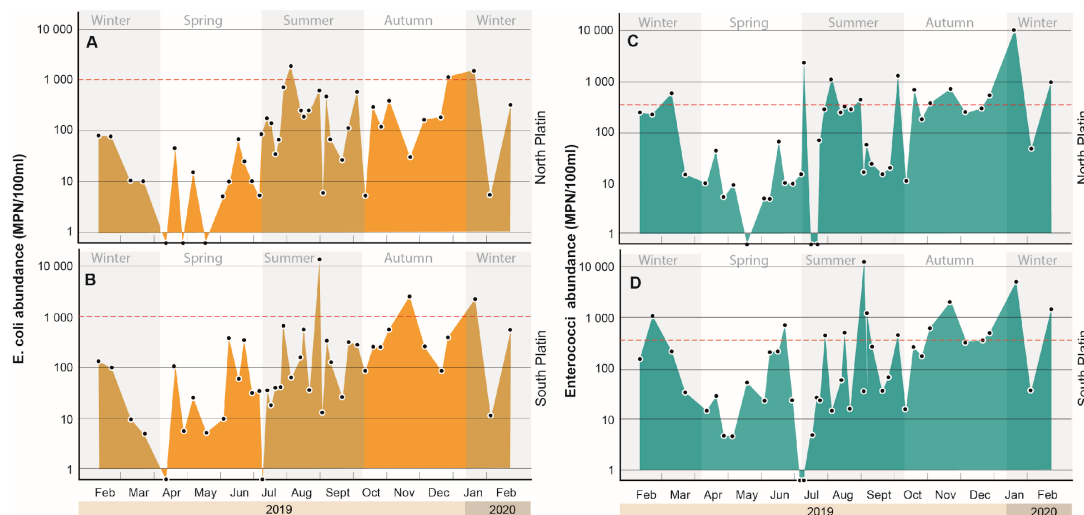


Figure 3: Annual variation in FIB abundances at the two sampling sites. A: *E. coli* abundance in water samples in North Platin. B: *E. coli* abundance in water samples in South Platin. C: Intestinal enterococci abundance in water samples in the North Platin. D: Intestinal enterococci abundance in water samples in South Platin. The black dots represent the average of the triplicate samples for each date, and the red dashed line indicate the European directive threshold for *E. coli*  $< 1000$  MPN/100 ml and intestinal enterococci  $< 370$  MPN/100 ml (MPN: most probable number).

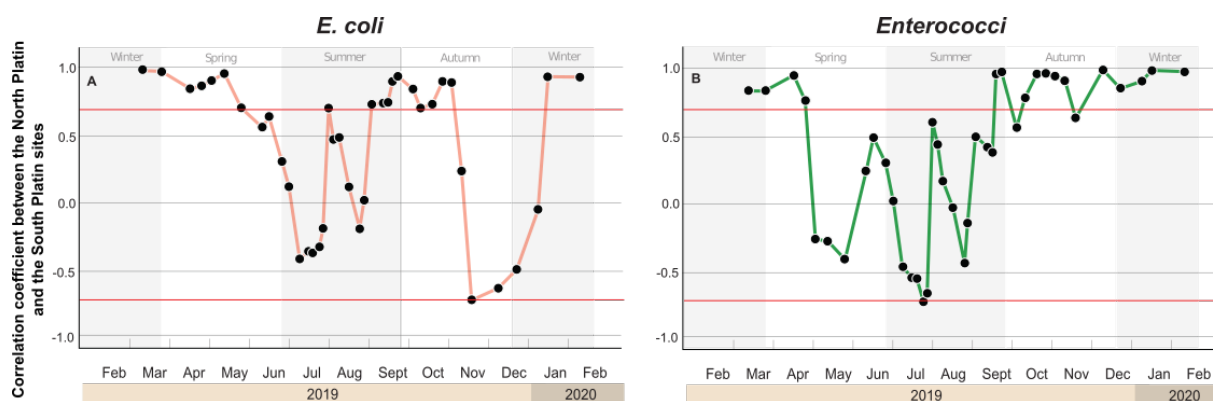


Figure 4 : Correlation coefficients (Pearson) of water samples for the abundance of FIB between the North and South Platin sites, over a sliding window of 5 consecutive sampling dates. A: Correlation coefficients for *E. coli* between the two sites. B: Correlation coefficients for intestinal enterococci between the two sites. The two red horizontal lines indicate thresholds for strong positive ( $> 0.7$ ) and negative ( $< -0.7$ ) correlations.

### 3.1.2. Correlations between FIB abundance and environmental parameters

The seasonal variation of the physico-chemical parameters of the environment is represented in the Supplementary Information (Figure 1 and Table 2). *E. coli* abundance in the North Platin was positively correlated with the  $\text{NO}_3$  ( $p < 0.05$ ),  $\text{SiO}_2$ , and  $\text{NH}_4$  ( $p < 0.01$ ) concentrations and negatively correlated with salinity ( $p < 0.05$ ). At this site, the abundance of intestinal enterococci was weakly correlated with VSS ( $p < 0.05$ ). There was a weak positive correlation between the 12-hours accumulated rainfall before sampling and the *E. coli* abundance ( $p < 0.05$ ) (Pearson correlation test, Table 1).

In the South Platin site, there was a high positive correlation between the FIB abundance and  $\text{PO}_4$ ,  $\text{NO}_3$ ,  $\text{NO}_2$ , and  $\text{SiO}_2$  concentrations ( $p < 0.01$ ,  $p < 0.001$ ). Water temperature was negatively correlated with both FIB types ( $p < 0.01$ ). Salinity was negatively correlated with intestinal enterococci abundance ( $p < 0.001$ ) and was positively correlated with *E. coli* abundance ( $p < 0.05$ ). In the North Platin, total bacterial abundance was negatively correlated with the *E. coli* ( $p < 0.05$ ) and intestinal enterococci ( $p < 0.05$ ) abundances. As in the North Platin, intestinal enterococci in the South Platin were weakly correlated with VSS ( $p < 0.05$ ). There was a strong positive correlation between FIB abundance and 12-hours and 24-hours accumulated rainfall before sampling ( $p < 0.001$ ,  $p < 0.05$ , respectively). Total bacterial abundance in the South Platin site was negatively correlated with both FIB abundance: *E. coli* ( $p < 0.05$ ) and intestinal enterococci ( $p < 0.01$ ) (Pearson correlation test, Supplementary Information Table 1).

Tableau 1: Pearson correlations in water between a set of environmental variables and the logarithm of the abundance of *E. coli* and intestinal enterococci, in the North and South Platin sites. Environmental variables: water temperature (Water temp), air temperature (Air temp), salinity, dissolved oxygen concentration (dO<sub>2</sub>), pH, phosphorous concentration (PO<sub>4</sub>), nitrite concentration (NO<sub>2</sub>), nitrate concentration (NO<sub>3</sub>), ammonium concentration (NH<sub>4</sub>), silicate concentration (SiO<sub>2</sub>), total organic carbon (TOC), total suspended solids (TSS), volatile suspended solids (VSS), 12-hours cumulated rainfall (12-hours rainfall) and 24-hours cumulated rainfall (24-hours rainfall), total bacteria abundance (Total bacteria), and chlorophyll-a concentration (Chloro a). Asterisks indicate a correlation significantly different from 0 (\*: p < 0.05; \*\*: p < 0.01; \*\*\*: p < 0.001).

Variables	North Platin		South Platin	
	<i>E. coli</i>	Intestinal Enterococci	<i>E. coli</i>	Intestinal Enterococci
Water temp	-0.02	0.01	-0.26**	-0.28**
Air temp	0.05	0.14	0.09	-0.24
Salinity	-0.21 *	0.01	0.22*	-0.31***
dO <sub>2</sub>	0.05	0.08	0.10	0.01
pH	0.00	0.00	-0.19*	0.09
PO <sub>4</sub>	0.00	0.09	0.26**	0.32***
NO <sub>2</sub>	0.17	0.15	0.31***	0.29***
NO <sub>3</sub>	0.27 **	0.09	0.28**	0.45***
NH <sub>4</sub>	0.19 *	0.11	0.07	0.73
SiO <sub>2</sub>	0.28 **	0.13	0.41***	0.47***
TOC	0.15	0.08	0.0	0.12
TSS	0.05	0.14	0.12	0.17
VSS	0.09	0.20*	0.12	0.18*
12-hours rainfall	0.17*	-0.00	0.36***	0.34***
24-hours rainfall	-0.02	0.00	0.21*	0.18*
Total bacteria	-0.19*	-0.24**	-0.24*	-0.29**

### 3.2. Sediment samples analyses

#### 3.2.1. *E. coli* and intestinal enterococci abundance in the North Platin and South Platin sites

For sediment, 22 samples were collected in triplicates at each site. In the North Platin site, the average abundance of *E. coli* over the year varied from 0.0 to  $0.76 \pm 1 \times 10^3$  MPN/10 g sediment (Figure 4A). The average abundance of intestinal enterococci varied from  $4.77 \pm 1.62 \times 10^1$  to  $7.95 \pm 4.51 \times 10^2$  MPN/10 g of sediment (Figure 4C). In the South Platin site, the average abundance of *E. coli* varied from  $7.67 \pm 4.04 \times 10^1$  to  $1.23 \pm 0.46 \times 10^2$  MPN/10 g sediment (Figure 4B). The mean abundance of intestinal enterococci varied from  $3.93 \pm 1.01 \times 10^1$  to  $0.81 \pm 1.02 \times 10^3$  MPN/10 g sediment (Figure 4D). At both sites, the abundance of intestinal enterococci was higher than the abundance of *E. coli*. The average abundance of total bacteria ranged from  $1.44 \pm 0.1 \times 10^9$  to  $1.85 \pm 0.5 \times 10^{10}$  cells/g at both sites (Supplementary Information Figure 2).

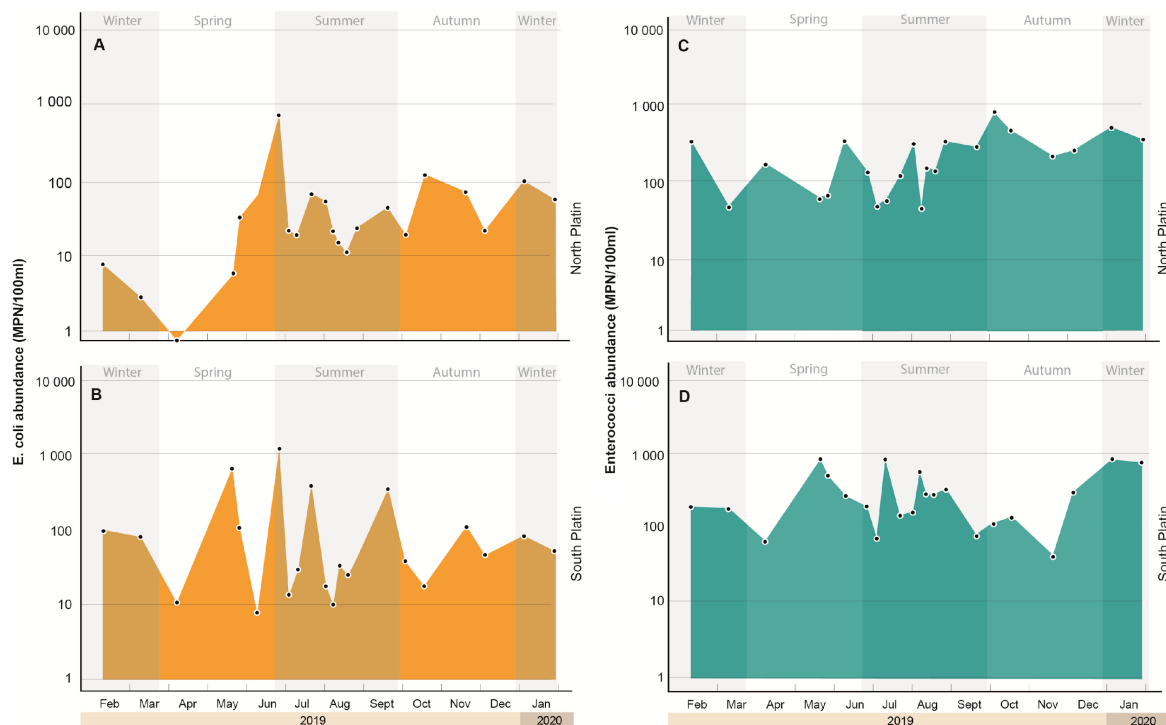


Figure 5: Annual variation in FIB abundances in the two sampling sites. A: *E. coli* abundance in sediment samples in North Platin. B: *E. coli* abundance in sediment samples in South Platin. C: Intestinal enterococci abundance in sediment samples in North Platin. D: Intestinal enterococci abundance in sediment samples in South Platin. The black dots represent the average of the triplicate samples for each date (MPN: most probable number).

The global correlation of FIB abundance over the year in the North and the South Platin sites performed by Pearson's test showed no significant correlation between the *E. coli* abundances ( $p > 0.05$ ) at the two sampling sites. The intestinal enterococci abundances were also uncorrelated between the two sites ( $p > 0.05$ ). Moreover, the temporal dynamics of FIB abundance between the North and South Platin sites performed along a sliding window of 5

consecutive sampling dates showed that the *E. coli* abundances between the two sampling sites were generally uncorrelated except in mid-summer (Figure 5A). The intestinal enterococci abundances were also uncorrelated between the two sampling sites and followed opposite trends in winter, spring, summer and autumn (Figure 5B).

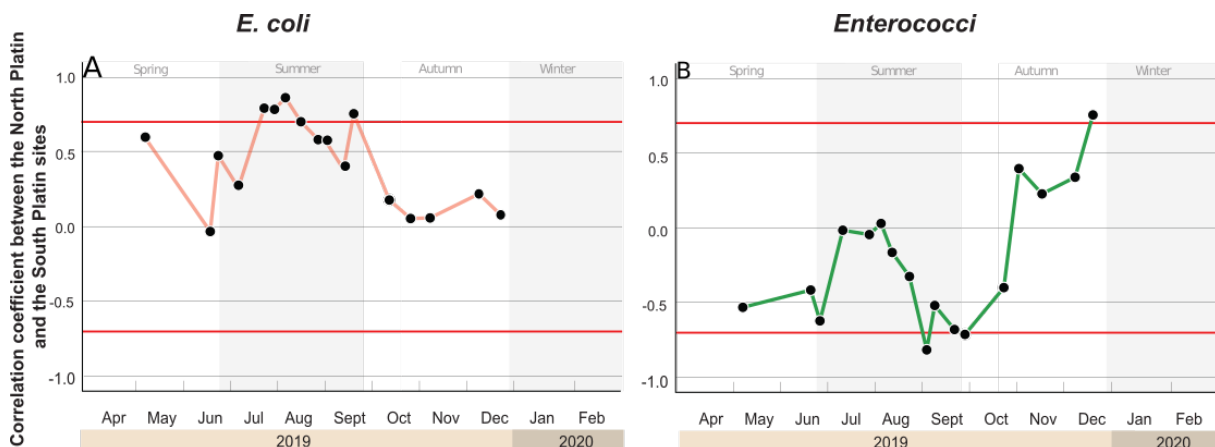


Figure 6: Correlation coefficients (Pearson) of sediment samples for the abundance of FIB between the North and South Platin sites, over a sliding window of 5 consecutive sampling dates. A: Correlation coefficients for *E. coli* between the two sites. B: Correlation coefficients for intestinal enterococci between the two sites. The two red horizontal lines indicate thresholds for strong positive ( $> 0.7$ ) and negative ( $< -0.7$ ) correlations.

### 3.2.2. Correlations between FIB abundance and environmental parameters

The seasonal variation of the physico-chemical parameters of the environment is represented in Supplementary Information (Figure 2 and Table 3). In the North Platin site, the *E. coli* abundance was negatively correlated with water temperature at the sediment surface ( $p < 0.01$ ) and with pH ( $p < 0.05$ ). The intestinal enterococci abundance was positively correlated with  $\text{NO}_2$  ( $p < 0.05$ ) and negatively correlated with sediment particle size ( $p < 0.05$ ). In the South Platin site, the *E. coli* abundance was negatively correlated with surface sediment temperature, as in the North Platin site ( $p < 0.01$ ) (Pearson correlation test, Table 2).

### 3.3. Relationship between FIB abundance in water and sediment samples at each site

In the North Platin site, the relationship between both FIB (*E. coli* and intestinal enterococci) abundances in water and sediment samples were correlated positively (Figure 6A). In the North Platin site, the relationship between both FIB (*E. coli* and intestinal enterococci) abundances in water and sediment samples were not correlated. In water, FIB abundances ranged from 0.0 to  $1 \times 10^4$  MPN/100 ml, while their concentrations in sediment were less than  $1 \times 10^3$  MPN/10 g (Figure 6B). The Pearson correlation between water and sediment samples was only significant

and positive for the North Platin site for both *E. coli* ( $p < 0.05$ ) and intestinal enterococci ( $p < 0.01$ ).

Tableau 2 : Pearson correlations in sediment between a set of environmental variables and the logarithm of the abundance of *E. coli* and intestinal enterococci, in the North and South Platin sites. Environmental variables: water temperature at the sediment surface (WS temp), air temperature (Air temp), salinity, pH, phosphorous concentration (PO<sub>4</sub>), nitrite concentration (NO<sub>2</sub>), nitrate concentration (NO<sub>3</sub>), ammonium concentration (NH<sub>4</sub>), silicate concentration (SiO<sub>2</sub>), total organic carbon (TOC), 12-hours cumulated rainfall (12-hours rainfall) and 24-hours cumulated rainfall (24-hours rainfall), total bacteria abundance (Total Bacteria), chlorophyll-a concentration (Chloro a) and particles size. Asterisks indicate a correlation significantly different from 0 (\*:  $p < 0.05$ ; \*\*:  $p < 0.01$ ; \*\*\*:  $p < 0.001$ ).

Variables	North Platin		South Platin	
	<i>E. coli</i>	Intestinal enterococci	<i>E. coli</i>	Intestinal enterococci
WS temp	-0.34**	-0.19	-0.33**	0.10
Air temp	0.28*	-0.24	-0.02	-0.00
Salinity	-0.17	-0.15	0.01	0.23
pH	-0.32*	-0.14	-0.07	-0.00
PO <sub>4</sub>	0.17	-0.17	0.02	-0.14
NO <sub>2</sub>	0.01	0.25*	-0.03	0.20
NO <sub>3</sub>	0.01	0.09	0.03	0.09
NH <sub>4</sub>	0.01	0.06	0.16	-0.09
SiO <sub>2</sub>	0.14	-0.14	0.16	0.00
TOC	0.05	0.10	0.00	0.17
12-hours rainfall	-0.03	-0.10	0.10	-0.22
24-hours rainfall	-0.06	0.02	0.14	-0.22
Total bacteria	0.13	-0.15	0.23	-0.14
Chloro a	0.07	0.01	-0.10	0.21
Particles size	-0.09	-0.33*	0.15	0.02

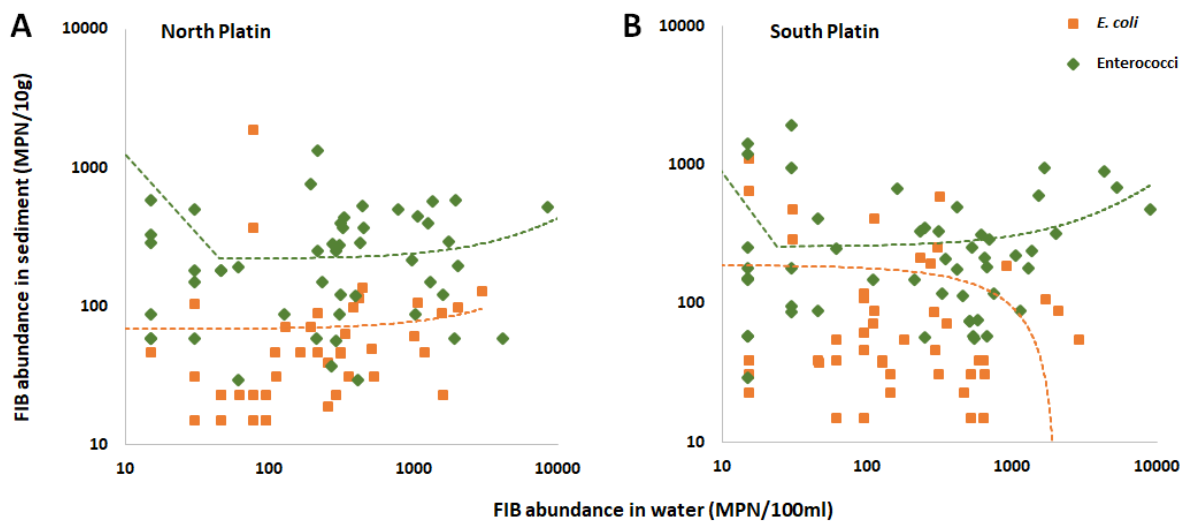


Figure 7: Relationships between FIB: *E. coli* (in orange) and enterococci (in green) concentrations in water and sediment samples. A: Relationships between FIB in North Platin: *E. coli* ( $r = 0.48$ ) and enterococci ( $r = 0.55$ ). B: Relationships between FIB in South Platin: *E. coli* ( $r = -0.32$ ) and enterococci ( $r = 0.10$ ). The orange and green dashed lines represent the correlation trend curves for *E. coli* and Enterococci respectively between sediment and water.

### 3.3. Relationships between FIB abundance and environmental factors

To summarize the relationship between FIB abundance and environmental parameters, PCA was applied with 18 biotic and abiotic variables for water samples (Figure 7). The PCA revealed clear clustering along mainly the primary (Axis 1) and secondary (Axis 2) axes, explaining a total of 50.1% and 47.85% of the variance observed in the North and South Platin sites, respectively. The PCA loading plots showed the relationship between biotic and abiotic parameters in the North Platin (Figure 7A) and in the South Platin (Figure 7B) sites. At both sites, FIB were positively associated with dissolved nutrients and accumulated rainfall data and negatively associated with salinity, water and air temperature.

For sediment samples, PCA was performed with 14 biotic and abiotic variables (Figure 7). The PCA revealed clear clustering along mainly the primary (Axis 1) and secondary (Axis 2) axes, explaining a total of 57.67% and 52.55% of the variance in the North and South Platin sites, respectively. The relationship between biotic and abiotic parameters in the North Platin (Figure 7C) and in the South Platin (Figure 7D) showed that the two FIB were positively associated with  $\text{NO}_2$ ,  $\text{NO}_3$ , and accumulated rainfall and negatively associated with salinity,  $\text{NH}_4$ ,  $\text{SiO}_2$ , water temperature at the sediment surface and air temperature.



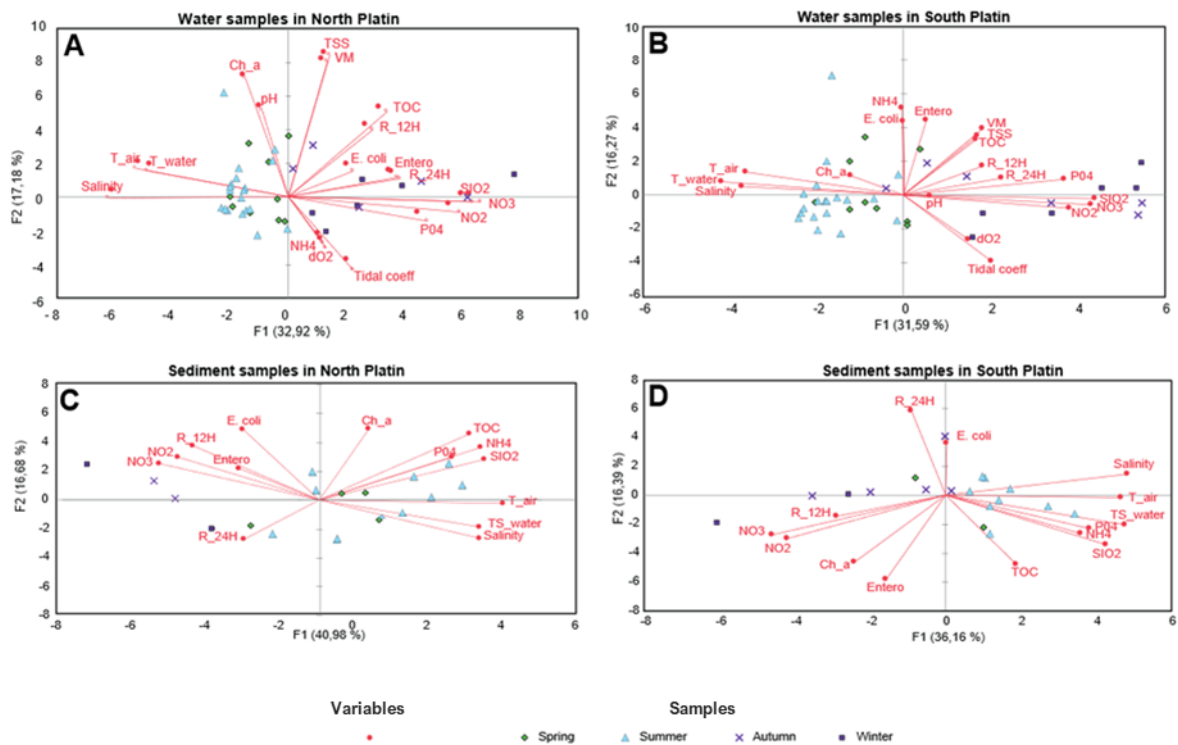


Figure 8 : Principal component analysis (PCA) of the microbial, physico-chemical and climatic data for water samples in North Platin (A) and South Platin (B), and for sediment samples in North Platin (C) and South Platin (D). The environmental parameters are indicated in red. The samples from spring are lozenges, samples from summer are triangles, samples from autumn are crosses, and samples from winter are squares. *E. coli* (*Escherichia coli*), Entero (intestinal enterococci), water temperature (T\_water), water temperature at the sediment surface (TS\_water), air temperature (T\_air), salinity, dissolved oxygen concentration (dO2), pH, phosphorous concentration (PO4), nitrite concentration (NO2), nitrate concentration (NO3), ammonium concentration (NH4), silicate concentration (SiO2), total organic carbon (TOC), total suspended solids (TSS), volatile suspended solids (VM), 12-hours cumulated rainfall (R\_12H), 24-hours cumulated rainfall (R\_24H), chlorophyll-a concentration (Ch\_a), tidal coefficient (tidal coeff).

In the sediment samples, the same associations as those in the water samples were globally observed, with high positives correlations between F1B, accumulated rainfall, NO<sub>2</sub>, and NO<sub>3</sub> and negative correlations with salinity, temperature, PO<sub>4</sub>, NH<sub>4</sub>, and SiO<sub>2</sub>.

The analysis of sample score plots showed a clear pattern: summer and spring samples were clustered according to water, air temperature and salinity for water samples. Winter and autumn samples were clustered according to the accumulated rainfall, PO<sub>4</sub>, SiO<sub>2</sub>, NO<sub>2</sub>, NO<sub>3</sub>, and TOC parameters (Figs 7A and 7B). For sediment, summer samples were clustered with the water temperature at the sediment surface, air temperature, salinity, PO<sub>4</sub>, NH<sub>4</sub>, SiO<sub>2</sub>, and TOC parameters, while autumn and winter samples were clustered with accumulated rainfall, NO<sub>2</sub>, and NO<sub>3</sub> (Figs 7C and 7D). The main environmental drivers in the water and sediment samples seemed to be water temperature and salinity during summer and spring, while they seemed to

be the 12-hours and 24-hours accumulated rainfall data and dissolved nutrients in autumn and winter, respectively.

## **4. Discussion**

### **4.1. Different patterns of FIB dynamics between water and sediment at the two sites**

In this study, we monitored the level of fecal contamination of Aytré Bay for a one-year period, as the beach has been closed to swimming since summer 2018 due to recurrent fecal contamination. FIB levels in water in the two sampling sites were generally low ( $<10^4/100$  ml) and similar to concentrations observed in prior studies in surface water, coastal streams or in catchment (Mauffret et al., 2012; Moreno et al., 2019; Savichtcheva et al., 2007; Viau et al., 2011). The determination of the microbiological quality of the two bathing sites showed that over the 45 sampling dates, the European regulatory thresholds were exceeded for 11 and 15 days for intestinal enterococci in the north and south parts of the bay, respectively, and for 3 different days for *E. coli* in each site. This fecal contamination can come from different sources as described in many studies. Fecal contamination in aquatic ecosystems mainly came from human activities (Jardé et al., 2018; Pommepuy et al., 2005) such as sewage discharges, failure to connect sewage systems and/or the presence of sewage outfalls (Mallin et al., 2007; Young et al., 2013). The presence of WWTPs and marinas near beaches could also constitute sources of fecal contamination (Kacar and Omuzbuken, 2017; Sobsey et al., 2003). In addition, animals neighboring bathing areas can contribute to fecal contamination, through agriculture activities, during walks of dogs or horses, and by the occurrence of wild animals such as birds (Alderisio and DeLuca, 1999; Nguyen et al., 2018; Pommepuy et al., 2006). As described previously, the Aytré Bay is very popular for recreational activities (swimming, walking dogs and horses) and is a resting place for sea birds. These different factors can therefore be significant sources of fecal contamination in this beach. The watershed of the Aytré Bay is made up of a marsh area and a lagoon treatment plant that can impact the temporal dynamic of FIB despite the regulation of their outflows into the bay during the summer periods (ASCONIT, 2017). Additionally, Aytré Bay is an enclosed beach with a gentle slope, protected from strong currents and waves by two islands and surrounded by marshes. These characteristics have been previously described as conducive to bacterial colonization, aggregation and regrowth in the sand, especially under some favorable environmental conditions, such as intermittent tidal-induced wetting and drying (Donahue et al., 2017; Litton et al., 2010; Yamahara et al., 2009).

Sediments play a major role in global biogeochemical cycling (Glud, 2008) and are increasingly considered as a naturalized secondary habitat for FIB and thus a source of waterborne fecal

bacteria (Byappanahalli et al., 2012; Whitman et al., 2006). Maximum FIB concentrations in sediment from both sampling sites were generally low ( $<10^3/10$  g). However, these concentrations were higher than concentrations observed in previous studies on beach sands (Bonilla et al., 2006; Halliday and Gast, 2012; Whitman et al., 2014; Yamahara et al., 2009) and coastal sediments (Luna et al., 2018; Perkins et al., 2014). In contrast, FIB concentrations in the two sampling sites were lower than the maximum concentrations ( $10^5$ - $10^6/100$  g) found in riverbank, wetland, and estuarine sediments (Desmarais et al., 2002; Evanson and Ambrose, 2006; Hassard et al., 2016). Generally, FIB abundance in sediment was several times higher in lakes, freshwater ecosystems (lake, river) than in the seawater (Ishii et al., 2006; Matthew et al., 2011; Whitman and Nevers, 2003). In aquatic ecosystems, several studies have shown that, in many cases, sediment resuspension can increase FIB concentrations in water with a greater effect than soil leaching (Palmer et al., 2020; Taylor et al., 2011; Whitman et al., 2014). In our study, the comparison between sediment and water performed by correlation analysis for each fecal indicator bacteria showed that FIB abundance appeared to be generally higher in water mainly during periods of contamination (exceeding regulatory thresholds). Even if the abundance of the two FIB between water and sediment were correlated positively, it seems that sediment is not a source of FIB for the water column. Studies showed that beach sand has high FIB counts suggesting that their leaching may have a greater impact than tidal sediment resuspension on recreational water quality (Bonilla et al., 2006; Halliday and Gast, 2012; Hartz et al., 2008; Matthew et al., 2011). For example, Whitman et al., (2006) found that *E. coli* is present in highly abundance in beach sand and at distances of at least 5 m within the shoreline. Others studies have attempted to analyze the growth of these bacteria in marine environments (Hartz et al., 2008; Heaney et al., 2015), mainly in intertidal zone with the extensive benthic-pelagic coupling (Nunnally, 2018; O'Mullan et al., 2019; Saint-Béat, 2012). FIB survival in sediments and sand may depend on the ability of the bacteria to store energy reserves and bind to sediment particles (Pommepuy et al., 2006) or the properties of beach sand to filter and trap microorganisms and organic matter, thus acting as a reservoir for fecal contamination and associated human pathogens (Hijnen et al., 2004). For example, enterococci have the ability to form biofilms through their extracellular polymeric substance (EPS), which could lead to adhesion to sediment particles or seaweed and resistance to environmental stresses (Byappanahalli et al., 2012). In addition, sediment particles were on average finer in the North Platin than in the South Platin. In the North Platin, particle size was negatively correlated with intestinal enterococci. As described in previous studies it appears that fine particles in the sediment favored the attachment of enterococci (Byappanahalli et al., 2012; Weiskerger et al.,

2019). As noted earlier, in the North Platin, enterococci could attach with their EPS to fine sediment particles giving them protection from environmental pressures. Furthermore, in water samples, intestinal enterococci were also the main indicator explaining the dynamics of fecal contamination. Our results are in agreement with the observations made by Signoretto et al. (2004) and Byappanahalli et al. (2012), who demonstrated that for aquatic ecosystems, enterococci were the appropriate indicators to predict the presence of fecal pathogens in marine waters.

The global comparison of FIB abundance showed that the abundance of each FIB (*E. coli* and intestinal enterococci) between the two sampling sites was positively correlated. However, the temporal dynamic analysis of the contamination at the two sites showed that both FIB followed different trends in summer. Moreover, the relationship of the two FIB between sediment and water was only significantly positive at the North Platin site. These results implied that the north and the south of the Aytré Bay are not exposed to the same fecal contamination sources. The main difference between the two sites is the occurrence of a stormwater lagoon and a freshwater discharge channel from the marsh areas in the south of the bay that may influence the fecal bacterial supply at this site (ASCONIT, 2017; CREOCEAN, 2005).

#### **4.2. Significant correlations between FIB abundance and some environmental parameters**

Some studies have indicated that environmental factor variations can influence FIB die-off rates through modifications of temperature, pH, nutrients, and dissolved oxygen concentration (Ackerman and Weisberg, 2003; Boehm et al., 2009; González-Fernández et al., 2021; Mau and Pope, 1999; Thi et al., 2016). *E. coli* can respond to signals from the environment, such as chemicals, pH, temperature, and osmolarity (Sumampouw and Risjani, 2014). Intestinal enterococci are a group of bacteria that optimally grow at temperatures between 10°C and 45°C with a salinity of 65 psu and a pH of 9.6 (Byappanahalli et al., 2012). As typical for most microorganisms, a higher *E. coli* concentration can be expected when the temperature is warmer (Palmer et al., 2020). However, our results did not show the same trend. Indeed, water temperature was negatively correlated with both FIB abundances in the South Platin site. In sediment samples, the temperature at the sediment surface was negatively correlated with *E. coli* abundance at both sites. Viau et al. (2011) also found a negative relationship between enterococci concentrations and water temperature. Similarly, in the study of Ishii et al. (2006), lower temperatures in spring limited the accumulation, survivability, and growth of *E. coli* in the nearshore beach sand environment, and higher concentrations of *E. coli* occurred later in the year as temperatures reached the seasonal maximums. Salinity concentration also seemed to regulate FIB abundance in Aytré Bay. The same negative associations were observed

between salinity and both FIB abundances in the global parameter relationship analysis. This parameter (ranging from 10.3 psu to 39.4 psu) negatively influenced FIB abundance only in water samples (*E. coli* in the North Platin site and intestinal enterococci in the South Platin site). Many studies have reported a negative relationship between salinity and the detection or survival of *E. coli* and enterococci. Atwill et al. (2007) and Mauffret et al. (2013) observed a trend of decreasing *E. coli* count with increasing salinity concentration. Carr et al. (2010) and Viau et al. (2011) found similar results for enterococci concentrations in tidally influenced Hawaiian streams (salinity range from 0.6 to 37.3 psu) and in coastal Mississippi waters (salinity range from 0.0 to 26.4 psu). In our study, salinity seemed to inhibit the growth and proliferation of FIB in Aytré Bay. Rainfall is a major environmental factor that can have an effect on fecal matter diffusion from soils to water through soil leaching and fecal degradation (Villemur et al., 2014). In the water samples, both FIB abundances were positively correlated with the 12-hours and 24-hours accumulated rainfall in the South Platin site, while in the North Platin site, only *E. coli* was positively correlated with the 12-hours accumulated rainfall. Our results corroborate many studies that found strong correlations between *E. coli* and enterococci with rainfall (Aragónés et al., 2016; González-Fernández et al., 2021; Jardé et al., 2018; Leight et al., 2018). In addition, Ackerman and Weisberg (2003) described an increase in ocean fecal bacterial concentrations associated with almost all storms larger than 6 mm. In addition, these authors showed that bacterial concentrations remained elevated for 5 days following rainfall events, although they generally returned to levels below water quality standards within 3 days. In this study, rainfall was on average lower compared to the previously cited study, but a strong positive correlation was observed between FIB abundance and rainfall at both bathing sites. These observations suggested that rainfall contributed to fecal beach contamination in the sampling sites mainly in South Platin, where there is a rainwater lagoon plant and the Vuhé outfall bringing fresh marsh water (ASCONIT, 2017) by washing lands and provoking inputs of allochthonous bacteria and nutrients into the bay. Dissolved nutrients were positively correlated with both FIB abundances in the South Platin site, while in the North Platin site, only *E. coli* was positively correlated with  $\text{NO}_3$ ,  $\text{SiO}_2$  and  $\text{NH}_4$ , mainly during winter. The highest nutrient concentrations were also quantified during the winter, as generally expected. Other studies showed the same trend. Khandeparker et al. (2020) showed that the abundances of *E. coli* and *Enterococcus faecalis* were significantly correlated with  $\text{PO}_4$ ,  $\text{NO}_3$ ,  $\text{NH}_4$ , and  $\text{SiO}_2$ . These positive correlations can be explained by the parallel effect of rainfall on increasing nutrient concentrations and thus fecal bacteria. The FIB abundances were negatively correlated with total bacterial abundance only in the water samples at the two sampling sites. As *E. coli*

and intestinal enterococci are used as indicators of pathogen presence, Leight et al. (2018) have highlighted complex positive and negative relationships between total bacteria and autochthonous or allochthonous pathogens. Our results revealed that in water, the FIB seemed to follow different trends compared to the total bacterial count. These results confirmed that the quantified FIB types constituted the fecal contamination and came from a punctual source.

Other low significant correlations were observed between the FIB and other environmental factors. As FIB are mostly organic, VSS are an indicator of organic-solid concentrations measured in samples of activated sludge to determine bacterial amounts (Guangyin and Zhao, 2017). At both sites, the abundance of intestinal enterococci correlated positively with VSS. This correlation shows that VSS could be a proxy for FIB presence even in natural ecosystems. The acidity of an environment is considered to control the rate of microorganism die-off (Reddy et al., 1981). In conclusion, these single correlations detected between FIB abundance and environmental parameters at the two sampling sites suggested that the FIB types have different dynamics at the two sampling sites. However, the interactions of physico-chemical parameters may explain the seasonal variations in FIB abundance (Ackerman and Weisberg, 2003).

#### **4.3. Environmental parameters as FIB drivers for seasonal samples**

Few studies have analyzed the seasonal variation of FIB and environmental parameters relationship during the different seasons of the year. Indeed, FIB can be brought and explained differently by environmental parameters based on season (Ferguson et al., 1996; Lipp et al., 2001; Weiskerger et al., 2019). The analysis of water and sediment sample distributions according to abiotic parameters suggested that FIB abundance was explained by temperature and salinity in spring in summer and by accumulated rainfall (12-hours and 24-hours) and dissolved nutrients (mainly  $\text{PO}_4$  and  $\text{SiO}_2$ ) during winter. Our findings supported González-fernández et al. (2021), who explained that rainfall, water temperature and salinity were the best predictors of microorganism concentrations in the ocean. Our results suggested that FIB occurrence in the two bathing sites had different temporal dynamics, and two scenarios may be proposed. (1) During fall and winter, by terrestrial runoff, rainfall increases FIB abundance and nutrient concentration. These results were in congruence with Sidhu et al.'s (2013) study conducted during the winter months, when the highest level of fecal contamination was observed as a consequence of stormwater runoff in the Onkaparinga River. Lipp et al. (2001) and Kacar and Omuzbiken (2017) also showed that higher indicator bacteria ratios were determined during periods of intense rainfall and low temperature corresponding to the winter parameters in our study. (2) During the summer, the high temperatures increased the salinity

concentration of the marine environment and were very close to the conditions found in the human digestive tract. These parameters would allow halotolerant and thermophilic intestinal bacteria to find favorable growth conditions, which would lead to their multiplication and therefore high concentrations of FIB in the marine environment.

## **Conclusion**

- The “poor quality” classification of the bathing water of the bay of Aytré was mainly due to high intestinal enterococci abundance.
- The abundance of *E. coli* and intestinal enterococci between water and sediment samples were correlated positively in the North Platin.
- During summer and spring, the main environmental drivers of the FIB dynamics were water temperature and salinity, while in fall and winter, the environmental drivers were rainfall and dissolved nutrients.
- From our results, it appeared that the fecal contamination observed at the two sites did not have the same temporal dynamics mainly in summer and therefore hypothetically came from different sources.
- The temporal monitoring performed in this study showed that the regulatory thresholds of the European bathing water directive were rarely exceeded at the two sites. However, future studies are required to determine the spatial variation in fecal contamination across the whole bay of Aytré.
- When relocated to a new environment, fecal bacteria seem to compete with resident bacteria, probably for essential nutrients. Future studies at the molecular level, such as metabarcoding and MST (microbial source tracking), are necessary to clearly identify the source of contamination and the functional role of fecal bacteria in the whole microbial ecosystem.

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**Contributions:**

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P. PINEAU: Formal analysis

B. SIMON-BOUHET, P. ORY: Statistical analysis, Writing – Review

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L.VACHER, T. AGION, M. TREILLES, H. MONTANIÉ: Conceptualization, Methodology, Supervision

H. AGOGUÉ: Conceptualization, Methodology, Writing, Review, Supervision.



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## Supplementary Information

Tableau 3 (Supplementary data T1): Water and sediment sampling frequency during the one-year at the two bathing sites of Aytré Bay.

Sampling sites	Samples type	Frequency												Total	
		Feb-19	Mar-19	Apr-19	May-19	Jun-19	Jul-19	Aug-19	Sept-19	Oct-19	Nov-19	Dec-19	Jan-20		Feb-20
North Platin	Water	2	2	3	3	4	8	8	4	4	2	2	2	2	46
North Platin	Sediment	1	1	1	1	2	4	4	2	2	1	1	1	1	22
South Platin	Water	2	2	3	3	4	8	8	4	4	2	2	2	2	46
South Platin	Sediment	1	1	1	1	2	4	4	2	2	1	1	1	1	22

Tableau 4 (Supplementary data T2): Variation of the environmental variables in the North and South Platin sites. Environmental variables are water temperature (Water temp), air temperature (Air temp), salinity, dissolved oxygen concentration (dO<sub>2</sub>), pH, phosphorous concentration (PO<sub>4</sub>), nitrite concentration (NO<sub>2</sub>), nitrate concentration (NO<sub>3</sub>), ammonium concentration (NH<sub>4</sub>), silicate concentration (SiO<sub>2</sub>), total organic carbon (TOC), total suspended solids (TSS), volatile suspended solids (VSS) and chlorophyll-a concentration (Chloro a).

Parameters	North Platin		South Platin	
	Min	Max	Min	Max
Water temp	7.5	28.9	6.7	29.3
Air temp	5.3	26.4	5.3	26.4
Salinity	20.5	36.6	17.1	36.7
dO <sub>2</sub>	3.9	12.6	5.1	13.2
pH	7.4	32.3	7.3	9.5
PO <sub>4</sub>	0.0	1.1	0.0	1.1
NO <sub>2</sub>	0.0	2.6	0.0	2.0
NO <sub>3</sub>	0.0	219.3	0.0	183.9
NH <sub>4</sub>	0.0	4.8	0.0	11.5
SiO <sub>2</sub>	0.0	72.7	0.0	64.9
TOC	0.0	2.4	0.0	2.7
TSS	5.5	483.3	5.2	480.0
VSS	0.0	78.3	0.0	89.3
Chloro a	0.0	45.1	0.0	20.6

Tableau 5 (Supplementary data T3): Variation of the environmental variables in the North and South Platin sites. Environmental variables are water temperature at the sediment surface (WS temp), air temperature (Air temp), salinity, pH, phosphorous concentration (PO<sub>4</sub>), nitrite concentration (NO<sub>2</sub>), nitrate concentration (NO<sub>3</sub>), ammonium concentration (NH<sub>4</sub>), silicate concentration (SiO<sub>2</sub>), total organic carbon (TOC), chlorophyll-a concentration (Chloro a), Sandy-clay size (Sandy-clay) and sand size (Sand).

Parameters	North Platin		South Platin	
	Min	Max	Min	Max
WS temp	17.8	28.0	4.8	26.5
Air temp	17.7	26.4	8.0	23.5
Salinity	29.2	47.4	10.3	39.4
dO <sub>2</sub>	1.8	9.1	5.2	12.5
pH	7.6	8.4	7.8	8.8
PO <sub>4</sub>	1.2	8.8	0.8	9.4
NO <sub>2</sub>	0.1	0.6	0.1	7.5
NO <sub>3</sub>	0.5	7.1	0.2	56.2
NH <sub>4</sub>	16.3	252.3	4.9	68.3
SiO <sub>2</sub>	17.4	211.4	12.5	172.1
TOC	1.9	13.7	0.7	5.4
Chloro a	2.3	20.9	5.3	21.7
Sandy-clay	0.0	0.8	0.0	0.7
Sand	0.1	1.0	0.0	1.0

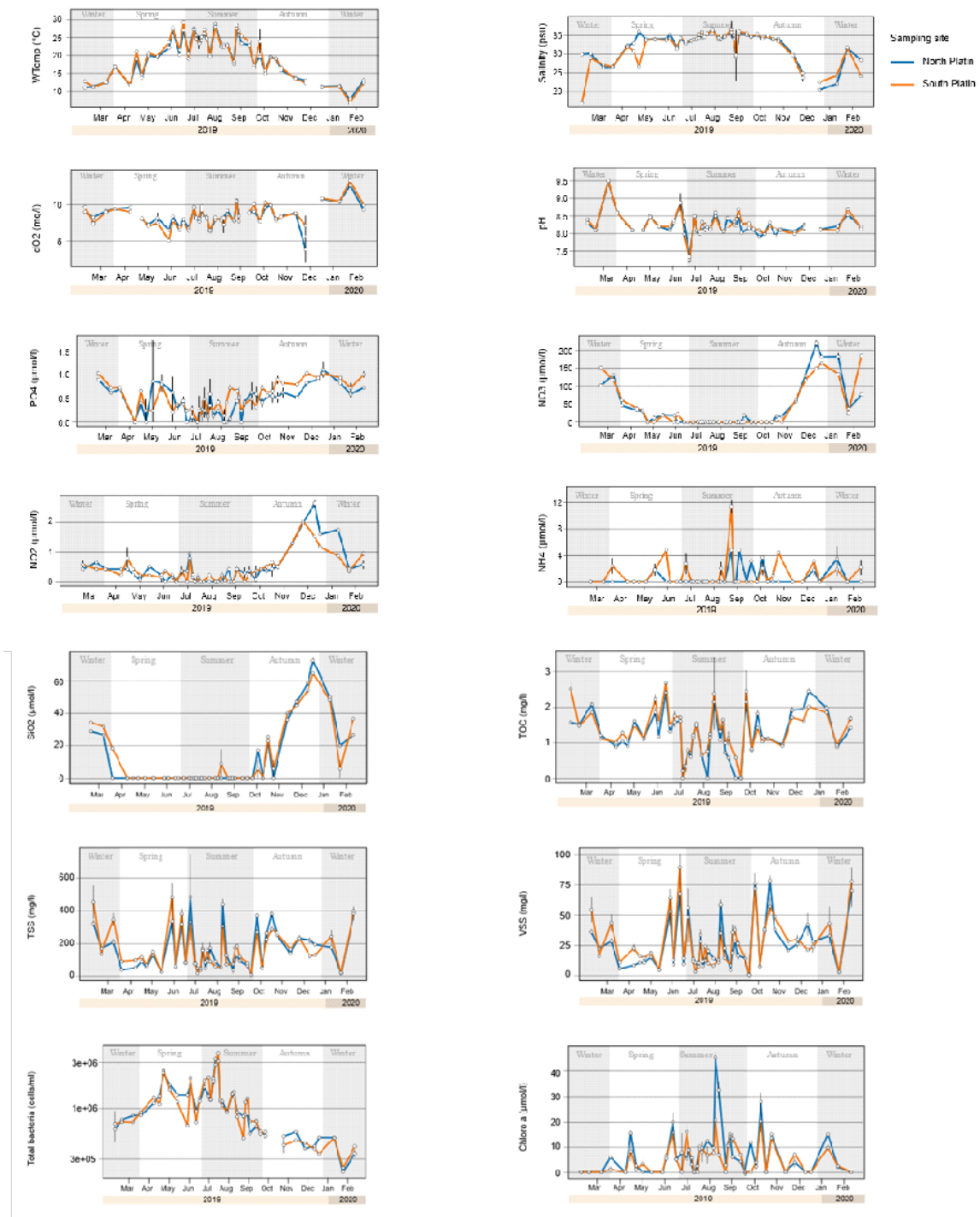


Figure 9 (Supplementary data F1): In situ physico-chemical parameters' variation in water samples in the North (in blue) and South (in orange) Platin sites. Water temperature [WTemp ( $^{\circ}\text{C}$ )], salinity, dissolved oxygen concentration [dO2 (mg/l)], pH, phosphorus concentration [PO4 ( $\mu\text{mol/l}$ )], nitrite concentration [NO2 ( $\mu\text{mol/l}$ )], nitrate concentration [NO3 ( $\mu\text{mol/l}$ )], ammonium concentration [NH4 ( $\mu\text{mol/l}$ )], silicate concentration [SiO2 ( $\mu\text{mol/l}$ )], total organic carbon [TOC (mg/l)], total suspended solids [TSS (mg/l)], volatile suspended solids [VSS (mg/l)], total bacteria abundance [total bacteria (cells/ml)], and chlorophyll-a concentration [Chloro a ( $\mu\text{mol/l}$ )]. The white dots represent the average concentration of the triplicate samples for each date. Error bars show standard deviations between triplicates.

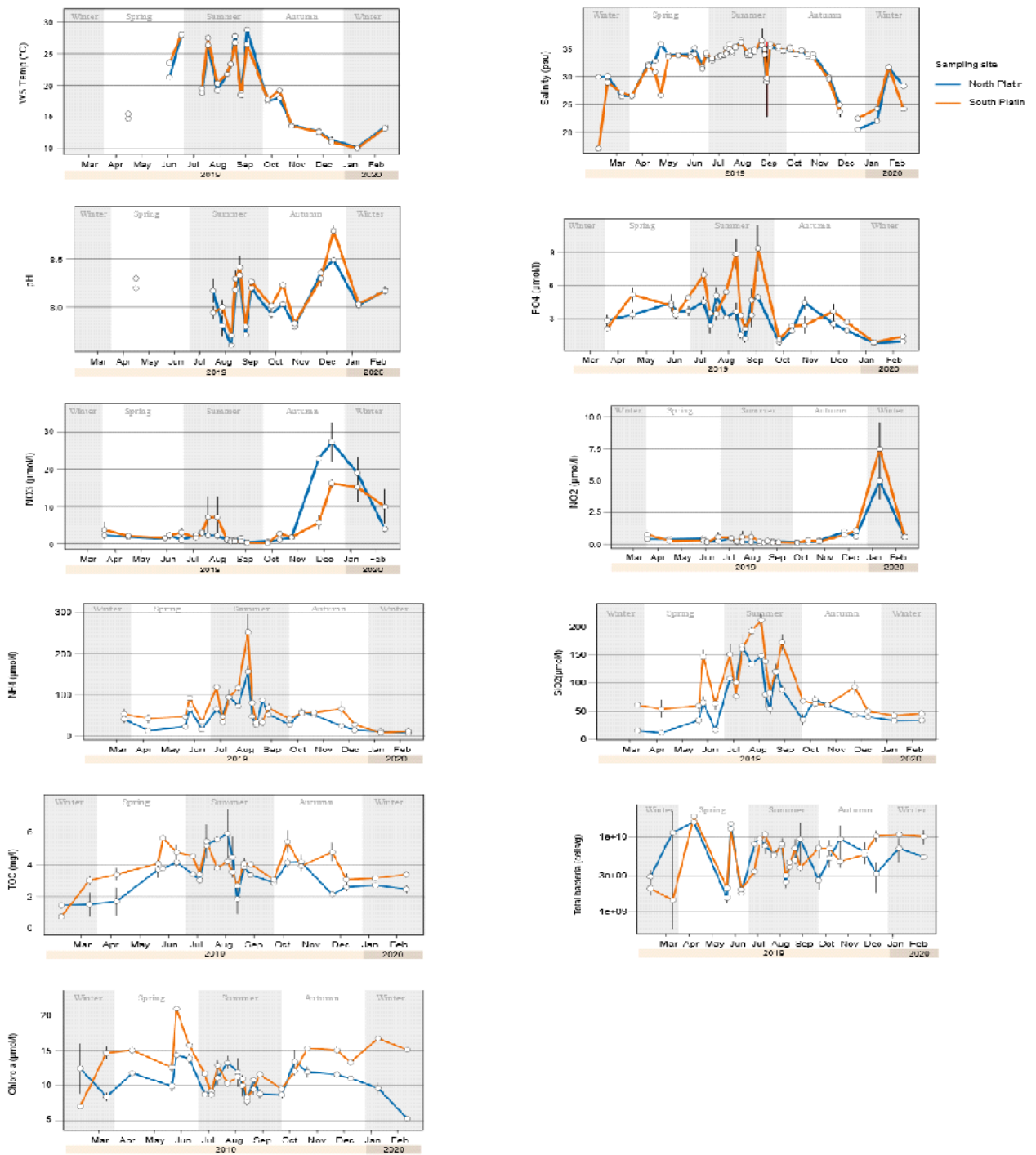


Figure 10 (Supplementary F1): In situ physico-chemical parameters' variation in sediment samples in the North (blue) and South (orange) Platin sites. Water temperature at the sediment surface [WS Temp ( $^{\circ}\text{C}$ )], salinity, pH, phosphorus concentration [ $\text{PO}_4$  ( $\mu\text{mol/l}$ )], nitrite concentration [ $\text{NO}_2$  ( $\mu\text{mol/l}$ )], nitrate concentration [ $\text{NO}_3$  ( $\mu\text{mol/l}$ )], ammonium concentration [ $\text{NH}_4$  ( $\mu\text{mol/l}$ )], silicate concentration [ $\text{SiO}_2$  ( $\mu\text{mol/l}$ )], total organic carbon [TOC ( $\text{mg/l}$ )], total bacteria abundance [total bacteria ( $\text{cells/g}$ )], chlorophyll-a concentration [Chloro a ( $\mu\text{mol/l}$ )]. The white dots represent the average concentration of the triplicate samples for each date. Error bars show standard deviations between triplicates.