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Marine Pollution Bulletin 57 (2008) 59–67
MARINE POLLUTION BULLETIN
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Faecal-indicator bacteria and sedimentary processes in estuarine mudflats (Seine, France)

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Abstract

Over a three-year period, quantification of faecal indicators and the molecular detection of *Escherichia coli* and *Salmonella* were monitored in sediments from three contrasting mudflats of the Seine estuary (France). The elevation of the mudflat surface was monitored concurrently using a high-resolution altimeter. During the period of the study, estuarine mudflats were areas of deposition for faecal-indicator bacteria and were mainly controlled by sedimentary processes. In the intertidal freshwater and subtidal mudflats, the highest abundances of faecal-indicator bacteria were counted during a depositional period. Maximum levels were observed in the freshwater mudflats during periods of high flow: thermotolerant coliforms: 3.9×10^4 cfu cm^{-2}, enterococci: 1.2×10^4 cfu cm^{-2}, *Clostridium perfringens* spores: 9.8×10^5 spores cm^{-2}. Loss of culturability of enteric bacteria in sediment microcosms demonstrated the remediatory capacity of the mudflats, even if they might be a secondary source of bacteria-forming spores to the water column through erosion and resuspension events.
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Keywords: Mudflats; Sediment; Estuary; Faecal indicators; Sedimentary processes; *Salmonella*

1. Introduction

The Seine estuary, located on the Northwestern European continental shelf, is highly anthropized, with a catchment area of 79,000 km^2 inhabited by 16 million inhabitants mainly located in urban areas. Forty percent of French agricultural and industrial activities are located in the estuary watershed. In this macrotidal estuary, sediment transport is highly variable and controlled by both tides and river flow. Fine-grained particles mainly associated with organic matter and chemical contaminants can be trapped in tidal mudflats (Bally et al., 2004; Billon et al., 2003; Le Hir et al., 2001; Lesourd et al., 2001). Sedimentary processes of the tidal mudflats of the Seine estuary were recently studied using a high frequency altimeter to measure the variation in mudflats bed-elevation (Deloffre et al., 2005). It was shown that during high river flow, both freshwater intertidal and subtidal mudflats were preferentially subjected to the load of solids carried by the river. On the intertidal mixing zone mudflats, the deposition was linked to the tidal range and the development of the maximum turbidity zone (Deloffre et al., 2006; Lesourd et al., 2003).

One of the major goals of the multidisciplinary program "Seine-Aval" is to investigate the role played by the estuarine mudflats related to the microbiological quality of the estuarine environment. It was shown that the microbial quality (i.e., abundance of thermotolerant coliform, (ThC)) of the Seine was greatly affected at the estuary entrance by effluent from a large wastewater treatment plant (WWTP) (8.5 million inhabitant equivalent from Paris and its suburbs), especially during periods of high flow (Georges et al., 2001; Servais et al., 1999). In contrast, during periods of low flow, the tributaries represent the

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0025-326X/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.marpolbul.2007.10.013

most important source of faecal coliforms in the Seine estuary (Garcia-Armisen et al., 2005). In this environment, faecal bacteria are generally attached to particles, and their behaviour is related to the dynamics of the suspended particulate matter (Crabill et al., 1999; Crump et al., 1999; Pires Coelho et al., 1999). In the estuarine environment, enteric bacteria undergo several environmental stresses such as variations in salinity, oxygen, temperature, and nutrients that lead to a loss of culturability and viability (Viable but Non Culturable, VNC) (Bordalo et al., 2002; Burkhardt III et al., 2000; Rozen and Belkin, 2001). It is thus not always possible to detect these bacteria in their recoverable form. Molecular methods have therefore been developed to determine if environmental samples have been contaminated by pathogenic bacteria (Kong et al., 2002; Petit et al., 1999; Touron et al., 2005).

The objective of this study was to determine to what extent the Seine estuary mudflats might store faecal indicators and pathogenic enteric bacteria (such as *Salmonella*). The microbial quality of sediments of three mudflats of the Seine estuary was investigated by estimating the abundance of bacterial indicators of faecal contamination: total coliforms (TC), faecal-thermotolerant coliform (ThC), enterococci, spores of sulphite-reducing anaerobes mainly corresponding to *Clostridium perfringens,* and *Escherichia coli* (molecular detection). In addition, the pathogenic bacteria *Salmonella* in sediment were studied by culture-based and molecular methods. These microbiological data were analysed in relation to the topographical evolution of the mudflats (erosion/deposition episodes). The results show that the estuary mudflats investigated have been or are still contaminated by faecal bacteria, but that this environment might not be favourable to the survival of these enteric bacteria in their culturable form.

2. Materials and methods

2.1. Site description and sediment sampling

Sediment samples were collected nine times from 2001 to 2003 on three mudflats of the Seine estuary (Fig. 1): the Oissel intertidal mudflat (49°20′288; 01°05′660), located in a freshwater zone; the Northern intertidal mudflat (49°26′823; 0°14′628), located in the mixing zone, at the mouth of the estuary; and a subtidal mudflat (March 2002: 49°26′000; 0°00′700; September 2002: 49°25′950; 0°00′730), located in the Bay of Seine. The intertidal mudflats of the Seine estuary consist of clay and silt deposits (<63 μm) originating from terrigenous material, and marine particles mainly in the middle and marine part of the estuary (Deloffre et al., 2005, 2006; Lesourd et al., 2003). Sediment cores (20-cm long) were collected, transported to the laboratory within three hours at 4 °C, and cut into ten 2-cm-thick slices. Bacteriological analyses were carried out within six hours. Subsamples (1 g, wet weight) of each section were frozen at −20 °C until subsequent molecular analysis could be performed (between one week to two months). The mean dry and wet densities, respectively, were estimated for each mudflat: 0.56 g cm^{-3} and 1.285 g cm^{-3} for the freshwater sediments, 0.766 g cm^{-3} and 1.464 g cm^{-3} for the mixing zone sediments, and 0.66 g cm^{-3} and 1.41 g cm^{-3} for the subtidal sediments.

2.2. Water sampling

Seine estuary water was sampled eight times by the Service de Navigation de la Seine (SNS), from 2001 to 2003, on a 153-km stretch between the Poses dam (km 202, the centre of Paris being taken as km zero by the French

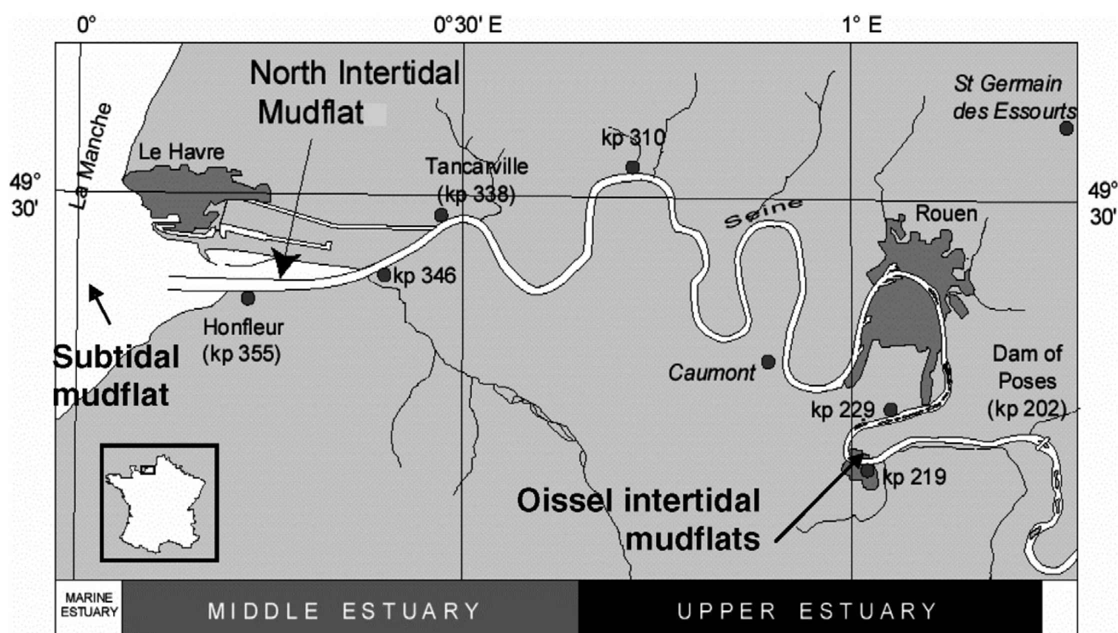


Fig. 1. Map of the Seine estuary (Guézennec et al., 1999). Arrows indicate the study sites: marine subtidal, intertidal mixing zone and freshwater intertidal mudflats.

agency of the "Seine Normandie" basin) and Honfleur (km 355.8). Water samples were collected at 1-m depth in 1-L sterile containers. Bacteriological analyses were carried out within six hours. The suspended load concentration was estimated by filtration trough a 47-mm cellulose acetate filter (0.45 μm).

2.3. Bacteriological analysis of water and sediments

Total coliforms (TC), thermotolerant coliforms (ThC), and enterococci were enumerated by plate count after membrane filtration according to standard international methods (ISO 9308-1, ISO 7899-2), with the following modification for sediment analysis: 2 g (wet weight) were added to 18 mL of NaCl 0.85% (w/v) supplemented with $Na_4P_2O_7$ (1 mM, final concentration) and mixed vigorously for 3 min to dissociate biofilms. Ten millilitre volumes of appropriate dilutions were filtered (0.45 μm, HA047, Millipore) and incubated on appropriate media (AES Laboratoire, Combourg, France). Under these conditions, threshold values for the enumeration of bacterial indicators in the sediments were 1×10^1 cfu g^{-1} for ThC, and less than 1×10^2 cfu g^{-1} from 0 to 8 cm and 1×10^1 cfu g^{-1} from 8 cm to 20 cm for TC and enterococci. *E. coli* were enumerated by toothpicking the ThC colony on RAPID *E. coli* 2 medium (BioRad, Marnes-la-coquette, France). Spores of sulphite-reducing anaerobes (mainly *C. perfringens)* were quantified on TSN agar (AES Laboratoire, Combourg, France) according to the ISO 6461 standard. *Salmonella* were isolated from 10 g of sediment after selective enrichment steps and growth on selective media (ASAP and XLT4 AES Laboratoire, Combourg, France), as recommended by ISO standards (ISO 6340). Confirmation of suspicious *Salmonella* was processed by PCR as described in the following section.

2.4. Nucleic acid extraction from environmental samples

Total DNA was extracted from a 0.5 g sediment sample (wet weight) using a Bio-101 FastDNA Spin kit in combination with the FastPrep FP120 bead beating system (Bio-101, USA) according to the manufacturer's instructions. Total crude DNA was purified by elution through Elutip-D column (Schleicher and Schuell, Dassel, Germany). The concentration of the resulting DNA was estimated by spectrophotometry (Gene Quant, Amersham).

2.5. PCR amplification

Molecular detection of *E. coli* was performed by PCR amplification of a 167 bp fragment of the *uidA* sequence coding the β-D-glucuronidase (Bej et al., 1991). DNA from sediment samples (1–50 ng) were used for PCR in a 50 μl final volume of the following reaction mixture: 1 × PCR reaction buffer (Eurogentec, Seraing, Brussels), 200 μM of each dNTP, 0.25 μM of each primer, 4 mM of $MgCl_2$ and 1 U of Red Goldstar *Taq* polymerase (Eurogentec, Seraing, Brussels). PCR amplifications were performed in a Perkin–Elmer thermocycler (GeneAmp PCR system 9700) as follows: 10 min at 95 °C for denaturation, 35-cycles of 1 min at 95 °C for denaturation, annealing of 1 min at 55 °C, 1 min at 72 °C for extension, and a final extension step at 72 °C of 10 min. Molecular detection of *Salmonella* was performed by a nested PCR amplification of a specific fragment 882 bp of the *fliC* sequence coding for a phase-one flagellin (Touron et al., 2005). Amplified fragments were visualized by electrophoresis in a 2% agarose gel (w/v) in 0.5 × TAE to confirm the expected size of PCR products. The detection limit or molecular amplification was, respectively 4–5 DNA copies for *Salmonella* and 28 copies for *E. coli*. DNA extracts from *E. coli* C600 or *S. Typhimurium* were used as a positive control. All negative samples, where no specific bacterial DNA could be amplified, were checked by amplifying the eubacterial 16S ribosomal DNA (16 S rDNA) sequence using primers pA (5′-AGTTTGATCATGGCTCAG-3′) and pHr (5′-GAGGTGATCCAGCCGCA_3′) (Edwards et al., 1989).

2.6. Microcosm experiments

Two strains of *E. coli* were used to inoculate the microcosm: SC11 (*E. coli* W3101 Nal^r) and SA100, previously isolated from water of the Seine estuary. Sediments (800 g, wet weight) were sampled in the upper 10 cm of the North intertidal mudflat and were checked for the absence of culturable *E. coli*. After sterile homogenization of the sediment, 24 subsamples of 20 g (wet weight) were conditioned in sterile polypropylene tubes. After a pre-incubation period of 4 days at 18 °C or 4 °C, 12 sediment microcosms were inoculated with *E. coli* (SC11 or SA100)

Table 1
Sediment characteristics of the three mudflats and river flows

Sampling date	River flow ($m^3 s^{-1}$)	Bed elevation (mm)	Age of superficial sediment (days)	Depositional conditions
Intertidal freshwater mudflat				
March 2002	1764	75	10–65	Deposit
May 2003	625	75	100–150	Deposit
July 2003	324		150–200	Erosion
Intertidal mixing zone mudflat				
May 2001	682		N.A	Erosion
September 2002	154	30	20 (fresh deposit)	Deposit
April 2003	294	55	6 (fresh deposit)	Deposit
July 2003	120		>85 days	Erosion
Subtidal mudflat				
March 2002	920	120	Deposit of fluid mud	Deposit
September 2002	185		Reworked sediment	Erosion

N.A: Not available.

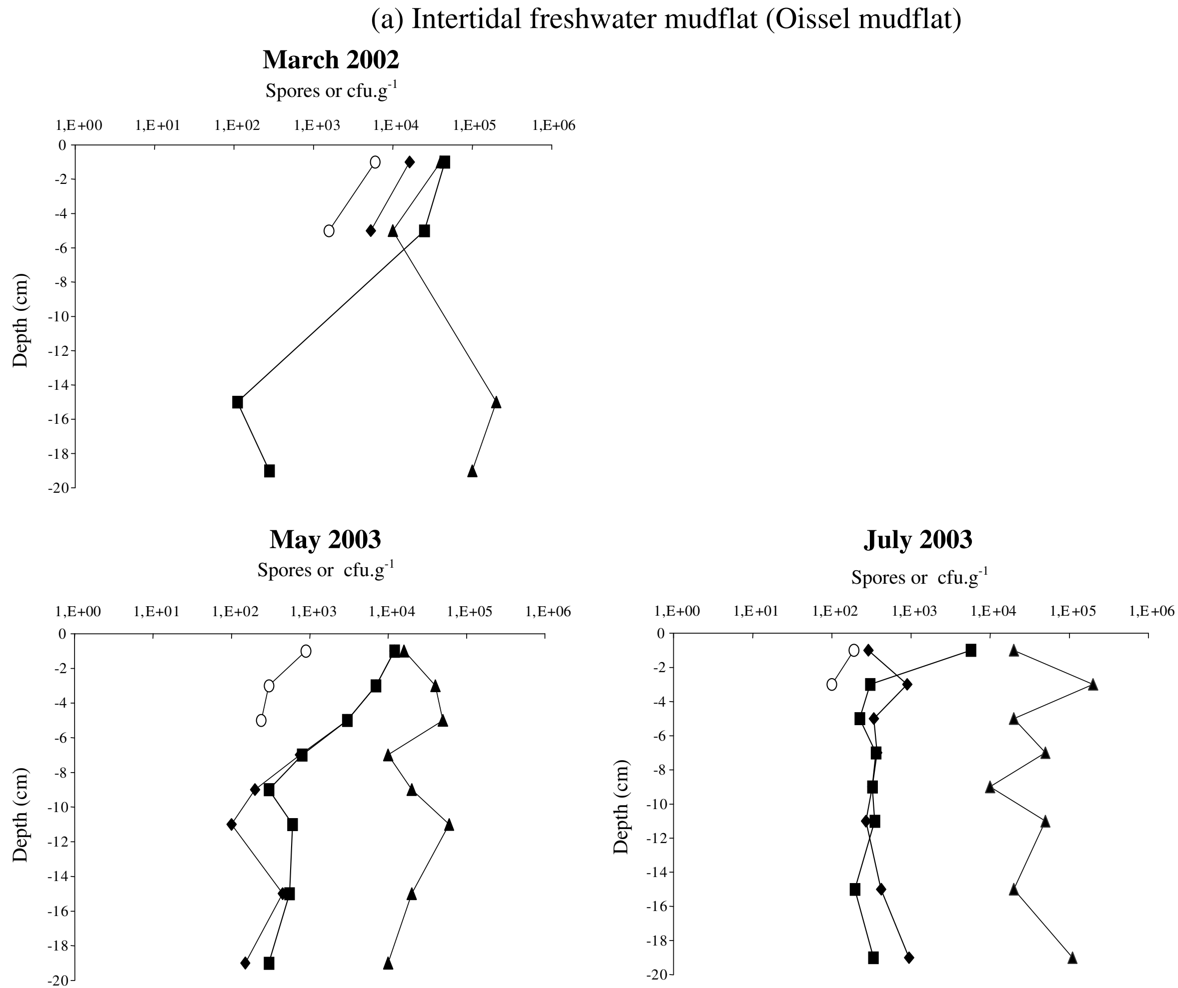


Fig. 2. Vertical distribution of faecal indicators TC, ThC, enterococci, and *C. perfringens* spores in the three mudflats from 2001 to 2003. (a) intertidal freshwater mudflat; (b) intertidal mixing zone mudflat; (c) subtidal mudflat. TC (■); ThC (♦); enterococci (○); *C. perfringens* spores (▲).

at a final density of 1×10^6 cells per gram ($t = 0$) and incubated at the same temperature in the dark for 41 days.

2.7. Topography

To determine periods of erosion or deposition, changes in mudflat elevation were monitored by an altimeter (Micrel ALTUS) with a resolution of ±2 mm at a high frequency (1 burst every 10 min) (Deloffre et al., 2005, 2006).

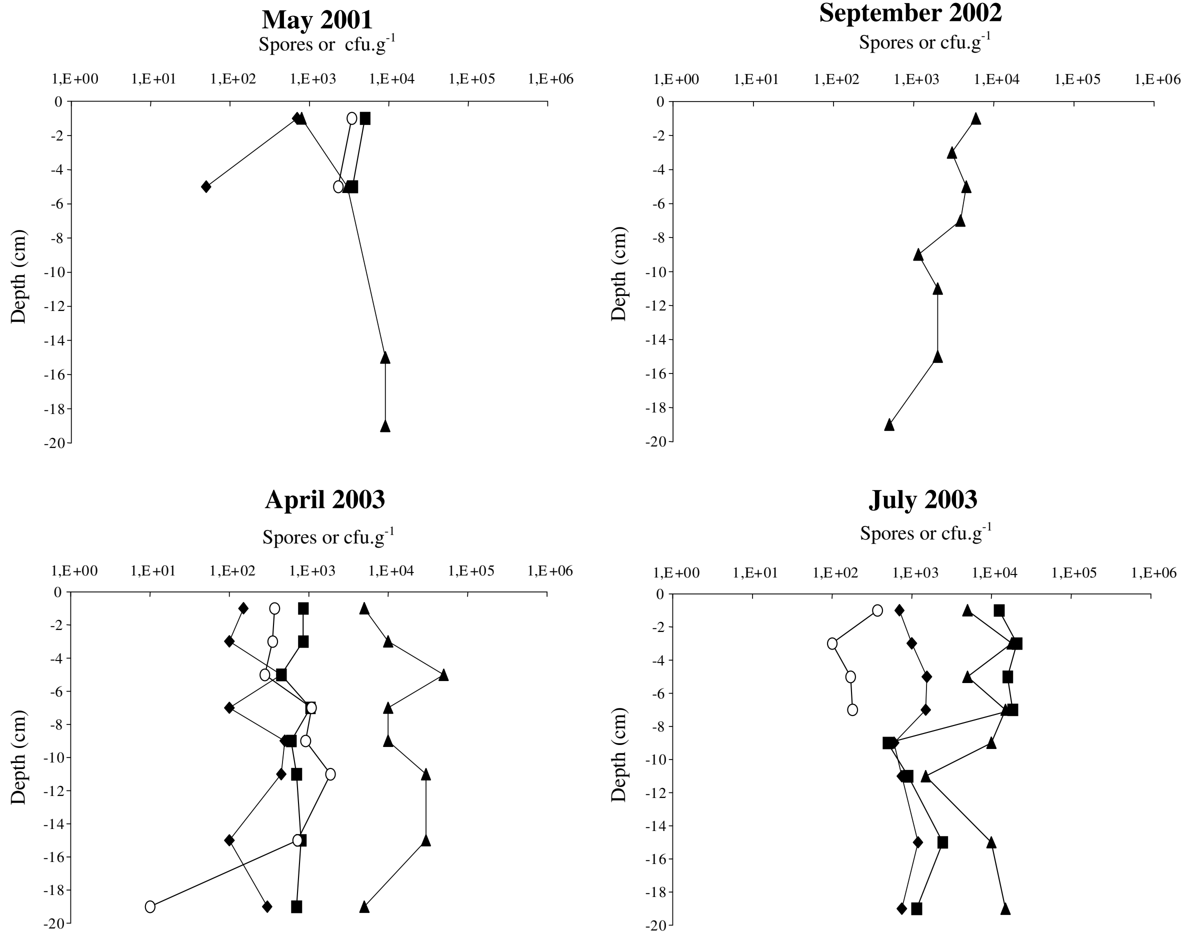
3. Results

3.1. Typical feature of faecal-indicator bacteria

The three mudflats sampled for this study showed contrasting physico-chemical characteristics: the intertidal Northern mudflat and the subtidal mudflat have marine sediment characteristics, while the Oissel mudflat has freshwater sediment characteristics (Deloffre et al., 2005). The Oissel intertidal mudflat is located in the freshwater part of the Seine estuary (Fig. 1). The March 2002 and May 2003 cores were collected during a period of high river flow corresponding to a depositional period; the surface sediments had been in place for 10–65 days (March 2002 core) and 100–150 days (May 2003 core) (Table 1). The July 2003 core was collected during a period of erosion. The Northern intertidal mudflat is located in the mixing zone of the Seine estuary, and is subjected to the influence of the tide and marine waters. For this mixing-zone mudflat, the September 2002 and April 2003 cores were collected after a depositional period; the surface sediments had been in place for 20 (September 2002 core) and 6 days (April 2003 core). In contrast, the cores collected in May 2001 and July 2003 were sampled after an erosional period (Table 1). Samples were collected during two periods in the subtidal mudflat: in March 2002, during a sedimentation period mainly caused by mud deposits, and in September 2002, during a low flow period when the accumulated sediment was reworked by waves and tidal currents (Deloffre et al., 2006).

The vertical distribution of faecal-indicator bacteria shows that the sediments of the three mudflats are affected by faecal contamination (Fig. 2). Culturable enterococci,

(b) Intertidal mixing zone mudflat



(c) Subtidal marine mudflat

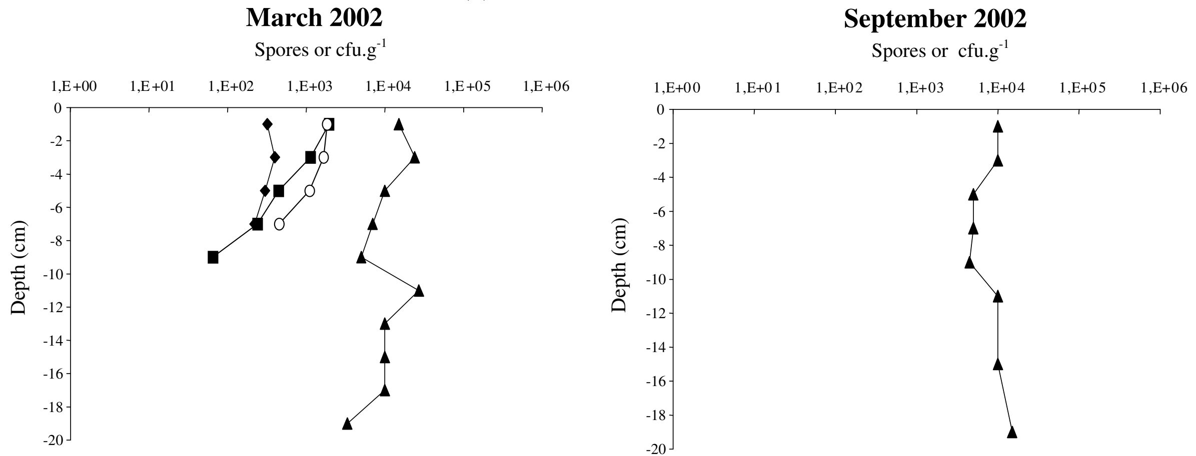


Fig. 2 (*continued*)

TC, and ThC were enumerated in seven of the nine cores, with the highest abundances observed in the top layers of sediment. In contrast, no faecal indicator bacteria were detected in cores sampled in September 2002 from both the mixing zone and the marine mudflats except spores of *C. perfringens*.

To estimate storage capacity and to compare faecal contamination of the three mudflats, data for the top 20 cm were integrated for each core (Table 2). However, it should be noted that such integration does not take into account the heterogeneity of the sediments. In the intertidal freshwater mudflat, TC, ThC, and enterococci were isolated in

Table 2
Comparison of faecal contamination of the three Seine estuary mudflats

	Integrated values of indicators (cfu or spores cm^{-2})[a]			
	Total coliforms (cfu cm^{-2})	Thermotolerant coliforms (cfu cm^{-2})	Enterococci (cfu cm^{-2})	*C. perfringens* (spores cm^{-2})
Intertidal freshwater mudflat				
March 2002	1.4×10^5	3.9×10^4	1.2×10^4	9.8×10^5
May 2003	2.1×10^4	2.0×10^4	1.1×10^3	3.1×10^5
July 2003	5.9×10^3	4.7×10^3	1.5×10^2	5.7×10^5
Intertidal mixing zone mudflat				
May 2001	2.6×10^4	1.3×10^3	1.7×10^4	1.5×10^5
September 2002	ND	ND	–	3.5×10^4
April 2003	1.0×10^4	3.6×10^3	1.0×10^4	2.2×10^5
July 2003	1.0×10^5	1.4×10^4	9.7×10^2	1.1×10^5
Subtidal mudflat				
March 2002	3.7×10^3	1.4×10^3	5.4×10^3	1.4×10^4
September 2002	ND	ND	–	1.0×10^5

ND: Not detected, below the detection threshold for enumeration of the bacterial indicator.
–: Not determined in these cores.
[a] Integrated values per cm^2 on a 20 cm long core.

every case. The highest values were observed in the sediment cores collected during a depositional period (March 2002, May 2003), with the highest of the indicators (TC: 1.4×10^5 cfu cm^{-2}, ThC: 3.9×10^4 cfu cm^{-2}, enterococci: 1.2×10^4 cfu cm^{-2}, *C. perfringens* spores: 9.8×10^5 spores cm^{-2}) corresponding to the maximum river flow (March 2002). The ratio of enterococci to ThC was lower (0.03–0.3) in the freshwater mudflat than in both the mixing zone and marine mudflats (0.07–13).

In the subtidal mudflat, sediments were less contaminated by at least one order of magnitude than the sediments of the two intertidal mudflats. Faecal-indicator bacteria were isolated during the depositional period of March 2002, whereas no indicator bacteria, except spores of *C. perfringens*, were detected for the erosional period of September 2002.

In the mixing-zone mudflat, the maximum values of TC and ThC were similar to or higher than those in the freshwater mudflats. For the mixing-zone mudflat, unlike the two other mudflats, maximum abundances of indicator bacteria were observed for erosional periods (May 2001 and July 2003). In contrast, the abundances of TC, ThC, and enterococci were below the detection threshold for the depositional period (September 2002) and no relation was observed with depositional or erosional periods for this mudflat.

C. perfringens spores were found at each depth in all cores in the same order of magnitude. In contrast, during these sampling periods, no *E. coli* was isolated but its DNA (*uidA* gene) was successfully amplified from seven of the nine cores (Table 3). These results suggest that, although the sediments of these three mudflats have been or still are contaminated by faecal bacteria, this environment might not be favourable for the survival of these enteric bacteria in their culturable form. Moreover, *Salmonella* was rarely detected by culturing methods (one of nine cores) although it was detected by molecular methods in four of nine cores (Table 3). *Salmonella* was isolated in the September 2002 samples but no TC or ThC were isolated, suggesting better survival of *Salmonella* than TC, ThC, or enterococci in the mudflat sediments.

3.2. Culturability of enteric bacteria in experiments

A microcosm experiment was performed to investigate the loss of culturability of enteric bacteria in mudflat sediments. The culturability of two strains of *E. coli* was compared at 4 °C and 18 °C. One strain was taken from the laboratory collection (SC11) and the second strain had been isolated previously from the Seine estuary (SA100). The decrease in culturability was greater at 18 °C than at 4 °C (Fig. 3). After 13 days of incubation at 4 °C, the culturability was around 50% for both strains and dropped to 21% (SA100) and 2% (SC11) after 41 days. The culturability of the strains at 18 °C was only 12% (SA100) and 5% (SC11) after 13 days incubation and less than 1% for both strains at the end of the experiment. The loss of culturability of SC11 thus is greater than that of SA100 at both temperatures, suggesting that the SA100, which was isolated from the Seine estuary, has a better physiological adaptation to this environment than the SC11 strain.

3.3. Faecal-indicator bacteria in the sediment mudflat versus in the water column

Faecal contamination was compared between the mudflat sediments and the water of Seine estuary (Table 4). Here, from 2001 to 2003, abundances of TC, ThC, enterococci, and *C. perfringens* spores are expressed as cfu (or spores) per dry gram of particulate matter, under the assumption that bacterial attachment to particles is similar within the water column and the mudflat. Geometric means of abundances of TC, ThC, and enterococci (cfu g^{-1}, dry weight) and occurrence of culturable

Table 3
Presence (+) or absence (−) of *Salmonella* and *E. coli* in mudflat sediments

Mudflat	Depth (cm)	*Salmonella*		*E. coli* PCR[d]
		Culturable	nmPCR[d]	
Intertidal freshwater mudflat[a]				
March 2002	00–02	−	+	+
	04–06	−	+	+
	14–16	−	+	+
	18–20	−	+	+
Intertidal mixing zone mudflat[b]				
May 2001	00–02	−	+	−
	04–06	−	−	−
	14–16	−	−	−
	28–30	−	−	−
September 2002	00–02	−	−	+
	02–04	+	+	+
	04–06	+	+	+
	06–08	−	−	−
	08–10	−	−	−
	10–12	−	−	+
	14–16	−	−	+
	18–20	−	−	+
Subtidal mudflat[c]				
March 2002	00–02	−	+	+
	02–04	−	+	+
	04–06	−	+	+
	06–08	−	−	+
	08–10	−	+	+
	10–12	−	−	+
	12–14	−	−	+
	14–16	−	−	+
	16–18	−	−	+
	18–20	−	+	+

[a] Cores sampled in May and July 2003: Molecular detection of *E. coli* (*uidA* gene) at each depth but no culturable *Salmonella* and no molecular detection of *fliC* gene.
[b] Cores sampled in April and July 2003: Molecular detection of *E. coli* (*uidA* gene) at each depth but no culturable *Salmonella* and no molecular detection of *fliC* gene.
[c] Cores sampled in September 2002: No culturable *Salmonella* and no molecular detection of *Salmonella* (*fliC* gene) and *E. coli* (*uidA* gene).
[d] Two independent molecular analyses were carried out for each sample. Positive and negative controls are described in Section 2.

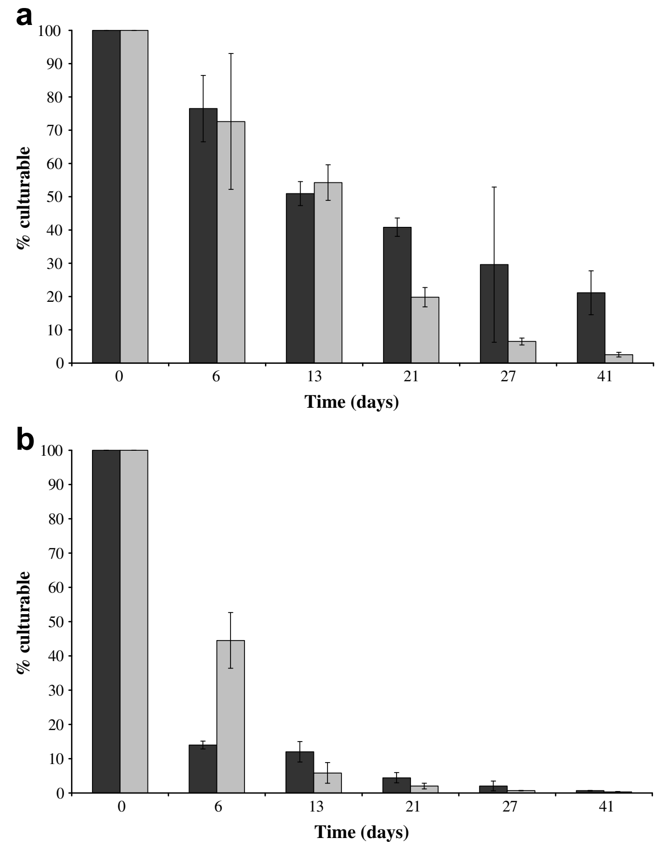


Fig. 3. Loss of culturability of two *E. coli* strains in sediment microcosms: percentage of culturablity at 4 °C (a), at 18 °C (b); strain SA100 (black) was previously isolated from the Seine estuary, and SC11 (grey) from laboratory collection. These results are the average of three independent experiments and the error bars indicate the standard error.

Salmonella were lower per unit of mudflat sediment than in the water column for all three mudflats (Tables 2 and 4). In contrast, *C. perfringens* was the only faecal indicator for which the highest abundances in the water column and in the mudflats were equal: in the freshwater zone, the abundances were 7.87×10^5 spores g^{-1} (dry weight) in the water column and 4.6×10^5 spores g^{-1} (dry weight) in the mudflat sediment, and in the saline zone, 1.47×10^5 spores g^{-1} (dry weight) in the water column and 1.0×10^5 spores g^{-1} (dry weight) in the mudflat sediment.

4. Discussion

Quantification of faecal indicators and the molecular detection of *E. coli* and *Salmonella* demonstrate that sediments of these three mudflats in the Seine estuary were areas of deposition of coliforms, enterococci, *Salmonella* and *C. perfringens* spores. This faecal contamination is resulting of the microbial quality of the estuarine water, the association of faecal bacteria with settling particles, the survival behaviour of these bacteria in this environment, and particle dynamics in the estuary. The systematically lower contamination of the mudflat sediments compared to those of the water column might result from the selective deposition of faecal bacteria attached to settling particles (Crump et al., 1999) combined with a different survival behaviour in sediment compared to water. The highest abundances of faecal-indicator bacteria generally were obtained from the upper layers of the sediments and decreased with depth, suggesting that this environment does not favour the growth or the survival of a viable culturable form of enteric bacteria. The ratio of enterococci to ThC might have been higher as a result of greater resistance of enterococci to osmotic stress in marine environment (Bordalo et al., 2002; Noble et al., 2003). Overall, in estuarine water and sediments, survival of enteric bacteria, *E. coli*, and *Salmonella* has mainly been discussed in terms of biotic factors and environmental stresses such as variations in salinity, temperature, and nutrients (An

Table 4
Faecal contamination of the water column along the Seine estuary (2001–2003)

	Station	km	Total coliforms[a]		Thermotolerant coliforms[a]		Enterococci[a]		Spores of *Clostridium perfringens*[a]	
			min–max[a]	Geom. mean	min–max[a]	Geom. mean	min–max[a]	Geom. mean	min–max[a]	Geom. mean
Upstream input	Poses	202	1.26×10^5–1.82×10^7	1.82×10^6	5.26×10^4–6.82×10^6	5.94×10^5	5.85×10^3–2.31×10^5	3.42×10^4	1.75×10^5–7.87×10^5	2.94×10^5
Urban area	Rouen	243	3.45×10^6–4.27×10^6	3.84×10^6	9.15×10^5–1.41×10^6	1.14×10^6	8.57×10^4–1.16×10^5	9.97×10^4	2.5×10^5–5.49×10^5	3.70×10^5
	Le Croisset	246.6	1.86×10^6–2.77×10^7	5.67×10^6	2.97×10^5–3.33×10^6	1.41×10^6	6.25×10^4–8.42 v10^5	1.61×10^5	1.88×10^5–4.59×10^5	2.97×10^5
	La Bouille	259.7	9.20×10^5–7.41×10^6	3.94×10^6	3.19×10^5–2.43×10^6	9.32×10^5	4.61×10^3–1.41×10^5	2.71×10^4	1.90×10^5–4.03×10^5	3.07×10^5
Estuary mouth	Tancarville	337	1.43×10^4–5.80×10^6	2.54×10^5	9.00×10^2–1.70×10^6	4.11×10^4	4.09×10^2–4.27×10^4	2.94×10^3	3.07×10^4–1.47×10^5	7.40×10^4
	Honfleur	355.8	1.53×10^4–5.99×10^5	6.63×10^4	3.08×10^2–5.54×10^5	1.95×10^4	4.08×10^1–5.43×10^4	1.66×10^3	1.57×10^4–6.17×10^4	2.99×10^4

[a] Results are expressed as cfu or spores g^{-1} of suspended matter (dry weight).

et al., 2002; Bordalo et al., 2002; Rozen and Belkin, 2001). Our results are consistent with other studies, and underline that pre-exposure to stresses can lead to a better chance of survival in the marine environment (Rozen and Belkin, 2001; Trousselier et al., 1998). Low temperatures might be an important factor in preserving culturable *E. coli* in estuarine sediments.

Spore-forming bacteria such as *C. perfringens* are able to survive for a long time in aquatic environments (Burkhardt III et al., 2000; Payment et al., 2000). In aquatic environments, *C. perfringens* spores are often considered to be a better indicator of sewage contamination than TC or ThC (Ferguson et al., 1996; Lipp et al., 2001; Skanavis and Yanko, 2001). In this study, *C. perfringens* spores were detected in all sediment samples, suggesting a continuous supply of faecal bacteria to the Seine estuary mudflats. Conversely, as described previously for rivers and lakes, the resuspension of sediments from Seine estuary mudflats might lead to higher densities of this faecal indicator in the water column (An et al., 2002; Obiri-Danso and Jones, 2000).

Faecal contamination of sediments in estuarine freshwater and subtidal mudflats might be related to patterns of sedimentation, with higher levels of faecal-indicator bacteria when sedimentation dominates. Deloffre et al. (2005) have demonstrated a direct correlation between the flux of suspended solids discharged by the river at the entrance of the Seine estuary and the thickness of deposits in the freshwater mudflats. However the relations between sedimentary processes and the seasonal dynamics of faecal contamination are more complicated in the mixing zone mudflats. The changes in this mudflat elevation were interpreted on the basis of the river flow, the maximum turbidity, the tidal range, the waves, and the tidal currents (Deloffre et al., 2005, 2006; Lesourd et al., 2001, 2003).

Here, we have shown that the estuarine mudflat of the Seine estuary are a depositional area for faecal-indicator bacteria, and is controlled by sedimentary processes among other factors. These mudflats might be a storage site for total coliforms, thermotolerant coliforms, and enterococci, but only ephemerally, as these faecal-indicator bacteria appear to lose their culturability in this environment. These mudflats thus have a remediatory capacity, even if they might also be a secondary source of faecal contamination of the water column, through the resuspension of *C. perfringens* spores under strong erosion.

Acknowledgments

This work was supported by the Seine-Aval 2 Scientific Research Program. The second author holds a research grant from the Region Haute-Normandie. We thank Mr A. Ficht (SNS, Rouen, France) for assistance with sampling, Dr Barbara Mahler for translation, Dr D. Boust for sediment density values and M. Ratajczak for technical assistance.

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