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Salmonella spp. and Fecal Coliform Loads in Coastal Waters from a Point vs. Nonpoint Source of Pollution

J. Baudart, J. Grabulos, J.-P.

Barusseau, and P. Lebaron*

ABSTRACT

Coastal areas are often contaminated by the dissemination of pathogenic bacteria from terrestrial inputs. In this study, we compared fecal coliforms (FC) and *Salmonella* spp. loads from a coastal Mediterranean river and from the submarine outfall of a coastal wastewater treatment station. A stratified sampling strategy was used to analyze storm events. Bacterial loads were estimated during a 16-mo period. *Salmonella* spp. loads from the river were high during storm events, and the annual loads were higher than those estimated from the coastal outfall. Bacterial loads from the river represented 3.0×10^6 FC yr⁻¹ and 6.9×10^6 *Salmonella* yr⁻¹, with at least 95% occurring during high waterflow (21 % of the year). Those from the submarine outfall represented 4.3×10^6 FC yr⁻¹ and 4.7×10^6 *Salmonella* yr⁻¹, with a regular temporal discharge. Bacterial loads from the river were associated with small clay particles (<2 µm), which originate from different reservoirs. These particles-sediment-trapped bacteria accumulated in the river-bed during the lowest water levels in the downstream part of the river and were resuspended during storm events. The quantitative relationship between both bacterial parameters vary depending on the source point of contamination. *Salmonella* spp. loads from the river are of great sanitary concern since high loads were recorded in summer (bathing activities) and autumn (oyster production and consumption). Our results should help water quality managers to define priorities to improve the bacterial quality of river discharges.

The *Salmonellae* are a large group of bacteria comprising more than 25(10 known pathogenic serotypes that can cause a range of symptoms from mild gastroenteritis to death (like typhoid fever). *Salmonella* spp. infection is one of the most commonly reported causes of food-borne disease in the Western world (McClelland and Pinder, 1994). *Salmonella* spp. are capable of infecting a variety of cold- and warm-blooded animals. They are ubiquitous enteric bacteria and also a prime example of water- and shellfish-transmitted pathogens. The increasing occurrence of *Salmonella* spp. in the environment increases the potential risk of illness from consuming shellfish.

Waterborne enteric pathogens are excreted, often in large numbers, in the feces of infected humans and animals at rates up to 10^{10} g⁻¹ of fecal matter (Sojka et al., 1974; Martel, 1985). Pathogens are common in municipality sewage (Morinigo et al., 1992; Salgado et al., 1993) and are released into rivers, streams, and coastal waters through the discharge of treated wastewater effluents.

The direct release of pathogenic bacteria from coastal

outfall of treated wastewater effluents is a permanent and point source of coastal water pollution. In range and pasture systems, the concentration of animal wastes is generally much more diffuse or dispersed. Range and pasture systems are nonpoint sources of contamination that contribute, mainly through storm and rainfall events, to the movement of soil particles into streams and rivers. The increasing turbidity is often associated with an increase in bacterial pollution since small particles are vectors of many enteric bacteria (Sherer et al., 1992; Irvine and Pettibone, 1996).

The mobilization of enteric bacteria from different nonpoint sources can cause a variety of problems. Fecal coliforms (FC) are used as indicators of the quality of shellfish and to classify the waters from which shellfish are harvested (Hood et al., 1983). Rainfall and storm events may result in the closing of bathing beaches due to temporarily high bacterial counts, indicating fecal pollution (Wyer et al., 1996).

The relative contribution of point and nonpoint pollution sources to the bacterial quality of coastal waters is relatively unknown. Water bacterial pollution is generally measured through the absolute measurement of enteric bacteria concentrations. However, concentration data alone may result in misinterpretations if not related to waterflow. Information on the temporal variability of discharge containing fecal indicators and *Salmonella* spp. would assist water quality managers in defining optimal treatment and risk management.

In the present study, we have quantified and compared fecal indicator and *Salmonella* spp. discharges from a submarine wastewater outfall and a Mediterranean coastal watershed characterized by various rearing activities (cattle [*Bos laurus*], poultry, boar [*Sus scrofa*]). The bacterial loads were compared with those for total suspended matter.

MATERIALS AND METHODS

Sampling Area

The Tech is a typical coastal river on the western Mediterranean coast with a drainage area of 780 km². From 1990 to 1996, the mean river annual discharge at Station 4 (5 km upstream from the mouth) was 3.9×10^9 m³ yr⁻¹. The river's source is located at an altitude of 2345 m and 78 km from the river's mouth. The river is characterized by a high declivity (8%) from the source to 20 km downstream, an intermediate slope (1.7%) along the next 30 km, and by a low slope close near the river's discharge (0.3%). The upstream area of the watershed is characterized by agriculture and forests with extensive rearing activities (cattle and boar). Some small poultry

Abbreviations: FC, fecal coliforms; CFU, colony forming unit; MPN, most probable number; TSM, total suspended matter; Q_d, daily mean; n waterflow; Q_s, daily sewage flow; 1_{FC}, fecal coliforms load; 1_{Sal}, *Salmonella* spp. load.

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farms (ducks [*Anas platyrhynchos*], chickens [*Gallus gallus*], geese [*Anser domesticus*]) are also located in this area. The Tech river has several tributaries in the western area (Vallespir region). Hydraulic conditions are defined by strong fluctuations of discharge rates with very low discharge rates in summer and flood events during the autumn and winter seasons. Numerous wastewater treatment stations are located within the watershed with their outfalls located in the river and its tributaries (Fig. 1a).

The wastewater treatment coastal station of Banyuls-sur-Mer (Fig. 1b) is a monitoring station with a physical and chemical treatment. From 1992 to 1995, the annual mean discharge was $5.6 \times 10^6 \text{ m}^3 \text{ yr}^{-1}$. The population varies from 5000 residents off season to 15 000 inhabitants during the summer time. The residence time of wastewater in the sewage station is 7.5 h. The sewage system combines rainfall waters and wastewaters, and effluents are discharged via a submarine outfall located 500 m offshore and 20 m depth below the surface.

Bacterial Analysis

Samples were collected in sterile flasks and analyzed within 4 h after sampling. Both spread plate (for analyses of wastewater from the coastal treatment station) and membrane filtration

(for analyses in river samples) procedures were used for the enumeration of fecal coliforms (FC). In each case, 10, 1, and 0.1 mL of water were filtered in duplicate through a 47-mm diameter membrane filter (Millipore Corp., Bedford, MA) with a pore size of 0.45 μm . The membranes were placed on TTC agar plates supplemented with Tergitol (Diagnostic Pasteur). For the spread plate procedure, serial dilutions were made in sterile physiological water (0.9% NaCl) and 100 μL of each were plated on TTC agar plates supplemented with Tergitol in duplicate. Incubation on TTC agar plates was at $44 \pm 0.5^\circ\text{C}$ for 24 h.

For the enumeration of *Salmonella* spp., two most probable number (MPN) procedures were used depending on the type of water (Vassiliadis, 1983; Baleux et al., 1988; Busse, 1995). Three volumes and triplicates were used to determine the MPN: 1 L, 100 mL, and 10 mL. In river samples, the presence of *Salmonella* spp. in 10 L was determined only for monthly samples. When the presence of *Salmonella* spp. was confirmed, the MPN value and confidence intervals of 95% were determined using the De Man table (De Man, 1975).

For river samples, *Salmonella* spp. counts were obtained after pre-enrichment in peptone water broth, without indole, (Diagnostic Pasteur) at 37°C for 24 h. Then, selective enrichment in Rappaport-Vassiliadis (R V 10) broth (Difco) was used

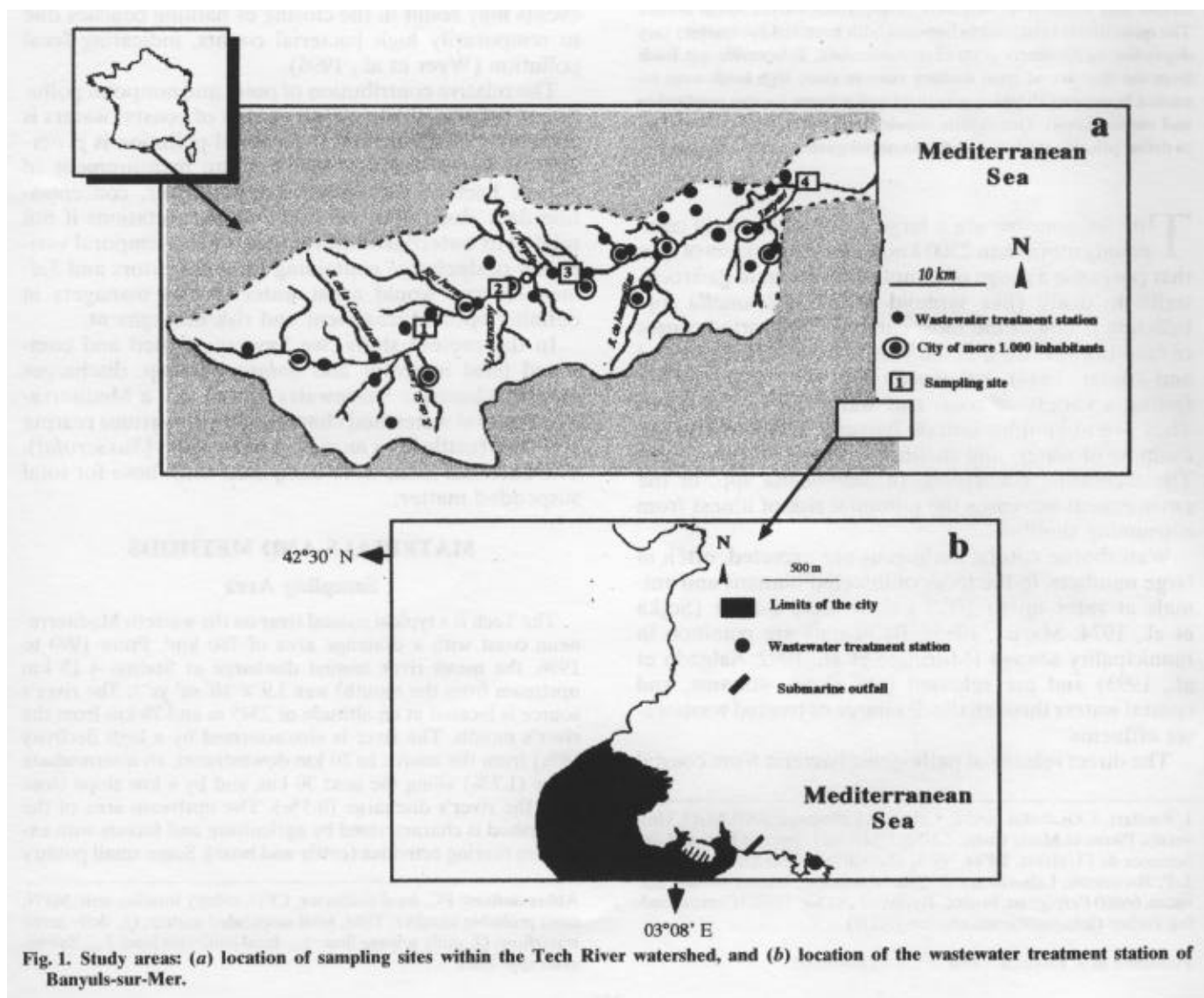


Fig. 1. Study areas: (a) location of sampling sites within the Tech River watershed, and (b) location of the wastewater treatment station of Banyuls-sur-Mer.

with an incubation at 43°C for 24 h. For wastewater samples, *Salmonella* spp. counts were obtained using the MPN technique proposed by Baleux et al. (1988). Briefly, filters were incubated in a selective Selenite F broth (BioMérieux) modified by the addition of novobiocine (45 mg L⁻¹; Sigma) and pril (200 mg L⁻¹; Henkel). After the selective enrichment step, colonies were isolated by spread plating aliquots onto *Salmonella-Shigella* agar (Diagnostic Pasteur) and incubating at 37°C for 24 to 48 h. Typical colonies of *Salmonella* spp. were confirmed by biochemical tests. Isolates were tested for their inability to produce oxidase (Oxidase Test, Difco), their ability to produce H₂S, degrade 4-methylumbelliferyl caprilate (Research Organics), and their inability to oxidize and ferment lactose (Aguirre et al., 1990; Freydiere and Gille, 1991). All *Salmonella* spp. isolates were confirmed by serological identification (CNEVA, Maisons-Alfort, Paris).

River Sampling Strategy

Four sampling stations (1-4) were chosen along the stream (Fig. 1a). Station 1 was located 20 km downstream from the source and was considered as a reference station of the upstream watershed. Station 2 was located 27 km downstream from the source and city of Arles-sur-Tech. Stations 2 and 3 were located upstream and downstream, respectively, from the wastewater effluents released for the city of Amélie-lesBains. Station 4, located 5 km upstream from the river's mouth, was considered as the downstream station where river discharges in the coastal zone were estimated (there was no additional water input to the river from this station to the mouth). Water samples ($n = 19$) were taken monthly at each station from June 1996 to October 1997 and bimonthly in September of each year.

A stratified sampling strategy was used to estimate bacterial loads to include fluctuations of both concentrations and discharge rates (Richards and Holloway, 1987). Three flood events (October 1996, December 1996, and June 1997) and a weak summer rainfall event (September 1997) were analyzed during the study period. Water samples were taken hourly during these events. The total number of samples analyzed during flood events depended on the time of each event. Unfortunately, it was not possible to use the same sampling frequency for both bacterial parameters because the

days were selected from May 1996 to November 1997, including 4 summer and 7 nonsummer sampling days. During each analyzed 24-h period, FC and *Salmonella* spp. were quantified every 3 and 6 h, respectively, for 24 h. The daily concentrations of both parameters were determined from mean values. These concentrations were used to calculate the mean concentration of both parameters during each seasonal period. Mean concentrations were multiplied by the mean daily sewage flow of each seasonal period to calculate bacterial loads.

Hydraulics Parameter

In the river, daily discharge rate (Q_d) was measured at the same stations used for water sampling. These stations were regularly gauged during the sampling period. For the wastewater treatment station the discharge values (Q_d) were obtained from an automatic recording system.

Sediments

Total suspended matter (TSM) were analyzed from river samples. Surface water samples (60 L) were collected monthly at Station 4 during the lowest flow period and hourly (20 L) during flood events. Total suspended matter was quantified and granulometric analyses were performed only during flood events for samples containing at least 3 g of TSM. Total suspended matter concentrations were measured after centrifugation at 20000 × g (CEPA centrifuge type Z41G, Bioblock Scientific) and drying at 40°C until stabilization of the dry weight. Granulometric analyses was performed with a Sedigraph 5100 to quantify the fraction of fine clay particles (<2 µm).

Statistical Analysis

Statistical comparison between different values of concentrations were determined using the Mann Whitney test and regression analysis (Scherrer, 1984). Coefficient of variation of FC/*Salmonella* ratio was determined from mean concentration values of each bacterial parameter. All analyses were made with the Statview software (version 1.03) and a LC630 Macintosh computer. For plotting data, *Salmonella* spp. concentrations (x) were first transformed to $(x + 1)$ to use a logarithmic scale.

Table 1. Hydraulic characteristic of each flood event and sampling frequencies of bacterial parameters.†

Flood event	$Q_{i \text{ max}}$	$Q_{d \text{ max}}$	D_{study}	Nb_{FC}	Nb_{Sal}
	m ³ s ⁻¹		h		
October 1996	170	96.5	144	30	14
December 1996	692	433	144	12	12
June 1997	206	206	24	3	3
September 1997	11.1	6.05	48	11	11

† $Q_{i \text{ max}}$ = maximum instantaneous discharge rate; $Q_{d \text{ max}}$ = maximum daily mean discharge rate; D_{study} = time of study; Nb = number of samples.

Table 2. p Values determined from the statistical comparison (Mann Whitney test) of mean bacterial counts between the summer ($n = 9$) and nonsummer ($n = 10$) periods. The comparison was made for FC and *Salmonella* spp.

Stations	FC	<i>Salmonella</i> spp.
1	0.37	0.41
2	<0.01*	0.41
3	0.016*	1.0
4	0.97	0.14

* Significance at the 5% level.

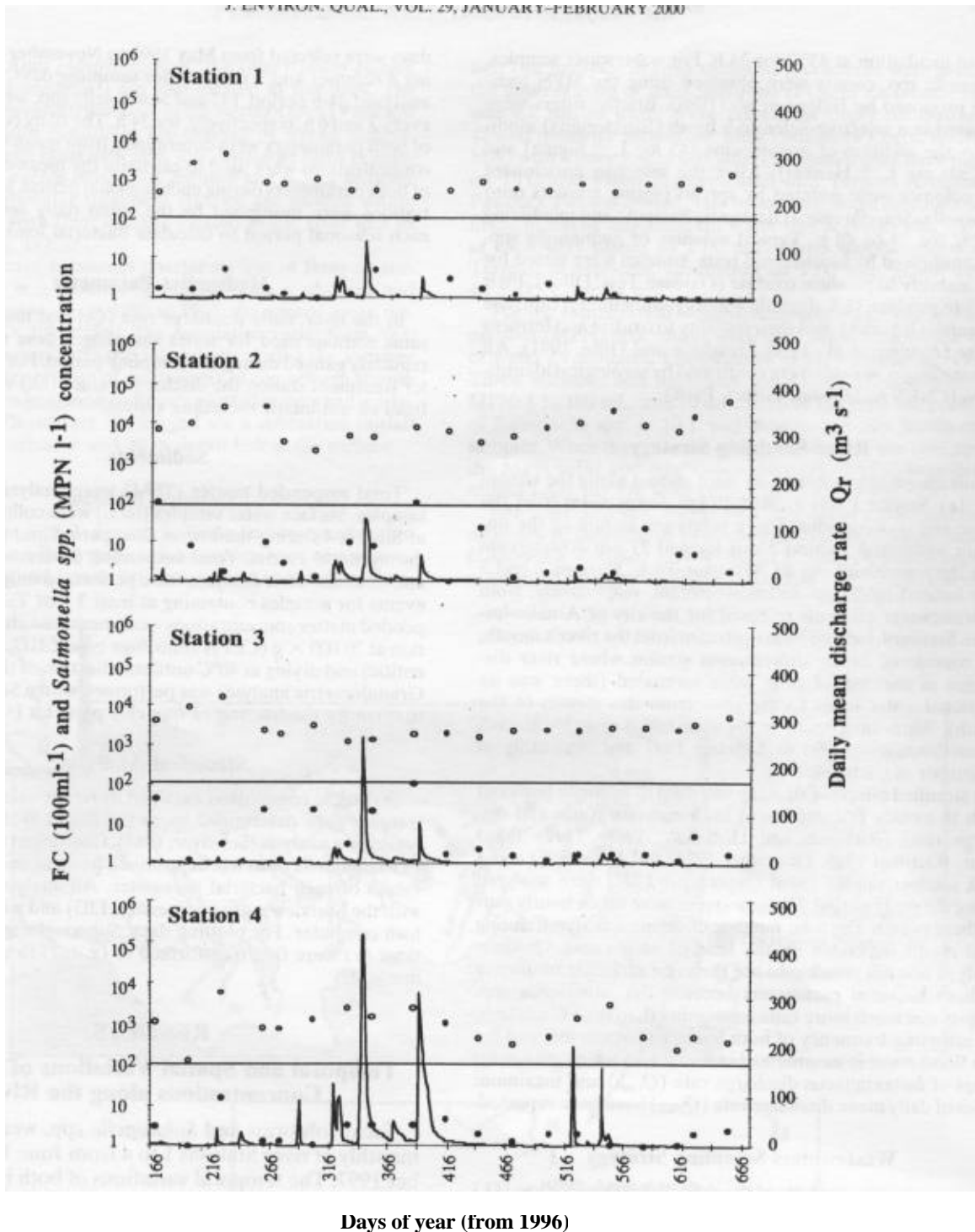


Fig. 2. Temporal variations of fecal coliform (open circles) and *Salmonella* spp. (closed circles) concentrations and daily mean discharge rates (Q_r) (solid line) from the monthly sampling at Stations 1, 2, 3, and 4. The horizontal line represents the European Guideline fecal coliforms

trations fluctuated between zero in I(1 L to 1.1×10^2 MPN L⁻¹). The highest concentrations were reached on the saure day at Stations 1, 2, and 3. The highest *Salmonella* spp. concentration at downstream Station 4 was 15 MPN L⁻¹.

To evaluate the effect of the summer season on the concentrations of both bacterial parameters, summer ($n = 9$) and nonsummer ($n = 10$) samples were grouped and mean values were compared (Table 2). For fecal coliforms, the concentrations were not significantly different during the summer season at Stations 1 and 4, but

were significantly higher during this period at Stations 2 and 3. For *Salmonella* spp., the concentrations were not significantly higher during the summer lime.

Figure 3 presents the results from samples collected during the stratified sampling at Station 4 over a 16-mo period (including low waterflow and flood events). High fluctuations of FC and *Salmonella* spp. concentrations were observed during this period. The numerous peaks of high discharge rates indicate the response to runoff events generated by rainfall. During the study, the daily mean discharge rate (Q_r) at Station 4 varied between

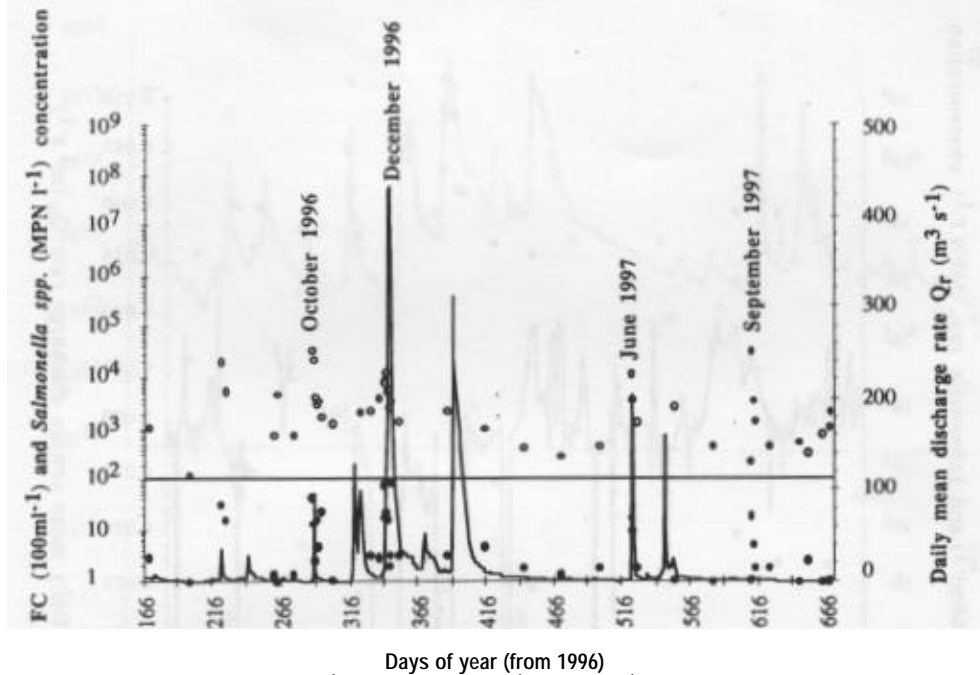


Fig.3. Temporal variations of fecal coliform (open circles) and *Salmonella* spp. (closed circles) concentrations and daily mean discharge rates (Q_r) (solid line) from the stratified sampling at Station 4. The horizontal line represents the European Guideline fecal coliforms for bathing waters.

1.08 and 433 $\text{m}^3 \text{s}^{-1}$. The maximum concentrations of FC and *Salmonella* spp. were 5×10^4 CFU 100 mL^{-1} and 1.1×10^7 MPN L^{-1} , respectively. The highest concentrations were observed during flood events (Fig. 3).

Temporal Variations of Bacterial Concentrations in Wastewater Effluents

A total of 11 sample days have been defined from May 1996 to November 1997 for sampling the effluents of the wastewater coastal station at Banyuls-sur-Mer. These periods were selected from the analysis of the sewage flow (Q_r) variations during the past 4 yr (1992-1995). The temporal fluctuations of FC and *Salmonella* spp. concentrations are reported in Fig. 4. Mean daily concentrations of FC fluctuated between 2.5×10^0 CFU 100 mL^{-1} and 1.4×10^1 CFU 100 mL^{-1} . The densities were significantly higher during the summer (Days of year: 185, 219, 596, and 632; $p = 0.014$). Mean daily concentrations of *Salmonella* spp. varied from 2.3 to 487 MPN L^{-1} . The highest values were recorded during the summer (Days of year: 219, 596, and 632), except the high value reported in March 1997 (Day of year 430; 487 MPN L^{-1}) (Fig. 4). These values, especially FC, are significantly higher than that of the river during low waterflow.

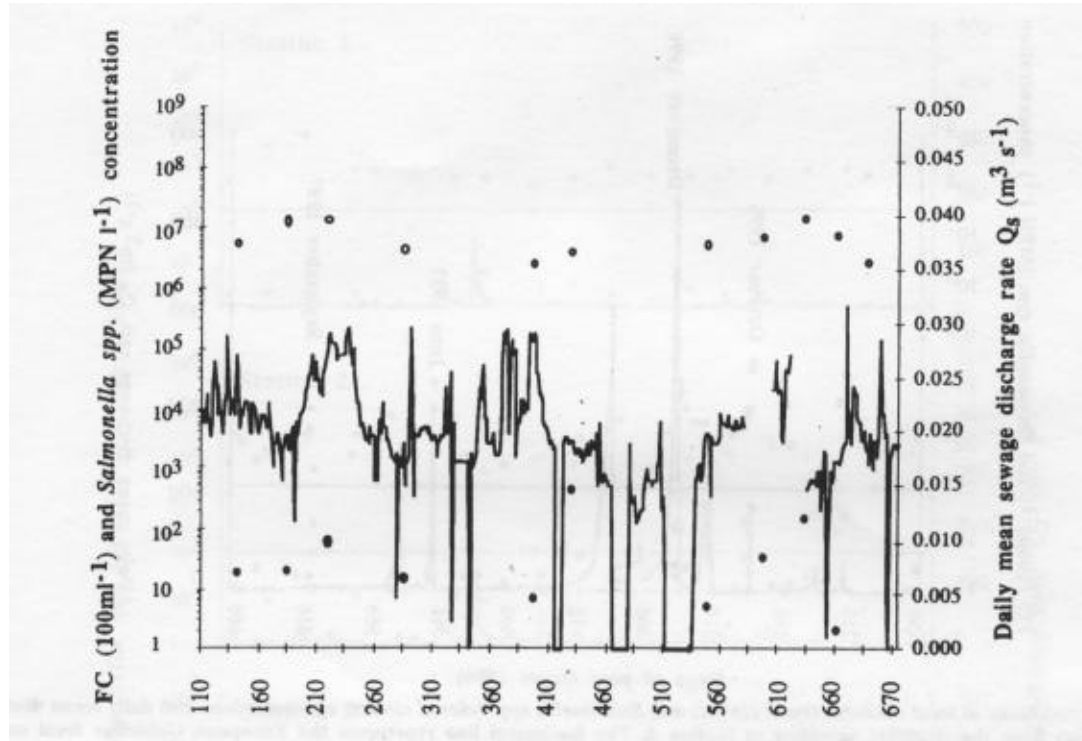
Estimation of Fecal Coliforms and *Salmonella* spp. Fluxes from Nonpoint and Point Pollution Sources

The estimation of bacterial flux is generally difficult because of the low frequency of sample collection compared with the continuous analysis of waterflow. In rivers, daily loads are generally estimated from the relationship between observed mean daily loads and discharge rates (Q_r) (Kao and Liu, 1996). Daily bacterial

loads of FC (L_{fc}) and *Salmonella* spp. (L_{sal}) were calculated by multiplying the daily stream waterflow by bacterial concentrations. Bacterial loads are well correlated with daily stream waterflow in a log-log plot (Fig. 5). The logarithmic-linear regressions are $\log L_{fc} = 1.43 \times \log Q_r + 11.86$ and $\log L_{sal} = 1.86 \times \log Q_r + 7.23$.

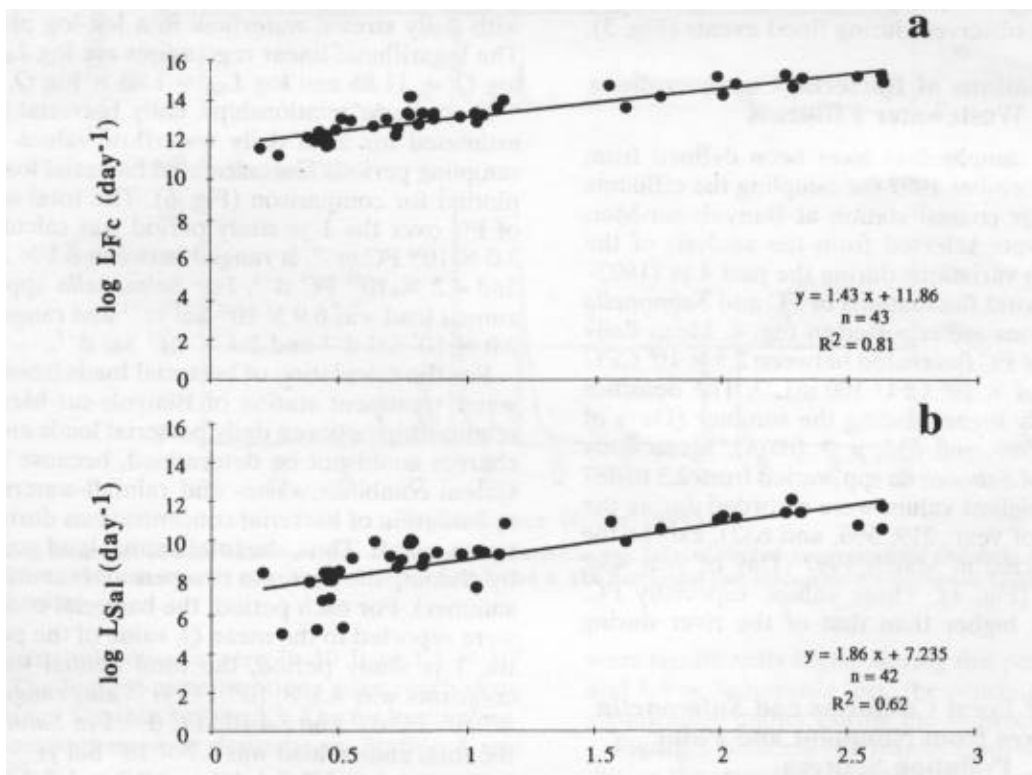
From these relationships, daily bacterial loads were estimated for each daily waterflow values during the sampling period. The calculated bacterial loads are also plotted for comparison (Fig. 6). The total annual load of FC over the 1-yr study period was calculated to be 3.0×10^6 FC yr^{-1} . It ranged between 8.1×10^4 FC d^{-1} and 4.2×10^5 FC d^{-1} . For *Salmonella* spp., the total annual load was 6.9×10^4 Sal yr^{-1} and ranged between 2.0×10^3 Sal d^{-1} and 1.4×10^5 Sal d^{-1} .

For the calculation of bacterial loads from the wastewater treatment station of Banyuls-sur-Mer, a similar relationship between daily bacterial loads and daily discharges could not be determined, because the sewage system combines waste- and rainfall-waters. It results in a dilution of bacterial concentrations during rain and storm events. Thus, the total annual load was estimated by dividing the year into two periods (summer and nonsummer). For each period, the bacterial concentrations were reported to the mean Q_r value of the period. Over the 1-yr study period, the total annual load of fecal coliforms was 4.3×10^6 FC yr^{-1} and ranged between 4.7×10^4 FC d^{-1} and 3.41×10^5 FC d^{-1} . For *Salmonella* spp., the total annual load was 4.7×10^4 Sal yr^{-1} and ranged



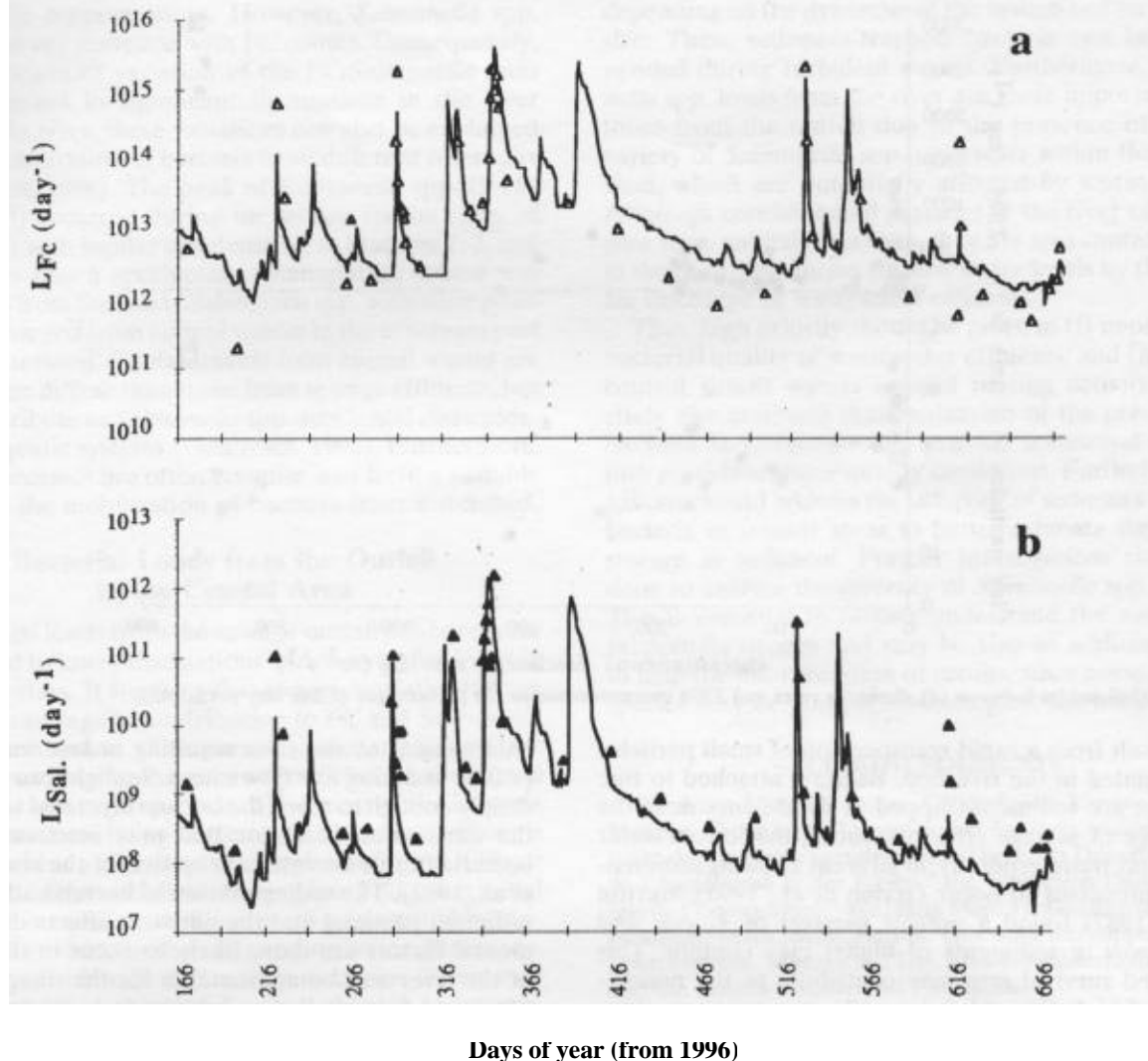
Days of year (from 1996)

Fig.4. Temporal variations of fecal coliforms (open circles) and *Salmonella* spp. (closed circles) concentrations and daily sewage discharges (Q_s) (solid line) at the wastewater treatment station of Banyuls-sur-Mer.



\log (daily mean discharge rate, Q_r) ($m^3 s^{-1}$)

Fig.5. Relationships between (a) daily mean discharge rates (Q_r) and daily fecal coliforms loads (L_m), or (h) daily *Salmonella* spp. loads (L_{sal})



Days of year (from 1996)

Fig. 6. Estimation of daily loads of (a) fecal coliforms and (b) *Salmonella* spp. The solid line is calculated from the discharge rate by the regression function and points are calculated from observed values.

range of river flow rates during flood events (Fig. 7). The highest TSM concentrations were recorded for waterflow values lower than $20 \text{ m}^3 \text{ s}^{-1}$ or higher than $400 \text{ m}^3 \text{ s}^{-1}$ (Fig. 7a). High proportions of small particles ($<2 \mu\text{m}$) were found for low flow rates below $20 \text{ m}^3 \text{ s}^{-1}$ and ranged between 25 and 45% of total dry weight of TSM (Fig. 7b). Discharge rate below $20 \text{ M}^3 \text{ s}^{-1}$ were measured during the first hours of flood events.

DISCUSSION

The aim of this study was to estimate and to compare the annual loads of FC and *Salmonella* spp. in coastal areas from two sources of bacterial pollution: (i) the direct release of effluents from a coastal wastewater treatment station through a submarine outfall, and (ii) a river discharge. Although the annual fluxes of FC were not very different between the two coastal pollution sources monitored in this study, bacterial loads were characterized by different temporal fluctuations and by the presence in river of associated particles. Further

more, the total discharge of *Salmonella* spp. from the river was more important and submitted to similar fluctuations.

Bacterial Loads from the River

Bacterial loads from the river varied greatly during the year and were highly related to storm events. Storm events contribution to FC and *Salmonella* spp. discharges in coastal waters represents 95 and 99% of annual loads, respectively. Wyer et al. (1996) reported similar results for different coastal river discharges to St. Aubin Bay in England. For these authors, the relation between contamination and storm events was due to an entrainment of sediments and associated microbes from bed and banks of channels during hydrographic events. In this study, the particle-size distribution during storm events showed that high percentages of fine particles ($<2 \mu\text{m}$) were resuspended during the first hours of storm events. This clay fraction was dominant during the increasing flow of each event up to $20 \text{ m}^3 \text{ s}^{-1}$ and

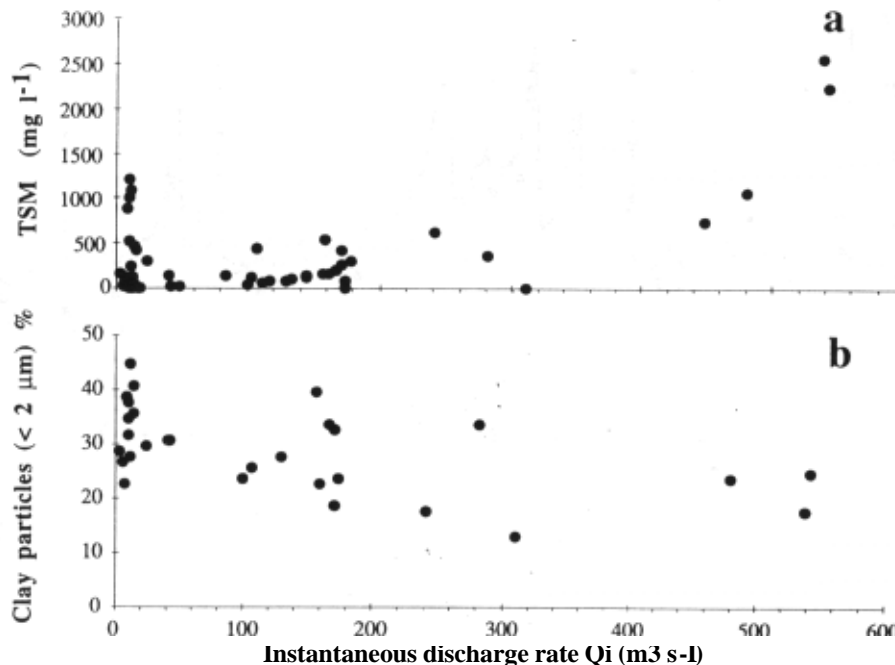


Fig. 7. Relationships between (a) discharge rates and TSM concentrations or (b) percentages of fine clay particles.

may result from a rapid resuspension of small particles accumulated in the riverbed. Bacteria attached to fine particles are sediment-trapped in *dead-zones* near the discharge of sewage effluents during the lowest water levels and, more generally, in all areas allowing sedimentation processes to occur (Irvine et al., 1995). Burton et al. (1987) found a greater survival of *E. coli* and *S. newport* in sediments of higher clay content. This increased survival rate may contribute to the maintenance of high FC and *Salmonella spp.* concentrations in sediment reservoirs.

Storm events occurring at the end of the summer season yielded high bacterial loads. For instance, the rainfall event in September 1997 occurred within 48 h and resulted in the discharge of 6.2×10^{10} *Salmonella spp.* into the coastal area. Bacteria were disseminated at a low flow ($Q_{me} = 6.05 m^3 s^{-1}$). Inversely, the storm event of October 1996 was longer in duration (144 h) with a 16-fold higher flow and resulted in a discharge of 3.9×10^{10} *Salmonella spp.*

In the Tech River, FC and *Salmonella spp.* concentrations were submitted to important variations throughout the year, but also between the four sampling stations. The densities generally increased at Stations 2 and 3 and then decreased downstream at Station 4. The increase of bacterial densities at Stations 2 and 3 was due to the discharge of effluents from different wastewater treatment stations in this section of the river. One of these effluents released near Arles-sur-Tech, (3 km upstream from Station 2) was not treated and discharged directly into the river (FC concentrations were 1.1×10^6 CFU $100 mL^{-1}$ in July 1997). In this upper part of the river, the slope is high (1.7-8%) and the low residence time of waters do not allow for natural self-purification processes to occur. Inversely, downstream from Station 3, the slope decreases (1.7-0.3%) concomitantly with an

enlargement of the river resulting in less water depth (< 0.80 m during low flow rates). Sunlight can penetrate deeply enough to reach the bottom layer and is probably the most effective factor that may inactivate enteric bacteria in this downstream section of the river (Curtis et al., 1992). The sedimentation of bacteria adsorbed to sediment particles and the negative effects of environmental factors are more likely to occur in this section of the river and be accountable for the disappearance of FC and *Salmonella spp.* from surface waters (Gannon et al., 1983; Curtis et al., 1992; Auer and Niehaus, 1993). Inversely, bacteria adsorbed to sediment particles survive better because they may be protected from the influence of irradiation and by the presence of organic matter (McFeters et al., 1974; Sherer et al., 1992; Davies et al., 1995). Sediments often contain 100 to 1000 times as many fecal indicator bacteria than the overlying water (Ashbolt et al., 1993). In this study, high concentrations were also reported in the sediments sampled in deadzones located in the downstream section (near Station 4). Among two different sediment reservoirs analyzed at 12 different times during the sampling period, FC concentrations varied in the range of 10^1 to 9×10^6 CFU per $100 cm^3$ of surface sediment. These sediment-trapped bacteria represent an important source of fecal pollution, because contaminated particles can be resuspended after river bottom disturbance by rainfall events (Howell et al., 1995; Wilkinson et al., 1995).

The discharge of both FC and *Salmonella spp.* cells by domestic and municipal sewage is well known and has been reported in different studies (Baleux et al., 1988; Morinigo et al., 1992; Oragui et al., 1993). A total of 13 municipal wastewater treatment stations and its tributaries routinely discharge directly into the Tech River. Thus, the variability of discharge rates may contribute to the spatio-temporal fluctuations of FC and

Salmonella concentrations. However, *Salmonella* spp. counts did not correlate with FC counts. Consequently, the coefficient of variation of the FC/*Salmonella* ratio was subjected to significant fluctuations in the river (299%). In river, these variations can also be explained by the mobilization of bacteria from different reservoirs (Geldreich, 1996). The peak of *Salmonella* spp. (>110 MPN L⁻¹) occurred during the winter season (Day of year: 386) with similar cell densities at Stations 1, 2, and 3 suggests that a accidental contamination source was upstream from Station 1. *Salmonella* spp. cells were probably discharged from animal wastes in the upstream part of the watershed. Contaminants from animal wastes are much more diffuse than those from sewage effluents, but may contribute to

Bacterial Loads from the Outfall in the Coastal Area

Bacterial loads from the sewage outfall offshore were submitted to lower fluctuations and to a regular input in coastal waters. It suggests that sewage outfalls offshore have a more regular contribution to FC and *Salmonella* spp. dissemination. The particle concentration of the released waters is low since most particles are eliminated during the treatment process. Thus, most of the bacteria released are free-living bacteria. Furthermore, effluents are released at a 20-m depth through a multiport diffuser at the end of the outfall discharge pipe. Following initial dilution, bacteria[contaminants are transported by currents and mixed with the surrounding water. Moreover, the survival of free-living bacteria in marine waters may be more limited in coastal waters than in sediments, as they are submitted to both osmotic and starvation stresses in oligotrophic coastal waters (Joux et al., 1997).

CONCLUSIONS

To our knowledge, this is the first study addressing the evaluation and comparison of *Salmonella* spp. pollution sources in terms of fluxes. The comparison of bacterial fluxes from point vs. nonpoint sources should be useful for water quality managers to develop remediation strategies for the improvement of bacterial quality of coastal waters.

On the one hand, sewage outfalls offshore participate in a regular and permanent discharge of bacteria in the coastal environment. These bacteria are released at a few hundred meters from the coast and most of these are free-living cells that probably rapidly lose their viability (Joux et al., 1997).

On the other hand, storm and rain events occur sporadically and contribute to major discharges of fecal

depending on the dynamics of the system and on particle size. Then, sediment-trapped bacteria can be resuspended during turbulent events. Furthermore, *Salmonella* spp. loads from the river are more important than those from the outfall due to the presence of a wide variety of *Salmonella* spp. reservoirs within the watershed, which are potentially affected by storm events. Although contaminated particles in the river can originate from animal reservoirs, they are also contaminated in the river-bed during the low water levels by the regular discharge of wastewater effluents.

Thus, high priority should be given to (i) improve the bacterial quality of wastewater effluents, and (ii) better control runoff waters around rearing activities. This study also confirms that evaluation of the presence of bacteria in sediment may provide additional insights into persistent water quality conditions. Further investigations should address the behavior of sediment-trapped bacteria in coastal areas to better estimate the risk of storage in sediment. Further investigations should be done to analyze the diversity of *Salmonella* spp. strains. This is essential to further understand the ecology of salmonella species and may be also an additional tool to help the interpretation of results, since some of these species may be good epidemiological markers.

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